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Synthetic biology advances for pharmaceutical production

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Synthetic biology enables a new generation of microbial engineering for the biotechnological production of pharmaceuticals and other high-value chemicals. This review presents an overview of recent advances in the field, describing new computational and experimental tools for the discovery, optimization and production of bioactive molecules, and outlining progress towards the application of these tools to pharmaceutical production systems.

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The synthetic biology revolution

Synthetic biology has seen rapid advances in the last couple of years. Initially focusing on proof-of-concept studies illustrating our ability of writing genetic code on a large scale and demonstrating the usefulness of introducing engineering concepts into biology, the field is now quickly moving towards industrial applications [1–7]. In particular, the engineering of microbial production systems for high-value small molecules is seen to hold great potential, aiming at compounds that range from flavours and fragrances to clinically relevant pharmaceuticals [8]. While just a few years ago, synthetic biology was seen as an avant-garde concept, its ideas and methods have now largely entered the mainstream of molecular biology and genetic engineering, so much so that the necessity for its ambitious engineering metaphors is already being debated [9,10]. In this review we provide a concise overview of some of the early achievements in the synthetic biology of pharmaceuticals and the advances in tools (molecular and computational) that are expected to drive this field forward rapidly.

Synthetic biology for pharmaceuticals

Pharmaceuticals have inspired some of the earliest success stories of synthetic biology for two main reasons: on the one hand, small-molecule drugs in current use (from aspirin to artemisinin) are very often derived from natural products, so that a return to microbial production systems is seen as relatively straight-forward. On the other hand, many natural biosynthetic pathways show a surprising level of built-in modularity at many levels, which can be exploited by the engineering approaches of synthetic biology [11,12]. This is particularly true for the large group of bioactive natural products that are active as antibiotics and related compounds: the evolutionary requirements for rapid diversification and for robust cross-species compatibility of pathways that are constantly exchanged between host organisms have shaped large modular assembly lines that can serve as starting points for synthetic biology. Cummings *et al.* [13] and Poust *et al.* [14] have recently reviewed the possibilities and limitations of this approach with a particular focus on polyketide synthases, and Kittleson *et al.* examine the challenges of modular genetic engineering from a broader systems biological perspective [15].

The synthetic biology of pharmaceuticals is further inspired by the recent avalanche of microbial genome and metagenome sequences which revealed an unexpected richness of unexplored biosynthetic capacities in almost every genome analysed [16,17]. For example, the recently published first comprehensive assessment of secondary metabolite diversity across microbial kingdoms, based on the computational analysis of more than 1100 complete genome sequences, detected more than 30 000 putative biosynthetic gene clusters (estimating a false-discovery rate of 5%) [18]. These were broadly distributed across the phylogenetic tree, with clusters of particular richness in, for example, actinomycetes, *Burkholderia* and *Pseudomonas*, but 'talented' strains of high predicted biosynthetic capacity being found in almost every larger bacterial group. A targeted analysis of Actinobacteria, combining genome mining and metabolomics, concluded that this group alone encodes for hundreds of thousands of possible drug leads [19••]. Under standard conditions, the majority of this biosynthetic potential is silent or cryptic, and synthetic biology is seen as a potential tool for awakening and mining this rich source of potential drug candidates on a large scale [17,20–24]. Additional genetic

engineering can then be applied to obtain variations and further diversity, with new or improved bioactivity [25,26].

Tools for the synthetic biology of pharmaceuticals

Synthetic biology, like all engineering disciplines, relies on the availability of powerful standardized tools for all steps along the design–build–test (and learn) cycle. Many of the required tools, for example genome synthesis, assembly and editing methods [27–29], are broadly generic. Others are specific to the field of small-molecule synthetic biology. These begin with methods for the comprehensive discovery and annotation of biosynthetic building blocks in newly sequenced genomes [30], as exemplified by the antiSMASH pipeline [31•,32]. In combination with advances in mass spectrometry-based analytics these annotations can be used to link genome information directly to bioactive compounds observed in bacterial cultures: this has recently been demonstrated for peptidic metabolites (both non-ribosomal peptides and Ribosomally synthesized and posttranslationally modified peptides, RIPPs), where incomplete and noisy sequence information derived from mass spectrometry can be used to successfully identify the gene clusters likely to produce them, and even to determine the actual structure of the end product [33,34••].

Another exciting source of biosynthetic building blocks is promised by recent advances in the de novo computational design of enzymes with activities that were previously unavailable in biologicals systems [35]. Enzyme engineering approaches also benefit from the increasing availability of genome sequences, which allow evolution-guided manipulation of existing enzymes, for example, for altered substrate specificity in molecular assembly lines for polyketides or non-ribosomal peptides [36•,37]. For example, identification of the recombination events underlying a major switch in substrate specificity during evolution of a bacterial non-ribosomal peptide synthase, allowed Crüsemann *et al.* [36•] to create enzyme variants that accepted alternative substrates, based on the inferred recombination points.

Analogous evolutionary strategies can also be applied at the pathway level, as demonstrated by the recent rational design of novel functional polyketide synthases by emulating the natural evolutionary processes underlying the diversification of this biosynthetic class [38•]. By examining the shared evolutionary history of the polyketides aureothin, spectinabilin and luteoreticulin, it was possible to develop a rational strategy of recombination and domain exchanges that reprogramme the aureothin polyketide biosynthetic pathway into a pathway that produced luteoreticulin. Protein engineering can also be very useful at the small scale, for instance creating enzymatic building blocks that are not affected by the end-product inhibition that is usually seen in natural enzymes. Schendzielorz *et al.* recently demonstrated the power of this 'mutein' approach in a case study on amino acid biosynthesis in *Corynebacterium glutamicum*, where high-throughput removal of inhibition allowed a massive increase in production titres [39••].

Other approaches to diversifying the pool of available chemical building blocks rely on a variety of experimental approaches. Walker *et al.* used synthetic biology to develop a library of new fluorinated building blocks for biomolecules [40••]. Starting from the fluoroacetate pathway of *Streptomyces cattleya*, the only known natural source of fluorinated biomolecules, they engineered acetate-based polyketide biosynthesis to incorporate the fluorinated precursor, via synthesis of fluoromalonyl-CoA as a modified extender unit. As many other natural products, ranging from isoprenoids to steroids and alkaloids, are also acetate-derived, this strategy offers potentially a general approach for expanding the chemical space around known pharmaceuticals.

The production of small molecules, whether awakened from genome information or optimized in a native or heterologous host, requires not only expression of the core biosynthetic pathway, but also sufficient supply of precursors and reduced competition from alternative reactions. Computational modelling has been useful for this purpose, and the specific challenges of modelling for secondary metabolite production have recently been reviewed [41]. The increasing availability of automated model construction and curation tools further increases the accessibility of the technology [42] and enables, for example, the comprehensive computational survey of potential production hosts for heterologous pathways [43].

Other computational tools allow the design of the actual pharmaceutical production system. A particularly ambitious recent example is the Retropath tool for the principled design of entire metabolic circuits [44••], based on constraining information about the metabolic capacities of the envisaged host organism (chassis) and the scope of available chemical reactions. In addition to the production modules, the Retropath framework also allows exploration of the design space for biosensing and regulation of the synthetic pathways, with the ultimate aim of enabling the construction of smart therapeutics, which integrate pharmaceutical synthesis and point-of-need delivery. Proof-of-concept examples of similar devices have recently been introduced, including engineered *Escherichia coli* that can sense and kill *Pseudomonas aeruginosa* biofilms by eavesdropping on the target quorum sensing signals and releasing antibiotic pyocin proteins in response [45].

While much of the actual DNA-level building of engineered systems for pharmaceutical production relies on