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Biofuel metabolic engineering with biosensors

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Metabolic engineering offers the potential to renewably produce important classes of chemicals, particularly biofuels, at an industrial scale. DNA synthesis and editing techniques can generate large pathway libraries, yet identifying the best variants is slow and cumbersome. Traditionally, analytical methods like chromatography and mass spectrometry have been used to evaluate pathway variants, but such techniques cannot be performed with high throughput. Biosensors — genetically encoded components that actuate a cellular output in response to a change in metabolite concentration — are therefore a promising tool for rapid and high-throughput evaluation of candidate pathway variants. Applying biosensors can also dynamically tune pathways in response to metabolic changes, improving balance and productivity. Here, we describe the major classes of biosensors and briefly highlight recent progress in applying them to biofuel-related metabolic pathway engineering.

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Introduction

Metabolic engineering of microbes holds the promise of producing many classes of chemicals, including fuels, from renewable feedstocks [1]. However, to compete with established production methods, engineered organisms must be highly productive, efficient, and robust at industrial scales. Many factors, such as the enzymes employed, regulatory proteins and genetic regulatory elements, can affect these phenotypes, and so a fundamental aspect of pathway engineering is identifying the complex genetic alterations required to create an optimized strain. While there are numerous ways to engineer genetically diverse strain libraries — in both random and/or directed fashions [2,3] — there are few assays that scale with the bandwidth of modern genetics (Figure 1). As such, it is critical to develop novel detection technologies in order to bring the full power of genetics to bear on metabolism.

An effective screening tool must be specific, high throughput, and sensitive to relevant metabolite concentrations. Most metabolites, except for special cases or by the use of exogenous chemical dyes (reviewed in [4]), cannot be measured using rapid optical methods. Chromatography and mass spectrometry (MS) are thus the only analytical tools available for measuring most biofuel-related metabolites despite their low throughput. Biosensors, genetically encoded components that respond to an input signal (e.g. metabolite concentration) and transduce that signal into a detectable output (e.g. fluorescence or gene expression), are emerging as a high-throughput alternative for measuring metabolite concentrations *in vivo*. Often adapted from natural proteins or aptamers, biosensors can be specific, sensitive, and non-destructive.

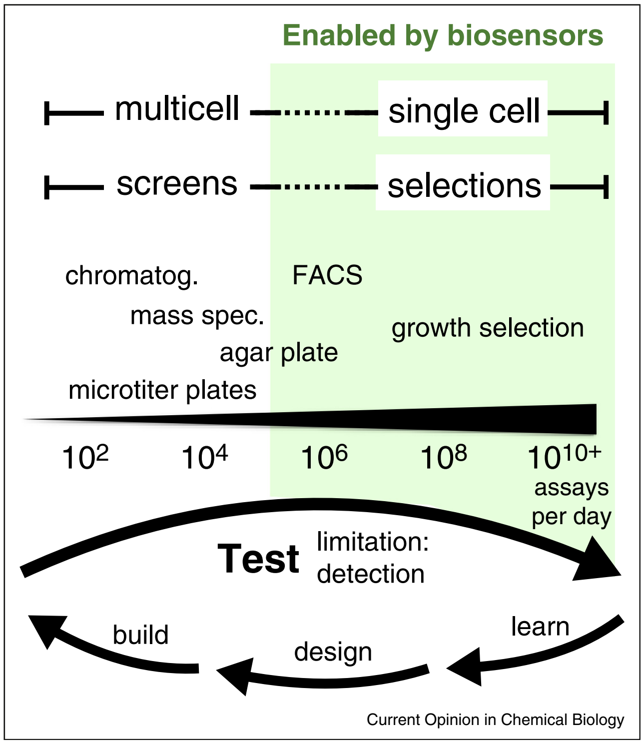
Here, we provide an introduction to biosensors and their use in modern metabolic engineering. As a point of comparison, we start with recent advances in analytical chemistry (reviewed in greater detail in [5]) and contrast this with the commonly employed classes of biosensors. We then focus in detail on the applications of specific biosensors to biofuel-related metabolic engineering.

State-of-the-art in analytical metabolite detection

Analytical chemistry methods, including chromatography and MS, are the gold standard for measuring metabolism. These methods are label-free, sensitive, and can detect many (e.g. 100+) metabolites in a single measurement [6]. However, these methods require time- and labor-intensive metabolite extractions that result in destructive, bulk measurements that are generally low throughput (10^1–10^3 per day). Two emerging MS-based platforms that may aid in overcoming these limitations are the RapidFire high-throughput MS system from Agilent Technology, Inc. [7] and surface-based MS techniques, such as Nanostructure-Initiator MS (NIMS) [8].

RapidFire uses robotics to automate the metabolomics workflow. Samples in microtiter plates are purified by solid-phase extraction and directly injected into an MS instrument. The instrument can processes a single sample

Figure 1



Biosensors enable rapid and single-cell quantitation of metabolites allowing for high-throughput evaluation of pathway variants and improving the rate-limiting 'test' step of the design-build-test-learn engineering cycle.

in less than 15 s, which is over 100× faster than traditional liquid-chromatography–MS measurements [7]. NIMS is a surface-assisted laser desorption/ionization technique that requires little sample preparation and uses a liquid 'initiator,' instead of a co-crystallization matrix, to produce spectra with high sensitivity and lower noise in the metabolite mass region. NIMS was recently used to screen >100 glycoside hydrolases (enzymes important for biomass hydrolysis) with a wide range of substrates and reaction conditions to generate more than 10,000 data points [9]. Although surface and automated MS techniques are not yet widely used, it is likely they will continue to increase in throughput and find applications in metabolic engineering.

Classes of genetically encoded biosensors

Biosensors are genetically encoded components that convert an input signal (e.g. metabolite concentration) into a measurable output like fluorescence or gene expression (Figure 2). In the following sections, we introduce common classes of biosensors constructed from fluorescent proteins, RNA, cytosolic transcription factors (TFs), G-protein-coupled receptors (GPCRs) as well as two-component systems and discuss their inherent advantages and disadvantages.

Fluorescent protein biosensors

Genetically encoded biosensors based on Förster resonance energy transfer (FRET) or single fluorescent proteins are promising tools for the analysis of metabolic pathways and their products. FRET biosensors consist of a ligand-binding domain (LBD) attached to a pair of fluorescent proteins that have overlap in their emission and excitation spectra, capable of FRET (Figure 2) [10]. Binding of a metabolite to the LBD alters the distance between the two fluorophores and changes the energy-transfer efficiency, measured as a ratio of fluorescence. While FRET biosensors have been developed for many different metabolites [11,12], they typically exhibit low dynamic ranges (e.g. tens of % change in signal) that significantly impede their use in screening applications. Single fluorescent protein biosensors (SFPBs) are fluorescent proteins that either directly detect input signals or are inserted within the primary sequence of a conformationally-labile LBD such that ligand binding affects fluorescence intensity (Figure 2) [13]. While genetically encoded SFPBs have high dynamic range (e.g. 10-fold) and are used in cell biology studies [14,15], they are not widely used in metabolic engineering [16••]. There are currently few available SFPBs due to the difficulty in engineering the coupling between an LBD and a fluorescent protein partner. Methods enabling rapid SFBP engineering may therefore be useful to increase the availability of this promising class of biosensors [17].

RNA biosensors

Riboswitches are naturally evolved ligand-responsive RNA elements that possess two components: a sensor (aptamer) domain that detects metabolite binding and a regulatory domain that converts binding-induced conformational changes into changes in gene expression (Figure 2) [18]. RNA-based biosensors also benefit from known techniques (e.g. SELEX) for generating aptamers against new metabolites [19] and have been adapted as biosensors for engineered pathways [20–22]. To date, however, use in metabolic engineering has been limited, likely from the challenges of recapitulating *in vitro* behavior within the cellular environment.

Cytosolic transcription-factor (TF) biosensors

TF-based biosensors detect environmental changes, such as metabolite levels, and alter gene expression in response (Figure 2). The most widely used are bacterial TFs, which are composed of an LBD that controls the engagement of a cognate DNA-binding domain to promoter/operator sites associated with target genes. Depending on the TF, DNA binding may lead to gene repression or activation. These biosensors can offer high sensitivity and dynamic range; small changes in ligand concentration are amplified through gene expression into large changes in protein abundance.

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