Systems biology prepares the ground for successful synthetic biology

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With the beginning of this century, the scope of Molecular Microbiology and Microbial Biotechnology has broadened by the advent of two major disciplines: systems biology and synthetic biology. In reality, one can consider them the two sides of the same agenda of understanding biological objects and phenomena with the quantitative tools of physics and the relational logic of engineering (de Lorenzo and Danchin, 2004). Historically, Systems Biology was born from the merger of model-based systems studies with the (sudden) access to massive *omics* data finally yielding quantitative system analysis (Westerhoff and Palsson, 2004). It aims at the holistic understanding of the whole cellular complexity investigating pro- and eukaryotic cells, tissues, organs and even humans. Systems biology is strong in analyzing complex, heterogeneous data, deciphering hidden information thus pinpointing to sensitive structures that execute control in the regulatory networks of interest. Systems Biology is thus the tool that unravels black boxes, i.e. fields of interest where our understanding is still poor despite the importance of the subject. As such, the mindset of systems biology acts as driver and solver at the same time to gain a comprehensive understanding of living systems.

Whereas systems biology serves as an integrator of the whole, synthetic biology basically follows the opposite roadmap to the same end: creating—even beyond natural systems, for the sake of understanding. To this end, the reductionist approach is taken to an extreme (Forster and Church, 2006) for breaking down the complexity of the cellular context into a limited set of interacting modules. This is in agreement with the engineering principle that a system should be decomposable and be understood in its smallest part to be called an engineered system. Synthetic biology follows this mindset by defining modules of interest and, important enough, to engineer the same and repurposing them for a human-designed functionality. Where systems biology yields the model-based integration of units in the host, synthetic biology focuses on these modules for engineering and implementing novel functions. Consequently, the key driver of synthetic biology is to create something new, whereas systems biology yields the comprehensive description of the status quo.

Figure 1: Systems biology yields the holistic understanding of systems without changing them. Accordingly a high complexity is covered in comprehensive studies. Synthetic biology follows novel approaches thereby creating numerous novel strains. Because single modules are often studied, the covered complexity is lower than in systems metabolic engineering. The later covers all levels of complexity

needed to engineer novel producers.

The ultimate reductionist approach is illustrated by the principle of 'orthogonality' that was introduced by synthetic biology (Lucks et al., 2008). Accordingly, modules are engineered (and characterized) that can be transferred from one host to the other executing the same functionality without interacting in the host system. Examples include novel promoters and regulatory circuits that represent self-organizing heterologous units in different cellular environments (Moon et al. 2012). Apparently, such systems closely follow the ideal of engineering – but do they mirror a universal principle or rather the rare exception?

In this context, the history of metabolic engineering may serve as a reference. In the 90s metabolic engineering was founded with the intention to engineer microorganisms with the help of recombinant technologies (Bailey, 1991). Early approaches concentrated on separated parts of the metabolism to amplify the production of native compounds or even accessing new products. Under 'lessons learned' Bailey (1999) summarized the likewise experiences by the statement that any change in the system will be answered by a multi-level response. Distinct, locally restricted effects on system modulations are unlikely to happen. On contrary, multi-level consequences including metabolic, transcriptional and translational control are often observed. This understanding became manifest in the current field of systems metabolic engineering (Lee et al., 2013; Woolston et al., 2013).

But what does this mean for systems biology and synthetic biology? Approximately 15 years after their birth, both disciplines have achieved respectable maturity. Consequently, questions arise how achievements can be translated into applied biotechnology. This special issue aims at supporting the translation by pinpointing on the interface between systems biology and synthetic biology. To be precise, reviewing topics have been selected that fulfill the double requirement of high relevance for strain engineering and profound data availability enabling systems biology studies. As such this special issue yields connecting both disciplines giving systems biology the task to provide the essential information finally enabling successful strain engineering with synthetic biology tools.

For this purpose this Curr Op Microbiol Section on 'Microbial systems biology' focuses on aspects of cellular organization, sensing, engineering and modeling, but keeping an eye on possible biotechnological applications. In fact, compared to pure chemical reactions, biotechnical processes using microbes possess an unbeatable, unique advantage: the biocatalyst is reproducing itself. Once engineered, microbial producers replicate the engineered code and pass it from one generation to the other. Apparently it is essential to understand the details of how DNA is replicated and how cells transfer this information to daughter cells. Therefore the *status quo* of cell cycle understanding is reviewed by *Lasker* referring to the well accepted model bacterium *Caulobacter crescentus*. It is shown that **spatial organization** is a prerequisite for successful cell cycling. By analogy this also holds true for the gene transcription machinery as outlined by *Govindarajan and Amster-Choder*. Likely because prokaryotes lack of obvious compartmentalised structures, cytoplasm was often considered as a 'mixed bioreactor' enabling close proteins/substrate contacts for catalyzing reactions of interest. However, biochemical logic suggests that the bacterial cell must be somehow domain-alised as to avoid massive chemical cross-reactivity (de Lorenzo et al., 2015), i.e spatial organization is much more conspicuous than formerly assumed. Consequently the too simplistic view of a mixed cellular tank is challenged.

In terms of engineering, bacteria are open systems exchanging mass and information with the environment. This holds also true for microbes as (recombinant) producers. According to the dreams of engineers, such microbial producers show maximum import, synthesis and export rates for substrates and products excluding any non-wanted interaction with cellular activity or product formation. Apparently, this ideal situation is rather the exception than the rule, in particular because

microbes are exposed to production conditions that are different to their native habitat. To create producers withstanding even harsh environments is a promising goal for strain engineering. In an interesting stocktaking effort towards this end, *Sandoval and Papoutsakis* reviewed our understanding of the bacterial cell wall as a toxicity barrier and outlined possibilities of further engineering.

The number of sulfur containing metabolites in microbes is rather limited. L-cysteine, L-homocysteine, L-methionine, S-adenosyl methionine, biotine and co-enzyme A are the most important protagonists and they are crucial for cellular growth. Microbial sulfur typically occurs as sulfide whereas sulfate is the common substrate used in fermentations. The necessary metabolic sulfur reduction is ATP intensive thus representing an inherent metabolic burden for microbial production processes. *Rückert* provides an overview about microbial sulfur reduction coming to the conclusion that large gaps of understanding still exist defining a promising research field for the future.

Engineering strains to create microbial producers requires for efficient molecular tools. Whereas singular genes or operons were in the focus of former genetic engineering, synthetic biology addresses the whole genome at once – called genome editing. *Csörgö et al.* review the current state-of-the-art outlining its potential for future applications. Given the awesome pace of papers reporting new and improved genome editing methods, this article risks being obsolete by the time of its publication! Yet, we expect it to remain as a witness of the state of affairs at this exciting stage of such an ever-faster field.

Complementary, synthetic biology yields implementing novel regulatory circuits for switching on/off non-native functions like the formation of targeted products. A most desirable tool for metabolic engineering involves metabolite-sensing and product-sensing biosensors that translate the presence of a given small molecule into a genetically tractable, and measurable phenotype *in vivo*. In this way, one can improve a production flowchart by adjusting many conditions to a maximum yield of the added-value molecule at stake. In this context, *Libis et al.* provides a review about the role of allosteric transcriptional factors that act as the core sensors of cellular devices for reporting the cellular response to chemicals of interest. In a further turn of screw, digital-type biological switches inspired by electrical engineering are wanted. Whether these are technically feasible and how future constructs may look like is reviewed by *Bradley et al.* Tightly linked to the implementation of non-native control units in microbial hosts is the question of its interaction with the microbial network. In essence, this question deals with the orthogonality paradigm of synthetic biology and how one can model and quantify the interplay between a human-designed genetic implant and the biochemical and regulatory *chassis* of the receiving microbial host– an issue addressed in the article by *Borkowski et al.*

Following the fundamentals of engineering, a system is fully comprehended only if we understand its smallest parts and we can model it for predicting its performance. Modeling systems dynamics is particularly challenging because it reveals non-appropriate model structures and assumptions very clearly. Accordingly, *Vasilakou et al.* have reviewed the state-of-the-art of dynamic metabolic modeling, with a focus on microbial systems. Conclusions are provided how related technologies can be used to efficiently engineer strains in the future.

In sum –and without pretending to be exhaustive, the reviews included in this Section represent a good palette of some of the most vibrant research areas through which Systems and Synthetic Biology is transforming contemporary research in Molecular Microbiology and Biotechnology. Instead of the traditional trial-and-error approaches that have dominated the field for a long time, the wealth of knowledge delivered by systemic approaches now guides the deep genetic engineering

of the biological properties at stake. And *engineering* thus stops being an *analogy* when applied to the live world to become a veritable *methodology* for creating new to Nature products and processes based on bacterial parts and devices.

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