A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals

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ABSTRACT

Cells perceive a wide variety of cellular and environmental signals, which are often processed combinatorially to generate particular phenotypic responses. Here, we employ both single and mixed cell type populations, pre-programmed with engineered modular cell signalling and sensing circuits, as processing units to detect and integrate multiple environmental signals. Based on an engineered modular genetic AND logic gate, we report the construction of a set of scalable synthetic microbe-based biosensors comprising exchangeable sensory, signal processing and actuation modules. These cellular biosensors were engineered using distinct signalling sensory modules to precisely identify various chemical signals, and combinations thereof, with a quantitative fluorescent output. The genetic logic gate used can function as a biological filter and an amplifier to enhance the sensing selectivity and sensitivity of cell-based biosensors. In particular, an *Escherichia coli* consortium-based biosensor has been constructed that can detect and integrate three environmental signals (arsenic, mercury and copper ion levels) via either its native two-component signal transduction pathways or synthetic signalling sensors derived from other bacteria in combination with a cell-cell communication module. We demonstrate how a modular cell-based biosensor can be engineered predictably using exchangeable synthetic gene circuit modules to sense and integrate multiple-input signals. This study illustrates some of the key practical design principles required for the future application of these biosensors in broad environmental and healthcare areas.

1. Introduction

Bacterial cells live in an ever changing environment and must therefore be equipped with specific genetically-encoded sensors and signalling networks to continuously perceive and react to the various environmental signals. Analogous to a typical electrome-chanical sensor, a cellular signalling network normally consists of three interconnected modules – the input sensors, internal processing and regulatory circuits and output actuators to allow signal sensing and timely adaptations in cell physiology (Lim, 2010; Wang and Buck, 2012) (Fig. 1). The input sensors are receptors, either embedded in the cell membrane (e.g. sensor kinases) or located freely in the cytoplasm (e.g. ligand responsive allosteric proteins) with which the cell can detect various extra- or intra-cellular signals, such as chemical molecules, metal ions, light, heat or antigens, and transduce them into differential

gene transcriptional levels. Downstream gene regulatory networks process and integrate such signals combinatorially for a logic decision to be made, mimicking the digital logic circuit in electronic circuitry. Decisions are signified by changes in the expression of output actuators: the relevant proteins and chemicals responsible for the final phenotypic changes in motility, growth and morphology etc.

Due to the inherent modular architecture of sensory systems, bacterial cells can be viewed as programmable living biosensors in which the three component modules are exchangeable (Fig. 1). For example, specific synthetic sensors can be developed from either the host's own genetic repertoire or that of other bacterial species with more relevant specialisations in sensing capabilities in order to detect for example particular environmental contaminants or disease-related signals. Informed by the advanced sensing capabilities of many environmental microbes, already a number of single-input bacterial biosensors (van der Meer and Belkin, 2010) have been constructed to detect various toxic pollutant such as arsenic (Joshi et al., 2009; Stocker et al., 2003; Trang et al., 2005), xylene and toluene (Paitan et al., 2004), DNT explosive (De Las Heras et al., 2008) and the human pathogen *Pseudomonas aeruginosa* (Saeidi et al., 2011) with fluorescence,

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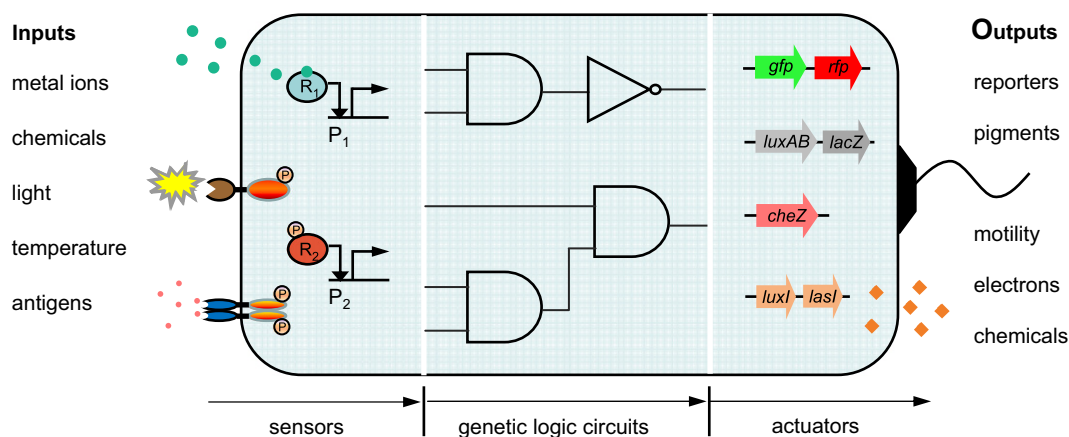 [2] Tel.: +44 20 7594 5442.

Fig. 1. Architecture of a synthetic modular cell-based biosensor. The cellular sensor comprises three interconnected and exchangeable modules, i.e. the input sensors, the internal genetic information processing circuits and the output actuators. The cells are engineered using various natural or synthetic sensors such as sensor kinases or intracellular receptor proteins to detect environmental signals and genetic circuits such as AND and NOT logic gates to modulate and integrate these multiple input signals. The programmed cells can then initiate customized responses by activating different output genes according to the logic decision transmitted upstream. Adapted with permission from Wang and Buck (2012).

luminescence or colorimetric pigments as outputs. However, these biosensors have been typically developed utilizing only the cell native sensory elements without additional layers of genetic processing and regulation, and thus can only detect one type of signal rather than a number of signals in a predefined combination. Instead, synthetic genetic logic circuits can be introduced through linking various cellular sensors and genetic actuators, to achieve customized cellular responses (Kobayashi et al., 2004) and sensing abilities (Khalil and Collins, 2010) in a designed and logical manner. In particular, multi-input cellular biosensors are essential for recognizing complex conditions, such as the cancer microenvironment normally specified by several signals in combination, where an enhanced sensing specificity and accuracy of the output response are necessary. For example, several genetic logic AND gates have been constructed to link inputs to pathogenicity-related signal responsive promoters (Nissim and Bar-Ziv, 2010), microRNAs (Xie et al., 2011) or proteins (Culler et al., 2010), and the output to a therapeutic suicide gene in order to achieve highly specific *in vivo* sensing and killing of diseased mammalian cells. These proof-of-concept examples demonstrate the great potential for using biological logic circuits to customize cell sensing and signalling for many useful applications (Khalil and Collins, 2010; Wang and Buck, 2012; Weber and Fussenegger, 2012).

Here, we employ both single cell type and multiple cell type populations, pre-programmed with engineered modular cell signalling and sensing circuits, as processing units to sense and integrate multiple environmental contamination associated signals. A modular, tightly-controlled and hypersensitive genetic AND gate was recently constructed in *Escherichia coli* that can integrate two genetic transcriptional inputs simultaneously (Wang et al., 2011). In the present work, we have engineered several *E. coli*-based logic-gated cellular biosensors by connecting the AND gate two inputs to a set of synthetic sensors for detecting and integrating the levels of arsenic, mercury, copper, zinc ions and bacterial quorum sensing molecules in an aqueous environment leading to a quantitative fluorescent output. The designed genetic logic circuits can act as a biological filter and an amplifier to enhance the sensing selectivity and sensitivity of whole cell-based biosensors. Moreover, a triple-input AND logic gated biosensor comprising two cell consortia was also constructed which can sense and integrate three environmental signals (As^{3+}, Hg^{2+} and Cu^{2+} levels) via a synthetic cell–cell communication module driving the cooperation between the two cell populations.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Plasmid cloning work and characterisation of the circuit constructs were all performed in *E. coli* TOP10 strain. Cells were cultured in LB (Luria-Bertani Broth) media (10 g/L peptone, 5 g/L NaCl, 5 g/L yeast extract). The antibiotic concentrations used were 50 μg/ml for kanamycin and 50 μg/ml for ampicillin. Cells inoculated from single colonies on freshly streaked plates were grown overnight in 5 ml LB in sterile 20 ml universal tubes at 37 °C with shaking (200 rpm). Overnight cultures were diluted into pre-warmed LB media at $OD_{600} = 0.025$ for the day cultures, which were induced and grown for 6 h at 37 °C prior to analysis, unless otherwise indicated. For the fluorescence assay by fluorometry, diluted cultures were loaded into a 96 well microplate (Greiner Bio-One, chimney black, flat clear bottom) by a repetitive pipette and induced with 5 μl (for single input induction) or 10 μl (for double input induction) or 15 μl (for triple input induction) inducers of varying concentrations to a final volume of 200 μl per well by a multichannel pipette. The microplate was covered with a transparent lid to counteract evaporation and incubated in the fluorometer (BMG FLUOstar) with continuous shaking (200 rpm, linear mode) between each cycle of repetitive measurements. All the chemical reagents used were analytical grade and purchased from Sigma Aldrich.

2.2. Plasmid circuit construction

Plasmid construction and DNA manipulations were performed following standard molecular biology techniques. The arsenic (J23101-rbs32-*arsR*-B0015-P_{arsR}) and mercury (J23115-rbs32-*merR*-B0015-P_{merT}) sensor fragments, *hrpR*-B0015 and *hrpS*-B0015 fragments and *hrpL* promoter were synthesised by GENEART and have been designed following the BioBrick standard (http://biobricks.org), i.e., eliminating the four restriction sites (EcoRI, XbaI, SpeI and PstI) exclusive for this standard by synonymous codon exchange and flanking with prefix and suffix sequences containing the appropriate restriction sites and RBS. The constitutive promoters J23101 and J23115 (http://partsregistry.org) were used to drive gene expression continuously as indicated. The double terminator B0015 was used to terminate gene transcription in all cases. The copper responsive P_{cusC}, zinc sensitive P_{zraP} and cadmium responsive P_{zntA} promoters