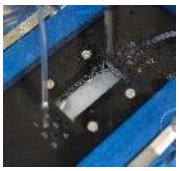

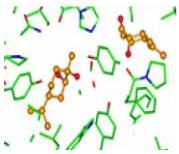
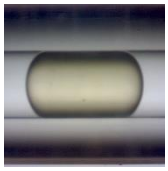

ESAB European Society of Applied Biocatalysis

ESAB-SBE Webinar

Synthetic Biology and Metabolic Engineering Tools and Methodologies

Friday, 22nd October 2021 at 16:00 – 18:00 CET

Chairs: Frangiskos Kolisis (National Technical University Athens)
Roland Wohlgemuth (Lodz University of Technology)

PROGRAMME

16.00 Homa Mohammadi-Peyhani[1,2] & Jasmin Hafner[1,2], Anastasia Sveshnikova[1], Victor Viterbo[1], Prof. Dr. Vassily Hatzimanikatis[1*], [1]Laboratory of Computational Systems Biotechnology, École Polytechnique Fédérale de Lausanne, EPFL, Lausanne, Switzerland
[2] These authors contributed equally.

Computational methods and resources for Synthetic Metabolism and Metabolic Engineering

Metabolic dark matter describes the gaps in today's knowledge of metabolic processes, which has been accumulated in the past decades of biochemical research. The continuous growth of biochemical reaction databases, the sustained discovery of novel natural products and the difficulty to predict the behavior of cellular metabolism strongly indicate that many metabolic components are currently missing from our biochemical record. Yet, these unknowns not only undermine our understanding of metabolism, they also hamper synthetic biology and metabolic engineering endeavors and therefore slow down the shift of the chemical industry towards greener, more sustainable biosynthesis processes. To our knowledge, no one has ever attempted to systematically map and fill the knowledge gaps in metabolism at the scale of global biochemical knowledge.

We herein present a comprehensive map of known, well-characterized and novel, predicted reactions between millions of known chemical, biochemical and bioactive compounds. It is the first attempt to systematically map and fill the knowledge gaps in metabolism at the scale of global biochemical reaction networks. Our work is based on the reaction prediction tool BNICE.ch, which condenses available biochemical knowledge about enzymatic reactions into generalized "reaction rules". We used these rules to integrate almost 2 million molecules from public databases (e.g. ChEMBL, PubChem, HMDB) including plant natural products, pharmaceuticals, and other molecules that are part of or interact with biological systems, into a global biochemical reaction network called ATLASx. Our technology can further propose biosynthetic routes for chemicals designed by synthetic chemistry. We additionally integrated our existing enzyme prediction tool BridgIT[1] to propose enzymes that may catalyze the novel, predicted reactions. A network analysis of ATLASx demonstrates how our predictions increase the interconnectivity of metabolic knowledge. To underline the biological relevance of our predictions, we present some practical examples that can be easily reconstructed using the ATLASx online platform. While this work is purely computational, several publications have demonstrated the application of our reaction prediction for the design of de novo synthetic pathways producing diverse benzylisoquinoline alkaloids[2], tropane alkaloid derivatives[3], and novel one-carbon assimilation pathways[4].

Overall, this work contributes to the fundamental understanding of biochemical processes in a global and systematic way, providing the scientific community with a rational estimate of the unexplored possibilities in biochemical research. ATLASx and the associated computational methods are the first collection of resources and tools of such scale and application range for exploring the known and predicted biochemical reaction space, and it should be of a useful and valuable resource for synthetic biology and metabolic engineering.

References
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2. Hafner, J., Payne, J., MohammadiPeyhani, H., Hatzimanikatis, V. & Smolke, C. A computational workflow for the expansion of heterologous biosynthetic pathways to natural product derivatives. Nature Communications 12, 1760 (2021).
3. Srinivasan, P. & Smolke, C. D. Engineering cellular metabolite transport for biosynthesis of computationally predicted tropane alkaloid derivatives in yeast. Proc Natl Acad Sci USA 118, e2104460118 (2021).
4. Yang, X., Yuan, Q., Luo, H., Li, F., Mao, Y., Zhao, X., Du, J., Li, P., Ju, X., Zheng, Y., Chen, Y., Liu, Y., Jiang, H., Yao, Y., Ma, H. & Ma, Y. Systematic design and in vitro validation of novel one-carbon assimilation pathways. Metabolic Engineering 56, 142–153 (2019).

16.30 Prof. Dr. Tobias Erb, Max Planck Institute for Terrestrial Microbiology, Marburg & LOEWE Center for Synthetic Microbiology (SYNMIKRO), Philipps University of Marburg, Germany

Re-thinking photosynthesis with synthetic biology: From natural photosynthesis to artificial chloroplasts and back

Carbon dioxide (CO_2) is a potent greenhouse gas that is a critical factor in global warming. At the same time atmospheric CO_2 is a cheap and ubiquitous carbon source. Developing new ways to capture CO_2 is key to create a sustainable world of tomorrow that will be able to feed an ever-increasing world-population and be based on a carbon-neutral economy.

While Nature provides the blue-print for developing such solutions, however, natural photosynthesis alone will not be sufficient to serve these needs. In my talk I will discuss the evolution and limitation of naturally existing CO_2 fixing enzymes and present strategies how to discover and engineer novel enzymes and pathways for the fixation of CO_2. I will further exemplify how lab automation and machine learning can be combined to realize and optimize these new-to-nature metabolic networks to outcompete the CO_2 fixation abilities of natural photosynthesis.

Finally, I will talk about the challenges of transplanting these new-to-nature pathways into natural and synthetic cells to create artificial chloroplasts, microbial cell factories and eventually also plants for the improved capture and conversion of CO_2.

17.00 Prof. Dr. Sven Panke, Swiss Federal Institute of Technology (ETH Zürich), D-BSSE & Molecular Systems Engineering, National Competence Center in Research (NCCR), Basel, Switzerland

Methods for strain and enzyme engineering

Despite the advances in rational design enabling the synthetic biology revolution, our ability to successfully reprogram strains and proteins remains strongly coupled to our ability to search though libraries of various sizes. The recent advances in DNA synthesis and microfluidics enable a re-thinking of the classical screening process in terms of library design, throughput, assay complexity, and assay output. I will use recent work from the BioprocessLab to illustrate some aspects of this ongoing transition for the development of improved bioproduction strains and enzymes.

17.30 Prof. Dr. Benjamin Woolston, Chemical Engineering, Northeastern University, Boston, MA, USA

Metabolic Engineering for Production of Biofuels and Bioproducts from Methanol

Single-carbon (C1) compounds including carbon monoxide, methanol and formic acid have emerged as promising feedstocks for biofuel and biochemical production. These substrates can be produced renewably from CO_2 through electrocatalysis or hydrogenation with renewable hydrogen, thus bypassing food security and land conversion concerns raised over traditional biofuel feedstocks. Methanol may be particularly advantageous due to its high energy density, ease of handling, and avoidance of the mass transfer challenges associated with gaseous C1 feedstocks. A major challenge to establishing a "methanol bioeconomy" is that, in contrast to platform microbes like *Escherichia coli* and *Saccharomyces cerevisiae*, a systems-level understanding of native methanol utilizers is lacking, and genetic tools for establishing heterologous product pathways in those microbes are underdeveloped. This leaves the metabolic engineer with a choice: to develop robust genetic tools to enable pathway engineering in native methylotrophs, or to engineer a more tractable organism like *E. coli* to grow methanol by importing and optimizing a methanol catabolic pathway. Both of these approaches have their benefits and challenges, and in this talk I will highlight these by presenting my work on both strategies. I will first discuss my efforts to engineer *E. coli* for methanol metabolism, with a particular emphasis on the identification and mitigation of pathway bottlenecks through rational metabolic engineering and isotopic tracer experiments. I will then discuss my group's more recent efforts on the native methylotroph *Eubacterium limosum,* an acetogenic microbe that converts methanol at very high carbon yields through the Wood-Ljungdahl pathway, presenting our work expanding the genetic toolbox, as well as metabolic flux analysis through isotopic tracer experiments we are conducting to more fully elucidate the underlying metabolic network topology under both unitrophic and mixotrophic growth conditions. The talk with conclude with some perspectives on the relative merits of both approaches for establishing the industrial-scale production of biofuels and bioproducts from methanol.

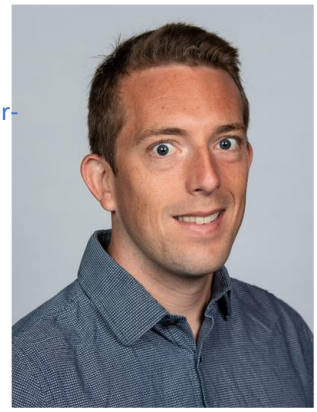
ABOUT THE SPEAKERS

Vassily Hatzimanikatis is Associate Professor of Chemical Engineering, Chemistry and Bioengineering at Ecole Polytechnique Federale de Lausanne (EPFL), in Lausanne, Switzerland. Dr Hatzimanikatis' research interests are on systems and synthetic biology, with focus on evolution and design of metabolism, integration of omics data, bioenergetics, and biochemical and biophysical kinetics. Dr Hatzimanikatis is a fellow of the American Institute for Medical and Biological Engineering (2010), he was a DuPont Young Professor (2001-2004), and he has also received the Jay Bailey Young Investigator Award in Metabolic Engineering (2000), the ACS Elmer Gaden Award (2011), and the Metabolic Engineering Award from the International Society of Metabolic Engineering (2014). He also serves as associate editor of the journals PLOS Computational Biology and Biotechnology & Bioengineering and, and Senior Editor of Biotechnology Journal.

Tobias J. Erb is a synthetic biologist and Director at the Max Planck Institute for terrestrial Microbiology in Marburg, Germany. Tobias Erb studied Chemistry and Biology at the University of Freiburg (D) and the Ohio State University (US). After stays at the University of Illinois (US) and ETH Zürich (CH), Tobi Erb moved to the Max Planck Institute in Marburg, where he was promoted to Director in 2017. Erb received numerous awards, among them the Heinz Maier-Leibnitz Prize of the German Research Foundation and the Otto Bayer Award. He was named one of 12 up- and coming scientists by American Chemical Society's C&EN and elected to the "Junge Akademie" at the National Academy of Science. Tobi Erb serves as expert on the working groups "Artificial Photosynthesis" and "Life Sciences" at the German National Academy of Sciences. Research in the lab of Tobi Erb centers on the discovery, function and engineering of novel CO_2 converting enzymes and their use in engineered and artificial photosynthesis, as well as the bottom-up design of synthetic chloroplasts and cells.

Sven Panke is Full Professor at the Department of Biosystems Science and Engineering of ETH Zürich, Basel, Switzerland (https://bsse.ethz.ch/bpl). Sven Panke received his Ph.D. in 1999 from ETH Zurich, Switzerland, for his work on the production of fine chemicals with recombinant bacteria. After a two year-stay in the biocatalysis group of the pharma product group of the Dutch chemical company DSM (Geleen, The Netherlands), he returned to ETH in 2001 as an Assistant Professor for Bioprocess Engineering. After receiving tenure in 2007, he moved to the newly founded ETH Department of Biosystems Science and Engineering in Basel. His main research topics include integrated reaction-separation systems, high-throughput screening, and synthetic biology. His work was awarded with the ETH Medal and the DSM Research Award. From his BioprocessLab originated the Biotech companies FGen, Memo-Therapeutics, Myria Biosciences, and Omne possibile

ABOUT THE SPEAKERS



Benjamin Woolston is Assistant Professor of Chemical Engineering at Northeastern University in Boston, MA. Dr. Woolston joined the Northeastern University Chemical Engineering department as an Assistant Professor in January 2020. He obtained his B.Sc. in Chemical Engineering with Honors at The Pennsylvania State University, where his undergraduate research, advised by Prof. Wayne Curtis, won the AIChE National Student Paper Competition. As an NSF Graduate Research Fellow, Dr. Woolston received his PhD in Chemical Engineering in 2017 from MIT under the guidance of Prof. Greg Stephanopoulos, where his research focused on the development of genetic tools to enable metabolic engineering in anaerobic CO2-fixing acetogens, and the establishment of a methanol utilization pathway in the model organism Escherichia coli. His Post-doctoral work was conducted in the laboratory of Prof. Emily Balskus in the Chemistry & Chemical Biology department at Harvard University, where he studied microbial metabolic pathways and enzymes that contribute to the stability of health-associated Lactobacilli in the human vaginal microbiota. At Northeastern, his research program – with funding from the US Department of Energy - combines approaches from his previous research training in metabolic engineering, synthetic biology, biochemistry and microbiology to engineer microbes for biofuel & biochemical production from single-carbon feedstocks, and as diagnostics and therapeutics in the Human gut microbiota. Since joining Northeastern, Dr. Woolston has taught a senior elective in Biochemical Engineering, and a graduate course in Kinetics & Reactor Design. He has authored 17 publications and one patent, and serves on the editorial board of Metabolic Engineering Communications and the Journal of Industrial Microbiology & Biotechnology. He is also the winner of the 2020 International Metabolic Engineering Society Jay Bailey Young Investigator award, and the 2021 Biotechnology and Bioengineering Daniel I.C. Wang award.

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ESAB aims to promote the development of Applied Biocatalysis and takes initiatives in areas of growing scientific & industrial interest in the field.

Schedule and Topics of next ESAB webinar:

19 Nov. 2021, 14.00-16.00 CET	Machine Learning, Computational, AI and IT Tools and Methodologies in Biocatalysis, organized by Jennifer Littlechild
10 Dec. 2021, 14.00-16.00 CET	Advances in Experimental Tools and Methodologies for Screening Enzyme Functions, organized by Roland Wohlgemuth

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