Making use of metabolic models – in silico driven design and engineering of industrial microorganisms

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Superior microorganisms that produce chemicals, materials and fuels from renewables are major drivers of the developing bio-based economy. Firstly, they hold the key to the optimized production of traditional bio-based products regarding key performance indicators such as titer, yield and productivity [1]. Secondly, they enable future production of important petrochemicals from green resources rather than from fossil fuels [2]. Without doubt, systems metabolic engineering is changing the way to design and optimize such microbial cell factories for industrial production: the integration of systems biology and systems biotechnology with new concepts from synthetic biology enables the global analysis and engineering of microorganisms–and bioprocesses at efficiency and versatility otherwise not accessible. In this regard, the lecture will highlight the integration of model-based prediction and design into industrial strain engineering: driven by massively increasing genomic information, stoichiometric models provide valuable insights into the properties of metabolic networks and give access to theoretical maximum yields, optimal pathways and preferred production hosts [3]. Most importantly, metabolic models can even guide smart and straightforward engineering of cells and processes [4]. The use of models for decision-making and strategic developments appears particularly useful for industrial application. Model-based approaches are fast, feasible at limited level of knowledge in early project stages and allow the investigation of different scenarios, all relevant for short development time and high performance required. Different examples from industrial microbiotechnology will demonstrate the power of using metabolic models: bio-based production of chemicals [5,6], polymers [7] and recombinant proteins [8]. Regarding industrial lysine production model-based design has provided synthetic strains, which reach the high performance of classical producers derived over the past fifty years [9].

References
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Despite the cellular process of metabolite efflux being ubiquitous to all microbial cells, we still do not know clearly how this process works and how it is regulated. It is believed that small metabolites, mainly those end products of fermentation are excreted through the plasma membrane passively, or they are secreted through specialised mechanisms such as vectorial reaction in response to hypo-osmotic stress, or uniport and symport transport systems. However, all of these mechanisms are based on the concept of “metabolic overflow”, which under specific metabolic conditions it is observed a massive excretion of some metabolic intermediates due to their intracellular accumulation. Although this concept seems appropriate to explain the secretion of some intracellular metabolites, it does not apply to many cases studied during continuous culture. Our metabolomics data obtained during different time-series studies of microbial growth under continuous and batch cultures confirm that the concept of “metabolic overflow” cannot be applied to explain the efflux of several intracellular metabolites found in the extracellular medium. Most of our studies indicate that microbial cells very often get rid of some intracellular metabolites in response to an environmental stimulus, even if these metabolites are key intermediates of central carbon metabolism.

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**O7-4**

**Towards genome-scale metabolic pathway analysis: metabolome integration allows efficient enumeration of elementary flux modes in metabolic networks**

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**Background:** Elementary flux mode (EFM) analysis is used to identify all non-decomposable steady state pathways in metabolic networks. EFMs represent minimal functional building blocks. They can be used to characterize metabolic phenotypes and cellular robustness or identify targets in metabolic engineering. Despite its potential EFM analysis is currently restricted to medium scale models as the number of EFMs in a network explodes with the network’s size.

**Method:** We present a novel computational method that integrates the cellular metabolome into an EFM analysis. As the metabolome directly maps to the Gibbs free energy surface of the reaction network, we are able to identify and remove thermodynamically infeasible EFMs during the runtime of an analysis without impacting any biologically relevant EFMs. Thermodynamic infeasibility is determined in the framework of network embedded thermodynamics. In this way we curb the explosion of the number of EFMs in large networks.

**Results:** We present details of our implementation and demonstrate that our new approach successfully tackles the major bottleneck strongly reduce the memory consumption. Thus an unbiased characterization of large-scale metabolic models is made possible. In fact, we present the first thermodynamically consistent EFM analysis of iJE660a, a genome-scale metabolic model of E. coli. For instance, our analysis confirms the inactivity of glutamate dehydrogenase in E. coli grown on glucose.

**Conclusion:** Thermodynamic EFM analysis successfully integrates the metabolome into an EFM analysis. It is solely based on first principles, and allows for an unbiased characterization of the metabolic capabilities in genome-scale metabolic networks.

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**O7-5**

**Genome scale metabolic modeling of recombinant protein producing yeasts: prediction of process parameters and metabolic engineering targets for efficient production**

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Genome scale metabolic models have been successfully applied to predict genetic interventions to redirect metabolic fluxes towards desired products of the primary and secondary metabolism. Complex polymeric products like heterologous proteins equally demand redistributions of primary metabolic fluxes, their rational design however is far less obvious. Therefore cell engineering for protein overproduction has concentrated mainly to transcription, codon usage, protein folding and secretion.

We have developed the first genome scale metabolic model for the yeast *Pichia pastoris* (PipMBEL1254), and integrated the synthesis of heterologous protein. The model could successfully predict the increase of protein production in oxygen limited conditions, as well as changes of central metabolic fluxes in production strains, as measured by $^{13}$C flux analysis. Metabolic engineering targets for enhanced protein productivity were predicted with FSEOF for gene overexpression and MOMA for gene deletion. After deleting or overexpressing the respective genes as predicted more than 50% of the interventions led to an enhanced production of cytosolic human superoxide dismutase (hSOD), and similarly of other proteins. Beneficial mutations were mainly related to reduction of the NADP/H pool and the deletion of fermentative pathways.

We demonstrate that genome scale metabolic modeling is suitable to describe metabolic changes in recombinant strains and can be successfully applied to design genetic interventions to the primary metabolism to increase recombinant protein production.

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**O7-6**

**Use of a novel combinatorial genetics platform to rapidly clone, express and select target biocatalytic activities for multigenic metabolic pathway optimization**

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Replacement of petroleum-based products and manufacturing processes with competitive bio-based alternatives is attracting