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Design and optimization of genetically encoded biosensors for high-throughput screening of chemicals

Hyun Gyu Lim[1,4], Sungho Jang[1,4], Sungyeon Jang[1], Sang Woo Seo[2] and Gyoo Yeol Jung[1,3]

Evolutionary engineering of microbes for the production of metabolites requires efficient screening methods to test vast mutant libraries. Genetically encoded biosensors are regarded as promising screening devices owing to their wide range of detectable ligands and great applicability to high-throughput screening and selection. Here, we reviewed the current progress in design and optimization of biosensors for high-throughput screening of chemicals. First, we summarized genetic parts of biosensors and strategies for their discovery and development. Next, we explained the properties of biosensors that are relevant to high-throughput screening. Finally, we described various methods for tuning biosensors to fulfill requirements of an efficient screening.

Addresses
[1] Department of Chemical Engineering, Pohang University of Science and Technology, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 37673, Republic of Korea
[2] School of Chemical and Biological Engineering, Institute of Chemical Process, Seoul National University, 1 Gwanak-Ro, Gwanak-Gu, Seoul 08826, Republic of Korea
[3] School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 37673, Republic of Korea

Corresponding authors: Seo, Sang Woo (swseo@snu.ac.kr), Jung, Gyoo Yeol (gyjung@postech.ac.kr)
[4] These authors contributed equally to this work.

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Introduction

Microbial engineering frequently relies on serendipitous discovery to improve chemical production due to the complexity of the biological system [1,2]. This strategy is based on the generation of libraries that contain rational or random mutations; particular variants with improved phenotypes are screened from the libraries. A major hurdle of this strategy is that the desired phenotype is mostly represented by the concentration of the metabolite produced, indicating that each producer should be experimentally evaluated. However, conventional methods (e.g., high performance liquid chromatography, gas chromatography, mass spectrometry, etc.) are not suitable for high-throughput screening because they are time-consuming and labor-intensive to assess the library space (Figure 1a) [3].

To address this issue, genetically encoded biosensors that are able to transduce the production of chemicals into easily detectable signals, such as color or fluorescence, have been developed (Table 1) [2,4]. These biosensors could significantly increase the screening throughput by determining the concentration of metabolite at the single-cell level and adapting it to various formats including fluorescence-activated cell sorting (FACS), microfluidic systems, and selection systems [5•,6,7•]. Furthermore, time and cost for screening can be greatly reduced since a producer cell serves as a sensor by itself.

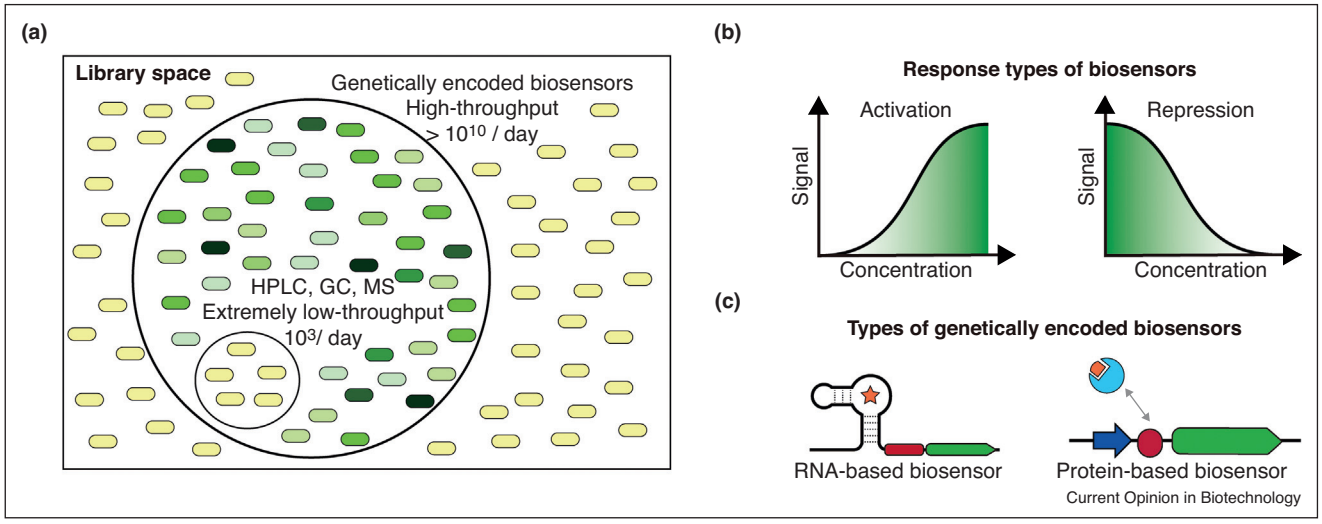
In this review, we briefly introduce the structural aspects of genetically encoded biosensors for their construction. Thereafter, we describe the features of biosensors and necessity of tuning. Finally, we describe recent strategies to optimize their behaviors for practical high-throughput screening applications.

Design of biosensors with diverse genetic elements

Screening of metabolite production requires sensor systems that detect target chemicals and transduce their concentrations into measurable outputs (Figure 1b). Genetically encodable biomaterials, namely, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein, have advantages for constructing such systems, as they can be easily installed and expressed in single cells of a variant of library [8,9]. Well-known examples of natural systems such as ligand-responsive riboswitches or transcription factors (TFs) raised the prospect of biomaterials as foundations for the sensor systems (Figure 1c). Therefore, numerous genetic parts have been employed to construct the genetically encoded biosensor systems with metabolite detectors, transducers, and output modules [10].

First, the metabolite detectors directly bind to cognate ligands through physical interactions. Both RNA and protein are capable of specific and tight binding to diverse

Figure 1



Genetically encoded biosensors for high-throughput screening of metabolite production. (a) Vast library space can be efficiently screened for the metabolite production by high-throughput methods based on biosensors. (b) Biosensors can either activate or repress the expression of outputs in response to increasing concentrations of the target metabolites. (c) Two types of genetically encoded biosensors according to the molecular composition. RNA-based biosensors directly bind to the target metabolites and control the expression of outputs that are also encoded in the RNA. Protein-based biosensors are made of allosteric transcription factors. The transcription factors bind to their target metabolite and regulate the expression of outputs that are under the control of cognate promoters.

metabolites. The most direct and feasible approach to obtain a detector for a target metabolite is by utilizing natural elements. Natural riboswitches and TFs are known to be responsive to numerous metabolites from amino acids to fatty acids and cofactors [11,12•]. Furthermore, detector modules for target metabolites could be newly discovered from nature. For example, RNA-based detector elements were discovered by utilizing a high-throughput method based on the next-generation sequencing [13••]. This method enabled genome-wide search for specific RNA sequence whose transcription termination is controlled by metabolites of interest. In addition, transcription factors and cognate promoters could be discovered based on the genome annotation and metabolite analysis in microbes [14]. Proteomics analysis was also applied to identify metabolite-responsive promoters [15]. When a suitable detector for the desired product could not be found, natural detectors could be engineered to alter their ligand specificity [16••,17]. Alternatively, artificial modules can be generated by *in vitro* selection method called systematic evolution of ligands by exponential enrichment (SELEX) or computational design for ligand binding [18,19,20,21•].

Next, the transducer regulates the expression of output module depending on the binding of the ligand to the detector. Molecular composition and mechanism of the transducer are highly associated with the nature of the detector. Transducers for RNA-based detectors are generally made of RNA which is directly attached to the 5′- and/or 3′-end of the detectors. The transducers enable ligand-dependent switching of the structure of the whole detector-transducer complex. These RNA transducers can regulate the expression of outputs during transcription, post-transcription, and translation [20••]. They could be designed rationally through computational approaches owing to their predictable base-pairings that largely contribute to the overall structure [22]. Otherwise, high-throughput screening and selection have been widely used for developing RNA transducers [23]. For protein-based detectors, inducible promoters with cognate operator sites can generally function as transducers [10]. Upon binding to a ligand, the protein-based detector adopts an alternative structure, dissociating from or binding to the operator site to initiate or to repress transcription, respectively.

Finally, the output module supports high-throughput screening and selection by expressing different amounts of detectable signals according to the metabolite concentration. One of the most preferred output modules is a fluorescent protein that can be easily combined with FACS and microfluidic systems. On the contrary, a selection marker gene renders the metabolite production a growth phenotype, allowing selection with theoretically unlimited throughput simply by the enrichment of a variant library under selection pressure [6]. Finally, an RNA aptamer called “Spinach” binds to a fluorophore to emit fluorescence without being translated into a protein [24]. This RNA-only output module could be coupled with other RNA aptamers to build chimeric RNA sensors that control their binding to the fluorophore, depending on the concentration of target metabolites [25].