Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Cell-free synthetic biology for environmental sensing and remediation

David K Karig[1,2]

The fields of biosensing and bioremediation leverage the phenomenal array of sensing and metabolic capabilities offered by natural microbes. Synthetic biology provides tools for transforming these fields through complex integration of natural and novel biological components to achieve sophisticated sensing, regulation, and metabolic function. However, the majority of synthetic biology efforts are conducted in living cells, and concerns over releasing genetically modified organisms constitute a key barrier to environmental applications. Cell-free protein expression systems offer a path towards leveraging synthetic biology, while preventing the spread of engineered organisms in nature. Recent efforts in the areas of cell-free approaches for sensing, regulation, and metabolic pathway implementation, as well as for preserving and deploying cell-free expression components, embody key steps towards realizing the potential of cell-free systems for environmental sensing and remediation.

Addresses
[1] Research and Exploratory Development Department, Johns Hopkins University Applied Physics Laboratory, 11100 Johns Hopkins Rd., Laurel, MD, USA
[2] Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA

Corresponding author: Karig, David K (David.Karig@jhuapl.edu)

Current Opinion in Biotechnology 2017, **45**:69–75

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

Edited by **Jan Roelof Van Der Meer** and **Man Bock Gu**

For a complete overview see the Issue and the Editorial

Available online 20th February 2017

http://dx.doi.org/10.1016/j.copbio.2017.01.010

Introduction

Microbes are found in nearly every realm on earth, ranging from thermal vents to Antarctic ice. The spectrum of sensing and metabolic activities that microbes exhibit to thrive in these environments has long inspired efforts to harness microbial biology for sensing and metabolic engineering applications. Sensing, for example, has been achieved with a wide range of different biological components, including enzymes, antibodies, receptor proteins, and nucleic acids [1]. Meanwhile, remediation has been accomplished even using natural microbes, although genetic engineering has also been used to improve metabolic efficiency of contaminant degradation [2].

To date, most biosensors utilize either a small set of purified biological components interfaced with a transducer, or whole cells that are simply modified to express reporter genes inserted downstream of ligand-activated promoters [1]. Most bioremediation efforts are similarly straightforward, focusing on either the use of natural cells or on the optimization of existing metabolic pathways. Synthetic biology offers transformative tools for improving both biosensing and bioremediation performance by expanding the range of sensor and remediation targets, and increasing the sophistication of sensor and regulator implementation. However, practical application of the resulting synthetic systems is hindered by safety concerns associated with the release of genetically modified organisms (GMOs) into the environment.

The emergence of cell-free synthetic biology offers a promising mechanism for circumventing GMO release [3,4], allowing deployment of gene networks and metabolic pathways without the risk of unbridled replication and spread of new microbial strains in the wild. Beyond safety, cell-free systems offer a host of other benefits as well. For instance, cell-free systems can operate in the presence of toxins that would inhibit or kill live cells. This means that key sensing and metabolic components, such as transcription factors and enzymes, can be produced in higher concentrations than in living cells, leading to improved sensitivity and efficiency. It also means that environmental chemicals are better tolerated, including those that are the target for sensing or remediation [5]. In addition, in cell-free platforms, all energy resources can be devoted to the engineered application, as opposed to supporting self-replication. Finally, the potential for evolution, which can undermine or even abolish engineered function, is largely removed in cell-free contexts.

Cell-free protein expression systems typically consist of a cell extract, which contains machinery essential for transcription and translation, as well as a number of components to fuel expression, including buffers, nucleotides, amino acids, and energy sources. Although cell-free protein expression systems have been used for decades to investigate biological phenomena and produce proteins

that are difficult to express in living cells, cost, yield and scale have historically prevented their adoption in sensing and bioremediation applications. Fortunately, these barriers have been recently removed thanks to new advances in cell-free preparations [6,7]. This has made possible a range of novel biosensing and bioremediation applications such as spill tracking, source pinpointing, and remediation *in situ*. The potential application space made possible by new advances in cell-free technology is the focus of the current review. First, we discuss sensing, including sensing modalities and integration of sensors into regulatory networks. We then touch on recent advances that facilitate the implementation of remediation pathways in cell-free systems. Finally, we discuss practical needs for applying cell-free systems, namely the unique challenges of cell-free systems as compared to living cells, as well as extension of shelf-life and the encapsulation of components for robustness in application contexts.

Sensing

Sensing modalities

Several different approaches for generating responses to ligands have been demonstrated in cell-free systems. These approaches include the use of receptors and other ligand responsive transcription factors [8], as well as an array of strategies based on leveraging DNA or RNA structures for regulation (*e.g.*, aptamers) [9]. The use of receptors is exemplified by the detection of bacterial quorum sensing signals using engineered genetic constructs in cell-free systems [10,11,12•,13]. These gene circuits express a bacterial quorum sensing receptor, which can form a complex with cognate quorum sensing molecules, subsequently enabling activation of a promoter expressing a reporter protein. This ability to detect chemical signatures of bacteria illustrates the potential for leveraging cell-free systems for pathogen detection. Besides quorum sensing receptors, other transcription factors that regulate downstream promoters upon ligand binding include the mercury binding transcription factor MerR [5], and the tetracycline binding transcription factor TetR [14].

While transcriptional regulator proteins offer robust performance, many sensing targets have no known regulator. By contrast, powerful selection procedures are available for identifying aptamers [15,16]. Therefore, a number of different cell-free sensing strategies have employed aptamers. In general, when a ligand binds an aptamer region, the aptamer changes conformation, resulting in a corresponding alteration in enzymatic activity, transcriptional efficiency, or translational efficiency, depending upon the precise implementation. Iyer and Doktycz, for example, demonstrated a DNA aptamer-based approach for engineering ligand responsive promoters in cell-free systems [17]. Specifically, they placed a DNA aptamer sequence near a T7 promoter such that ligand binding to the aptamer regulated transcription. The majority of approaches, however, rely on RNA aptamers (*e.g.*, riboswitches). For instance, Ogawa presented an approach for designing riboswitches that function in eukaryotic cell-free systems and demonstrated responses to theophylline, FMN, tetracycline, and sulforhodamine B [18]. In addition to DNA and RNA aptamer approaches, more recently, a novel RNA regulation approach was designed for sensing specific RNA sequences [19]. Pardee *et al*. utilized this method to detect Ebola [20] and Zika [21] RNA in *Escherichia coli* extracts.

Few direct comparisons have been made to date between cell-free sensors and their counterparts in more traditional sensors (*e.g.*, nano-bio sensors or whole cell sensors) in terms of sensitivity and specificity. A cell-free theophylline riboswitch in a cell-free translation system [18] and an aptamer-based electrochemical biosensor for theophylline [22] exhibited different, yet overlapping dynamic ranges of detection (3–100 μM vs. 0.2–10 μM). Similarly, cell-free and whole cell receptor-based sensors have been compared and exhibited fairly similar response characteristics [13]. As more cell-free biosensors are constructed and characterized in the future, the key determinants of sensitivity and specificity may be elucidated for each sensing modality. Meanwhile, by comparison to whole cell biosensors, the cell-free context may offer several sensitivity advantages. First, it may be possible to produce key receptors in higher concentrations than can be achieved in living cells. Second, it has been shown that cell-free systems can avoid problematic false negatives that arise in whole cell biosensors when ligands reach levels that are toxic to cells [5].

Collectively, the diversity of sensing options that have been demonstrated in cell-free systems suggests that sensors can be developed for a wide variety of targets. Future approaches may additionally leverage the amenability of cell-free protein expression systems for producing other components such as membrane receptors [23] and antibodies [24].

Complex regulation

The above sensing modalities offer basic sensing and response function; however, the deeper potential of synthetic biology lies in leveraging gene circuits to implement complex regulation. This regulation may be used to process multiple inputs and correspondingly regulate one or more outputs (reporters or remediation products). In addition, specificity may be generated through digital logic. For instance, a logical AND gate of multiple sensors with imperfect specificity may generate a response with an overall high specificity. This approach to improving specificity is analogous to the recent use of dual aptamers, whereby two aptamers were used to target different sites of a ligand in order to achieve highly specific detections, in a nanoparticle hybrid sensor [25]. While few dual