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Research review paper

Enabling tools for high-throughput detection of metabolites: Metabolic engineering and directed evolution applications

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ABSTRACT

Within the Design-Build-Test Cycle for strain engineering, rapid product detection and selection strategies remain challenging and limit overall throughput. Here we summarize a wide variety of modalities that transduce chemical concentrations into easily measured absorbance, luminescence, and fluorescence signals. Specifically, we cover protein-based biosensors (including transcription factors), nucleic acid-based biosensors, coupled enzyme reactions, bioorthogonal chemistry, and fluorescent and chromogenic dyes and substrates as modalities for detection. We focus on the use of these methods for strain engineering and enzyme discovery and conclude with remarks on the current and future state of biosensor development for application in the metabolic engineering field.

1. Introduction

Metabolic Engineering efforts seek to rewire organisms for the industrially-relevant production of value-added products from renewable feedstocks (Liu et al., 2013). In doing so, the product may be a native, endogenous metabolite or the result of de novo biosynthesis through the use of heterologous enzymes and pathways. Either way, the great power of this approach is the ever-expanding chemical diversity inherent in and reachable from cellular metabolism. However, the intrinsic complexity of biological systems imposes long and costly Design-Build-Test (DBT) cycle during research and development. Fortunately, recent breakthroughs are beginning to change this picture. Specifically, advances in the adjacent fields of DNA sequencing and synthesis (Esvelt and Wang, 2013), directed and in vivo evolution (Crook et al., 2016; Esvelt et al., 2011; Ravikumar et al., 2014; Wang et al., 2009), targeted transcriptional regulation (Bikard et al., 2013; Deaner and Alper, 2017; Gilbert et al., 2013; Mali et al., 2013a; Perez-Pinera et al., 2013; Qi et al., 2013), and precision genome editing (Cong et al., 2013; DiCarlo et al., 2013; Jiang et al., 2013; Jinek et al., 2013; Mali et al., 2013b) enable more rapid prototyping of cell factories. However, these advances have clearly exposed the Test phase as the rate limiting step in the development process. To reach parity, the Test phase needs to match the high and increasing throughput of strain engineering and synthetic biology. However, unlike DNA, RNA, and protein detections that rely on complementarity and/or a finite set of building-blocks (like amino acids and nucleotides), metabolites display a very wide chemical diversity. As a result, many applications rely on either “one-off” screens and selections or spectrometric analysis. Thus, substantial improvements in the areas of metabolite sensing are required to achieve high throughput in a way that is not just brute-force replication of slow technologies (i.e. requiring chromatography). The field of metabolic sensing and high throughput detection is a highly explored area. This review serves to compile and highlight the diverse modalities used to construct biosensors for high-throughput metabolic detection and provide examples of their application in high throughput strain engineering workflows.

2. Platforms for high throughput screening

To maximally harness the power of large DNA and evolution libraries (often 10^7–10^9 variants/week), equally high throughput platforms are required for assessing the phenotype of interest. The easiest and least equipment-dependent method used is to link the desired phenotype with a growth-based selection (Dragosits and Mattanovich, 2013). However, this approach inherently selects solely for growth rate enhancement, a complex and non-specific trait that is often counterproductive to high level biosynthesis of the desired product. For many cases, growth-based selections are either not optimal or impossible due to the required coupling of growth and production via endogenous or synthetic routes. In such cases, traditional methods for small molecule

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quantification are used including high pressure liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and well-plate based spectrophotometric assays. However, these approaches generally cannot achieve the throughput needed to effectively and efficiently screen very large libraries (Dietrich et al., 2010). Massive parallelization, automation, and miniaturization of well-plate assays has partially bridged this gap (especially for screening pharmacologically active compounds) (Janzen and Bernasconi, 2002), but wide deployment often demands high capital costs and specialized skills.

In contrast to selections and traditional approaches, biosensors enable high throughput quantification by transducing the concentration of the target molecule into an easily measurable signal such as fluorescence, luminescence, or absorbance. In this regard, screening for product concentration can leverage single cell, high throughput analysis techniques such as fluorescence activated cell sorting (FACS) (Mattanovich and Borth, 2006) or droplet-based microfluidic sorting (Agresti et al., 2010). While there is great power in such an approach, sensing metabolites is notoriously challenging. Unlike nucleic acid detection and sequencing technologies based on relatively predictable thermodynamic binding between complementary bases (Aebersold and Mann, 2003), a generalizable technique to detect wide varieties of small molecules at very high throughput is not available. Specifically, the microbial metabolome and resulting chemical space span nearly every measurable molecular property (Fernie and Stitt, 2012; Fernie et al., 2004). This reality has led to a proliferation of different biosensor modalities and approaches that are each tailored to specific classes of molecules. The remainder of this review focuses on describing these various sensing modalities (along with their advantages and disadvantages) and their applications in the area of metabolite overproduction. We conclude with a summary of the state-of-the-art and perspectives for moving forward.

3. Modalities for metabolite sensing

3.1. Intrinsic properties of chemicals

In particular applications, the product itself serves as a scalable biosensor. Specifically, the chemical nature of absorbance or fluorescence of product molecules can be used as an inherent screening technique (Fig. 1). Numerous metabolic engineering products have chromphores or fluorophores. As examples, flavonoids and carotenoids are natural pigments that display a wide range of colors, spanning from red to violet (Tanaka et al., 2008; Verpoorte and Alfermann, 2000). Molecules such as β-carotene, apigenin, anthocyanin, are colored in red-orange, yellow, and blue, respectively, and these compounds can be thus be selected for via simple colorimetric assays. Several other products have innate fluorescent capability, with the capacity to absorb light or electromagnetic radiation of a particular wavelength and then emit a signal at a different wavelength. As examples, riboflavin, betaxanthin, and camptothecin exhibit excitation/emission at 450/518, 470/510, and 370/434 nm, respectively (Chandrakuntal et al., 2006; Gandia-Herrero et al., 2005; Loh and Ahmed, 1990). Table 1 summarizes instances and some applications where intrinsic spectral properties of target molecules have been used for engineering or evolution. As an example, metabolic engineering efforts have focused on the production of lycopene, and important antioxidant (Seren et al., 2008). The intrinsic property of this red pigment allows for rapid strain identification. This trait was explored in conjunction with global stoichiometric modeling and transposon-mediated mutagenesis to identify 8.5- and 2-fold increases in titers over wild type and engineered parental strains (Alper et al., 2005). Likewise, to improve $_{L-}$tyrosine production in *E. coli*, global transcription machinery engineering (gTME) was employed to perturb gene expression and strains were selected on the basis of melanin accumulation (a brown pigment) on plates. The result was a strain with a 114% increase in tyrosine production (Santos and Stephanopoulos, 2008b; Santos et al., 2012). While these applications were of relative ease due to inherently detectable product attributes, most molecules of interest do not possess such intrinsic detection capacity.

3.2. Protein-based biosensors

The proteome responds to and detects metabolites naturally using a variety of modes (Fernie and Stitt, 2012) which makes proteins a natural choice for the molecular recognition of many small molecules of interest to metabolic engineering and synthetic biology. Moreover, proteins and their constituent domains can be rewired and modularized to establish biosensors. These protein-based biosensors can be imported into a wide variety of host organisms for in vivo application, and sometimes can be purified for effective in vitro function as well (de los Santos et al., 2016; Li et al., 2017). Among the many different classes of protein-based biosensors available, this review will focus on the most common types, including transcription factors, engineered fluorescent proteins, and G protein-coupled receptors.

3.2.1. Transcription factors

Transcription factors (TFs) are proteins that can often natively react to stimuli such as small molecules and then transduce an easily

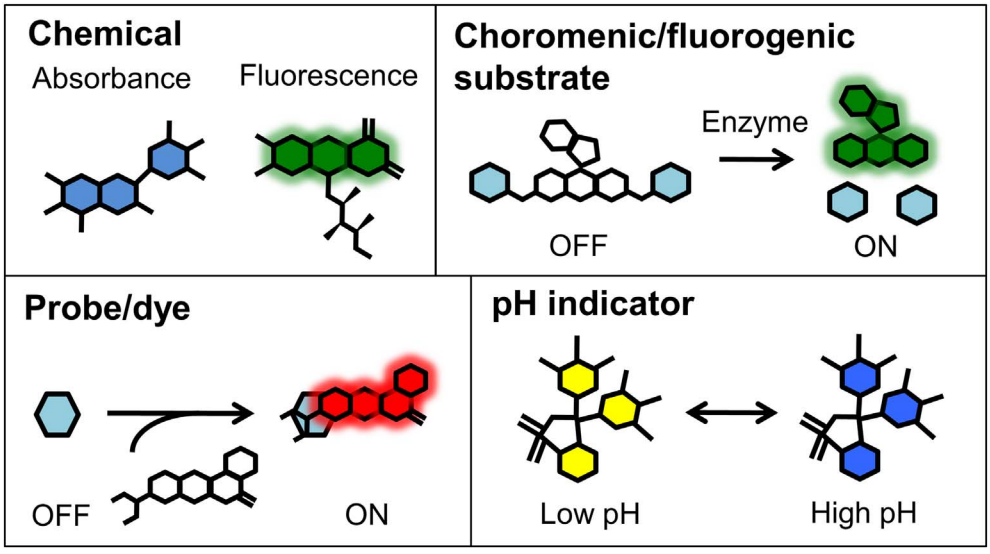

Fig. 1. Chromogenic and fluorogenic compounds, and mechanisms of activation. The physicochemical properties can be activated by enzymatic hydrolysis, chemical interactions, and pH change. Abbreviation: Enz., enzyme.