2014 ME-X Conference Program Book

Contents

Program Overview

Sunday, June 15		
3:30-7:00 PM	Registration Check In	Bayshore Grand Foyer
4:45-5:30 PM	Opening Reception and Poster Session (Poster Group 1)	Bayshore Salon DEF
5:30-6:15 PM	Plenary Lecture: Christophe Schilling, Genomatica	Bayshore Salon ABC
6:15-7:00 PM	Plenary Lecture: Vineet Rajgarhia, Total	Bayshore Salon ABC
7:00-7:30 PM	Opening Reception Continued	Bayshore Salon DEF
Monday, June 16		
7:00 AM-12:00 PM	Registration Check In	Bayshore Grand Foyer
7:30-8:00 AM	Breakfast	Stanley Park Ballroom
8:00-10:05 AM	**Session 1: Parts to Protein Scale Engineering** Session Chairs: Timothy Lu, MIT and Fernando Valle, Codexis	Bayshore Salon ABC
10:05-10:30 AM	Break	Bayshore Grand Foyer
10:30 AM-12:35 PM	**Session 2: Pathway Scale Engineering** Session Chairs: Michelle Chang, UC Berkeley, George Chen, Tsinghua University and John Dueber, UC Berkeley	Bayshore Salon ABC
12:35-2:00 PM	Lunch	Stanley Park Ballroom
2:00-4:05 PM	**Session 3: Genome Scale Engineering** Session Chairs: Hal Alper, University of Texas at Austin and Zach Serber, Zymergen	Bayshore Salon ABC
4:00-4:45 PM	Free Time	
4:45-5:30 PM	**Rapid Fire Poster Session (Poster Group 1)**	Bayshore Salon ABC
5:30-6:30 PM	Poster Session (Poster Group 1) Session Chairs: Irina Borodina, Novo Nordisk Biosustainability Center, Michael Jewett, Northwestern University, Julius Lucks, Cornell University, Caroline Peres, DuPont	Bayshore Salon DEF
6:30-7:00 PM	Buses to Earl's	In front of hotel
6:45-8:00 PM	Reception at Earl's in Yaletown	
7:00 AM-12:00 PM	Registration Check In	Bayshore Grand Foyer
Tuesday, June 17		
7:00 AM-12:00 PM	Registration Check In	Bayshore Grand Foyer
7:30-8:00 AM	Breakfast	Stanley Park Ballroom
8:00-10:05 AM	**Session 4: Systems Biology and Engineering** Session Chairs: Iman Famili, Intrexon, Hiroshi Shimizu, Osaka University and Jamey Young, Vanderbilt University	Bayshore Salon ABC
10:05-10:30 AM	Break	Bayshore Grand Foyer
10:30 AM-12:35 PM	**Session 5: Computational Methods and Design** Session Chairs: Anthony Burgard, Genomatica and Costas Maranas, Penn State University	Bayshore Salon ABC
12:35-2:00 PM	Lunch	Stanley Park Ballroom
2:00-4:00 PM	**Session 6: Rational Design-Build-Test Era** Session Chairs: Maciek Antoniewicz, University of Delaware, Akihiko Kondo, Kobe University and Michael Lynch, Duke University	Bayshore Salon ABC
4:00-4:45 PM	Free Time	
4:45-5:30 PM	Rapid Fire Poster Session (Poster Group 2)	Bayshore Salon ABC
5:30-6:30 PM	Reception and Poster Session (Poster Group 2)	Bayshore Salon DEF

Wednesday, June 18		
7:00 AM- 12:00 PM	Registration Check In	Bayshore Grand Foyer
7:30-8:00 AM	Breakfast	Stanley Park Ballroom
8:00-10:05 AM	**Session 7: Industrial Applications Related to Chemicals and Fuels** Session Chairs: Ramon Gonzalez, Rice University and Kristala Jones Prather, MIT	Bayshore Salon ABC
10:05-10:30 AM	Break	Bayshore Grand Foyer
10:30 AM-12:35 PM	**Session 8: Metabolic Engineering Applications to Impact Health** Session Chairs: Christian Metallo, UC San Diego and Nicola Zamboni, ETH	Bayshore Salon ABC
12:35-2:00 PM	Lunch	Stanley Park Ballroom
2:00-4:05 PM	**Session 9: Emerging Technologies** Session Chairs: Emily Leproust, Twist Biosciences	Bayshore Grand Foyerr
4:05-5:00 PM	IMES General Assembly Meeting	Bayshore Salon ABC
5:00-6:00 PM	Poster Session and Reception (Poster Group 2)	Bayshore Salon DEF
6:00-7:00 PM	Awards Presentation and Lecture	Bayshore Salon ABC
7:00-9:00 PM	Dinner	Stanley Park Ballroom
Thursday, June 19		
7:15-8:30 AM	Registration Check In	Bayshore Grand Foyer
7:30-8:00 AM	Breakfast	Stanley Park Ballroom
8:00-11:00 AM	**Workshop — Tools for Commercial Bioprocess Design and Economic Analysis**	Salon ABC

Conference Organizers

Conference Chair

- Ryan Gill, University of Colorado, Boulder

Conference Co-Chairs

- Todd Peterson, Synthetic Genomics, Inc.
- Chris Voigt, Massachusetts Institute of Technology
- Stefan Wildt, Novartis

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- Jens Nielsen, Chalmers University of Technology
- Vassily Hatzimanikatis, École Polytechnique Fèdèrale De Lausanne
- Sang Yup Lee, Korea Advanced Institute of Science and Technology
- James Liao, University of California, Los Angeles
- Gregory Stephanopoulos, Massachusetts Institute of Technology

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Organizing Society

imes
INTERNATIONAL METABOLIC ENGINEERING SOCIETY

The International Metabolic Engineering Society (IMES) seeks to promote and advance metabolic engineering as an enabling science for bio-based production of materials, pharmaceuticals, food ingredients, chemicals and fuels. It is a technological community of the Society for Biological Engineering. IMES aims to:

- Raise interest, understanding, and recognition of engineers' and scientists' roles in metabolic engineering.
- Promote metabolic engineering as an enabling science for development of sustainable and environmentally friendly bio-based production of chemicals, liquid fuels, energy and materials.
- Organize international conferences in the field of metabolic engineering and industrial biotechnology, here under the bi-annual Metabolic Engineering conference.
- Coordinate activities in the field of industrial biotechnology with other organizations (such as the World Council on Industrial Biotechnology, BIO and EUROPA-BIO).
- Infuse awareness to the public and civil society at large on metabolic engineering with both opportunities and risks.
- Drive for scientific excellence in the field of metabolic engineering through establishment and maintenance of prestigious awards for both young and senior researchers.
- Foster and promote peer-review of research on metabolic engineering through publications, journals and and/or conferences.

Welcome Address

Greetings!

We want to welcome you to beautiful Vancouver, British Columbia and the Metabolic Engineering X (ME-X) conference, brought to you by the International Metabolic Engineering Society (IMES) and the Society for Biological Engineering (SBE) – AIChE Technological Communities.

The field of metabolic engineering has rapidly emerged as an important area of research whose effects range from the environment to medicine. ME-X is one of the few forums that bring together biologists, chemists, and engineers to discuss ways in which their disciplines can collaborate to find novel solutions to fundamental and applied problems that are limiting the development of new technologies and therapies.

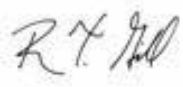
We hope you're ready for a tremendous conference. On Sunday, you'll have the opportunity to hear from Vineet Rajgarhia, the VP of Biotech R&D at Total New Energies, and Christophe Schilling, the CEO of Genomatica. Throughout the week we have top notch speakers in all of the following sessions. Monday's talks will focus on Parts to Protein Scale Engineering, Pathway Scale Engineering, and Genome Scale Engineering. Tuesday will start with Systems Biology and Engineering and will be followed by Computational Methods and Design and the Rational Design-Build-Test Era. Wednesday will look to the industrial applications of metabolic engineering in fuels, chemicals and health. Thursday will feature a half day workshop on Tools for Commercial Bioprocess Design and Economic Analysis.

In addition to oral presentations, the conference will also feature over 220 posters and approximately 50 rapid fire presentations. Breakfasts, lunches, receptions and dinners at this Gordon-style conference will ensure that you have the opportunity to hear every speaker while also having time to interact with your peers.

A lot of work has gone into making this conference a success. We would not have been able to do this without the contributions of my co-chairs Todd Peterson (Synthetic Genomics), Chris Voigt (MIT) and Stefan Wildt (Novartis). We also acknowledge the contributions of our Organizing Committee and contributions of our organizing committee. We also acknowledge the contributions of our Scientific Advisory Board, who were instrumental in selecting our invited speakers. Our Session Chairs deserve our thanks for their careful deliberations in order to select an outstanding selection of presenters for contributed talks from among a very strong set of abstracts. We extend additional thanks to all of our corporate sponsors, academic supporters, and media partners without whom the conference could not happen.

Finally, we would like to thank you for attending the conference. We hope these five days will be pleasant, educational, and inspiring.

Sincerely,



Ryan Gill
ME-X Conference Chair

Todd Peterson, ME-X Conference Co-Chair
Chris Voigt, ME-X Conference Co-Chair
Stefan Wildt, ME-X Conference Co-Chair

Technical Program

Sunday, June 15	
4:45-5:30 PM	**Opening Reception and Poster Session (Poster Group 1)**
5:30-6:15 PM	Plenary Lecture: A 15-Year Journey to Figure out How to Derive the Most Economic Value from Metabolic Engineering — Christophe Schilling, Genomatica
6:15-7:00 PM	Plenary Lecture: Perspectives on Creating & Scaling-up Better Energy Solutions — Vineet Rajgarhia, Total
7:00-7:30PM	Opening Reception Continued
Monday, June 16	
7:30-8:00 AM	Breakfast
8:00-10:05 AM	**Session 1: Parts to Protein Scale Engineering** Session Chairs: Timothy Lu, MIT and Fernando Valle, Codexis
8:00-8:25 AM	Invited Speaker: New Enzymes by Evolution: Expanding Nature's Catalytic Repertoire — Frances Arnold, Caltech
8:25-8:50 AM	Synthetic Enzymes for Synthetic Biology — Alexandre Zanghellini, Arzeda
8:50-9:15 AM	Invited Speaker: *In Vivo* Evolution of Enzymes and Metabolic Pathways for the Production of Chemicals — Philippe Soucaille, University of Toulouse
9:15-9:40 AM	Development of Adaptable Synthetic Promoters to Optimize Protein Scale Engineering in Yeast — Ian Fotheringham, Ingenza, Ltd.
9:40-10:05 AM	Invited Speaker: Protein Engineering of Isoprene Synthase Enables High Titer Isoprene Production — Maggie Cervin, E.I. du Pont de Nemours
10:05-10:30AM	Break
10:30AM-12:35 PM	**Session 2: Pathway Scale Engineering** Session Chairs: Michelle Chang, UC Berkeley, George Chen, Tsinghua University and John Dueber, UC Berkeley
10:30-10:55 AM	Invited Speaker: Engineering Organisms and Processes for Cost-Effective Lipid Production — Greg Stephanopoulos, MIT
10:55-11:20 AM	Pathway Engineering Meets Evolution: New Approaches for Improving Pentose Catabolism in Yeast — Hal Alper, University of Texas at Austin
11:20-11:45 AM	Invited Speaker: Rewriting Pathways to Life for Carbon Conservation — Jim Liao, UCLA
11:45 AM - 12:10 PM	Engineering Static and Dynamic Tuning of Malonyl-CoA Derived Metabolite Biosynthesis in Microorganisms — Mattheos Koffas, RPI
12:10-12:35 PM	Invited Speaker: Novel Biosynthetic Pathways for Production of Acids and Alcohols — Kristala Jones Prather, MIT
12:35-2:00 PM	Lunch
2:00-4:05 PM	**Session 3: Genome Scale Engineering** Session Chairs: Hal Alper, University of Texas at Austin and Zach Serber, Zymergen
2:00-2:25 PM	Invited Speaker: Comprehensive characterization of genotype to phenotype relationships in *E.coli* — Morten Sommer, Technical University of Denmark
2:25-2:50 PM	Metabolic Engineering via RNAi-Assisted Genome Evolution (RAGE)- Huimin Zhao, University of Illinois
2:50-3:15 PM	Genetically Encoded Biosensors + FACS + Recombineering: Versatile Tools for Strain and Enzyme Development — Michael Bott, Forschungzentrum Juelich
3:15-3:40 PM	A Unique Bioengineering Platform for the Efficient Optimization of Metabolic Pathways — Kedar Patel, DNA2.0 Inc.
3:40-4:05 PM	Flux Response of Glycolysis and Storage Metabolism during Rapid Feast/Famine Conditions in Penicillium Chrysogenum Using Dynamic 13C Labeling — S. Ajoscha Wahl, DUT, Kluyver Netherlands
4:00-4:45 PM	Free Time
4:45-5:30 PM	Rapid Fire Poster Session (Poster Group 1)
5:30-6:30 PM	**Poster Session (Poster Group 1)** Session Chairs: Irina Borodina, Novo Nordisk Biosustainability Center, Michael Jewett, Northwestern University, Julius Lucks, Cornell University, Caroline Peres, DuPont
6:30-7:00 PM	Buses to Yale Town
6:45-8:00 PM	Reception

Tuesday, June 17	
7:30-8:00 AM	Breakfast
8:00-10:05AM	**Session 4: Systems Biology and Engineering** Session Chairs: Iman Famili, Intrexon, Hiroshi Shimizu, Osaka University and Jamey Young, Vanderbilt University
8:00-8:25 AM	Invited Speaker: Systems Metabolic Engineering of Microorganisms for the Production of Polymers and Monomers — Sang Yup Lee, KAIST
8:25-8:50 AM	Advances in 13C Metabolic Flux Analysis: Complete-MFA, Co-Culture MFA and Dynamic MFA — Maciek Antoniewicz, University of Delaware
8:50-9:15 AM	Invited Speaker: Integrating Kintetic Models of Metabolism with k-OptForce for Strain Design- Costas Maranas, Penn State University
9:15-9:40 AM	Design of Terpenoid Producing Synthetic Microbial Cell Factories by Integrative *in silico* Modeling and "Omics" Data Analysis — Dong-Yup Lee, National University of Singapore
9:40-10:05 AM	Invited Speaker: Opening Pandora's Box — Lars Nielsen, Australian Institute for Bioengineering & Nanotechnology
10:05-10:30 AM	Break
10:30 AM-12:35 PM	**Session 5: Computational Methods and Design** Session Chairs: Anthony Burgard, Genomatica and Costas Maranas, Penn State University
10:30-10:55 AM	Invited Speaker: Integration of *in silico* Design and Experimental Evaluation for Creation of Microbial Cell Factories — Hiroshi Shimizu, Osaka University
10:55-11:20 AM	Fast Enumeration of Smallest Metabolic Engineering Strategies in Genome-Scale Networks — Steffen Klamt, Max Planck Institute
11:20-11:45 AM	Mapping Photoautotrophic Carbon Metabolism Using the INCA 13C Flux Analysis Platform — Jamey Young, Vanderbilt University
11:45 AM - 12:10 PM	An Integrated Multi-Omics and Computational Characterization of Seven Unique *Escherichia coli* Production Chassis Commonly-Used in Industrial Biotechnology — Adam Feist, UC San Diego/Technical University of Denmark
12:10-12:35 PM	Deepak Chandran, Autodesk is the last spot in the Computational Methods session
12:35-2:00PM	Lunch
2:00-4:00 PM	**Session 6: Rational Design-Build-Test Era** Session Chairs: Maciek Antoniewicz, University of Delaware, Akihiko Kondo, Kobe University and Michael Lynch, Duke University
2:00-2:30 PM	Invited Speaker: Using Systems Biology for Identification of Novel Metabolic Engineering Targets — Jens Nielsen, Chalmers University of Technology
2:30-3:00 PM	Invited Speaker: Chris Voigt, MIT
3:00-3:30 PM	Invited Speaker: Programming Biological Operating Systems: Genome Design, Assembly and Activation — Daniel Gibson, J. Craig Venter Institute and Synthetic Genomics, Inc.
3:30-4:00 PM	TBD
4:00-4:45 PM	Free Time
4:45-5:30 PM	Rapid Fire Poster Session (Poster Group 2)
5:30-6:30 PM	Reception and Poster Session (Poster Group 2)
Wednesday, June 18	
7:30-8:00 AM	Breakfast
8:00-10:05 AM	**Session 7: Industrial Applications Related to Chemicals and Fuels** Session Chairs: Ramon Gonzalez, Rice University and Kristala Jones Prather, MIT
8:00-8:25 AM	Invited Speaker: Metabolic Engineering of Yarrowia lipolytica to Produce High Value Products — Quinn Zhu, DuPont
8:25-8:50 AM	Harnessing Metabolism for Sustainable Production of Chemicals: From Systems Biology to Commercial Scale — Mark Burk, Genomatica
8:50-9:15 AM	Designing Microorganisms for Production of Diverse Biopolymers — George Chen, Tsinghua University
9:15-9:40 AM	Design of Microbial Cell Factories for Lignocellulosic Biorefinery — Akihiko Kondo, Kobe University
9:40-10:05 AM	Invited Speaker: Metabolic Engineering of Complex Natural Product Pathways in Bacteria — Ajikumar Parayil, Manus Biosciences

Technical Program

10:05-10:30 AM	Break
10:30 AM-12:35 PM	**Session 8: Metabolic Engineering Applications to Impact Health** Session Chairs: Christian Metallo, UC San Diego and Nicola Zamboni, ETH
10:30-10:55 AM	Invited Speaker: Prosthetic Networks — Synthetic Biology-Inspired Treatment Strategies For Metabolic Disorders — Martin Fussenegger, ETH
10:55-11:20 AM	Ovarian Cancer Metabolism: Systems-Scale Dynamics as a Platform for Identifying Therapeutic Targets — Mark Styczynski, Georgia Tech
11:20-11:45 AM	Invited Speaker: Metabolic Strategies to Enhance the Toxicity of Nitric Oxide in Pathogens — Mark Brynildsen, Princeton University
11:45 AM - 12:10 PM	Prediction and Quantification of Bioactive Microbiota Metabolites in Murine Gut — Kyongbum Lee, Tufts University
12:10- 12:35 PM	Invited Speaker: The Gene Regulatory Network of Mycobacterium tuberculosis, v1.0 — David Sherman, Seattle Biomedical Research Institute
12:35-2:00 PM	Lunch
2:00-4:05 PM	**Session 9: Emerging Technologies** Session Chair: Emily Leproust, Twist Biosciences
2:00-2:25 PM	Invited Speaker: Expression of Heterologous Sigma Factors in *Escherichia coli* to Explore the Heterologous Genomic Space for Building, Stepwise, Complex, Multicomponent Phenotypes — Eleftherios 'Terry' Papoutsakis, University of Delaware
2:25-2:50 PM	Robust Technology for Antibiotics-Free Contamination Resistance — A. Joe Shaw, Novogy
2:50-3:15 PM	Invited Speaker: Establishing Cell-Free Metabolic Engineering for Pathway Debugging and the Production of Sustainable Chemicals — Michael Jewett, Northwestern University
3:15-3:40 PM	High Throughput Screening of Antibody Secretion in CHO Cells Using Split-GFP and Droplet Microfluidics — Johan Rockberg, KTH-Royal Institute of Technology
3:40-4:05 PM	Invited Speaker: Transforming Yeast from Moonshiners into Oil Barons: Lessons from the Industrialization of Metabolic Engineering — Sunil Chandran, Amyris
4:05-5:00 PM	IMES General Assembly Meeting
5:00-6:00 PM	**Poster Session and Reception (Poster Group 2)**
6:00-7:00 PM	Awards Presentation and Lecture: Mathematical Modeling and Computational Analysis and Design of Metabolic Reaction Networks — Vassily Hatzimanikatis, EPFL
7:00-9:00 PM	Dinner
Thursday, June 19	
7:30-8:00 AM	Breakfast
8:00-11:00 AM	Workshop: Tools for Commercial Bioprocess Design and Economic Analysis
8:00-8:05 AM	Introduction — Jeff Lievense, Genomatica
8:05-8:40 AM	Reverse the Direction in Bioprocess Development: From Full Scale to Synthetic Biology- Sef Heijnen, TU Delft
8:40-9:40 AM	Economic Analysis of Fermentation Processes (Feedstock, Capital, Production Costs) — Jeff Lievense and Alex Patist, Genomatica
9:40-9:50 AM	Break
9:50-10:10 AM	Engineering Cost Model — Alex Patist
10:10-10:40 AM	Case Studies (3-hydroxypropionic acid, undecanoic acid) — Jeff Lievense and Alex Patist
10:40-10:55 AM	Process Omics: Chemical Advantage Dashboard — Jeff Lievense
10:55-11:00 AM	Closing Remarks - Alex Patist

Rapid Fire Presentation Schedule

Group 1— Monday, June 16 4:45-5:30 PM

Presentation Number	Poster Number	Title	Name
1	9	Production of 4-Hydroxybutyric Acid By Metabolically Engineered Mannheimia Succiniciproducens and Its Conversion to Gamma-Butyrolactone By Acid Treatment	Junho Bang
2	18	Novel Acetyl-CoA Transfer Route in *Saccharomyces Cerevisiae*	Yun Chen
3	22	Production of Phenol from Glucose in *Escherichia coli* through Metabolic Engineering Approach	Hannah Chung
4	23	Towards Synthetic Phototrophy: Engineering Proton-Pumping Rhodopsins into *E. coli*	Nico J. Claassens
5	33	Metabolic Engineering of Photorespiratory Bypass Pathways to Enhance Novel Biofuel Production in Transgenic Plants	Sheba Goklany
6	49	Comparative Cross-Strain Analysis of Stress Resistance Mechanisms Revealed By Transposon Insertion Sequencing	Rebecca M. Lennen
7	51	Development of a Yeast Cell Factory for Resveratrol Production	Mingji Li
8	55	Comprehensive Study of Metabolic Flux Rewiring in *E. coli* Knockout Strains	Christopher P. Long
9	61	Developing an Integrated Systems and Synthetic Biology Platform for Gas Fermenting Acetogens	Esteban Marcellin
10	62	Rational Genome Engineering with Genetically Encoded Biosensors at Single-Cell Scale	Jan Marienhagen
11	65	K-Optforce: Strain Design Using Kinetic Information	Thomas Mueller
12	68	Synthesis of Nylon 6,5 from Biologically Prepared 5AVA By Metabolically Engineered *Escherichia coli*	Young Hoon Oh
13	75	Rapid Evaluation of Itaconic Acid Production Strategies in *Saccharomyces Cerevisiae*	Hans Roubos
14	78	Ubiquinone Accumulation Improves Osmotic-Stress Tolerance in *Escherichia coli*	Daniel C. Sevin
15	79	Transport and Metabolism of Fumaric Acid in *Saccharomyces Cerevisiae*	Mihir Shah
16	80	Implementing the Formose Pathway for Conversion of Electricity and CO_2 to Biofuel Precursors Via Formate in *Escherichia coli*	Amanda Lee Smith
17	82	Systems Metabolic Engineering of *Escherichia coli* for the Production of Fumaric Acid	Chanwoo Song
18	92	Cytosolic Acetyl-CoA Synthesis By Pyruvate-Formate Lyase in Yeast	Harmen M. van Rossum
19	95	Single-Cell Bioreactors Boost Bioprocess Development: New Insights into Cellular Metabolism	Wolfgang Wiechert
20	99	Construction of Fast Xylose-Fermenting Yeast Based on Industrial Ethanol-Producing Diploid *Saccharomyces Cerevisiae* By Rational Design and Adaptive Evolution	Junjie Yang
21	100	Bacterial Cell Factory for Production of Scyllo-Inositol, a Potential Therapeutic Agent for Alzheimer's Disease	Ken-ichi Yoshida
22	108	Cooperative Co-Culture of Escherichia coli and *Saccharomyces Cerevisiae* for Overproduction of Paclitaxel Precursors	Kang Zhou

Rapid Fire Presentation Schedule

Group 2 – Tuesday, June 17, 4:45 - 5:30 PM

Presentation Number	Poster Number	Title	Name
1	110	Engineering a Balanced Mevalonate Pathway in *E.coli*	Jorge Alonso-Gutierrez
2	111	Yeast Mitochondrial Engineering: Targeting the Powerhouse of the Cell for Advanced Biofuel Production	Jose L. Avalos
3	112	Combining Elementary Mode Analysis with a Network Embedded Thermodynamic Approach for Analysis of Microbial Adipic Acid Production	Nils J. H. Averesch
4	114	Development of Next Generation Yeast Strains for Ethanol Production from Lignocellulosic Feedstocks	Viktor Boer
5	117	Understanding and Overcoming Monoterpene Toxicity in Yeast for the Production of Renewable Jet Fuels	Timothy Brennan
6	125	Splitting the *E. coli* Metabolism for the Production of Fructose-6-P Derived Chemicals	Pieter Coussement
7	130	Expression Optimization of Multi-Enzyme Pathways for Xylose Utilization and Chemical Production	John E. Dueber
8	134	Novel Biosensors for Optimizing Yeast Cell Factories	David Florian
9	135	13C Metabolic Flux Analysis of Co-Culture Systems: A Novel Approach	Nikodimos A. Gebreselassie
10	142	Using Protein Scaffolds to Redirect Photosynthetic Reducing Power for Biosynthesis of Natural Products	Maria Henriques de Jesus
11	147	Lysate of Engineered Escherichia coli Supports Conversion of Glucose to 2,3-Butanediol with Near-Theoretical Yields and Ultrahigh Productivity	Jennifer E. Kay
12	153	Replacement of the *Saccharomyces Cerevisiae* Acetyl-CoA Synthetases By cetylating Acetaldehyde Dehydrogenase for Cytosolic Acetyl-CoA Synthesis	Barbara U. Kozak
13	162	The Impact of Orthogonal Gene Expression on Heterologous Pathway Productivity	George H. McArthur
14	163	Tools to Resolve Compartmentalized Metabolism in Mammalian Cells	Christian M. Metallo
15	171	Computing Proteome Abundance and Activity with a Genome-Scale Model of Metabolism and Gene Expression	Edward J. OBrien
16	187	Tools for Advancing Genome Engineering on the Protein, Pathway, and Genome Scale	T. Steele Reynolds
17	188	Ultra-High-Throughput Screening of Enzyme Libraries with Droplet-Based Microfluidics	Philip Romero
18	213	Quorum-Sensing Linked RNAi for Dynamic Pathway Control in *Saccharomyces Cerevisiae*	Thomas C. Williams
19	214	Balancing Flux through Secondary Metabolic Pathways in Plant Culture Systems	Sarah Wilson
20	215	Optimization of Multi-Gene Biological Systems Using High-Throughput DNA Assembly, Sequencing, and Model-Guided Search Strategies	Lauren B. A.Woodruff

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Opening Reception

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Awards **Wednesday, June 18 6:00-7:00 pm**

International Metabolic Engineering Award — Presented to: Vassily Hatzimanikatis, École Polytechnique Fédérale de Lausanne

The 2014 International Metabolic Engineering Award has been given to Dr. Hatzimanikatis for his contributions to the field of metabolic engineering, through pioneering development of methods to model and analyze large metabolic networks, in particular demonstration of how such networks can be used to compute novel biosynthetic pathways and how kinetics and thermodynamics can be incorporated to improve the predictive strength of genome-scale metabolic modeling. Dr. Hatzimanikatis is well known for his many seminal contributions to metabolic modeling of many different microorganisms, and his work has impacted the development of several industrial processes. He will be presenting an award lecture Tuesday evening on "Mathematical Modeling and Computational Analysis and Design of Metabolic Reaction Networks." The full presentation abstract is on page 50.

Dr. Vassily Hatzimanikatis is currently Associate Professor of Chemical Engineering and Bioengineering at Ecole Polytechnique Federale de Lausanne (EPFL), in Lausanne, Switzerland. He received a PhD and an MS in Chemical Engineering from the California Institute of Technology, and his Diploma in Chemical Engineering from the University of Patras, in Greece. After the completion of his doctoral studies, he held a research group leader position at the Swiss Federal Institute of Technology in Zurich (ETHZ), Switzerland. Prior to joining EPFL, Dr. Hatzimanikatis was Assistant Professor at Northwestern University, at Illinois, USA, and he worked for three years at DuPont and Cargill.

Dr. Hatzimanikatis is Editor-in-Chief of Metabolic Engineering Communication, associate editor of the journals Metabolic Engineering, Biotechnology & Bioengineering and Integrative Biology, and Senior Editor of Biotechnology Journal. He has published over 70 articles and he is co-inventor in three patents and patent applications.

Dr. Hatzimanikatis is a fellow of the American Institute for Medical and Biological Engineering (2010) and he is in the founding Board of Director of the International Metabolic Engineering Society. He was a DuPont Young Professor (2001-2004), and he received the Jay Bailey Young Investigator Award in Metabolic Engineering (2000), and the ACS Elmar Gaden Award (2011).

The International Metabolic Engineering Award is intended to recognize an outstanding career contributor to the field of Metabolic Engineering and is awarded by the International Metabolic Engineering Society at its bi-annual conference. Previous awards winners are: James E. Bailey (2000), Gregory Stephanopoulos (2002), Jens Nielsen (2004), James Liao (2006), Sang Yup Lee (2008), Eleftherios Papoutsakis (2010), Jay Keasling (2012). The 2014 award is co-sponsored by DSM.

Jay Bailey Young Investigator Award in Metabolic Engineering — Presented to: Hal Alper, University of Texas at Austin

Dr. Hal Alper is an Assistant Professor at The University of Texas at Austin in the Department of Chemical Engineering, and a Fellow of the Paul D. & Betty Robertson Meek Centennial Professorship. His lab focusses on metabolic and cellular engineering in the context of biofuel, biochemical and biopharmaceutical production with a strong emphasis on fungal host systems. Moreover, his work applies and extends the approaches of related fields such as synthetic biology, systems biology, and protein engineering. Dr. Alper is the recipient of the Camille and Henry Dreyfus New Faculty Award (2008), the Texas Exes Teaching Award (2009), the DuPont Young Investigator Award (2010), the Office of Naval Research Young Investigator Award (2011), the UT Regents' Outstanding Teaching Award (2012), the Biotechnology and Bioengineering Daniel I.C. Wang Award (2013), and the Camille Dreyfus Teacher-Scholar Award (2014). Dr. Alper received his B.S. in Chemical Engineering from The University of Maryland College Park (2002) and his Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology (2006).

This award is based on a 2012 publication in Metabolic Engineering that demonstrates xylose transporter capacity (and subsequently catabolic rates) can be altered through the directed evolution of molecular transporter proteins. This paper is the first to demonstrate that molecular transporter proteins can be improved for biotechnological applications. Moreover, this work establishes the principle for "molecular transporter engineering" which can be applied not just to carbohydrate transporters, but also to an array of influx and efflux pumps. This work continues to expand the scope of metabolic engineering, particularly at the interface of protein engineering. In addition, this work established the foundation for subsequent rewiring of sugar transporter preference.

This award was instituted in honor of Jay Bailey, a visionary of future directions in biotechnological research and a brilliant contributor to the founding and advancement of the field of metabolic engineering. The purpose of the award is to recognize outstanding research accomplishments in the field of metabolic engineering by a young investigator. The recipient will have advanced the frontiers of metabolic engineering through originality and creativity of experimental or computational concept application. The 2014 award is cosponsored by Elsevier's Metabolic Engineering Journal and Metabolic Engineering Communications.

Poster Awards

Metabolic Engineering X will be presenting three graduate students with poster awards, sponsored by Manus Biosynthesis.

Opening Plenary: A 15-Year Journey to Figure out How to Derive the Most Economic Value from Metabolic Engineering

Christophe Schilling, CEO, Genomatica
Sunday, June 15, 5:30-6:15 pm

As a founder and CEO, Christophe Schilling has led Genomatica from a developer of metabolic engineering tools to a position of leadership in biotech processes for the production of major chemicals – with multiple customers, $125 million in equity investments, and numerous awards for their technology, commercialization results and corporate culture.

Please join Christophe as he takes you on a 15 year (and counting) personal tour of his experiences. How did Genomatica go from pioneers in the commercial application of computational modeling of metabolism for the life sciences to the choice of a specific business strategy focused on developing and licensing process technology for chemical production, and the addition of experimental capabilities, process engineering and other core strengths? Christophe will speak to the technical, business and people issues that come up when starting with an idea, building a company, raising money, getting customers, and more. He'll also share his personal view of where metabolic engineering can 'most readily' turn into economic value – and what it takes to do so.

Christophe holds a Ph.D. in bioengineering from the University of California, San Diego. He received a B.S. in biomedical engineering from Duke University and was honored as a Distinguished Young Alumnus in 2010. He serves on the World Economic Forum Global Agenda Council on Biotechnology. Christophe founded Genomatica in 1998. Since being named CEO in May 2009, he has led Genomatica to widespread recognition as a leader in industrial biotech, with a commercialized first process, top-tier licensees, strong investors - and an unrivaled string of awards for engineering (Kirkpatrick), science (EPA Presidential Green Chemistry), industry leadership (voted #1 Hottest three years in a row by Biofuels Digest), and company culture (The Scientist as a Best Place to Work). Christophe frequently speaks on industry trends at major conferences, and serves as a member of the World Economic Forum Global Agenda Council on Biotechnology.

Opening Plenary: Perspectives on Creating & Scaling-up Better Energy Solutions

Vineet Rajgarhia, Vice President of Biotech R&D,
Total New Energies
Sunday, June 15, 6:15-7:00 pm

Vineet has been involved in developing Biobased Fuels and Chemicals for over 16 years since receiving his Ph.D. in microbiology from the Ohio State University for polyketide metabolic engineering. Vineet started his industrial career with Cargill Biotech and NatureWorks where his team and he created Cargill's low pH, cellulose utilizing, industrial biocatalysts and their award-winning low pH lactic acid producing biocatalysts. He has also been fortunate to lead some incredible scientists while developing Consolidated Bioprocessing of biomass to produce fuels and chemicals at Mascoma and seaweed to fuels at BAL. Vineet is currently the VP of Biotech R&D at Total New Energies, USA, leading Total's endeavor in industrializing biobased solutions to meet our energy and chemical needs in a sustainable manner. Vineet also holds an MBA from Columbia University, NY / London Business School, UK.

Workshop: Tools for Commercial Bioprocess Design and Economic Analysis

Chair: Jeff Lievense, Genomatica
Thursday, June 19, 8:00-11:00 am

The purpose of this workshop is to share strategies and tools for commercial bioprocess design and economic analysis. Sef Heijnen will describe, with modest information, how to visualize the outcome – a full scale fermentation process – and the fundamental importance of doing so before a research program is started in order to deliver production microbes that scale successfully. Jeff Lievense and Alex Patist will provide a set of tools for deriving full scale plant capital and production costs given basic information on the product, biochemical stoichiometries, feedstocks, product recovery approach, plant size and location. A quantitative dashboard will be introduced as a means of tracking process development progress and its competitiveness. Three case studies will be presented during the workshop.

8:00-11:00 AM	**Workshop—Tools for Commercial Bioprocess Design and Economic Analysis**
8:00-8:05 AM	Introduction— Jeff Lievense, Genomatica
8:05-8:40 AM	Reverse the Direction in Bioprocess Development: From Full Scale to Synthetic Biology Sef Heijnen, TU Delft
8:40-9:40 AM	Economic Analysis of Fermentation Processes (Feedstock, Capital, Production Costs) Jeff Lievense and Alex Patist, Genomatica
9:40-9:50 AM	Break
9:50-10:10 AM	Engineering Cost Model — Alex Patist
10:10-10:40 AM	Case Studies (3-hydroxypropionic acid, undecanoic acid) — Jeff Lievense and Alex Patist
10:40-10:55 AM	Process Omics: Chemical Advantage ashboard Jeff Lievense
10:55-11:00 AM	Closing Remarks — Alex Patist

Presenter Instructions

Speakers

Speakers should plan to meet the session chair at least 15 minutes prior to the session. Please sit in the front of the room during your session. Personal laptops will not be connected to the projector for individual presentations. Unless otherwise noted in the program, speakers will have 25 minutes for their talk. This block includes time for questions. Please help us keep to the schedule.

Presentations at the upcoming Metabolic Engineering X Conference will be handled by our on-site presentation management system, LaunchPad. As a presenter at the meeting, please review the following guidelines for uploading and presenting your talk:

1. File Preparation

LaunchPad supports virtually any file type and does not place restrictions on size. For best results, bring your files to the conference in their original format (not converted) on a USB flash drive to the on-site technician located at the tech table in the back of the session room. If you plan to use multimedia such as videos, please bring the source files as well, even if you have previously embedded the files in your presentation. **Please submit your presentation files to the LaunchPad technician at least an hour before your session. Please submit your presentation files to the LaunchPad technician at least an hour before your session.**

2. Upload and Testing

The technician will assist you with uploading your files using an upload kiosk. In most cases this process takes just a few minutes. Here you can look through your presentation and make any last-minute edits. Please note that the podium computer uses "presenter mode" in supported applications and presenter notes ARE visible if you choose to include them.

3. Presenting

Launching your talk on LaunchPad is very straightforward. On the screen you will see a list of the presenters in your session, labeled by their number and last name (e.g. 01 Smith). Click on your name, and your presentation will launch automatically into full screen. There will be a combination wireless remote / laser pointer at the podium with which to advance your slides, or you can use the keyboard / trackpad if you prefer. When you are finished, instead of closing your presentation just press the glowing "RESET" button to the left of the laptop. This will close your presentation and return to the main LaunchPad screen for the next speaker. Similarly, if the presenter before you did not reset the podium and you do not see the list of speakers, pressing "RESET" will take you back to the main LaunchPad screen.

Rapid Fire Presenters

Rapid fire presenters will have 90 seconds to present 1 slide with no animation summarizing their poster. The rapid fire presentation schedule can be seen in section 11 of the program book.

Poster Presenters

There will be 2 separate groups of poster presenters. Poster sessions will take place in Bayshore Salon DEF. Poster Group 1 will be presenting in the poster sessions on Sunday, June 15th and Monday, June 16th. Presenters in this group can set up their posters starting at 12:30 pm on Sunday. All posters in this group should be taken down by 10:30 am on Tuesday. Poster Group 2 will be presenting in the poster sessions on Tuesday, June 17th and Wednesday, June 18th. Presenters in this group can set up their posters starting at 12:35 pm on Tuesday. All posters in this group should be taken down by 8:30 am on Thursday. You can see your poster number in section 11 of the program book.

Contact Information

SBE staff will be available at registration check-in at the Bayshore Grand Foyer during the following times:

Sunday, June 15h 3:30-7:00 pm
Monday, June 16th 7:00 am-12:00 pm
Tuesday, June 17th 7:00 am-12:00 pm
Wednesday, June 18th 7:00 am-12:00 pm
Thursday, June 19th 7:15-8:30 am

Following the conference, you may reach SBE's Technical Product Associate, Evan Flach, by email at evanf@aiche.org or by phone at 646.495.1381.

Session Chairs: Irina Borodina, Novo Nordisk Biosustainability Center; Michael Jewett, Northwestern University; Julius Lucks, Cornell University; Caroline Peres, DuPont

Poster Group 1 –
Poster sessions Sunday and Monday, unshaded.

Poster Group 2 –
Poster sessions Tuesday and Wednesday, shaded

1. The Genetic and Metabolic Regulation of Rhamnolipids Biosynthesis in Pseudomonas Aeruginosa Reveals New Engineering Strategies for Heterologous Expression.

A. M. Abdel-Mawgoud, F. Lepine and E. Déziel*
INRS-Institut Armand-Frappier, Laval, QC, Canada

2. Isotopically Nonstationary 13C Flux Analysis of Isobutyraldehyde Production in Synechococcus Elongatus.

*A. Adebiyi**
Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN

3. Characterization of LDH Genes for L-Lactic Acid Production in Pichia Pastoris.

*P. B. A. Almeida**
Molecular Biology, Universidade de Brasilia, Brasilia, Brazil

4. Identification of Key Metabolite Concentrations and Enzyme Saturations Determining the Physiological States of Glucose-Fed *E. coli* for the Production of 1,4-Butanediol.

S. Andreozzi[1,2], L. Miskovic[1,2] and V. Hatzimanikatis[1,2]*
[1]Swiss Institute of Bioinformatics, Lausanne, Switzerland
[2]Laboratory of Computational Systems Biotechnology, EPFL, Lausanne, Switzerland

5. New Advances in 13C Metabolic Flux Analysis: Complete-MFA, Co-Culture MFA and Dynamic MFA.

*M. R. Antoniewicz**
Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

6. Parallel Labeling Experiments: A Novel Approach for Validating Metabolic Network Models.

J. Au and M. R. Antoniewicz*
Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

7. Synthetic Microbial Metabolism Refactoring for the Production of a Chemical Synthon, 2,4-Dihydroxybutyric Acid.

C. Auriol and J. M. Francois*
Toulouse White Biotechnology, Toulouse, France

8. Implementation of a Disassociated Fatty Acid Synthase System (FAS type II) in *Saccharomyces cerevisiae* for Fatty Acid and Wax Ester Production.

F. Azevedo and B. Johansson*
CBMA - Center of Molecular and Environmental Biology / Department of Biology, University of Minho, Braga, Portugal

9. Production of 4-Hydroxybutyric Acid By Metabolically Engineered Mannheimia Succiniciproducens and Its Conversion to Gamma-Butyrolactone By Acid Treatment.

J. Bang, S. Choi1, H. U. Kim, W. J. Kim and S. Y. Lee*
Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea,

10. Challenges in Reverse Engineering of Industrial Fermentation Strains.

K. R. Benjamin, A. Tsong, C. Reeves, B. Kaufmann, A. Singh, J. Ubersax, M. Fleck, L. Pickens, M. Durot and D. Platt*
Amyris Inc., Emeryville, CA, United States of America

11. Metabolic Engineering for Ricinoleic Acid Production in Y. Lipolytica.

A. Beopoulos[1], J. Verbeke[1], F. Bordes[2], M. Guicherd[2], M. Bressy[2], A. Marty[2] and J. M. Nicaud[1]*
[1]Micalis, INRA, France
[2]LISBP, INSA, Toulouse, France

12. Fatty Acid Overproduction in a Genetically Engineered Puryvate Decarboxylase-Negative Strain of *Saccharomyces cerevisiae*.

A. Bergman, V. Siewers and J. Nielsen*
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

13. Isolation, Characterization and Metabolic Engineering of a Thermophilic Bacillus for Green Chemical Production.

E. F. Bosma[1], A. H. P. van de Weijer[1], J. van der Oost[1], W. M. de Vos[1] and R. van Kranenburg[1,2]*
[1]Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands
[2]Corbion, Gorinchem, Netherlands

14. Controlled Protein Degradation for Development of Metabolite Valves.

I. Brockman and K. Prather*
Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

15. Flexible and User Friendly Tools for the Incorporation of Fluxomics Data into Metabolic Models.

R. Carreira[1], M. Pont[2], J. F. Tomb[2], S. G. Villas-Bôas[3], M. Rocha[1] and I. Rocha[1]*
[1]Centre of Biological Engineering, Department of Biological Engineering, University of Minho, Braga, Portugal
[2]E.I. du Pont de Nemours and Company, DE
[3]Centre for Microbial Innovation, School of Biological Sciences, University of Auckland, Auckland, New Zealand

16. Unraveling the Inhibitory Effects of Acetate on Ethanol Production in Cen.PK.

A. Chakrabarti[1,2], A. Kiparissides[1,2], K. C. Soh[1,2], B. R. B. H. van Rijsewijk[3], J. W. Hickman[3], T. S. Najdi[3], D. Halim[3], V. A. Portnoy[3], M. A. Lorenz[3], R. Oseguera[3], A. M. Burja[3], L. Miskovic[1,2] and V. Hatzimanikatis[1,2,4]*
[1]Laboratory of Computational Systems Biotechnology, EPFL, Lausanne, Switzerland
[2]Swiss Institute of Bioinformatics, Lausanne, Switzerland
[3]BP Biofuels, Global Technology Centre (GTC), San Diego, CA
[4]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland

17. Understanding and Optimizing Free Fatty Acid Production in Synechocystis Sp. PCC 6803.

Y. E. Cheah and C. A. M. Peebles*

Chemical and Biological Engineering, Colorado State University, Fort Collins, CO

18. Novel Acetyl-CoA Transfer Route in Saccharomyces cerevisiae.

Y. Chen, Y. Zhang and J. Nielsen*
Chemical and Biological Engineering, Chalmers University of Technology, Gothneburg, Sweden

19. Microbial Production of Short-Chain Alkanes.

S. Y. Choi, Y. J. Choi and S. Y. Lee*
Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

20. High-Throughput Screening System and Its Biotechnological Applications.

J. H. Choi, K. C. Ko, Y. Han, D. E. Cheong, J. S. Kim and J. J. Song*
Korea Research Institute of Bioscience and Biotechnology (KRIBB), South Korea

21. Overcoming Inefficient Cellobiose Fermentation By Cellobiose Phosphorylase in the Presence of Xylose.

K. Chomvong[1], V. Kordic[2], X. Li[3], S. Bauer[4], A. E. Gillespie[3], S. J. Ha[5], E. J. Oh[5], J. M. Galazka[3], Y. S. Jin[5] and J. H. D. Cate[3]*
[1]Plant and Microbial Biology, University of California at Berkeley
[2]Chemistry, University of California at Berkeley
[3]Molecular and Cell Biology, University of California at Berkeley
[4]Energy Biosciences Institute
[5]Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL

22. Production of Phenol from Glucose in *Escherichia coli* through Metabolic Engineering Approach.

H. Chung[1], B. Kim[1], H. Park[1], D. Na[2] and S. Y. Lee[1],*
[1]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea,
[2]School of Integrative Engineering, Chung Ang University, Seoul, South Korea

23. Towards Synthetic Phototrophy: Engineering Proton-Pumping Rhodopsins into *E. coli*.

N. J. Claassens[1], M. Volpers[2,3], V. A. P. Martins Dos Santos[2], J. van der Oost[1] and W. M. Vos[1,4]*
[1]Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands
[2]Laboratory of Systems and Synthetic Biology, Wageningen University, Wageningen, Netherlands
[3]Center for Biological Systems Analysis, University of Freiburg, Freiburg, Germany
[4]Department of Bacteriology and Immunology, Helsinki University, Helsinki, Finland

24. Engineering Anaerobic Amino Acid Production in *Saccharomyces cerevisiae*: Alanine As Case of Study.

H. F. Cueto-Rojas, J. J. Heijnen, A. Goel, N. Milne, J. M. Daran and A. Wahl*
Department of Biotechnology, Delft University of Technology, Delft, Netherlands

25. Cytosolic Acetyl-CoA Platform in Yeast for Biochemicals Production.

Z. Dai, Y. Zhang, A. Krivoruchko, V. Siewers and J. Nielsen*
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

26. Toward a Biosynthetic Route to Sclareol and Amber Odorants.

*L. Daviet**
Biotechnology R&D, Firmenich SA, Geneva, Switzerland

27. Advanced Production of Faee in a *S. cerevisiae* Cell Factory.

B. W. de Jong[1], S. Shi[2], V. Siewers[1] and J. Nielsen[1]*
[1]Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[2]Metabolic Engineering Research Laboratory, Institute of Chemical Engineering and Sciences, Singapore, Singapore

28. Metabolic Engineering of Cyclic Triterpenoid Production in *Saccharomyces cerevisiae*.

B. E. Ebert[1], K. Walter[1], C. Lang[2] and L. M. Blank[1]*
[1]iAMB - Institute of Applied Microbiology, RWTH Aachen University, Aachen, Germany
[2]Organobalance GmbH, Germany

29. Development & Exploitation of Gene Tools for Metabolic Engineering in Saccharolytic Clostridia.

M. Ehsaan, K. Winzer and N. Minton*
School of Life Sciences, The University of Nottingham, Nottingham, United Kingdom

30. Novel Methods to Investigate Solvent Toxicity in Bacteria.

E. Fletcher[1] and C. French[2]*
[1]Systems and Synthetic Biology, Chalmers University of Technology, Göteborg, Sweden
[2]Synthetic and Systems Biology Centre (SynthSys), University of Edinburgh, Edinburgh, United Kingdom

31. Targeted Proteomics Enabled Metabolic Engineering of *Clostridium Cellulolyticum* for n-Butanol Production.

S. M. Gaida, A. Liedtke, A. H. W. Jentges and S. Jennewein*
Industrial Biotechnology, Fraunhofer IME, Aachen, Germany

32. Isobutanol Production By an Industrial *Saccharomyces cerevisiae* Strain.

W. C. Generoso, H. Dietz, M. Oreb and E. Boles*
Institute of Molecular Biosciences, Goethe University Frankfurt am Main, Frankfurt am Main, Germany

33. Metabolic Engineering of Photorespiratory Bypass Pathways to Enhance Novel Biofuel Production in Transgenic Plants.

S. Goklany[1], Y. K. Kim[2], H. Ma[2], Y. C. Liu[2], E. Takahashi[3], D. Ort[3], J. Yuan[2] and J. Chappell[1]*
[1]Pharmaceutical Sciences, University of Kentucky, Lexington, KY
[2]Plant Pathology and Microbiology, Texas A&M University, TX
[3]Plant Biology, University of Illinois at Urbana-Champaign, IL

34. Metabolic Activities and Their Control at the Mitochondria-Cytosol Interface in CHO Cells.

E. Heinzle[1], J. Wahrheit[2] and A. Nicolae[2]*
[1]Biochemical Engineering, Saarland University, Saarbrucken, Germany
[2]Saarland University , Saarbrucken, Germany

35. General and Specific Stress Responses Towards Short Even-Chain Alcohols in Lactic Acid Bacteria Provide Clues for Improving Second Generation Biorefineries.

A. M. Hviid[1], P. R. Jensen[2] and M. Kilstrup[1]*
[1]Institute for Systems Biology, Technical University of Denmark

[2]National Food Institute, Technical University of Denmark

36. Isotopically Nonstationary 13C Metabolic Flux Analysis of Arabidopsis thaliana Rosettes at Altered Light Conditions.

L. J. Jazmin[*1], F. Ma[2], D. K. Allen[2,3] and J. D. Young[1]*
[1]Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN
[2]Donald Danforth Plant Science Center, St. Louis, MO,
[3]USDA-ARS, St. Louis, MO

37. Biotechnical Production of Ethylene in *S. cerevisiae* - Insights from Metabolic Modeling, Cultivation Studies and Enzyme Engineering.

N. Johansson[*1], K. Persson[1], P. Quehl[1], G. Vieira[2], S. Heux[2], J. Norbeck[1] and C. Larsson[1]*
[1]Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[2]LISBP - INSA de Toulouse, Toulouse, France

38. Microbial Production of Cis, Cis-Muconic Acid By *Klebsiella Pneumoniae*.

H. M. Jung[*1], J. H. Kim[2] and M. K. Oh[1]*
[1]Deparment of Chemical and Biological Engineering, Korea University, Seoul, South Korea
[2]Department of Chemical Engineering, Dong-A University, Busan, South Korea

39. Effects of Pyruvate Formate Lyase Inactivation in *Klebsiella Pneumoniae* and Its Application to Diol Production.

M. Y. Jung and M. K. Oh*
Department of Chemical and Biological Engineering, Korea University, Seoul, South Korea

40. Bio-Hydrogen Production By Continuous Culture of Hyperthermophilic Archaeon from Carbon Monoxide.

T. W. Kim[*1], S. S. Bae[1], J. G. Na[2], H. S. Lee[1], J. H. Lee[1] and S. G. Kang[1]*
[1]Marine Biotechnology Research Division, Korea Institute of Ocean Science and Technology, Ansan, South Korea
[2]Biomass and Waste Energy Laboratory, Korea Institute of Energy Research, Daejeon, South Korea

41. Prediction and Design of Novel Metabolic Pathways for the Production of Desired Chemicals.

D. I. Kim, A. Cho, H. Yun, J. H. Park, S. Y. Lee and S. Park*
Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

42. Integration of Transcriptomic Data in Genome-Scale Metabolic Models Predicts *in vitro* Intracellular Central Carbon Metabolic Fluxes with High Correlation in *Escherichia coli* and *Saccharomyces cerevisiae*.

M. K. Kim[*1] and D. Lun[2]*
[1]Rutgers University
[2]Computer Science, Rutgers University

43. Model-Driven Metabolic Engineering of *Escherichia coli* for Improving Conversion of Lignocellulose-Derived Sugars to Ethanol.

J. Kim[*1,2], M. Tremaine[1], R. Landick[1,3,4], P. Kiley[1,5] and J. L. Reed[1,2]*
[1]DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI
[2]Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, WI
[3]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI
[4]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI
[5]Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, WI

44. Yeast Cell Factories for Production of Biobutanol.

A. Krivoruchko, V. Siewers and J. Nielsen*
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

45. Engineering of a Stable, Syntrophic Microbial Coculture for Enhanced H2 Production.

B. LaSarre, A. L. McCully, A. L. Posto and J. B. McKinlay*
Department of Biology, Indiana University, Bloomington, IN

46. Redirecting Photosynthetic Reducing Power into Light-Driven Biosynthesis of Bioactive Natural Compounds.

L. M. M. Lassen, A. Z. Nielsen, C. E. Olsen, B. L. Møller and P. E. Jensen*
Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Copenhagen, Denmark

47. Biosynthesis of 2-Hydroxyacid Containing Polyhydroxyalkanoates in Metabolically Engineered Ralstonia Eutropha.

S. J. Park[*1], Y. H. Oh[*2], J. E. Yang[3], S. Y. Choi[3], S. H. Lee[*2] and S. Y. Lee[*3]*
[1]Department of Environmental Engineering and Energy, Myongji University, Yongin, South Korea
[2]Korea Research Institute of Chemical Technology, Daejeon, South Korea
[3]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

48. Biosynthesis of Polyhydroxyalkanoates in Recombinant Ralstonia Eutropha Engineered to Utilize Sucrose As a Carbon Source.

S. J. Park[*1], Y. H. Oh[*2], J. E. Yang[3], S. Y. Choi[3], S. H. Lee[*2] and S. Y. Lee[*3]*
[1]Department of Environmental Engineering and Energy, Myongji University, Yongin, South Korea
[2]Korea Research Institute of Chemical Technology, Daejeon, South Korea
[3]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

49. Comparative Cross-Strain Analysis of Stress Resistance Mechanisms Revealed By Transposon Insertion Sequencing.

R. M. Lennen and M. J. Herrgård*
The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark

50. Construction of a Efficient Xylose Metabolic Pathway in *Saccharomyces cerevisiae* for Ethanol Production.

Y. Li[*1] and T. Tan[2]*
[1]Beijing Key Lab of Bioprocess, Beijing University of Chemical Technology, Beijing, China
[2]Beijing University of Chemical Technology, Beijing, China

51. Development of a Yeast Cell Factory for Resveratrol Production.

M. Li[*1], K. R. Kildegaard[1], E. A. R. Prado[1], C. B. Jendresen[1], S. G. Stahlhut[1], I. Borodina[1] and J. Nielsen[1,2]*

[1]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark
[2]Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

52. Modelling Population Dynamics of Pseudomonas Putida KT2440 Under Various Growth Conditions.

S. Lieder[*1], M. Jahn[2] and R. Takors[1]
[1]Institute for Biochemical Engineering, University of Stuttgart, Stuttgart, Germany
[2]Environmental Microbiology, UfZ Leipzig, Leipzig, Germany

53. Carbon Flux-Associated Redox Rebalancing By Static and Dynamic Control.

J. H. Lim[*1] and G. Y. Jung[1,2]
[1]School of Interdisciplinary Bioscience and Bioengineering (I-Bio), POSTECH, Pohang, South Korea
[2]Department of Chemical Engineering, POSTECH, Pohang, South Korea

54. Enhanced Utilization of Non-Favored Sugars from Marine Biomass By Re-Designed *Escherichia coli*.

H. G. Lim[*1], J. H. Lim[2] and G. Y. Jung[1,2]
[1]Department of Chemical Engineering, POSTECH, Pohang, South Korea
[2]School of Interdisciplinary Bioscience and Bioengineering (I-Bio), POSTECH, Pohang, South Korea

55. Comprehensive Study of Metabolic Flux Rewiring in *E. coli* Knockout Strains.

C. P. Long and M. R. Antoniewicz*
Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

56. Metabolic Engineering of Pseudomonas Putida KT2440 for the Production of Compounds Derived from the Shikimic Acid Pathway.

S. Lorenz[*1], N. Trachtmann1, N. Graf[2], J. Altenbuchner[2] and G. A. Sprenger[1]
[1]Institute of Microbiology, University of Stuttgart, Stuttgart, Germany
[2]Institute of Industrial Genetics, University of Stu, Stuttgart, Germany

57. Metabolic Engineering of Yeast for Commercial Production of Succinic Acid.

A. Los, R. Verwaal, B. den Dulk, M. Jansen, Z. Zhao, T. Geurts and R. Bovenberg*
DSM Biotechnology Center, Delft, Netherlands

58. Vitamin Analogs As Antiinfectives: Occurrence, Mode of Action, Metabolism and Production.

*M. Mack**
Biotechnology, Mannheim University of Applied Sciences, Mannheim, Germany

59. Genome-Scale Strain Designs Based on Regulatory Minimal Cut Sets.

R. Mahadevan[*1], A. von Kamp[2] and S. Klamt[3]
[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada
[2]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
[3]Analysis and Redesign of Biological Networks, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany

60. A Bayesian Design of Experiments for Ensemble Modelling of Metabolic Networks.

E. Manesso and R. Gunawan*
Institute for Chemical and Bioengineering, ETH Zurich, Zurich, Switzerland

61. Developing an Integrated Systems and Synthetic Biology Platform for Gas Fermenting Acetogens.

E. Marcellin[*1], M. Koepke[2], W. Mitchel[2], S. Segovia[2] and L. K. Nielsen[1]
[1]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia
[2]LanzaTech

62. Rational Genome Engineering with Genetically Encoded Biosensors at Single-Cell Scale.

J. Marienhagen, S. Binder, G. Schendzielorz, L. Eggeling and M. Bott*
Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Juelich, Juelich, Germany

63. An Integrated Multi-Omics and Computational Characterization of Seven Unique *Escherichia coli* Production Strains Commonly-Used in Industrial Biotechnology.

*J. M. Monk**
University of California, San Diego, La Jolla, CA

64. Computationally Guided Characterization of Carboxylic Acid Reductases for Expanding Aldehyde Bioproduction.

M. Moura, S. Lenzini, D. Pertusi, L. J. Broadbelt and K. E. J. Tyo*
Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL

65. K-Optforce: Strain Design Using Kinetic Information.

*A. Chowdhury, A. Khodayari, C. D. Maranas and T. J. Mueller**
Chemical Engineering, Pennsylvania State University, University Park, PA

66. Rational Design & Optimization of a Synthetic Entner-Doudoroff Pathway for Improved & Controllable NADPH Regeneration.

C. Y. Ng[*1], I. Farasat[1], C. D. Maranas[1] and H. Salis[2]
[1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA
[2]Chemical Engineering / Biological Engineering, Pennsylvania State University, University Park, PA

67. Strategies for Improving Renewable Phenol Biosynthesis in Engineered *Escherichia coli*.

*B. Thompson and D. R. Nielsen**
Chemical Engineering, Arizona State University, Tempe, AZ

68. Synthesis of Nylon 6,5 from Biologically Prepared 5AVA By Metabolically Engineered *Escherichia coli*.

Y. H. Oh[*1], S. J. Park[2], B. K. Song[1], J. Jegal[1], S. H. Lee[1] and S. Y. Lee[3]
[1]Korea Research Institute of Chemical Technology, Daejeon, South Korea

[2]Department of Environmental Engineering and Energy, Myongji University, Yongin, South Korea
[3]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

69. Metabolic Flux Analysis of Isopropyl Alcohol-Producing *Escherichia coli*.

N. Okahashi[*1], K. Yoshikawa[1], Y. Matsumoto[2], T. Shirai[3], F. Matsuda[1], T. Hirasawa[1,4], C. Furusawa[1,5], M. Wada[2] and H. Shimizu[1]*
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[2]Synthetic Chemicals Laboratory, Mitsui Chemicals Inc.
[3]Biomass Engineering Program, Riken, Kanagawa, Japan
[4]Bioscience and Biotechnology, Tokyo institute of Technology
[5]Quantitative Biology Center, Riken

70. The D494G Point Mutation in the Bifunctional Alcohol and Aldehyde Dehydrogenase (adhE) of Clostridium Thermocellum Leads to Improved Ethanol Production.

*D. Olson**
Thayer School of Engineering, Dartmouth College, Hanover, NH

71. Identifying Bottlenecks in Engineering Efficient Cellobiose Metabolism (Evidence for putative promoters within operon and TCA cycle imbalance).

V. Parisutham and S. K. Lee*
School of life sciences, Ulsan National Institute of Science and Technology, Ulsan, South Korea

72. Building Metabolic Engineering Tools to Better Understanding Product Production from Microbial Sources: Using the Cyanobacterium Synechocystis Sp. PCC 6803 for Astaxanthin Production.

S. Albers[1] and C. A. M. Peebles[*2]*
[1]Department of Cell and Molecular Biology, Colorado State University, Fort Collins, CO
[2]Chemical and Biological Engineering, Colorodo State University, Fort Collins, CO

73. Steering Prokaryotic Gene Expression Using Engineered Riboswitches.

G. Peters[*1], J. Maertens[1], J. Beauprez[1], J. Lammertyn[2] and M. De Mey[1]*
[1]Inbio.be, Department of Biochemical and Microbial Technology, Ghent University, Ghent, Belgium
[2]MeBioS, Biosystems Department, University of Leuven, Leuven, Belgium

74. Sensor-Selector Strategy for Directed Evolution of Biosynthetic Pathways.

S. Raman[*1] and G. Church[2]*
[1]Synthetic Biology, Wyss Institute, Harvard University, Boston, MA
[2]Department of Genetics, Wyss Institute, Harvard University, Boston, MA

75. Rapid Evaluation of Itaconic Acid Production Strategies in *Saccharomyces cerevisiae*.

*Z. Zhao, B. Meijrink, B. Gielesen, B. Ozalp, R. van der Hoeven, L. Wu, R. Bovenberg and H. Roubos**
DSM Biotechnology Center, Delft, Netherlands

76. Production of Aromatic Compounds in *E. coli* Strains Lacking Interconversion of PEP and Pyr When Glucose and Acetate Are Coutilized.

A. Sabido[*1], J. C. Sigala*[*2], G. Hernández-Chávez[1], N. Flores[1], G. Gosset[1] and F. Bolívar[1]*
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[2]Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, D. F., Mexico

77. Metabolic Transistor Strategy for Controlling Electron Transfer Chain in *Escherichia coli*.

H. Wu[1,2], L. Tuli[1], G. N. Bennett[4] and K. Y. San[*1]*
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[2]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China
[3]Biochemistry and Cell Biology, Rice University, Houston, TX

78. Ubiquinone Accumulation Improves Osmotic-Stress Tolerance in *Escherichia coli*.

D. C. Sevin and U. Sauer*
Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland

79. Transport and Metabolism of Fumaric Acid in *Saccharomyces cerevisiae*.

M. Shah, J. J. Heijnen and W. Gulik*
Department of Biotechnology, Delft University of Technology, Delft, Netherlands

80. Implementing the Formose Pathway for Conversion of Electricity and CO_2 to Biofuel Precursors Via Formate in *Escherichia coli*.

A. L. Smith[*1], J. Siegel[2,3], A. Wargacki[2], J. Bale[4], D. Baker[2,3] and M. E. Lidstrom[1,5]*
[1]Chemical Engineering, University of Washington, Seattle, WA
[2]Biochemistry, University of Washington, Seattle, WA
[3]Biomolecular Structure and Design Program, University of Washington, Seattle, WA
[4]Molecular and Cellular Biology, University of Washington, Seattle, WA
[5]Microbiology, University of Washington, Seattle, WA

81. Engineering the Valine Assimilation Pathway to Produce Biochemicals and Fuels in *S. cerevisiae*.

K. Solomon, E. Ovadia and M. A. O'Malley*
Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA

82. Systems Metabolic Engineering of *Escherichia coli* for the Production of Fumaric Acid.

C. Song, S. Choi, D. I. Kim, J. W. Jang and S. Y. Lee*
Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

83. Engineering the Glycolytic Pathway of *E. coli* K12 Mutants By Gene Deletions and Introduction of a Fructose 6-Phosphate Aldolase.

*M. Wolfer, K. Gottlieb, N. Trachtmann and G. A. Sprenger**
Institute of Microbiology, University of Stuttgart, Stuttgart, Germany

84. The Potential of Lactic Acid Bacteria As Microbial Factory for Pentanol Isomer Production.

K. I. Starlit[*1], B. Koebmann[1], J. Martinussen[2], S. T. Joergensen[1] and P. R. Jensen[3]*
[1]Bacterial Strain Technology, Novozymes A/S, 2880 Bagsværd, Denmark

[2]Dept. of Systems Biology, Technical University of Denmark, 2800 Lyngby, Denmark
[3]National Food Institute, Technical University of Denmark

85. Engineering *Saccharomyces cerevisiae* for the Production of Hexadecanol and Octadecanol.

D. Stuart, X. D. Liu and I. Wong*
Biochemistry, University of Alberta, Edmonton, AB, Canada

86. L-Methionine Production with Recombinant *E. coli*.

R. Takors[1], M. Rahnert[1], A. Teleki[1], H. Priefert[2] and B. Bathe[3]*
[1]University of Stuttgart, Institute of Biochemical Engineering, Stuttgart, Germany
[2]Evonik Industries AG, HN-BP-RD, Halle, Germany
[3]Health & Nutrition, R&D Bioproducts, Halle, Germany

87. Engineering of Artificial Enzyme Complexes Mediated By Heterospecific Coiled-Coil Zippers or Synthetic Protein-Protein Interaction Domains in *Saccharomyces cerevisiae*.

C. Mignat, T. Thomik*, E. Boles and M. Oreb*
Institute of Molecular Biosciences, Goethe University Frankfurt am Main, Frankfurt am Main, Germany

88. Driven By Demand Metabolic Engineering — Recombinant Rhamnolipid Synthesis in Pseudomonas Putida As an Example.

T. Tiso[1], A. Wittgens[2], F. Rosenau[2] and L. M. Blank[1]*
[1]iAMB - Institute of Applied Microbiology, RWTH Aachen University, Aachen, Germany
[2]Institute of Pharmaceutical Biotechnology, Ulm University, Ulm, Germany

89. SMET: Systematic Multiple Enzyme Targeting for Rational Design of Optimal Strains.

C. T. Trinh[1] and A. Thompson[2]*
[1]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, Knoxville, TN
[2]Energy Science and Engineering, University of Tennessee, Knoxville, TN

90. Mathematical Modelling of Apoptosis for GS-NS0 Cell Culture Secreting Monoclonal Antibody: Linking Gene to Growth, Metabolism and Metabolic Stress.

C. Usaku[1], E. N. Pistikopoulos[2] and A. Mantalaris[1]*
[1]Biological Systems Engineering Laboratory, Department of Chemical Engineering, Imperial College London, London, United Kingdom
[2]Dept. of Chemical Engineering, Centre for Process Systems Engineering, Imperial College London, London, United Kingdom

91. Strong Reduction of Acetate Overflow in *Escherichia coli* By Systems Metabolic Engineering.

K. Valgepea[1,2], R. Nahku[1,2], K. Peebo[1,2], P. J. Lahtvee[1,2] L. Arike[1,2], G. Riis[1,2], M. Oun[1,2], K. Adamberg[1,2] and R. Vilu[1,2]*
[1]Tallinn University of Technology
[2]Competence Centre of Food and Fermentation Technologies

92. Cytosolic Acetyl-CoA Synthesis By Pyruvate-Formate Lyase in Yeast.

H. M. van Rossum[1], B. U. Kozak[1], K. R. Benjamin[2], L. Wu[3], J. M. G. Daran[1], J. T. Pronk[1] and A. J. A. van Maris[1]*
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[2]Amyris Inc., Emeryville, CA94608, United States of America
[3]DSM Biotechnology Center, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands

93. Itaconic Acid Production in *Escherichia coli* By Overexpression of Citrate Synthase, Aconitase, and Cis — Aconitate Decarboxylase.

K. Vuoristo[1], A. Mars[2], J. Springer[2], G. Eggink[2], J. Sanders[2] and R. Weusthuis[1]*
[1]Bioprocess Engineering, Wageningen University, Wageningen, Netherlands
[2]Biobased Products, Wageningen University and Research Centre, Wageningen, Netherlands

94. Metabolic Engineering of *Klebsiella Pneumoniae* for 1-Butanol Production By Using Crude Glycerol.

M. Wang[1] and T. Tan[2]*
[1]Beijing key lab of bioprocess, Beijing University of Chemical Technology, Beijing, China
[2]Beijing University of Chemical Technology, Beijing, China

95. Single-Cell Bioreactors Boost Bioprocess Development: New Insights into Cellular Metabolism.

W. Wiechert, D. Kohlheyer, A. Grünberger, C. Probst, S. Helfrich, J. Frunzke, L. Eggeling, K. Nöh and S. Noack*
IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany

96. Corynebacterium Glutamicum Engineered As a Designer Bug for the Production of Pyruvate and Succinate.

S. Wieschalka[1], B. Blombach[2] and B. J. Eikmanns[3]*
[1]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark
[2]Institute of Biochemical Engineering, University of Stuttgart, Germany
[3]Institute of Microbiology and Biotechnology, University of Ulm, Germany

97. Systems Metabolic Engineering of Corynebacterium Glutamicum to Overcome the Cellular Toxicity Derived from Cellulosic Hydrolysate.

H. S. Park[1,2], Y. Um1, S. J. Sim[2,3] and H. M. Woo[1,2]*
[1]Clean Energy Research Center, Korea Institute of Science and Technology, Seoul, South Korea
[2]Green School, Korea University, Seoul, South Korea
[3]Department of Chemical and Biological Engineering, Korea University, Seoul, South Korea

98. Development of Genetic Tools for the Metabolic Engineering of the Thermophilic Acetogen Moorella Thermoacetica.

B. Woolston, D. Currie, H. Rismani-Yazdi and G. Stephanopoulos*
Chemical Engineering, MIT, Cambridge, MA

99. Construction of Fast Xylose-Fermenting Yeast Based on Industrial Ethanol-Producing Diploid Saccharomyces cerevisiae By Rational Design and Adaptive Evolution.

L. Diao, Y. Liu, F. Qian, J. Yang, Y. Jiang and S. Yang*
CAS Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

100. Bacterial Cell Factory for Production of Scyllo-Inositol, a Potential Therapeutic Agent for Alzheimer's Disease.

K. Tanaka[1], Y. Toya[2], S. Takenaka[3], H. Shimizu[4] and K. I. Yoshida[3]*
[1]Organization of Advanced Science and Technology, Kobe University, Kobe, Japan
[2]Graduate School of Information Science and Technology, Osaka University, Suita, Japan

[3]Department of Agrobioscience, Kobe University, Kobe, Japan
[4]Department of Bioinformatic Engineering, Osaka University, Suita, Japan

101. Construction of a Hybrid Pathway for Selectively Removing Nitrogen Atom from Carbazole.

*J. Hou and B. Yu**
Institute of Microbiology, Chinese Academy of Sciences

102. Protein Design for a De Novo Synthetic Pathway of Microbial Production of 1,3-Propanediol from Sugar.

*F. Geng, Z. Chen and A. P. Zeng**
Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, Hamburg, Germany

103. Systematic Characterization of Protein_Protein Interface for the Development of Artificial Biomachinery for Metabolic Engineering.

*C. W. Ma and A. P. Zeng**
Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, Hamburg, Germany

104. Dynamic Control of Metabolism through Engineering Ligand-Induced Allosteric Regulation Based on a New Concept of Thermodynamic Cycle of Protein Dynamics.

*C. W. Ma, F. Geng, S. Rappert and A. P. Zeng**
Institute of Bioprocess and Biosystems Engineering, Hamburg University of Technology, Hamburg, Germany

105. Development of the First Scalable Rubbery Polyester.

K. Zhang and M. Xiong*
Chemical Engineering and Materials Science, University of Minnesota, MN

106. Co-Culture Based Modular Engineering for Aromatic and Aromatic-Derived Compounds Production in *E. coli*.

H. Zhang, B. Pereira and G. N. Stephanopoulos*
Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

107. A Fast Metabolic Sensor for *in vivo* Cytosolic Phosphate Concentration in *Saccharomyces cerevisiae*.

J. Zhang[*1,2], J. J. Heijnen[1,2] and S. A. Wahl[1,2]*
[1]Department of Biotechnology, Delft University of Technology, Delft, Netherlands
[2]Kluyver Centre for Genomics of Industrial Fermentation, Delft, Netherlands

108. Cooperative Co-Culture of *Escherichia coli* and *Saccharomyces cerevisiae* for Overproduction of Paclitaxel Precursors.

K. Zhou, K. Qiao, S. Edgar and G. Stephanopoulos*
Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

109. Systematic Engineering of Lipid Metabolism for Fatty-Acid-Based Biofuel Production.

Y. Zhou, B. Klaas, V. Siewers and J. Nielsen*
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

110. Engineering a Balanced Mevalonate Pathway in *E. coli*.

*J. Alonso-Gutierrez**
Fuel Synthesis, Joint Bioenergy Institute (JBEI), Emeryville, CA

111. Yeast Mitochondrial Engineering: Targeting the Powerhouse of the Cell for Advanced Biofuel Production.

J. L. Avalos[*1,2], G. Fink[2] and G. Stephanopoulos[1]*
[1]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA
[2]Whitehead Institute, Cambridge, MA

112. Combining Elementary Mode Analysis with a Network Embedded Thermodynamic Approach for Analysis of Microbial Adipic Acid Production.

N. J. H. Averesch[*1], V. S. Martínez[2] and J. O. Krömer[1]*
[1]Centre for Microbial Electrosynthesis (CEMES) within the Advanced Water Management Centre (AWMC), The University of Queensland, St Lucia, Australia
[2]Australian Institute for Bioengineering and Nanotechnology (AIBN)

113. Metabolic Reconstruction of Clostridium Acetobutylicum for Enhanced Production of Butyric Acid.

J. Bang, Y. S. Jang and S. Y. Lee*
Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea

114. Development of Next Generation Yeast Strains for Ethanol Production from Lignocellulosic Feedstocks.

P. de Waal[1], W. Hartman[1], H. de Bruijn[1], A. Teunissen[1], R. de Jong[1], P. Klaassen[1], W. Wisselink[2], J. T. Pronk[2], T. van Maris[2] and V. Boer[1]*
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[2]Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

115. Identifying the Source of Strain-to-Strain Variability in Isoprenoid Production Capacity of *E. coli* Using a Systems Biology Approach.

M. Bongers, J. B. Y. H. Behrendorff, C. E. Vickers and L. K. Nielsen*
Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

116. Rational Metabolic Engineering of Baker's Yeast for Production of 3-Hydroxypropionic Acid.

*I. Borodina**
The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark

117. Understanding and Overcoming Monoterpene Toxicity in Yeast for the Production of Renewable Jet Fuels.

T. Brennan[1], J. Kroemer[2] and L. K. Nielsen[3]*
[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, Brisbane, Australia
[2]Centre for Microbial Electrosynthesis (CEMES), The University of Queensland, Brisbane, Australia
[3]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

118. Molecular Approaches to Improve 1-Butanol Tolerance and Production in *Escherichia coli*.

L. M. Bui, Z. Rahman, A. Geraldi, K. H. Kang, J. H. Lee and S. C. Kim*
Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea

119. Metabolic Engineering for Production of 5-Aminovalerate and Glutarate Using *Escherichia coli*.

T. U. Chae[1], S. J. Park[2], E. Y. Kim[3], W. Noh[3], H. M. Park[1], Y. H. Oh[3], S. H. Lee[3], B. K. Song[3], J. Jegal[3] and S. Y. Lee1 [1]*

Poster Presentations

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[2]Department of Environmental Engineering and Energy, Myongji University, Yongin, South Korea
[3]Korea Research Institute of Chemical Technology, Daejeon, South Korea

120. Synthetic Regulatory Small RNAs for Genome-Wide |Metabolic Engineering.

T. U. Chae[*1], D. Na[2], S. M. Yoo[1], H. Chung[1], H. Park[1] and S. Y. Lee[1]*
[1]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea
[2]School of Integrative Engineering, Chung Ang University, Seoul, South Korea

121. Analysis of Aerobic-to-Anaerobic and Anaerobic-to-Aerobic Switches in *E. coli* Using Large-Scale Dynamic Metabolic Models.

A. Chakrabarti[*1,2], G. Fengos[1,2], M. Ataman1[1,2], K. C. Soh[1,2], L. Miskovic[1,2]and V. Hatzimanikatis[1,2,3]*
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[2]Swiss Institute of Bioinformatics, Lausanne, Switzerland,
[3]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

122. Direct Fermentation for Isobutene, Butadiene and Propylene Production : A Highway to Renewable Plastics, Synthetic Rubber and Fuels.

*R. Chayot**
Strain Construction, Global Bioenergies, EVRY, France

123. Biosynthesis of Lactate-Containing Polymers in Metabolically Engineered *Escherichia coli*.

S. Y. Choi[*1], Y. K. Jung[1], S. J. Park[2] and S. Y. Lee[1]*
[1]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea
[2]Department of Environmental Engineering and Energy, Myongji University, Yongin, South Korea

124. Production of Native-Sized Spider Dragline Silk Protein through Metabolic Engineering Approach in *Escherichia coli*.

H. Chung[*1], X. X. Xia[2] and S. Y. Lee[1]*
[1]Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea
[2]School of Life Sciences and Biotechnology, Jiao Tong University, Shanghai, China

125. Splitting the *E. coli* Metabolism for the Production of Fructose-6-P Derived Chemicals.

P. Coussement, J. Maertens, J. Beauprez, M. Duhamel, W. Van Bellegem, D. Van Herpe, M. De Mey and W. Soetaert*
Inbio.be, Department of Biochemical and Microbial Technology, Ghent University, Ghent, Belgium

126. Engineering *S. cerevisiae* Metabolism for Efficient Production of Acetyl-CoA Derived Products.

*C. Denby**
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127. Engineering Yeast to Produce Fatty Acid-Derived Fuels and Chemicals.

L. d'Espaux, W. Runghphan and J. D. Keasling*
Lawrence Berkeley National Lab, CA

129. Engineering a Functional Deoxyxylulose Phosphate (DXP) Pathway in *Saccharomyces cerevisiae*.

K. Dietzel, E. Antipov, G. Wichmann, N. Moss, P. Jackson, S. Gaucher, S. Gottlieb, J. D. Newman and L. Zhao*
Amyris Inc, Emeryville, CA

130. Expression Optimization of Multi-Enzyme Pathways for Xylose Utilization and Chemical Production.

J. E. Dueber[*1], M. Lee[1] and L. Latimer[2]*
[1]Bioengineering, U.C. Berkeley, Berkeley, CA
[2]Chemical Biology, U.C. Berkeley, Berkeley, CA

131. The Importance of the Lipid Biosynthetic Pathway for Glycolipids Production in Engineered *E. coli* Cells.

N. Mora-Buyé, M. Faijes and A. Planas*
Institut Químic de Sarriá - Universitat Ramon Llull

132. Micrornas and Apoptosis in Cell Culture - Application for Enhanced Biological Production and Cancer Treatment.

*J. Shiloach**
Biotechnology Lab, NIDDK /NIH, Bethesda, MD

133. Implications of the Assumptions on Intracellular Metabolic Operational States in Metabolic Control Analysis.

G. Fengos[*1,2], A. Chakrabarti[1,2], K. C. Soh[1,2], L. Miskovic[1,2] and V. Hatzimanikatis[1,2,3]*
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[2]Swiss Institute of Bioinformatics, Lausanne, Switzerland
[3]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

134. Novel Biosensors for Optimizing Yeast Cell Factories.

F. David, V. Siewers and J. Nielsen*
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

135. 13C Metabolic Flux Analysis of Co-Culture Systems: A Novel Approach.

N. A. Gebreselassie and M. R. Antoniewicz*
Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE

136. Targeted Omics Informed Engineering to Improve C5 Alcohol Production in *E. coli*.

*K. W. George**
Fuels Synthesis, Joint Bioenergy Institute (JBEI), Emeryville, CA

137. A Novel Design of a Translation Coupling-RNA Scaffold System to Improve the Efficiency of Molecular Chaperone on Recombinant Proteins Solubilization.

A. Geraldi, L. M. Bui, Z. Rahman, K. H. Kang, J. H. Lee and S. C. Kim*
Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea

138. 2-Butanol and Butanone Production in *Saccharomyces cerevisiae* through the B12 Dependent Dehydratase Pathway Using a Tev-Based Expression System.

P. Ghiaci[*1], J. Norbeck[2] and C. Larsson[2]*
[1]Chemical and Biological Engineering, Systems and Synthetic Biology, Chalmers University of Technology, Gothenburg, Sweden

[2]Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

139. Using Metrxn for Flux Elucidation and Model Reconstruction.

A. Kumar*[1], S. Gopalakrishnan*[2] and C. D. Maranas[2]
[1]The Huck Institutes of the Life Sciences, Penn State, State college, PA
[2]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

140. Use of Transporter Plug-Ins for Enhanced Productivity and Reduced Byproduct Formation of Bioalkanes and Related Compounds.

C. Grant*, P. Morris and F. Baganz
Advanced Centre for Biochemical Engineering, University College London, London, United Kingdom

141. Engineering Efficient Xylose Metabolism Using Synthetic Biology.

R. Hennessy*[1], H. I. Neves[1], P. Krabben[2], E. Jenkinson[2] and G. H. Thomas[1],
[1]Dept. of Biology, University of York, York, United Kingdom
[2]Green Biologics Ltd, Abingdon, United Kingdom

142. Using Protein Scaffolds to Redirect Photosynthetic Reducing Power for Biosynthesis of Natural Products.

M. Henriques de Jesus*, A. Z. Nielsen, B. L. Møller and P. E. Jensen
Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Copenhagen, Denmark

143. Assessing Metabolic Response to Increased Substrate Loading Rate in Mixed-Culture Fermentation of Waste Water.

R. D. Hoelzle*[1], B. Virdis[1,2] and D. J. Batstone[1]
[1]Advanced Water Management Centre, University of Queensland, Brisbane, Australia
[2]Centre for Microbial Electrosynthesis, University of Queensland, Brisbane, Australia

144. Genetic Engineering to Produce Higher Alcohols in Yeast *Saccharomyces cerevisiae*.

J. Ishii*[1], F. Matsuda[2], A. Kondo[3] and K. Ida[3]
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145. Directed Evolution of Terpene Synthases Using High-Throughput Colorimetric Screening Based on Substrate Consumption.

M. Iwasaki*[1], A. Fujii[1], M. Furubayashi[1], S. Wang[1] and D. Umeno[1,2]
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146. Enhanced Biofuel Production through Coupled Consumption of Acetic Acid and Cellulosic Sugars By Engineered Yeast.

N. Wei[1], G. Zhang[2], J. C. Quarterman[2], S. R. Kim[3], J. H. D. Cate[4] and Y. S. Jin*[2]
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[4]Molecular and Cell Biology, University of California at Berkeley

147. Lysate of Engineered *Escherichia coli* Supports Conversion of Glucose to 2,3-Butanediol with Near-Theoretical Yields and Ultrahigh Productivity.

J. E. Kay*[1] and M. C. Jewett[2]
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[2]Northwestern University, Evanston, IL

148. Evolution Reveals a Glutathione-Dependent Mechanism of 3-Hydroxypropionic Acid Detoxification.

K. R. Kildegaard*[1], B. M. Hallström[2], T. H. Blicher[3], N. Sonnenschein[1], N. B. Jensen[1], S. Sherstyk[1], S. J. Harrison[1], J. Maury[1], M. J. Herrgård[1], A. S. Juncker[1], J. Förster[1], J. Nielsen[1,4] and I. Borodina[1]
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[3]The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark
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149. Optimality of Microbial Metabolism with Biosynthetic Heterologous Reactions.

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150. Deciphering Thermodynamics in Metabolic Networks: A Priority List of Candidates for Metabolomics.

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151. Malic Acid Production By Aspergillus Oryzae.

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152. Feasibility Studies of New Strategy for Ultra-High-Throughput Screening (uHTS) of Novel Enzyme By *in vitro* Compartmentalization (IVC) Using Microbeads from Metagenomic Resources.

K. C. Ko*, B. Lee, D. E. Cheong, J. J. Song and J. H. Choi
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153. Replacement of the *Saccharomyces cerevisiae* Acetyl-CoA Synthetases By Acetylating Acetaldehyde Dehydrogenase for Cytosolic Acetyl-CoA Synthesis.

B. U. Kozak*[1], H. M. van Rossum[1], K. R. Benjamin[2], L. Wu[3],

J. M. G. Daran[1], J. T. Pronk[1] and A. J. A. van Maris[1]
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154. Decorating Bacterial Surfaces By Designer Molecules Advances the Fundamental Knowledge about Bacterial Growth.

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155. Metabolic Modulation in Response to Chemical-Induced Signal Transduction in Chlamydomonas Reinhardtii.

J. E. Lee[*1], D. Y. Lee[1], J. J. Park[2] and O. Fiehn[3]*
[1]Kookmin University
[2]Washing State University
[3]Genome Center, UC Davis

156. Metabolically Engineered *Escherichia coli* for Isoprene Biosynthesis.

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[2]Beijing University of Chemical Technology, Beijing, China

157. Application of a Genetically-Encoded Metabolite Sensor for Single Cell Analysis and Development of Production Strains.

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158. On the Effects of Phenotype Prediction Methods over Strain Design Algorithms. a Multi-Objective Approach.

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159. Exploring Bacterial Microcompartments to Establish Orthogonal Metabolism.

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160. Redemption: Reduced Dimensional Ensemble Modeling and Parameter Estimation.

Y. Liu, E. Manesso and R. Gunawan*
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161. Metabolic Engineering of Yeast Central Metabolism for Higher Alcohol Production.

F. Matsuda[*1,2], J. Ishii[3], S. Nishino[1], K. Morita[1], A. Kondo[2,4] and H. Shimizu[1]*
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162. The Impact of Orthogonal Gene Expression on Heterologous Pathway Productivity.

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163. Tools to Resolve Compartmentalized Metabolism in Mammalian Cells.

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164. Identification of the Missing Enzymes for the Heterologous Production of n-Butanol at High Yield.

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166. Engineering Improved Productivity of 1,4-Butanediol in *E. coli* _ a Kinetic Modeling Approach.

S. Andreozzi[1,2], A. Chakrabarti[1,2], K. C. Soh[1,2], A. Burgard[3], S. Van Dien[3], L. Miskovic[*1,2] and V. Hatzimanikatis[1,2,4]*
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167. Designing a Nitrogen Fixation Circuit in an Oxygenic Photosynthetic Organism.

A. Balassy[1], A. G. Hoynes-O'Connor[1], C. M. Immethun[1], D. Liu[2], T. J. Mueller[*3], R. Saha[3], Y. Xiao[1], T. S. Moon[1], F. Zhang[1], C. D. Maranas[3] and H. B. Pakras[1,2]*
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168. Effect of CO Transfer on Hydrogen Production By Carboxydotrophic Archeon *Thermococcus Onnurineus* NA1.

J. G. Na[*1], H. S. Jeong[1], T. W. Kim[2], S. G. Jeon[1], G. H. Kim[1], S. H. Chung[1] and S. G. Kang[2]*
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169. Streamlining Central Catabolism for _ La Carte Activation of Glycolysis.

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170. Towards a Chassis Organism for Synthetic Biology.

S. Noack[*1], S. Unthan[1], M. Baumgart[1], M. Herbst[2], G. Seibold[3], C. Rückert[4], V. Wendisch[2] and W. Wiechert[1]*
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171. Computing Proteome Abundance and Activity with a Genome-Scale Model of Metabolism and Gene Expression.

E. J. OBrien, A. Ebrahim, J. U. Carreri, J. A. Lerman and B. O. Palsson*
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172. Deciphering Dynamic Regulation Patterns of Cellulose-Degrading Enzymes in Anaerobic Fungi.

K. Solomon[1], J. K. Henske[1], C. Haitjema[1], D. Borges-Rivera[2], D. A. Thompson[2], A. Regev[2] and M. A. O'Malley[*1]*
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173. *Aspergillus Terreus* Isolated from the Brazillian Diversity: Genomic Variability Associated to Lovastatin Production.

N. Parachin, N.S.[*1], F. Mullinari[1], K. Assis1, B. Magalhães[2], G. Fernandes[2] and M. S. Felipe[1]*
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174. Regulation of Ancillary Reactions Around the Central Carbon Metabolism of *E. coli*.

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175. Examining the Complex Transcriptional Response of Perturbing Anthranilate Synthase in the Terpenoid Indole Alkaloid Pathway in *Catharanthus Roseus*.

*J. Sun and C. A. M. Peebles**
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176. Discovery of Gene Overexpression Targets for Biofuel Product Tolerance in Yeast.

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177. Microbial Production of Renewable Monoethylene Glycol.

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[1]Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA
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178. Improving the Internal Flux Distributions from Genome Scale Metabolic Models of *S. cerevisiae*.

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179. The Yeast Pathway Kit: A Method for Rational or Combinatorial Metabolic Pathways Design in *Saccharomyces cerevisiae*.

F. Pereira[*1], N. S. Parachin[2], B. Hähn-Hagerdal[3], M. F. Gorwa-Grauslund[3] and B. Johansson[4]*
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[3]Department of Applied Microbiology, Lund University, Lund, Sweden
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180. Efficient Searching and Annotation of Metabolic Networks Using Chemical Similarity.

D. Pertusi, A. Stine, L. J. Broadbelt and K. E. J. Tyo*
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181. Development of a Highly Efficient Gene Delivery System for Syngas Fermenting Clostridia.

*G. Philipps**
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182. Retooling Glycolysis in *Saccharomyces cerevisiae* for More Efficient Isoprenoid Production.

L. Pickens, K. Hawkins, Y. Tsegaye, A. Meadows, E. Antipov, L. Xu, M. Dasika, A. Tai, T. Mahatdejkul-Meadows, S. Ganesan, J. Lai, L. Chao, P. Westfall, Y. Kim, T. Gardner and A. Tsong*
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183. Systematic Characterization of Intracellular Metabolic States through Flux Directionality Profile Analysis.

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184. Trans-Regulatory Elements As Tools for Metabolic Engineering.

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185. Multiplex Amino Acid Metabolism Engineering for Increased Production of L-Ornithine in Yeast.

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186. Improved N-alkanes Production in *Escherichia coli* By Spatial Organization of Alkane Biosynthetic Pathway Enzymes.

Z. Rahman[*1], L. M. Bui[1], B. H. Sung[2], A. Geraldi[1], K. H. Kang[1], J. H. Lee[1] and S. C. Kim[1]*
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187. Tools for Advancing Genome Engineering on the Protein, Pathway, and Genome Scale.

E. Freed, T. J. Mansell, G. Pines, T. S. Reynolds and R. T. Gill*
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188. Ultra-High-Throughput Screening of Enzyme Libraries with Droplet-Based Microfluidics.

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189. Kinetic Reconstruction and Analysis of Sphingolipid Metabolism.

G. Savoglidis[*1,2], I. Riezman[3], A. X. S. Santos[3], H. Riezman[3] and V. Hatzimanikatis[1,2]*
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190. De Novo Production of Monoterpenoic Acids with Pseudomonas Putida.

J. Schrader, M. Buchhaupt, J. Mi, P. Lubuta and D. Becher*
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191. A Computationally-Driven Metabolic Engineering Strategy to Increase Cellulose Production in Plants.

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[1]Biological Systems Engineering, Virginia Tech, Blacksburg, VA
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192. Metabolic Engineering of an Endogenous Pathway for n-Butanol Production in *Saccharomyces cerevisiae*.

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193. Development of an *in silico* Tool of Novel Metabolic Pathway Designs for Microbial Productions.

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194. Production of Fatty Acid Derived Biofuels in *Saccharomyces cerevisiae*.

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195. Rapid One-Step Inactivation of Single or Multiple Genes in *Escherichia coli*.

C. Song and S. Y. Lee*
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196. Pigment-Based, Low-Cost, Portable Micronutrient Status Tests Using Engineered Bacteria.

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197. The Role of Trehalose Metabolism in *Saccharomyces cerevisiae* from a Quantitative Approach.

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198. Characterization of Anaerobic Central Metabolism to Improve Succinate Production in Enterobacter Aerogenes.

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[4]Business Strategy and Development Department, Ajinomoto Co.
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199. Expanding Biosynthetic Pathways Based on Thermodynamic Preferences.

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200. Fatty-Acid Production in Yeast through Reversal of the Beta-Oxidation Cycle.

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[2]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark

201. Increased 3-Hydroxypropionic Acid Production from Glycerol Based on the Genome-Scale Metabolic Simulation in *Escherichia coli*.

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203. Improving Dipicolinic Acid Production By *Bacillus Subtilis* during Stationary Phase Based on ^{13}c-Metabolic Flux Analysis.

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204. Construction of *E. coli* Reporter Strains for the Study of Stress-Answer Regulator Proteins.

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205. Rational Design of Modular Cells for Efficient Combinatorial Biosynthesis of Designer Bioesters.

C. T. Trinh, Y. Liu and D. Layton*
Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, Knoxville, TN

206. Design and Construction of an Artificial Nonmevalonate Operon of *Escherichia coli*.

K. Tsuge, T. Togashi, M. Hasebe, M. Tomita and M. Itaya*
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207. Lactic Acid Production from Xylose By Engineered *Saccharomyces cerevisiae*.

T. L. Turner[1], G. Zhang[1], V. Subramaniam[2], D. Steffen[2] and Y. S. Jin[1]*
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[2]Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL

208. Model Driven Mechanistic Analysis of Adaptive RNAP Mutations.

J. Utrilla[1], E. O'Brien[1], D. McCloskey[1], A. M. Feist[1,2] and B. O. Palsson[1,2]*
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209. Carbon Dioxide Fixation By Calvin-Cycle Enzymes Improves Ethanol Yield in Yeast.

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210. Rational Engineering of the Methylerythritol Phosphate Pathway through Metabolic Control Analysis.

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211. Comprehensive Evaluation of Two Genome-Scale Metabolic Network Models of Scheffersomyces Stipitis.

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[3]Auburn University, Auburn, AL

212. Genome Scale Metabolic Modeling Reveals New Insights into Biomass Production in the Marine Sponge Amphimedon Queenslandica.

J. Watson[1], R. Palfreyman[2], S. Fernandez-Valverde[1], T. Brennan[2], L. K. Nielsen[3], B. Degnan[1], S. Degnan[1] and J. O. Krömer[4]*
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213. Quorum-Sensing Linked RNAi for Dynamic Pathway Control in *Saccharomyces cerevisiae*.

T. C. Williams[1], N. J. H. Averesch[2], N. Lekieffre[2], G. Winter[2], C. E. Vickers[1], L. K. Nielsen[1] and J. O. Krömer[2],*
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214. Balancing Flux through Secondary Metabolic Pathways in Plant Culture Systems.

S. Wilson[1] and S. Roberts[2]*
[1]Chemical Engineering, University Of Massachusetts Amherst, Amherst, MA
[2]University of Massachusetts, Amherst

215. Optimization of Multi-Gene Biological Systems Using High-Throughput DNA Assembly, Sequencing, and Model-Guided Search Strategies.

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[2]Synthetic Biology Center, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA

216. Evaluation of Biosynthetic Pathways for Conversion of Natural Gas to Liquid Fuels.

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217. High-Efficiency Scarless Genetic Modification Method in *Escherichia coli* without Counterselection.

J. Yang, B. Sun, H. Huang, Y. Jiang, L. Diao, B. Chen, C. Xu, X. Wang, J. Liu, W. Jiang and S. Yang**
Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

218. Metabolic Engineering of a Biosensor-Based Screening Platform in Yeast.

J. Zhang[1], M. K. Jensen[1] and J. D. Keasling[1,2,3,4,5]*
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[5]Department of Bioengineering, University of California, Berkeley, Berkeley, CA

Poster Presentations

219. Metabolic Engineering for Production of Valuable Chemicals Based on *Escherichia coli* Strains Designed *in silico*.

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[3]DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI

220. Hy-Dynfba: A Software Platform to Build Large Models, Hybrid in Time Scale.

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221. Production of Enantiomerically Pure (S)-3-Hydroxybutyrate Using Metabolically Engineered *Saccharomyces cerevisiae*.

E. J. Yun and K. H. Kim*
Department of Biotechnology, Korea University Graduate School, Seoul, South Korea

222. Characterization of High Ethanol Producing Properties of Recombinant *Saccharomyces cerevisiae* ETS3 Transformed with a Mutated SPT15 Gene.

H. Park[*1], Y. J. Seong[1], K. H. Kim[2] and Y. C. Park[1]*
[1]Department of Bio and Fermentation Convergence Technology, Kookmin university, Seoul, South Korea
[2]Department of Biotechnology, Korea University Graduate School, Seoul, South Korea

223. Comparative Metabolomic Study of Anaerobic and Aerobic Processings of Metabolite Sample Preparation for Clostridium Acetobutylicum.

S. Kim[*1], S. H. Lee[*1,2] and K. H. Kim[1]*
[1]Department of Biotechnology, Korea University Graduate School, Seoul, South Korea
[2]R&D Center, GS Caltex Corporation, Daejeon, South Korea

224. Identification and Characterization of a Processive Endoglucanase from a Marine Bacterium As a Means to Substitute Cellulbiose Hydrolases.

H. J. Lee, I. J. Kim, H. Youn and K. H. Kim*
Department of Biotechnology, Korea University Graduate School, Seoul, South Korea

225. Engineering of a protein translocation system in Rhodococcus jostii RHA1 for the secretion of ligninases.

R. Roccor and L. D. Eltis*
Department of Microbiology and Immunology, The University of British Columbia, Vancouver, Canada

226. Synthetic Design of Pathways and Organelles for Photosynthetic Terpene Production.

Y. K. Kim[*1], H. Ma[1], Y. Wang[2], S. Goklany[3], E. Takahashi[4], Y. C. Liu[1], S. Dai[5], D. Ort[4], J. Chappell[3], X. G. Zhu[2] and J. Yuan[1]*
[1]Plant Pathology and Microbiology, Texas A&M University, TX
[2]PICB, Chinese Academy of Science
[3]Pharmaceutical Sciences, University of Kentucky, Lexington, KY
[4]Plant Biology, University of Illinois at Urbana-Champaign, IL
[5]Department of Veterinary Pathobiology, Texas A&M University, TX

227. Production of Anteiso-Branched Fatty Acids in *Escherichia coli*, Next Generation Biofuels with Improved Cold-Flow Properties.

R. W. Hauschalter[*1], W. Kim[1], T. A. Chavkin[2], L. The[2], M. E. Garber[2], L. Katz[2] and J. D. Keasling[1,2]*
[1]Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA
[2]QB3 Institute, University of California, Berkeley, Emeryville, CA

228. A Functional Rect Gene for Recombineering of Clostridium.

H. Dong, Y. Zhang and Y. Li*
Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

229. Enhancement of Hyaluronic Acid Molecular Weight By Re-Direction of Metabolic Fluxes in Engineered *Lactococcus Lactis* Cultures.

M. Kaur and G. Jayaraman*
Department of Biotechnology, Indian Institute of Technology-Madras, Chennai, India

230. High-Throughput Screening of Metabolite Producers Using Synthetic Suicide Riboswitch in *Saccharomyces cerevisiae*.

S. W. Lee and M. K. Oh*
Department of Chemical and Biological Engineering, Korea University, Seoul, South Korea

231. A Computational Method to Construct an Extensive Metabolic Pathway Database.

M. Araki[1], H. Makiguchi[2], T. Ogawa[2], K. Miyaoku[*3], T. Taniguchi*[*3], R. S. Cox III[4], M. Nakatsui[4] and A. Kondo[5]*
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[4]Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, Kobe, Japan
[5]Graduate School of Engineering, Department of Chemical Science and Engineering, Kobe University, Kobe, Japan

232. Development and Analysis of High-Light Stress Tolerant Strain of *Synechocystis* Sp. PCC 6803.

K. Yoshikawa[*1], K. Ogawa[1] and H. Shimizu[2]*
[1]Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, Japan
[2]Graduate School of Information Science and Technology, Osaka University, Suita, Japan

233. Transport Proteins for Itaconic Acid Production in Aspergillus Niger.

M. G. Steiger[*1,2,3], P. J. Punt[2,4], A. F. J. Ram[2], D. Mattanovich[1,4] and M. Sauer[1,4]*
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[2]Institute of Biology, University Leiden, Leiden, Netherlands
[3]Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria
[4]TNO Microbiology and Systems Biology, Zeist, Netherlands

234. A Strategy for Design, Redesign, and Optimization of Ethylene Production in *E. coli*.

S. Lynch[1,2], C. Eckert[*3,1], J. Yu[1], P. C. Maness[1] and R. Gill[2]*
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[2]Chemical and Biological Engineering, University of Colorado, Boulder, CO
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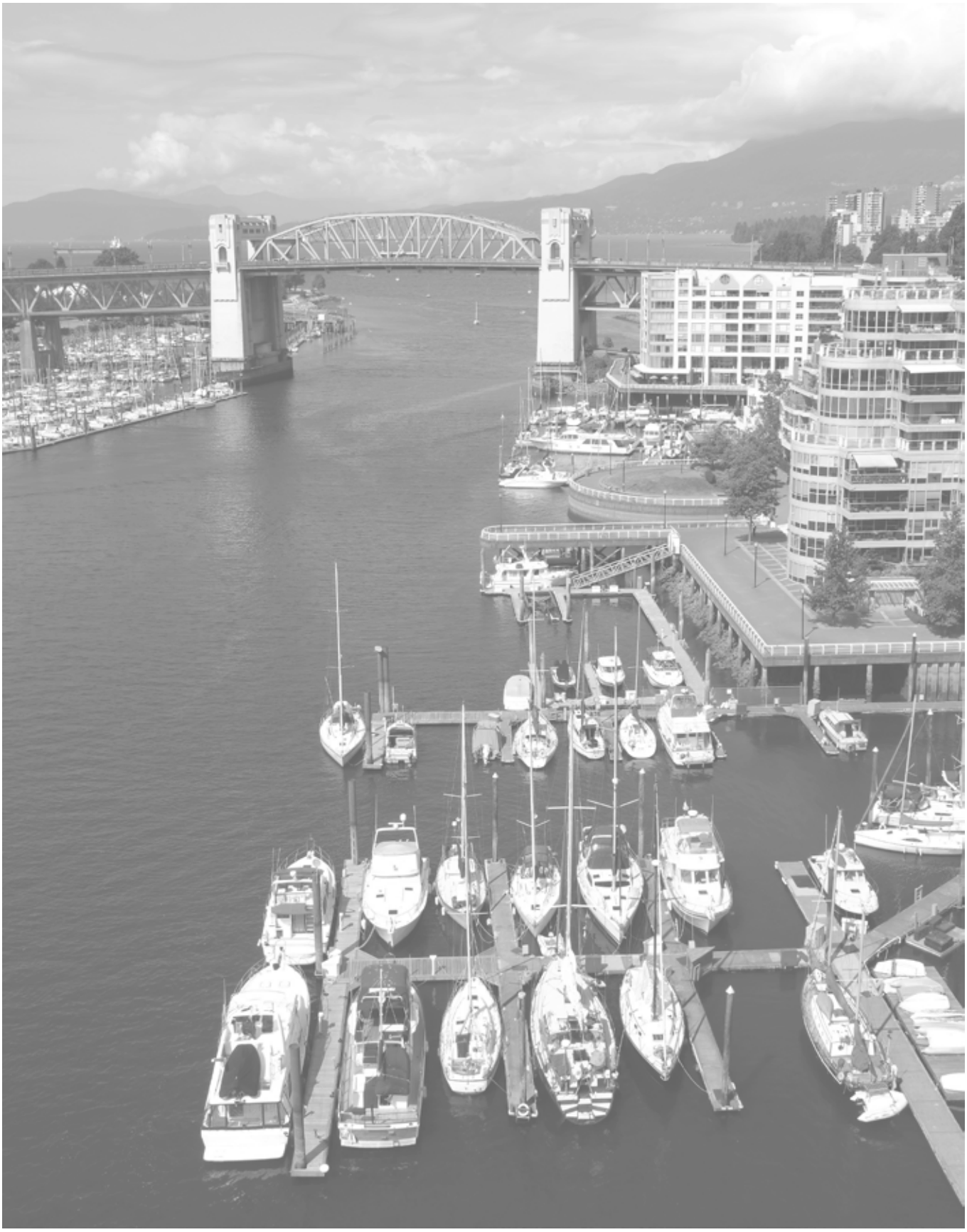

Oral Presentation Abstracts

Opening Plenary — Sunday, June 15

A 15-Year Journey to Figure out How to Derive the Most Economic Value from Metabolic Engineering

Christophe Schilling
CEO, *Genomatica, San Diego, CA*

As a founder and CEO, Christophe Schilling has led Genomatica from a developer of metabolic engineering tools to a position of leadership in biotech processes for the production of major chemicals — with multiple customers, $125 million in equity investments, and numerous awards for their technology, commercialization results and corporate culture.

Please join Christophe as he takes you on a 15 year (and counting) personal tour of his experiences. How did Genomatica go from pioneers in the commercial application of computational modeling of metabolism for the life sciences to the choice of a specific business strategy focused on developing and licensing process technology for chemical production, and the addition of experimental capabilities, process engineering and other core strengths? Christophe will speak to the technical, business and people issues that come up when starting with an idea, building a company, raising money, getting customers, and more. He'll also share his personal view of where metabolic engineering can 'most readily' turn into economic value — and what it takes to do so.

Perspectives on Creating & Scaling-up Better Energy Solutions

Vineet Rajgarhia
Vice President, *Biotech R&D, Total New Energies USA, Emeryville, CA*

Session 1 — Monday, June 16
Parts to Protein Scale Engineering

Chairs: Timothy Lu, Massachusetts Institute of Technology and Fernando Valle, Codexis

Invited Speaker: New Enzymes by Evolution: Expanding Nature's Catalytic Repertoire.

F. H. Arnold*
Chemistry & Chemical Engineering, California Institute of Technology, Pasadena, CA

Enzymes are admired for their ability to selectivity catalyze myriad transformations. Not satisfied with nature's vast catalytic repertoire, we are constantly searching for new ones to add to the toolkit of genetically encoded chemistry. My group uses the one proven algorithm for biological design—evolution—to optimize existing catalysts and create whole new ones. A powerful approach to engineering useful biological molecules, directed evolution both circumvents and underscores our profound ignorance of how sequence encodes catalytic function. I will describe various ways we have used evolution (contaminated with a little chemical intuition) to generate new catalysts starting from one of nature's most impressive, the cytochrome P450 monooxygenase. I will share several examples of engineered versions that catalyze important synthetic reactions not known in nature.

Synthetic Enzymes for Synthetic Biology

Alexandre Zanghellini*[1], Kyle Medley[2], Michal Galdzicki[1,2], Rudesh Toofanny[1], Yih-En Andrew Ban[1] and Herbert Sauro[2]
[1]R&D, Arzeda, Seattle, WA
[2]Bioengineering, University of Washington, Seattle, WA

Our ability to design cell factories to produce valuable chemicals requires the "recombination" not only of existing but also designer enzymes into novel metabolic pathways to achieve entirely new metabolic function. This poses two distinct challenges that need to be solved in an integrated way if we ambition to fully deliver on the promise of synthetic biology. The first challenge deals with the rapid design of novel enzymes with high level of activities for reactions not known to be catalyzed in nature. We will review Arzeda's published and unpublished successes in the computational de novo design of synthetic enzymes with entirely new catalytic sites[1–5]. The practical impact of our technology will be highlighted through case studies of our recent industrial collaborations, including the repurposing of existing natural active sites to catalyze novel reactions and the design of novel enzymes for the fermentation of key chemical building blocks.

The second challenge is that of finding optimal ways to arrange natural and designed enzymes to biosynthetize a small-molecule of interest. To illustrate our progress towards solving this challenge, we will discuss a novel tool that Arzeda has been developing for the automated design of novel biosynthetic pathways. Inspired by retrosynthetic methods in organic chemistry, our software draws on databases of known natural enzymatic reactions as well as reactions that can be catalyzed by computationally designed enzymes to exhaustively enumerate biosynthetic routes from a set of desired metabolites down to any small molecule of interest. Pathways are ranked based on thermodynamic feasibility, designability of each enzymatic step (with specific metrics

relating to our computational enzyme design software) and overall complexity and predicted yield of the synthetic route. We will present applications of industrial relevance in the field of fine and bulk chemicals.

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Invited Speaker: *In vivo* Evolution of Enzymes and Metabolic Pathways for the Production of Chemicals

Philippe Soucaille*, *LISBP, INSA, University of Toulouse, Toulouse, France*

The development of efficient microorgansims for the production of chemicals by fermentation relies most of the time on the construction of genetically optimized microorganisms based on a rational metabolic engineering approach. However, improved enzymes or new enzyme activities are frequently needed and it is time and money consuming to obtain them either by rational protein engineering or by the now classical evolutionary methods like error prone PCR or DNA shuffling.

We have developed and patented an efficient *in vivo* molecular evolution method to rapidly evolve enzymes or metabolic pathways. The simple principle of this method is to rationally design a microorganism to link the activity of the enzyme(s) or metabolic pathway to be evolved to the growth of the host organism. When such a strain is constructed, it is then easy to evolve the enzyme(s) (thanks to the "natural mutation frequency" of living organisms) by selecting for growth improvement in a continuous culture. To highlight efficiency of this approach, we will present five examples where in less than a month improved or new enzymes were successfully obtained. In the context of developing new bioprocesses involving fermentations or bioconversions, we will demonstrate how this method can really speed up the time to market of new "biochemicals".

Development of Adaptable Synthetic Promoters to Optimize Protein Scale Engineering in Yeast.

Ian Fotheringham*, *Ingenza, Ltd., Roslin, United Kingdom*

We have developed a protein production system for use in engineered yeast strains that significantly improves upon currently available alternatives. Our new system incorporates novel synthetic promoter constructs that have been rationally designed to drive optimal gene expression. The resulting synthetic system addresses significant limitations in the current state-of-the-art for yeast-based protein expression. Our system is applicable towards multiple opportunities across industry sectors including metabolic engineering of biochemical pathway intermediates and the production of therapeutic protein end products. This work combines innovations in the areas of synthetic promoter design, expression element library generation and screening, and yeast-based expression optimization. The rapid design, manufacture and testing of this system's components was enabled by the application of Ingenza's combinatorial genetics platform (inABLE®). This combinatorial genetics platform was used to rapidly clone, express, select and optimize target promoter function across many separate assays, from thousands of independent gene expression control elements derived from bioinformatics-driven synthetic promoter design. This unique combination of technologies and capabilities allowed us to realize a platform with the flexibility to address an even broader range of challenges across yeast-based protein expression.

Invited Speaker: Protein Engineering of Isoprene Synthase Enables High Titer Isoprene Production.

Maggie Cervin*, *Industrial Biotechnology, E.I. du Pont de Nemours, Palo Alto, CA*

DuPont and The Goodyear Tire & Rubber Company are developing a BioIsoprene™ product alternative to petroleum derived isoprene. Isoprene is a key chemical required to produce a diverse range of industrial products including a wide variety of specialty elastomers used in surgical gloves,

rubber bands, golf balls, shoes and tire manufacture. The annual world market potential for polymer grade isoprene is approximately 2 billion dollars. We envision the biological production of isoprene (BioIsopreneTM monomer) as a sustainable solution that can address the lack of supply and price instability of this valuable commodity.

The biosynthetic production of isoprene presents significant metabolic engineering challenges that include overcoming pathway enzyme regulation, channeling carbon to product, balancing redox and energy requirements of the cell, and selection of appropriate enzymes. The key enzyme, isoprene synthase, has poor kinetic parameters and was a major hurdle to production of isoprene. Using rational protein engineering and PCR mutagenesis in combination with *in vitro* and *in vivo* screening techniques we identified variants that enabled isoprene to be produced at commercially relevant metrics. Analysis has demonstrated that the BioIsoprene™ product is >99% pure prior to recovery and purification. Pre-pilot work with the developing process has lead to the manufacture of prototype passenger car tires demonstrating the functional process from start to finish.

Session 2
Pathway Scale Engineering

Chairs: Michelle Chang, University of California, Berkeley; George Chen, Tsinghua University; John Dueber, University of California, Berkeley

Invited Speaker: Engineering Organisms and Processes for Cost-Effective Lipid Production

Gregory Stephanopoulos*
Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

Lipids are an important product that can be used for the production of biofuels such as biodiesel. The latter is presently produced from vegetables and seed oils, however, its long-term cost-effective production should utilize carbohydrate feedstocks for optimal land use. To this end, we have engineered an oleaginous yeast for the high yield conversion of carbohydrates to lipids and accumulation at high titers. The engineering strategy followed a push-pull approach whereby the fatty acid synthesis pathway was enhanced through the overexpression of Acetyl-CoA-Carboxylase (ACC1) along with the downstream lipid storage pathway for lipid sequestration in lipid bodies. We found that both pathways are important for optimal conversion yield and productivity, which is further obtained by minimizing citrate byproduct formation and maximizing pathway throughput. Pathway throughput is maximized by reducing or eliminating regulation of fatty acid synthesis, a goal that was achieved through the modulation of various target desaturase-expressing genes. This strategy yielded sharp increases in overall growth rate and lipid synthesis in small-scale flasks and bioreactor runs. We also examined lipid synthesis from acetate and other volatile fatty acid (VFA) substrates that can be obtained from inexpensive feedstocks such as synthesis gas or municipal solid waste by anaerobic digestion. We show that, using our engineered strains, VFAs can be converted efficiently to lipids at high yields. Additionally, using efficient cell recycle systems, dilute VFA streams can be converted to lipids that, as intracellular products, accumulate in the yeast cells at high titers. Our analysis shows that efficient lipid and biofuel production processes depend critically on feedstock cost and process yield, however, our results, taken together, suggest that these products are possible to produce in a cost-effective manner and be used at scale for biofuel production.

Pathway Engineering Meets Evolution: New Approaches for Improving Pentose Catabolism in Yeast

Hal Alper*, *Department of Chemical Engineering, The University of Texas at Austin, Austin, TX*

Blending pathway engineering with directed evolution and adaptive strain engineering enables a powerful approach for rapid metabolic engineering. Here, we demonstrate the power of this blended approach through three examples of improving pentose catabolism engineering that each demonstrates different stages of the design cycle. First, we couple bioprospecting, directed evolution, and ultimately rational design to rewire molecular transporter proteins. Specifically, sugar transport preference and kinetics can be rewired through the programming of a specific sequence motif. This rewiring can convert hexose transporters into pentose transporters and can be used to improve overall pentose catabolic rates. Second, we couple pathway engineering with directed evolution and adaptive evolution to obtain a strain of yeast with superior xylose catabolic rates. Initially, we evolved xylose isomerase enzyme for improved function in yeast. When this enzyme is coupled with strain engineering and adaptive evolution, xylose catabolic rates equivalent to and exceeding those using the oxidoreductase pathway can be achieved. In this instance, the resulting strain of yeast achieves yields of 0.49 g ethanol / g xylose and is among the best utilizers of xylose reported to date. Third, we report on a complete design cycle from strain identification to bioprospecting to evolution and optimization. Specifically, we isolated a fungal

strain with unique pentose catabolic potential. Through genome sequencing and complementation, we establish novel pentose catabolic pathways for xylose, arabinose, and xylan. When coupled with directed evolution, strains were capable of utilizing and converting xylose, arabinose, and xylan as sole carbon sources in minimal medium conditions. These evolved pathways and cells highlight the potential of this blended approach and establish novel, functional pathways. Each of these examples demonstrates the potential of a "metabolic pathway protein engineering" blended approach.

Invited Speaker: Rewriting Pathways to Life for Carbon Conservation

James C. Liao*, *Department of Chemical and Biomolecular Engineering, University of California, Los Angeles*

Production of chemicals and fuels using biological method is a desirable goal that has been pursued for decades, if not centuries. Although success stories have been increasing recently, biological processes are still difficult to compete with traditional chemical processes. One of the major limitations of biological processes lies in the central pathways that support all life processes on earth. In particular, glycolysis, a fundamental metabolic pathway in life that exists in almost all organisms to decompose sugars, proceeds in a way that loses 1/3 of the carbon to CO_2 when producing most of the fuels and chemicals. As a result, almost all biofuel and biochemical production processes suffer a significant loss in yield. The pathway proceeds through partial oxidation and splitting of sugars to pyruvate, which in turn is decarboxylated to produce acetyl-coenzyme A (CoA) for various biosynthetic purposes. The decarboxylation of pyruvate loses a carbon equivalent, and limits the theoretical carbon yield to only two moles of two-carbon (C2) metabolites per mole of hexose. This native route is a major source of carbon loss in biorefining and microbial carbon metabolism. In this talk, we will discuss the design and construction of a non-oxidative, cyclic pathway that allows the production of stoichiometric amounts of C2 metabolites from hexose, pentose, and triose phosphates without carbon loss. This pathway, termed Non-Oxidative Glycolysis (NOG) enables complete carbon conservation in sugar catabolism to acetyl-CoA, and can be used in conjunction with CO_2 fixation and other one-carbon (C1) assimilation pathways to achieve 100% carbon yield to desirable fuels and chemicals.

Engineering Static and Dynamic Tuning of Malonyl-CoA Derived Metabolite Biosynthesis in Microorganisms

Mattheos A. G. Koffas*[1] and Peng Xu[2]
[1]*Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY*
[2]*Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA*

Our research goal is to utilize the richness, versatility but also simplicity of microbial organisms in order to make them ideally suited to convert cheap, renewable resources into either high-value or commodity chemicals.

For the purpose of reprogramming the cellular network in order to achieve optimal phenotypes supporting high-yield production, we have developed in silico model of the genome-wide metabolism *Escherichia coli*. Through the application of Metabolic Flux Analysis, we can predict genetic modifications such as deletions and gene expression attenuations that lead to dramatic increases in production levels. Such Systems Biology approaches, in combination with traditional genetic engineering have resulted in robust production levels that can result in the commercially viable processes for the synthesis of important molecules, in particular ones that derive from malonyl-CoA. We also report the engineering of both positive and negative feedback controls for dynamic tuning of metabolic flux in *E. coli*. Specifically, we have identified a dual transcriptional regulator that can act either as an activator or a repressor for two different promoters. The level of activation or repression is dependent on the level of intracellular malonyl-CoA. As a proof of concept, we demonstrated that the expression of two reporter proteins can be exclusively switched between the on and off state. By engineering this synthetic malonyl-CoA controller, we envision that both the malonyl-CoA source pathway and the malonyl-CoA sink pathway can be dynamically modulated so that carbon flux can be efficiently redirected to synthesize our target compounds. Implementation of this dynamic control will maintain the intracellular malonyl-CoA at the optimal level and improve both the productivity and yield of value-added metabolites in *E. coli*.

Invited Speaker: Novel Biosynthetic Pathways for Production of Acids and Alcohols

Kristala Jones Prather*, *Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA*

Microbes are being increasingly used as "microbial chemical factories" to produce compounds that include bulk and specialty chemicals, materials, pharmaceuticals, and biofuels. As new products are desired through biological conversion, new enzyme catalysts are needed to mediate these transformations. We have exploited the promiscuous activity of several different enzymes in order to build a versatile platform pathway for the synthesis of various 3-hydroxyalkanoic acids, fatty acids and alcohols. Examining the product profile *in vivo* has led to greater understanding of the constituent enzymes in the absence of access to the substrates needed for *in vitro* analysis. I will discuss our findings to date with this pathway, as well as challenges that we face towards linking *in vitro* characterization to *in vivo* performance, and opportunities to use experimental data to guide the selection of additional enzyme variants that may have higher target activities.

Sssion 3
Genome Scale Engineering

Chairs: Hal Alper, University of Texas at Austin and Zach Serber, Zymergen

Invited Speaker: Comprehensive characterization of genotype to phenotype relationships in *E. coli*.

Morten Sommer* *Department of Systems Biology, Technical University of Denmark, Denmark*

Genome engineering has revolutionized our ability to construct diverse cell libraries opening up for the possibility of conducting comprehensive studies on the relationship between genotype and phenotype. We have used multiplex automated genome engineering (MAGE) to construct libraries for assessing the impact on specific genetic variants on the translation and transcription of genes in E.coli. Using microarray derived oligos we demonstrate that libraries can be engineered to introduce artificial promoters driving the expression of a majority of non-essential genes in E coli. Furthermore, we use these approaches to comprehensively quantify the dependence of the Shine-Dalgarno sequence on protein expression and use this information to modulate the translation rate of all transcription factors in E.coli. We show that these approaches can be used to identify causal mutations underlying improved tolerance phenotypes as well as to efficiently explore phenotypic space to accelerate cell factory development.

Metabolic Engineering Via RNAi-Assisted Genome Evolution (RAGE)

Tong Si, Han Xiao, Xiong Xiong, and Huimin Zhao Departments of Chemical and Biomolecular Engineering, Chemistry, and Biochemistry, Bioengineering, Institute for Genomic Biology, and Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL*

Engineering complex traits such as inhibitor tolerance and temperature tolerance remains a main challenge in metabolic engineering. In this talk we report the development of RNA interference (RNAi)-assisted genome evolution (RAGE) as a generally applicable method for engineering complex traits in yeast. Through iterative cycles of creating a library of RNAi induced reduction-of-function mutants coupled with high throughput screening or selection, RAGE can continuously improve target trait(s) by accumulating multiplex beneficial genetic modifications in an evolving yeast genome. We demonstrated RNAi screening in *Saccharomyces cerevisiae* for the first time by identifying two known and three novel suppressors of a telomerase-deficient mutation yku70 . We then showed the application of RAGE for improved acetic acid tolerance, a key trait for microbial production of chemicals and fuels. Three rounds of RAGE led to the identification of three gene knockdown targets that acted synergistically to confer an engineered yeast strain with one of the highest reported levels of acetic acid tolerance. In addition, we used RAGE to improve the furfural tolerance, another key trait for microbial production of chemicals and fuels from cellulosic materials. We discovered that disruption of SIZ1 gene encoding an E3 SUMO-protein ligase by knockdown or deletion conferred significantly higher furfural tolerance compared to other previously reported metabolic engineering strategies in *S. cerevisiae*. As a third example, we used RAGE to engineer a thermotolerant *S. cerevisiae* strain that grows well under 42C, a temperature at which the wild type strain grows poorly. Finally, coupled with *in vivo* biosensors, we used RAGE to improve the titers of polyhydroxybutyrate (PHB) and fatty acids, respectively. Taken together, RAGE represents a novel high throughput genome-scale engineering tool for engineering complex traits and improving production of chemicals and fuels in yeast.

Genetically Encoded Biosensors + FACS + Recombineering: Versatile Tools for Strain and Enzyme Development

Michael Bott*, Georg Schendzielorz, Stephan Binder, Solvej

Siedler, Stephanie Bringer, Jan Marienhagen, Julia Frunzke and Lothar Eggeling, *Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Juelich, Juelich, Germany*

Transcriptional regulators usually function as sensors and regulators, as their activity is controlled by specific stimuli, such as metabolites, redox status, toxic compounds, or inorganic ions. We recently have exploited this property for the construction of biosensors, which are able to detect the concentrations of amino acids such as L-lysine or L-methionine in single bacterial cells and convert this information into a fluorescence output by using EYFP or other autofluorescent proteins as reporter. The response was linear in a certain concentration range (Binder et al. 2012; Mustafi et al. 2012). The lysine sensor was used to isolate single producer cells from a library of randomly mutagenized cells of Corynebacterium glutamicum by fluorescence-activated cell sorting (FACS). Characterization of these clones led to the identification of known, but also of novel mutations triggering lysine overproduction (Binder et al. 2012).

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More recently, we also developed a biosensor for the NADPH/$NADP^+$ ratio based on the transcriptional regulator SoxR of *Escherichia coli*. SoxR is a homodimer with each subunit containing a [2Fe-2S] cluster. Only when oxidized to $[2Fe–2S]2^+$ they confer transcriptional activity to SoxR, which in turn results in expression of soxS. SoxS then activates expression of the SoxRS regulon, which mediates the cellular response to superoxide, to diverse redox-cycling drugs like paraquat, or to nitric oxide and includes e.g. the genes for superoxide dismutase (sodA), glucose 6-phosphate dehydrogenase (zwf), or fumarase C (fumC). Inactivation of SoxR involves its NADPH-dependent reduction catalyzed by the rsxABCDGE and rseC products. The observation that soxS expression was strongly increased during the NADPH-dependent reductive biotransformation of methyl acetoacetate to (R)-methyl 3-hydroxybutyrate by an alcohol dehydrogenase (Siedler et al. 2014a) suggested that SoxR is responsive to the NADPH/$NADP^+$ ratio, which was already indicated in previous studies (Liochev and Fridovich 1992; Krapp et al. 2011). By placing the eyfp gene under the control of the soxS promoter, correlations of the specific fluorescence intensity with the period of high NADPH demand and with alcohol dehydrogenase activity were found. In a proof-of-principle study, the pSenSox sensor was used to isolate via FACS an alcohol dehydrogenase variant with improved activity for the substrate 4-methyl-2-pentanone out of mutant library. Thus, this sensor is suitable for HT-screening of NADPH-dependent enzymes (Siedler et al. 2014b).

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ACS Synth Biol 3:21-29

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A Unique Bioengineering Platform for the Efficient Optimization of Metabolic Pathways

Kedar G. Patel*, Mark Welch, Sridhar Govindarajan and Claes Gustafsson, *DNA2.0 Inc., Menlo Park, CA*

Metabolic engineering enables the conversion of microbial cells into factories for the production of drug molecules and other valuable chemical products. Modulation and optimization of metabolic pathways is accomplished by several complementary approaches that influence the presence, catalytic properties and abundance of pathway enzymes.

We have developed a bioengineering platform that is based on the seamless integration of synthetic biology, efficient gene synthesis and modern machine learning to address whole bio-system optimization. The platform enables simultaneous exploration of a large number of variables ranging from synonymous mutations via amino acid substitutions and DNA/protein parts all the way through pathway replacement and genome editing at the chromosomal level while minimizing the sample numbers needed. We manage the large size of possible sequence space by condensing the dimensionality and capturing correlating variables. Causal variables are identified and their relative contribution quantified by iterative rounds of systematic exploration. The technology is generic and broadly applicable in biology and can be used within existing QbD (Quality by Design) processes to capture and interrogate design information much upstream of typical industrial scale QbD.

We will describe several independent unpublished case studies illustrating the efficiency and power of the technology. Examples includes the engineering of polyketide synthase pathways for producing novel compounds, maximizing bio-production yield of commercial small molecules as well as therapeutic.

Flux Response of Glycolysis and Storage Metabolism during Rapid Feast/Famine Conditions in Penicillium Chrysogenum Using Dynamic 13C Labeling

Lodewijk de Jonge[1], Alessandro Abate[2] and S. Aljoscha Wahl*[3,4]
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The physiology, growth and product formation of a cellular system are the results of a complex interaction between the extracellular environment and the cellular metabolic and regulatory mechanisms[1]. Thus, the production capacity of an organism depends strongly on environmental conditions that could be a reason for unexpected scale-up behaviour. The scale-up of substrate limited cultivation processes results in a series of differences in environmental conditions that have their origin in transport limitations and non-ideal mixing. Especially relevant are oxygen limitation[2], increase in carbon dioxide concentrations and substrate starvation in certain areas of the large-scale reactor[1, 3, 4.] Cells circulating through the broth experience alternating environments with different substrate availability[5] and the cellular environment becomes highly dynamic (at the time scale of the mixing time), reaching from substrate excess to substrate starvation, henceforth called the feast/famine regime. These dynamic conditions can lead to reduced biomass yield and reduced product formation.

For *Penicillium chrysogenum*, intermittent feeding in 360 s cycles with 36 s feeding with fresh medium and 324 s of no feed were applied to mimic large-scale bioreactor conditions. This regime leads to a 50% decrease in Penicillin G production[5]. Concentration measurements of intracellular metabolites over the feast/famine cycles have shown drastic intracellular dynamics in several metabolite levels, including nucleotides[5]. The concentration of the penicillin G pathway precursors was lower than continuous cultures, but could not explain the 50% decrease in productivity. Also, enzyme activities were comparable in both conditions. De Jonge et al[5] therefore concluded that the dynamics and the kinetics of the reactions have a major impact on the penicillin production flux. Additionally, other pathways could consume more energy, reducing the amount of ATP available for penicillin G production. One hypothesis was an increased synthesis and degradation of storage compounds to buffer the extracel-

lular dynamics in substrate supply. A cycle of synthesis and degradation results in net energy consumption (futile cycle).

Intracellular fluxes that form a cycle cannot be estimated from extracellular measurements and intracellular concentration measurements alone, but additional labeling measurements are required. Here we apply a recently developed metabolic dynamic approach using hybrid systems theory to identify dynamic flux profiles during the feast/famine cycle[6] from dynamic concentration and 13C labeling measurements.

Results:

The intermittent feeding led to fluctuations in the extracellular glucose concentration between 400 mM down to 6.5 mM at the end of the cycle. The intracellular metabolite concentrations responded strongly and showed up to 100 fold changes.

When comparing steady state and the feast/famine feeding regime, significant differences in the amount of storage metabolites were observed. The average trehalose concentration increased from 59.1 µmol/gDCW to an average of 178 µmol/gDCW under dynamic conditions. In contrast to the increase in trehalose, the mannitol concentration decreased from 374 µmol/gDCW to 83 µmol/gDCW when dynamic conditions were applied.

The changes in concentration during a single cycle (360s) were small compared to the measurement accuracy. Mass isotopomer measurements are relative measurements and the accuracy increases at high metabolite concentration as the signals become stronger. Trehalose reaches an enrichment of 2.5% (per carbon) within 360s and 4.5% after three cycles.

The trehalose enrichment increased from 1.1% to about 2.4% within 210 s. An increase was only seen after about 100 s, which is also in agreement with the observation that the precursor Tre6P enrichment rose after 40-70 s. In contrast to this late enrichment, the first intermediate of the trehalose/glycogen branch G1P had a labeling pattern very similar to G6P. It can be assumed, that the phosphoglucomutase (PGM) connecting G6P and G1P was operating close to equilibrium. The concentration of UDP-Glc increased after the addition of substrate, and also 13C enrichment was immediately measured. The enrichment of UDP-Glc and Tre6P reached a maximum at about 210 s and began to slowly decrease again. At this time point the extracellular substrate concentration already dropped to 15 µmol/L.

The average concentration of mannitol was lower (83 µmol/gDCW). But, the pool reached a labeling enrichment of 7.0% after 360s, which corresponds to an approximated production flux equivalent to 6.3% of the added glucose. After 200 s a decrease in concentration was observed and the labeling enrichment remained constant, indicating that there was no further production of mannitol. The pathway intermediate M6P seems to be at fast equilibrium with F6P – concentration profile and labeling enrichment were very similar.

Based on the concentration and labeling measurements, a dynamic flux estimation was performed using piece-wise affine flux functions in time. For the upper glycolytic flux, it was observed that the rate of glucose uptake rapidly increased to 0.41 µmol/gDCW/s at 18 s and reached a maximum of 0.48 µmol/gDCW/s at 100 s and then decreased in steps to its starting value 0.02 µmol/gDCW/s. The following reaction (PGI) increased rapidly and reached a value of 0.40 µmol/g/s at 18 s. For the next interval, the flux decreased, in contrast to the uptake rate – a trend also observed for the following glycolytic reaction steps.

A significant amount of the carbon was entering storage metabolism and to a smaller extent the non-oxidative Pentose-Phosphate pathway. Thus, the differences in the first steps of glycolysis clearly indicate that the connected fluxes, PPP and storage metabolism, have to be taken into account.

Looking at the average flux, about 18% of the carbon entering the cell was recycled in the trehalose node. Additionally, 11% of the incoming carbon was recycled via glycogen. Mannitol cycling reached 9.1% of the inflow. Thus, the synthesis of these pools added up to 38% of the carbon uptake. The average inflow into the oxidative pentose-phosphate pathway was estimated at only 1.7% of the uptake rate. The maximum flux through the oxidative pentose phosphate pathway was observed after 100 s (10 nmol/gDCW/s), which is lower compared to steady-state conditions[7], suggesting that NADPH production might originate from another reaction.

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Session 4 — Tuesday, June 17 Systems Biology and Engineering

Chairs: Iman Famili, Intrexon; Hiroshi Shimizu, Osaka University; Jamey Young, Vanderbilt University

Invited Speaker: Systems Metabolic Engineering of Microorganisms for the Production of Polymers and Monomers

Sang Yup Lee* *Chemical & Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea*

Due to our increasing concerns on environmental problems, there has recently been much interest in developing bio-based processes for the production of chemicals, fuels and materials from renewable non-food biomass. Polymers derived from fossil resources through chemical processes are important materials being used in industries and our everyday life, and thus are used in large amounts. In this lecture, systems metabolic engineering strategies employed for the production of various polyesters will be reported. Also, metabolic engineering strategies employed for the production of chemicals that are used as monomers of polymers of industrial importance will be reported. The example products will include natural and non-natural polyesters, diacids, diamines, and others. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries of Ministry of Science, ICT & Future Planning.]

Advances in 13C Metabolic Flux Analysis: Complete-MFA, Co-Culture MFA and Dynamic MFA

Maciek R. Antoniewicz, *Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE*

Measuring fluxes by 13C metabolic flux analysis (13C-MFA) has become a key activity in metabolic engineering, biotechnology and medicine. In this talk, I will present three major new advances in the field of 13C-MFA that are extending the range of biological systems that can be analyzed with this technique and the types of biological questions that can be addressed. The three major advances that I will discuss are: 1) COMPLETE-MFA (or complementary parallel labeling experiments technique for metabolic flux analysis), which improves the precision and accuracy of flux estimates by about one-order-of-magnitude; 2) co-culture MFA, which allows metabolic fluxes to be measured in multi-cellular systems; and 3) dynamic MFA, which extends flux analysis to dynamically changing biological systems.

1. The COMPLETE-MFA methodology that we have developed is based on combined analysis of multiple isotopic labeling experiments, where the synergy of using complementary tracers greatly improves the precision of estimated fluxes. Here, I will demonstrate the COMPLETE- MFA approach using all singly labeled glucose tracers, [1-13C], [2-13C], [3-13C], [4-13C], [5-13C], and [6-13C] glucose to determine precise metabolic fluxes for wild-type *E. coli*, and using all [1-13C], [2-13C], [3-13C], [4-13C], and [5-13C] xylose to determine precise metabolic fluxes for Thermus thermophilus. In these studies, cells were grown in multiple parallel cultures on defined medium with glucose and/or xylose as the only carbon source. Mass isotopomers of biomass amino acids were measured by gas chromatography-mass spectrometry (GC-MS) and the data from all experiments were then fitted simultaneously to a single flux model to determine accurate intracellular fluxes. In all cases, we obtained a statistically acceptable fit with more than 300 redundant measurements. As I will demonstrate, the flux maps that we have determined here are the most precise flux results obtained thus far (by about order-of-magnitude) for any biological system.

2. Microbial communities play an important role in biofuel production, biomedical research, food production, and waste water treatment. Co-culture systems particularly have unique advantages in optimizing product yield as a result of synergistic interactions. To gain insight into these systems we have developed the first methodology for measuring metabolic fluxes in multi-cellular systems. Here, I will demonstrate our novel co-culture 13C-MFA framework that does not require any physical separation of cells or proteins. Specifically, we have developed a new computational approach for modeling isotopic labeling in biological systems that allows fluxes in individual populations to be computationally deconvoluted from the overall co-culture 13C-labeling data.

We show that the overall 13C-labeling data has abundant information not only to estimate the fluxes in multiple populations, but also to determine the fraction of each cell population in the co-culture (e.g. to visualize co-culture dynamics). I will demonstrate the co-culture flux analysis methodology using a co-culture system of two *E. coli* knockout strains, Δzwf (knockout of the first step in the pentose phosphate pathway) and Δpgi (knockout of the first step in glycolysis pathway), using a yeast/*E. coli* co-culture, and using a thermophilic co-culture system. The new flux analysis methodology that we have developed for analyzing co-culture systems adds a new dimension to the field of 13C-MFA and provides an enormous resource to the metabolic engineering and biotechnology communities.

3. Finally, we have developed new methods for dynamic 13C-MFA, for biological systems that are not at metabolic steady state. The 13C-DMFA methodology is based on integrating time-series of metabolite measurements and non-stationary 13C-labeling data to quantify flux changes in time. Thus, this allows us to measure for the first time dynamically changing metabolic fluxes, for example, in fed batch fermentations. Three key advantages of our 13C-DMFA method are: 1) time-series of metabolite concentration and labeling data can be applied directly for estimating dynamic fluxes, making data smoothing unnecessary; 2) characteristic metabolic phases during a culture are identified automatically by the algorithm; 3) 13C-labeling data provides insights into transients in fluxes of parallel and cyclic pathways that cannot be observed without labeling.

Invited Speaker: Integrating Kinetic Models of Metabolism with k-OptForce for Strain Design

Costas D. Maranas*, *Department of Chemical Engineering, The Pennsylvania State University, University Park, PA*

Computational strain-design approaches relying solely on knowledge of model stoichiometry cannot capture the effect of enzyme activity levels and substrate-level enzyme regulation on metabolic flux redirection. In this talk, we apply the recently developed k-OptForce procedure which integrates the available kinetic descriptions of metabolic reactions with stoichiometric models, to sharpen the prediction of intervention strategies for improving the bio-production of chemicals of interest. The suggested interventions are comprised of both direct enzymatic activity changes (for reactions with available kinetics) and indirect reaction flux manipulations (for reactions with only stoichiometric information). In some cases, additional modifications are needed to overcome the substrate-level regulations imposed by the representative kinetic model, while in other cases, kinetic expressions shape flux distributions so as to favor the overproduction of the desired product requiring fewer direct interventions. k-OptForce requires as input kinetic expressions that accurately capture the substrate-level regulation of metabolic fluxes. To this end, we constructed a kinetic model of *E. coli* core metabolism that satisfies the fluxomic data for wild-type and seven mutant strains by making use of the recently introduced Ensemble Modeling (EM) concepts. This model consists of 138 reactions, 93 metabolites and 60 substrate-level regulatory interactions and accounts for glycolysis/gluconeogenesis, pentose phosphate pathway, TCA cycle, major pyruvate metabolism, anaplerotic reactions and a number of other reactions. Parameterization of the model was performed using a formal optimization algorithm that minimizes uncertainty-scaled discrepancies between model predictions and flux measurements. Application of k-OptForce for overproduction of bio-chemicals recapitulated existing intervention strategies, while identifying additional and alternate manipulations often distal to the point of regulations. This framework paves the way for an integrated analysis of kinetic and stoichiometric models and enables elucidating system-wide metabolic interventions while capturing regulatory and kinetic effects.

Design of Terpenoid Producing Synthetic Microbial Cell Factories By Integrative *in silico* Modeling and "-Omics" Data Analysis

Dong-Yup Lee*[1], Meiyappan Lakshmanan[1] and Seon-Won Kim[2]
[1]*Chemical and Biomolecular Engineering, National University of Singapore, Singapore, Singapore*
[2]*Division of Applied Life Science, Gyeongsang National University, Jinju, South Korea*

Terpenoids are a set of diverse, low molecular-mass plant secondary metabolites with several important applications in human health and nutrition. Nevertheless, its low yields from the natural sources, i.e. plants, have demanded the search for an effective alternate production strategy. In this regard, metabolic engineering is an effective tool to synthesize terpenoids in bulk quantities using fast-growing microbes such as *E. coli* and *S. cerevisiae* by creatively customizing their innate metabolic capabilities by up-, down-regulating, inserting and/or deleting numerous metabolic genes at the same time. To this end, we herein present an in silico model-driven

systematic framework for the rational design of microbial cell factories for synthetic terpenoids production. Guided by the genome-scale metabolic modeling and "-omics" data profiling of plants, Arabidopsis and rice, we first analyzed the metabolic organization, gene expression pattern and the putative transcriptional mechanisms involved in terpenoids synthesis. These studies include the comparison of terpenoid-backbone synthetic pathways, 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway and mevalonic acid (MVA) pathway, effect of different colored lights in terpenoid synthetic gene expression and their relevant regulatory mechanisms. These analyses revealed that the MEP pathway is slightly better than MVA pathway in terms of IPP yield and the stimulating effects of light, especially blue color, in terpenoid gene expression. Based on these observations, we chose to manifest these characteristics into *E. coli* for synthetic terpenoid production as it has MEP pathway naturally and well characterized genetic tools. Further, again with the guide of genome-scale model, identified relevant gene knockout targets to enhance the carbon flux through MEP pathway. Additionally, we also found that terpenoids synthesis in host organisms is often limited due to the limited availability of intracellular NADP(H) concentrations through in silico simulations. To resolve this issue, we propose a novel computational algorithm, cofactor modification analysis (CMA), which identifies the plausible enzyme targets for cofactor engineering. The identified enzyme target was subsequently modified for cofactor specificity from NAD(H) to NADP(H) using the mutational targets identified from computational molecular modeling and analysis. Apart from engineering the innate metabolic capabilities of host organism to synthesize terpenoids, expressing the foreign plant genes in microbes at an optimal level is also a major challenge. In the proposed framework, this bottleneck can be fully addressed by resorting to the CC optimization framework, optimizing the individual codon usage and codon context of the host organism. In general, this study outlines an in silico model-driven approach for designing microbial cell factories to synthesize non-native compounds optimally.

Invited Speaker: Opening Pandora's Box

Lars Nielsen*, *Australian Institute for Bioengineering & Nanotechnology, The University of Queensland, Australia*

Secondary metabolite processes developed using classical strain and process engineering continue to account for the majority of the biotech market. Convoluted fermentation processes in highly complex media with highly sensitive strains are the rule rather than the exemption. Systems level analysis and design has immense potential to domesticate and refine these processes. Genomics alone has proven unhelpful and simple contrasts fail to capture the complexity of these processes. Recent advances in omics, however, enables the generation of high-density, high-quality data that are truly informative and can guide rational design. In this talk, I will demonstrate how quantitative, multi-omics studies of several clostridia and actinomycetes have been converted into operational models and used for the rational design of superior strain and processes.

Session 5
Computational Methods and Design

Chair: Anthony Burgard, Genomatica and Costas Maranas, Penn State University

Invited Speaker: Integration of *in silico* Design and Experimental Evaluation for Creation of Microbial Cell Factories

Hiroshi Shimizu* *Graduate School of Information Science and Technology, Osaka University, Suita, Japan*

Integration of in silico and experimental approaches is highly desired for creation of microbial cellfactories. Recently, on the basis of whole-genome information, the genome-scale metabolic reaction models (GSM) of cells have been reconstructed for many organisms. Using the GSMs, a reliable prediction of metabolic fluxes is possible by using Flux Balance Analysis (FBA). We reconstructed GSMs of industrially a useful microorganism Corynebacterium glutamicum [Shinfuku et al., 2009] and cyanobacterium Synechocystis sp. [Yoshikawa et al., 2011]. And then, the constructed GSMs are applied to prediction of metabolic fluxes under several environmental conditions and to design of genetic modification for valuable compounds production. A multiple deletion algorithm, FastPros [Ohno, 2013] and a non-native metabolite production design approach, ArtPathDesign [Chatsurachai et al., 2013] are also introduced.

Metabolic flux analysis (MFA) based on quantification of 13C-labeling patterns of metabolites by mass spectroscopy (MS) is a powerful tool to quantify fluxes experimentally in a metabolic network of microorganisms. In this approach, after cells are cultivated using a 13C-labeled substrate, metabolites are extracted from the cells for use in MS analyses. Metabolic fluxes are determined using stoichiometric constraints coupled with extracellular measurements and 13C-labeling patterns of the metabolites. We experimentally determined

metabolic fluxes of C. glutamicum and Synechocystis and analyzed states of metabolic pathways. *In Synechocystis sp*., metabolic fluxes under different environmental conditions of photon and carbon source supply is analyzed. Combination of the these *in silico* and experimental flux analysis with other omics analyses such as transcriptomics and metabaolomics are also discussed.

Fast Enumeration of Smallest Metabolic Engineering Strategies in Genome-Scale Networks

Steffen Klamt* and Axel von Kamp, *Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

One ultimate goal of metabolic network modeling is the rational modification of biochemical networks to optimize the bio-based production of certain compounds. Although several constraint-based optimization techniques have been proposed for this purpose, there is still a need for computational approaches allowing an effective systematic enumeration of efficient intervention strategies in large-scale metabolic networks.

Here we present the MCSEnumerator approach by which a large number of the smallest genetic intervention strategies (with fewest targets) can be readily computed in genome-scale metabolic models [1]. The algorithm builds upon an extended concept of Minimal Cut Sets (MCSs) which are minimal combinations of reaction (or gene) deletions leading to the fulfillment of a predefined intervention goal. It exploits the fact that smallest MCSs can be calculated as shortest elementary modes in a dual network and uses an improved procedure for shortest elementary-modes calculation. Recently, MCSEnumerator was extended to allow also for the computation of regulatory MCSs which are minimal combinations of reaction knockouts and up and downregulations enforcing a desired behavior (see Abstract of Mahadevan et al.).

Realistic application examples demonstrate that our algorithm is able to list thousands of the most efficient intervention strategies for various intervention problems in genome-scale networks. We used MCSEnumerator to compute strain designs for growth-coupled synthesis of different products by heterotrophic as well as photoautotrophic organisms. We found numerous new engineering strategies partially requiring fewer interventions and guaranteeing higher product yields than reported previously. In contrast to many other approaches, our method does not require the assumption of optimal growth since MCSs can be computed in such a way that they guarantee growth-coupled product synthesis for any growth rate. Generally, a broad spectrum of intervention problems can be considered: one only needs to provide a description of the desired and undesired behaviors (flux distributions) by means of linear inequalities. With this flexible definition, MCSEnumerator can also be employed for other purposes, e.g., for enumerating synthetic lethals.

In summary, the presented approach can quickly calculate a large number of smallest engineering strategies with neither network size nor the number of required interventions posing major challenges. Given its unprecedented speed and high flexibility in formulating intervention problems, we expect MCSEnumerator to become an important tool for Metabolic Engineering.

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Mapping Photoautotrophic Carbon Metabolism Using the INCA 13C Flux Analysis Platform

Jamey D. Young*, *Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN*

Although steady-state 13C -labeling experiments are widely used to quantify fluxes in heterotrophic organisms, autotrophs assimilate carbon solely from CO_2 and therefore produce a uniform steady-state 13C-labeling pattern when grown on $13CO_2$. This makes steady-state 13C metabolic flux analysis (MFA) ineffective for studying autotrophic metabolism. However, transient measurements of isotope incorporation following a step change from unlabeled to labeled CO_2 can be used to estimate photoautotrophic fluxes by applying isotopically nonstationary MFA (INST-MFA). We have recently developed a package of MATLAB routines called INCA that automates the computational workflow of INST-MFA. INCA is the first publically available software package that can perform INST-MFA on networks of arbitrary size and complexity. To establish proof of concept, we first applied INCA to map fluxes in the model cyanobacterium Synechocystis sp. PCC 6803 growing under photoautotrophic conditions. Our study relied on both GC-MS and LC-MS/MS to quantify labeling trajectories of 15 intracellular metabolites following administration of 13C-labeled bicarbonate to a photobioreactor culture. Overall, we were able to precisely quantify the rates of all Calvin cycle and TCA pathway reactions, as well as several “wasteful” side reac-

tions that contribute to suboptimal photoautotrophic growth of Synechocystis.

We next adapted our INST-MFA model to a terrestrial plant system. We performed *in vivo* isotopic labeling of Arabidopsis thaliana leaves with $13CO_2$, measured the transient labeling of 30 metabolite fragment ions using mass spectrometry, and estimated fluxes throughout leaf photosynthetic metabolism using INCA. Leaves were exposed to either 200 or 500 µmol m-2s-1light, with or without prior acclimation. Approximately 1,200 independent mass isotopomer measurements were regressed to estimate 110 fluxes under each condition. Photorespiration flux was significantly increased under high light conditions, despite concomitant increases in carboxylation flux that led to enhanced sucrose production. Interestingly, we observed an inverse relationship between intermediate pool sizes and Calvin cycle fluxes as light intensity increased. Additionally, we identified enhanced hexose exchange between the chloroplast and cytosol as a potential short-term adaptation to high light that was suppressed by acclimation. Taken together, these studies have established 13C INST-MFA and the INCA software package as a comprehensive platform to map carbon fluxes in cyanobacteria, plants, and other photoautotrophic organisms.

An Integrated Multi-Omics and Computational Characterization of Seven Unique *Escherichia coli* Production Chassis Commonly-Used in Industrial Biotechnology

Adam M. Feist*, *DTU-Center for Biosustainability, Hørsholm, Denmark, Bioengineering, UCSD, La Jolla, CA*

A major challenge in industrial biotechnology is the selection of an appropriate platform organism to be engineered in order to maximize the production of the desired chemical in as little time as possible. Often times, researchers resort to their in-house favorite strain due to a lack of a standardized and functional comparison across strains within a species. In this study, we set out to clarify some of the strain-specific differences in the most common laboratory organism and production chassis,*Escherichia coli*. Specifically, we performed an integrated functional characterization of seven commonly used industrialEscherichia coli strains (BL21 (DE3), C, Crooks, DH5 , MG1655, W and W3110) through phenomics, transcriptomics, and genome-scale modelling under both aerobic and anaerobic conditions. Genome-scale metabolic models were generated for each of the strains and integrated with the phenomic data to determine reactions that carry high flux in each of the strains. High-flux reactions and their encoding genes were compared to differentially expressed genes in each of the strains, as well as across the strains, to characterize shared and strain-specific behaviour. Furthermore, the wild-type phenotypes of each of the strains were compared to production phenotypes for a variety of industrial compounds to make a prediction on what strains are inherently better suited to produce a given compound or class of compounds. The result of the study is a classification that can be used to guide selection of a given strain for the biosynthesis of a desired product.

Session 6
The Rational Design-Build-Test Era

Chairs: Maciek Antoniewicz, University of Delaware; Akihiko Kondo, Kobe University; Michael Lynch, Duke University

Invited Speaker: Using Systems Biology for Identification of Novel Metabolic Engineering Targets

Jens Nielsen[*1, 2]*
[1]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, Denmark
[2]Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

The yeast *Saccharomyces cerevisiae* is widely used for production of fuels, chemicals, pharmaceuticals and materials. Through metabolic engineering of this yeast a number of novel new industrial processes have been developed over the last 10 years. Besides its wide industrial use, *S. cerevisiae* serves as an eukaryal model organism, and many systems biology tools have therefore been developed for this organism.

Despite our extensive knowledge of yeast metabolism and its regulation we are still facing challenges when we want to engineer complex traits, such as improved tolerance to toxic metabolites like butanol and elevated temperatures or when we want to engineer the highly complex protein secretory pathway. In this presentation it will be demonstrated how we can combine directed evolution with systems biology analysis to identify novel targets for rational design-build-test of yeast strains that have improved phenotypic properties. Examples will be on identifying targets for improving tolerance towards butanol and increased temperature and for improving secretion of heterologous proteins.

Invited Speaker

Chris Voigt*, *Massachusetts Institute of Technology*

Invited Speaker: Programming Biological Operating Systems: Genome Design, Assembly and Activation

Daniel Gibson*, *J. Craig Venter Institute, La Jolla, CA; Synthetic Genomics, La Jolla, CA*

The DNA technologies developed over the past 20 years for reading and writing the genetic code converged when the first synthetic cell was created 4 years ago. An outcome of this work has been an extraordinary set of tools for synthesizing, assembling, engineering and transplanting whole bacterial genomes. Technical progress, options and applications for bacterial genome design, assembly and activation are discussed.

Session 7 — Wednesday, June 18 Industrial Applications Related to Chemicals and Fuels

Chairs: Ramon Gonzalez, Rice University and Kristala Jones Prather, Massachusetts Institute of Technology

Invited Speaker: Metabolic Engineering of Yarrowia lipolytica to Produce High Value Products.

Quinn Zhu*, Bjorn Tyreus and Ethel Jackson, *Biotechnology, Central Research Development, E.I. du Pont de Nemours and Company, Wilmington, DE*

The oleaginous yeast Yarrowia lipolytica has been used as a host for production of carotenoids, resveratrol, omega-3 and omega-6 fatty acids by metabolic engineering at DuPont. Two commercial products have been developed, “New Harvest”, a clean, renewable and vegetarian oil with eicosapentaenoic acid (EPA, C20:5, omega-3 fatty acid) at 55% (weight/weight) for human nutrition supplement and “VerlassoR”, a premium branded salmon which is sustainably farmed due to incorporating EPA-rich Yarrowia biomass in the feed The yeast triacylglyceride oil has a unique fatty acid profile with less than 5% as saturated fatty acids and more than 55% as EPA. The oil with this fatty acid profile provides significant health benefits for human and animals. A most advanced Yarrowia production strain has been generated to produce EPA at >25% dry cell weight, a titer that is 67% higher than was obtained with the strain we previously reported (Nature Biotechnology, 2013, 8: 734-740). We will describe how the strain was generated and outline the learnings of our journey from lab research to commercial products.

Harnessing Metabolism for Sustainable Production of Chemicals: From Systems Biology to Commercial Scale Manufacturing

Mark Burk*, Anthony Burgard, Stephen Van Dien and Harry Yim, *Genomatica, Inc., San Diego, CA*

Oil and natural gas are used as the primary raw materials for manufacturing a wide range of large volume chemicals, polymers, and other products. Growing concerns over the environment and volatile fossil energy costs have led to efforts to develop more sustainable processes that can result in these same products made from renewable feedstocks with lower cost, energy consumption, and greenhouse gas emissions. Metabolic engineering of microorganisms is a powerful approach to address this need.

Genomatica has developed a complete bioprocess for the production of 1,4-butanediol (BDO) from carbohydrate feedstocks. BDO is a chemical intermediate (> 3B lbs/yr) that goes into a variety of products including automotive, electronics and apparel (such as spandex), and is currently produced commercially through energy-intensive petrochemical processes using hydrocarbon feedstocks.

An enabling feature of Genomatica’s process is the microorganism. *E. coli* has been engineered for high level production of BDO from a range of sugars with high rate and titer and very few by-products. Achieving such high level performance involved detailed engineering of the pathways and the metabolic network in order to entice the organism to channel its precious cellular resources into BDO production. For example, eliminating by-products sounds straightforward (simply delete genes in the by-product pathway), but often perturbs metabolism in unexpected ways. Our systems-based approach to organism engineering provided critical insights about redox and energy metabolism and revealed the path forward to higher level BDO production and a much more robust strain. The importance of taking a step back and performing well-designed diagnostic experiments will be emphasized. These efforts have culminated in commercial scale production of over 5 M lbs of BDO from dextrose.

Conventional sugars such as corn dextrose and sugar cane-derived sucrose are considered staples of the food chain. Accordingly, non-food biomass-derived sugars have emerged as an important feedstock for chemicals and fuels. However, uptake and utilization of biomass sugars such as xylose, arabinose, and prominent hydrolysate polysaccharides requires different metabolic pathways with a unique set of constraints. Again, our systems-based platform proved invaluable in deciphering the metabolic bottlenecks and opening the gates to improved performance.

This presentation will cover the development of a complete bioprocess for commercial scale production of BDO. Systems biology and diagnostic experiments will be emphasized to highlight the importance of rational metabolic engineering approaches for achieving high level strain performance.

Designing Microorganisms for Production of Diverse Biopolymers

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Microbial polyhydroxyalkanoates (PHA) have been developed as bioplastics for the past many years. PHA have diverse structures, at least 150 monomers have been found in the PHA polymer chains. However, it has been very difficult to precisely control the PHA polymer structures. In this presentation, all the PHA synthesis pathways have been studied, microorganisms have been designed with synthetic pathways allowing precise control of the PHA structures. The synthetic organisms can now make various PHA homopolymers, random copolymers, block copolymers, even graft polymers can be produced combined microbial and chemical synthese. For example, commercial PHA are normally poly-3-hydroxybutyrate (PHB), copolyesters of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx), copolyesters of 3-hydroxybutyrate and 4-hydroxybutyrate (P3HB4HB), as well as copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV). The designed organisms produced a series of novel PHA including homopolymers, random copolymers and block copolymers. For example, poly-3-hydroxypropionate (P3HP), poly-4-hydroxybutyrate (P4HB), poly-3-hydroxydecanoate (P3HD) and poly-3-hydroxydodecanoate (P3HDD) et al. Random copolymers containing defined monomer compositions can also be microbially produced. More importantly, block copolymerization containing various block such as diblock copolymers of PHB-b-P4HB, PHB-b-PHHHx, P3HP-b-P4HB, PHBHHx-b-PHDD et al have been produced. They remarkably increased the diversity of PHA structures and properties. By manipulating the block compositions, the polymer properties can be easily controlled. Now, PHA industry will be entering a functional polymer era, which allows PHA competitiveness not by the low cost but by its functionality. We will soon experience ultra-strong, shape memory, gas selective permeability and other environmentally responsive PHA.

Design of Microbial Cell Factories for Lignocellulosic Biorefinery

Akihiko Kondo*

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The biorefinery manufacturing process for producing chemicals and liquid fuels from biomass is a promising approach for securing energy and resources. Establishing economically feasible fermentation processes requires markedly increasing final product titers due to the high energy demands of subsequent product recovery steps, as well as the capital and production costs associated with biorefinery equipment. Although high-yield production of target compounds by metabolically optimized microbes is necessary, achieving higher titers inevitably requires increased loading of solid lignocellulose in the SSF and CBP processes. Increase in the solid concentration results in corresponding increases in chemical production. However, by increasing the lignocellulose content in the bioreactor, the concentration of fermentation inhibitors released during the pretreatment of biomass would reach higher levels. Accordingly, microorganisms that are resistant to inhibitors are a prerequisite for the high-titer production of fuels and chemical products. To further engineer cellulolytic recombinant and native strains for use in CBP, system-wide modifications of intracellular metabolic pathways using advanced engineering tools such as minimal hosts, vectors, genetic controllers, and characterized enzymes are needed, which would improve the potential of not only target productivities but cell growth and viability during the fermentation. The integration of cellulolytic capabilities with metabolic systems specified for targeted chemical production will allow customized CBP microorganisms to be developed using advanced gene manipulation technologies.

Invited Speaker: Metabolic Engineering of Complex Natural Product Pathways in Bacteria

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As the “low hanging fruit” of metabolic engineering, primary metabolites were chosen as the initial targets for commercial production in microbial systems. Researchers have since begun to leverage the lessons learned during development of these metabolic engineering approaches with the aim of

producing specialized metabolites, which are more complex and challenging targets. Specialized (secondary) metabolites, which are products of complex biochemistries found in nature, include a vast number of chemical candidates (>200,000) for a myriad of applications such as drugs, food additives, consumer products and industrial chemicals. Most of these natural products are chemically complex and heavily functionalized molecules with multiple chiral centers. Nature builds these intricate molecules through multistep biosynthesis using complex promiscuous enzymes such as cytrochrome P450's. In addition to the low accumulation of these molecules in nature (at ppm levels), the structural complexity of natural products precludes the development of economical synthetic routes to these molecules. Therefore, developing tools and technologies for the rapid and efficient construction of multi-step biosynthetic pathways enabling the creation of microbial strains capable of producing specialized natural products is a high priority research area for the metabolic engineering community.

We developed a new metabolic engineering approach, multivariate modular metabolic engineering (MMME), for systematically engineering such complex pathways. Recently, we expanded the scope of our approach to enable quick construction and optimization of orthologous pathways for the selection of the best variant biosynthetic routes to desired specialized products. These approaches enabled not only rapid construction of microbial strains for synthesizing complex biochemicals in useful accessible quantities, but provided several key insights on natural product biosynthesis and the origins of biosynthetic diversity of these specialized products. Here, we focus on the application of pathway engineering, targeted proteomics, metabolomics and transcriptomics in the context of MMME for optimizing the multi-step pathways for terpenoid synthesis in bacteria. This enabled the rapid construction of hundreds of strains, with lines capable of reaching multigram per liter production of several key natural product chemicals.

Session 8
Metabolic Engineering Applications to Impact Health

Chair: Christian Metallo, University of California, San Diego and Nicola Zamboni, Eidgenössische Technische Hochschule Zürich

Invited Speaker: Prosthetic Networks — Synthetic Biology-Inspired Treatment Strategies For Metabolic Disorders

Martin Fussenegger*, *Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*

Since Paracelsus' (1493-1541) definition that the dosing makes the drug the basic treatment strategies have largely remained unchanged. We continue to use a precise prescribed dose of a small-molecule drug, a protein therapeutic or a therapeutic transgene to constitutively modulate or complement the activity of a disease-relevant target. However, this treatment concept does neither consider the metabolic dynamics nor the interdependence of the most important pathophysiologies of the 21stcentury such as obesity, diabetes and cardiovascular disorders. Synthetic biology-inspired prosthetic networks may act as metabolic prostheses that provide the dynamic interventions, the immediate pre-disease action and the multi-target capacity required to meet with the treatment challenges of the future. Prosthetic networks consist of synthetic sensor-effector gene circuits that (i) seamlessly operate in implanted designer cells, (ii) constantly sense, monitor and score metabolic disturbanes in peripheral circulation, (iii) process OFF-level concentrations of pathologic metabolites, and (iv) coordinate an adjusted therapeutic response in an (v) automatic and self-sufficient manner. We will present our latest generation of synthetic mammalian gene circuits and provide a few examples of prosthetic networks operating in animal models of prominent human diseases to highlight the challenges and impact of synthetic biology on future biomedical applications.

Ovarian Cancer Metabolism: Systems—Scale Dynamics As a Platform for Identifying Therapeutic Targets

Mark P. Styczynski*, *School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA*

While response to first-line treatment of ovarian cancer (the most deadly gynecological cancer) is high, most patients relapse, and five-year survival rates have remained constant for decades. A significant cause of this relapse and resultant mortality is believed to be a class of cells referred to as "cancer initiating cells", a chemoresistant subpopulation believed to be able to reconstitute the entire tumor.

Though metabolic dysfunction is becoming increasingly accepted as a hallmark of cancer, efforts to exploit and manipulate metabolism to fight cancer (analogous to metabolic engineering) are not yet pervasive. To move towards that goal, it is imperative to have a more comprehensive, systems-scale understanding of cancer metabolism than just (for example)

aerobic glycolysis and glutaminolysis. To this end, we have performed system-wide metabolite profiling (metabolomics) of intracellular ovarian cancer metabolism along three important axes: bulk vs. cancer initiating cells, microenvironment-relevant perturbations (e.g., hypoxia, decreased glucose, chemotherapeutics), and temporal dynamics.
This work is the first-ever metabolic characterization of a cancer initiating cell line and its isogenic parental cell line, allowing for unique insight into the metabolic differences specific to cancer initiating cells that could be exploited in treatment. The cell types display significant, time-dependent differential responses to environmental perturbations. There are also more general differences between these cell types even in the absence of perturbations, including a pathway and two of its metabolites (proline and putrescine) that are typically accumulated in cancer, but are consistently lower in cancer initiating cells. These molecules also have documented roles in differentiation, suggesting a relationship between their levels and a delicate balance between proliferation and potency. We believe these differences (and others) can be leveraged to identify novel therapeutic targets, or perhaps even leads based on endogenous metabolites, that would actively target cancer initiating cells.

Invited Speaker: Metabolic Strategies to Enhance the Toxicity of Nitric Oxide in Pathogens

Mark P. Brynildsen*, *Chemical and Biological Engineering, Princeton University, Princeton, NJ*

Nitric oxide (NO•) is an antimicrobial used by immunity to neutralize pathogens. The importance of NO• to immune function is evidenced by the many pathogens, including Mycobacterium tuberculosis, Neisseria meningitides, Vibrio cholerae, Salmonella enterica, and enterohemorrhagic *Escherichia coli* (EHEC), that depend on NO• detoxification to establish an infection[1-7]. Inhibitors of NO• defense systems are under investigation as next-generation antibiotics[8,9], and direct delivery of NO• has shown potential for treating infections when antibiotics fail[10-17]. However, these efforts have been hampered by a narrow concentration window within which bacteria are neutralized and host cells remain unharmed, and a lack of effective agents that enhance NO• toxicity in pathogens[9,14]. A quantitative understanding of NO• cytotoxicity, and the adaptive responses mounted by bacteria would aid in identifying targets to sensitize pathogens toward host- or therapeutic-derived NO•. Due to the complexity of the NO• biochemical reaction network, where NO• directly reacts with Fe-S clusters, O2, and $O2^{\bullet-}$, and its autoxidation products (e.g., N_2O_3, $ONOO^-$) damage thiols, tyrosine residues, and DNA bases[18-20], coupled with the metabolic requirements of NO• defense systems (e.g., NADH, ATP), computational approaches are required to understand how bacteria process and respond to NO• stress.
Here I will discuss our work on the construction, experimental validation, and systems-level exploration of a detailed kinetic model of NO• metabolism and stress in *E. coli* [21]. This model has provided accurate predictions of NO• distributions among its reactions pathways under both aerobic and microaerobic conditions, enabled the discovery of a novel kinetic dependency of a major NO• detoxification system, and most recently been used to systematically identify the mechanism by which deletion of a protease produces major defects in NO• detoxification. Further, I will discuss how we have translated the model to EHEC, and used it to investigate NO• control of virulence factor expression in this dangerous food-borne pathogen. These results demonstrate the utility of quantitative metabolic modeling to the study of NO• stress in bacteria, and further, identify novel targets that when inhibited sensitize bacteria toward NO•.

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Prediction and Quantification of Bioactive Microbiota Metabolites in Murine Gut

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Increasing evidence suggest that the metabolites produced by the gastrointestinal (GI) tract microbiota are important modulators of human health and disease. However, only a handful of bioactive microbiota metabolites in the GI tract have been identified. The microbiota has the potential to carry out a diverse range of biotransformation reactions that are unavailable to the mammalian host, and to produce a broad spectrum of metabolites nonnative to the host. Isolating and characterizing individual bacteria to identify metabolites is intractable, as many species present in the GI tract cannot be cultured under standard laboratory conditions. Untargeted metabolomics approaches have been useful in characterizing the metabolic impact of perturbed microbiota by profiling bodily fluids and samples directly connected to GI tract, but are not well suited to resolving the origin of a metabolite as either bacterial or host metabolism. We present here a novel metabolomics strategy that integrates in silico analysis with targeted metabolomics to facilitate identification and quantification microbiota metabolites. We model the microbiota as an integrated metabolic system comprising 149 different species reported to be present in the mammalian GI tract, and represent this system with a metabolic reaction network. Of the 2,409 distinct reactions in the microbiota network, approximately 50 % are strictly bacterial, i.e. absent in the murine host, with the largest number of reactions involved in amino acid metabolism. Focusing on tryptophan (TRP) as a representative, diet-derive amino acid, we utilize a probabilistic pathway construction algorithm to predict potential metabolic derivatives present in the murine GI tract, while also discriminating between microbiota- and host-specific derivatives. We validate the model-based predictions using multiple reaction monitoring (MRM), a quantitative mass spectrometry technique, on cecum and fecal samples from control and antibiotic treated mice. We find that antibiotic treatment significantly alters the levels of the predicted TRP metabolites. To demonstrate the potential for the predicted and confirmed TRP derivatives to play a physiological role, we characterize

these metabolites as ligands for the aryl hydrocarbon receptor (AhR), a nuclear receptor that regulates xenobiotic transformation as well as inflammation in host cells.

Invited Speaker: The Gene Regulatory Network of Mycobacterium tuberculosis, v1.0

David Sherman*
Seattle Biomedical Research Institute

The bacterial pathogen *Mycobacterium tuberculosis* (MTB) infects 30% of all humans and kills someone every 20 – 30 seconds. In the push for new interventions, we have recently taken the first steps towards a complete reconstruction of the *Mycobacterium tuberculosis* (MTB) gene regulatory network. We developed a high-throughput system based on ChIP-Seq for mapping transcription factor (TF) binding, and assayed genome-wide expression following induction of each TF. Using this method we report on the DNA binding and transcriptional regulatory profile of ~80% of all predicted MTB DNA binding proteins (>150 genes). We identify many strong candidate interactions for direct transcriptional regulation associated with DNA binding; however, we also note significant DNA binding that cannot be linked directly with transcriptional control. We propose a model in which TFs act on a spectrum from specific local control of gene expression to widespread binding with little or no direct impact on proximal genes, and suggest that many prokaryotic TFs bind DNA prolifically yet still home to and directly regulate a limited number of targets. This work updates current concepts of prokaryotic transcriptional control and also should form the basis for iterative rounds of modeling, prediction and refinement in pursuit of badly needed new TB therapies.

Session 9
Emerging Technologies

Chair: Emily Leproust, Twist Biosciences

Invited Speaker: Expression of Heterologous Sigma Factors in *Escherichia coli* to Explore the Heterologous Genomic Space for Building, Stepwise, Complex, Multicomponent Phenotypes

Eleftherios. T. Papoutsakis*, Stefan M. Gaida, Sergios A. Nicolaou, Nicholas R. Sandoval, Kyle Zingaro and Yongbo Yuan, *Chemical and Biomolecular Engineering, University of Delaware, Newark, DE*

A key limitation in using heterologous genomic or metagenomic libraries in functional genomics and genome engineering is the low-level expression of heterologous genes in screening hosts, such as *Escherichia coli*. To overcome this limitation, we constructed *E. coli* strains capable of recognizing heterologous promoters by expressing sigma factors from the phylogenetically distant *Lactobacillus plantarum* and *Bacillus subtilis*. Such strains were employed for screening heterologous DNA libraries using the promoter GFP-trap concept. We show greatly increased transcription from single and combined genomic libraries of *L. plantarum, B. subtilis*, *Clostridium pasterianum, C. acetobutylicum* and *Deinococcus radiodurans*, thus enlarging the genomic space that can be functionally sampled in *E. coli*. We show two applications. In one, we show that screening fosmid-based *L. plantarum* genomic libraries in an *E. coli* strain with a chromosomally integrated *L. plantarum* rpoD (coding for the major sigma factor) allowed the identification of *L. plantarum* genetic determinants imparting 14-fold increased survivability to 7% v/v ethanol compared to the control in *E. coli*. In the second, we demonstrate the concept for a sequential, iterative assembly strategy for building multigenic traits by exploring the synergistic effects of genetic determinants from broader genomic spaces. Specifically, building upon the success of our recently reported semi-synthetic stress response system expressed off plasmid pHSP, we probed the genomic space of the solvent tolerant *L. plantarum* to identify genetic determinants that impart solvent tolerance in combination with pHSP. Using two targeted enrichments, one for superior viability and one for better growth under ethanol stress, we identified several beneficial heterologous DNA determinants that act synergistically with pHSP. In separate strains, a 209% improvement in survival and an 83% improvement in growth over previously engineered strains based on pHSP were thus generated. We then developed a composite phenotype of improved growth and survival by combining the identified *L. plantarum* genetic fragments. The best performing strain produced a 3.7-fold improved survival under 8% ethanol stress, as well as a 32% increase in growth under 4% ethanol. We also show that this strain can significantly improve ethanol productivity in a practically significant Melle-Boinot like fermentation process.

Robust Technology for Antibiotics-Free Contamination Resistance

A. Joe Shaw*[1], Maureen A. Hamilton[1], Johannes P. van Dijken[2], Gregory N. Stephanopoulos[3] and Colin R. South[1]
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Microbial contamination is a liability for industrial bioprocesses affecting yield, productivity, and operability. Mitigation often increases overall cost or results in undesired process steps, such as antibiotic use. For scale-up of new bioprocesses, contamination can be the single largest barrier to successful operation.

Traditional control methods, such as sterilization, operation at low pH, or application of antimicrobial compounds kill or inhibit undesired microbes. The ROBUST principle focuses instead on creating an environment where only the desired microorganism has access to essential growth nutrients, leaving contaminants unable to reproduce. To create such an environment, we have engineered metabolic pathways that enable degradation of nitrogen and phosphorous containing chemicals not commonly encountered in nature. When cultured in media with the desired chemical as sole nitrogen or phosphorous source, engineered organisms rapidly outcompete contaminants, resulting in a broadly applicable alterative to antibiotic use for creation of selective fermentation environments.

Invited Speaker: Establishing Cell-Free Metabolic Engineering for Pathway Debugging and the Production of Sustainable Chemicals

Michael Jewett*, *Northwestern University*

Rapid population growth, a rise in global living standards, and climate change concerns have intensified the need for sustainable, low-cost production of bioenergy, commodity chemicals, and natural products. Industrial biotechnology is one of the most attractive approaches for addressing this need, particularly when large-scale chemical synthesis is untenable. While the number of microbial metabolic engineering success stories is rapidly growing, the fraction of biochemicals amenable to economical production is still limited because engineering whole-cell microorganisms with biosynthetic pathways remains costly and slow. Common problems afflicting the current state-of-the-art include low volumetric productivities (g/L/h), build-up of toxic intermediates or products, byproduct losses via competing pathways, and constraints arising from the fact that microbial growth and adaptation objectives are often diametrically opposed to the overproduction and release of a single product. To overcome these limitations, we are expanding the scope of the traditional bioengineering model by using cell-free systems to harness ensembles of catalytic proteins prepared from crude lysates, or extracts, of cells for the production of target products. In this presentation, I will discuss our efforts to demonstrate ultra-high productivities and titers for metabolic conversion and also perform design-build-test (DBT) iterations without the need to re-engineer organisms. This experimental approach holds great promise to increase our ability to debug and optimize modular construction of pathways in cellular lysates through the use of simple, well-defined experimental conditions. We anticipate that cell-free systems will open new frontiers for biomanufacturing when cellular toxicity limits commercial feasibility of whole-cell fermentation.

High Throughput Screening of Antibody Secretion in CHO Cells Using Split-GFP and Droplet Microfluidics

Yunpeng Bai, Magnus Lundqvist, Anna-Luisa Volk, Mathias Uhlen, Helene Andersson Svahn, Haaken N. Joensson and Johan Rockberg*, *Proteomics and Nanobiotechnology, KTH- Royal Institute of Technology, Stockholm, Sweden*

The commercial and clinical success of antibodies, both as tools in research and diagnostic and as therapeutics, has convincingly been shown in recent years.

Production of monoclonal antibodies is predominantly done by overexpression of antibody genes in Chinese hamster ovary (CHO) cells for their ability to generate correctly folded and glycosylated proteins a large scale. To meet the growing demands of high-yielding and cost-effective production strains numerous rational, undirected or combinatorial approaches have been employed to enhance level of antibody secretion. Access to scalable and fast ways of monitoring product secretion is however limiting the ability for a complete and efficient screening of such libraries of strains.

Here we describe a novel high throughput screening system allowing for single cell characterization and sorting of antibody secreting CHO cells using droplet microfluidics and split-GFP complementation. A short tag, comprising the 11-strand of GFP, was genetically fused to the monoclonal antibody Herceptin and expressed in CHO as fusion proteins. We demonstrate the ability to encapsulate and cultivate such single CHO cells in mono-disperse droplets in a microfluidic device. By adding GFP 1-10 complement to the droplets we further show the ability to monitor secretion of tagged antibody by detection of GFP fluorescence upon complementation. We also show the ability to enrich for CHO

secreting tagged highly fluorescent antibodies in a library with a background of wild type Herceptin secreting cells.

Using this generic technology we anticipate a better ability to perform fluorescent screenings of libraries for the identification of CHO strains with improved antibody secretion.

Invited Speaker: Transforming Yeast from Moonshiners into Oil Barons: Lessons from the Industrialization of Metabolic Engineering

Sunil Chandran*, *Research Programs, Amyris*

Amyris has transformed ethanologenic baker's yeast into farnesene oil producers. Farnesene is a 15-carbon hydrocarbon derived from the sterol synthesis pathway (the same root pathway that make cholesterol). Its branched structure makes it ideal for processing into base-oils for cosmetics (replacing shark liver oil), synthetic engine lubricants, diesel fuel, and high-performing polymers for tires. In four years, Amyris has improved its yeast from producing only a single microliter in a test tube, to making thousands of liters in a full-scale fermentations at unprecedented yields and productivities. I will share lessons learned from the challenges of strain development and scale-up at Amyris, and how those lessons might be applied to accelerate and industrialize genetic and metabolic engineering.

International Metabolic Engineering Award Lecture

Mathematical Modeling and Computational Analysis and Design of Metabolic Reaction Networks

Vassily Hatzimanikatis, *Chemical Engineering and Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Metabolic engineering is the science of analyzing and designing of complex metabolic and cellular processes. The fundamentals of metabolic engineering are based on chemical reaction engineering and process systems engineering. Mathematical modeling and computational analysis are essential components of these fundamentals. We will discuss the contributions of mathematical modeling and computational modeling to the field of metabolic engineering and we will present some of our recent work in this area.

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