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Current Opinion in Biotechnology

Metabolomics tools for the synthetic biology of natural products

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Metabolomics plays an increasingly central role within the Design–Build–Test cycle of synthetic biology, in particular in applications targeting the discovery, diversification and optimised production of a wide range of natural products. For example, improved methods for the online monitoring of chemical reactions accelerate data generation to be compatible with the rapid iterations and increasing library sizes of automated synthetic biology pipelines. Combinations of label-free metabolic profiling and ^{13}C-based flux analysis lead to increased resolution in the identification of metabolic bottlenecks affecting product yield in engineered microbes. And molecular networking strategies drastically increase our ability to identify and characterise novel chemically complex biomolecules of interest in a diverse range of samples.

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Current Opinion in Biotechnology 2018, **54**:114–120

This review comes from a themed issue on **Analytical biotechnology**

Edited by **Hiroshi Shimizu** and **Fumio Matsuda**

https://doi.org/10.1016/j.copbio.2018.02.015

Synthetic biology of natural products

Synthetic biology facilitates the biosynthesis of pharmaceutical ingredients and other high-value chemicals by employing the Design–Build–Test cycle of engineering to guide the systematic enhancement of microbial factories [1–4]. Exemplary successful applications of synthetic biology to natural product production include a one-pot method for menthol biosynthesis in *Escherichia coli* [5], the modular extension of a styrene biosynthesis pathway to produce 2-phenylethanol [6], cannabinoid biosynthesis in yeasts [7], and the heterologous production of antibiotics using extensively refactored biosynthetic gene clusters: myxobacterial α-pyrone antibiotics in *Myxococcus xanthus* [8] and kasugamycin (an aminoglycoside antibiotic isolated from *Streptomyces kasugaensis*) in actinomycetes [9].

A recent review by Smanski and colleagues [10] provides details of recent advances in the technologies underpinning the Build aspects of the synthetic biology cycle, including pathway construction and pathway screening, while a complementary review by Chen *et al.* [11] focuses on the modelling approaches for the construction and optimisation of cell factories for bio-production, which cover a large part of the Design activities. In the present review, we will in turn focus on the Test component of synthetic biology, focusing in particular on advances in metabolomics as a discovery and debugging tool for metabolically enhanced microbial systems.

Test analytics — appropriate technologies

Mass spectrometry (MS) coupled to chromatography remains the domineering technology used for the quantification of natural product targets and is also the most widely used platform for the global profiling of the impact of an engineered biosynthetic pathway on the microbial metabolome. The challenge for the analytical technologies is to achieve the acquisition speed and sensitivity required to meet the high-throughput needs of a synthetic biology-based pipeline. Traditionally, products are measured directly from an aliquot of cell culture medium or — in the case of volatile products — they are captured in solvent overlays and transferred to vials or multi-well plates for analysis. These approaches are often slow (tens of minutes per sample plus preparation time) and provide only a snap shot of what is occurring at a given time.

To overcome this limitation, much effort has been invested into the development of improved methods for the online monitoring of chemical reactions; which would provide greater control of sampling and provide dynamic results with regard to product turnover. Definitions of the analytical terminology described herein are summarised in Table 1. In recent work by Yan *et al.*, desorption electrospray ionisation (DESI) coupled to ion mobility MS (IM-MS) was used for the high-throughput screening of biocatalysis directly from bacterial colonies on agar plates [12], which can in principle be applied to a broad range of substrates and products, including free amines, carboxylic acids, alkaloids and phenols; multiple analytes can be detected in a single analysis thus allowing for the screening of diverse strain libraries with complex

Table 1

Glossary of analytical technologies

Technique/approach	Full name	Description
Metabolomics	—	The untargeted, non-biased detection and identification of all low-molecular weight compounds (metabolites) present within a biological sample or system.
MS	Mass spectrometry	Analytical technique based on the ionisation of analytes (e.g. by DESI, MALDI, PTR or SIFT; see below), the subsequent separation of ions according to mass/charge ratio, and their detection and quantification.
MSI	Mass spectrometry imaging	Mass spectrometry is conducted in a spatial manner thus permitting the visualisation of the two-dimensional localisation of analytes within a sample, for example across a microbial colony growing on an agar plate.
DESI-MS	Desorption electrospray ionisation mass spectrometry	Ambient ionisation technique using a nebulised electrospray. Highly charged microdroplets collect analytes from the surface of the sample prior to secondary droplets carrying the analyte to the MS. This ionisation technique is particularly suitable for MSI.
IM-MS	Ion mobility mass spectrometry	A variant of MS, with additional separation of ions according to the time it takes for them to travel through a drift tube with a homogeneous, continuous electric field in the presence of a neutral gas. This leads to separation of ions according to size and shape (collision cross section), complementing the mass/charge information available in traditional MS.
MALDI-MS	Matrix assisted laser desorption ionization mass spectrometry	Ionisation approach whereby a matrix (an energy-absorbing small organic compound) is applied to/mixed with a sample. A laser applied to the matrix:sample mix excites the matrix molecules and leads to the generation of volatilised ions which subsequently enter the MS. This technique is suitable for MSI.
PTR-MS	Proton transfer reaction mass spectrometry	A soft ionisation technique using an ion beam of protonated water molecules, H_3O^+, as an ion source to protonate (and thus ionise) volatile analytes. This technique permits for real-time monitoring of organic molecules in the gas phase.
SIFT-MS	Selected-ion flow-tube mass spectrometry	Similar to PTR-MS, this soft ionisation technique uses precursor ions in the gas phase to ionise volatile analytes. The precursor ions are generated by a microwave plasma ion source, and a single ion species can be selected (H_3O^+, NO^+ or O_2^-) to perform as reactant ion. Neutral volatile analyte molecules react with the precursor ions and undergo ionisation. This technique permits for real-time monitoring in the gas phase.
Molecular networking	—	A computational method for MS data analysis that allows for the identification of sets of spectra from chemically related molecules (spectral networks), based on similarities in molecular fragmentation patterns, even in the cases when the spectra are not matched to any known compounds.

product profiles. DESI-MS was also applied to the rapid analysis of enzyme kinetics by Cheng and co-workers [13], who measured product formation in a buffered aqueous medium, explored the possibility of adjusting the pH and solvent composition of the DESI spray to quench the enzymatic reaction and thus improved the accuracy of the kinetic measurements by preventing post-ionisation reactions.

As an alternative to DESI, matrix-assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) has readily been applied towards the large-scale phenotyping of bacteria [14,15]. A related optically guided MALDI-MS strategy has recently been implemented for the profiling of microbial colonies for rapid screening of natural product analogue libraries [16••]. This impressive development used optical imaging of microbial colonies to direct the laser coordinates for an automated MALDI-MS screening of approximately 1000 colonies directly from an imprinted glass slide with an MS sampling rate of about one colony per second. Reaction products were screened *in situ* and results overlaid with the optical images; integration of results allowed for subsequent colony picking and recovery of the desired mutant strains. The majority of commercially available MALDI-MS instrumentation permit a spatial resolution of >100 μm. However, the group of Bernhard Spengler has recently dramatically pushed this boundary towards much better lateral resolutions down to 1.4 μm [17], thus further advancing the technique towards single cell resolution and even higher throughput [18].

The coupling of microreactors or continuous flow chemical reactors directly to the mass spectrometer provides an enhanced ability to characterise unstable reaction products and reduces the sample volume required (albeit