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Bacterial signaling systems as platforms for rational design of new generations of biosensors

Susana K Checa[1], Matias D Zurbriggen[2] and Fernando C Soncini[1]

Bacterial signal-responsive regulatory circuits have been employed as platform to design and construct whole-cell bacterial biosensors for reporting toxicity. A new generation of biosensors with improved performance and a wide application range has emerged after the application of synthetic biology concepts to biosensor design. Site-directed mutagenesis, directed evolution and domain swapping were applied to upgrade signal detection or to create novel sensor modules. Rewiring of the genetic circuits allows improving the determinations and reduces the heterogeneity of the response between individual reporter cells. Moreover, the assembly of natural or engineered modules to biosensor platforms provides innovative outputs, expanding the range of application of these devises, from monitoring toxics and bioremediation to killing targeted cells.

Addresses
[1] Instituto de Biología Molecular y Celular de Rosario, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Consejo Nacional de Investigaciones Científicas y Técnicas. Suipacha 590, S2002LRK Rosario, Argentina
[2] Faculty of Biology and BIOSS Centre for Biological Signalling Studies, Schänzlestrasse 18, 79104 Freiburg, Germany

Corresponding author: Checa, Susana K (checa@ibr.gov.ar)

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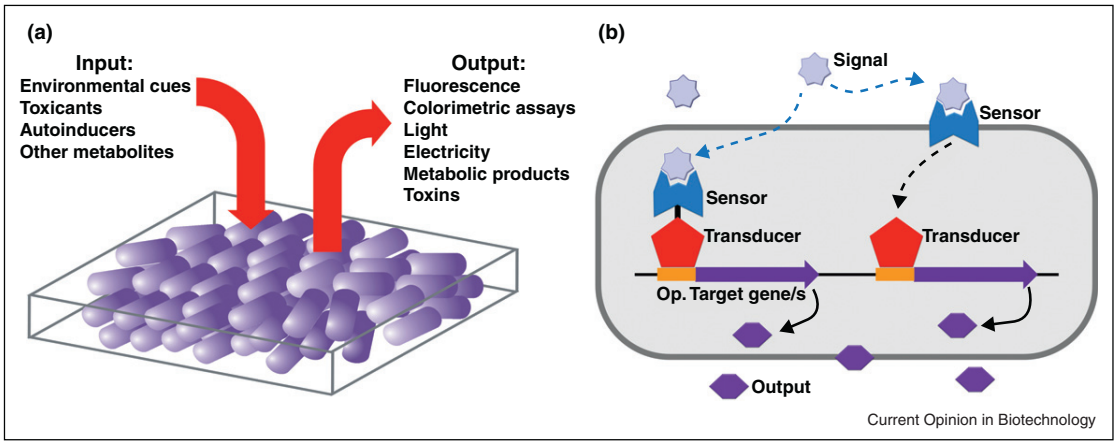
Introduction

Bacteria are constantly monitoring their surroundings to rapidly detect an environmental perturbation. Survival in any given condition depends on the coordinated action of a number of transcriptional, post-transcriptional and post-translational regulatory circuits that sense diverse, sometimes transient, environmental cues and mount the appropriate response by modulating the expression or activity of specific effectors. In sensing circuits that modulate the output at the transcriptional level, the external perturbation affects gene-promoters activities by causing a conformational change in a regulatory factor. This allosteric modification activates or inhibits its binding to the operator sequence or distorts the promoter structure [1,2] resulting in induction or repression of gene expression. The structural and functional characterization of different bacterial sensory/response circuits facilitates their engineering to carry out specific tasks. In the simplest case, bacteria are equipped with a sensory device to couple the detection of a physical or chemical perturbation (the signal) to the production of a quantifiable output reporter, usually an enzyme like β-galactosidase or luciferase, or a fluorescent protein [3,4]. From the pioneer work of King *et al.* in the early 1990s reporting the first bacterial biosensor for naphthalene [5], an increasing number of bioreporters have been designed to monitor a wide range of environmental toxicants, for example, heavy metals, benzene derivates, polycyclic aromatic hydrocarbons, among others. These devices are emerging as easy, rapid and cheap alternatives to analytical techniques owing to their capability to detect only the bioavailable fraction of a given signal, providing a more realistic estimation of its actual impact on the ecosystem. Besides, bacterial reporter platforms offer the possibility of performing online determinations in a dose-responsive manner, and in virtue of their nature, are ideal candidates for developing miniaturized assays as well as for designing portable instruments [4,6].

In the last decade, the application of synthetic biology concepts to biosensor design highly improved the performance and broadened the range of application of these devices [7–10]. Synthetic biology is a new area of biological research and technology applying basic engineering principles like modularization, rational design and modeling to the construction of complex biological networks with desired properties and functionalities. The approach involves the design and generation of new biological parts from natural existing components, that is, the building blocks necessary for the construction of such higher order systems, including genetic circuits, synthetic metabolic pathways and signaling systems [11–13]. This conceptual and experimental approach is applicable to the re-wiring of existing systems to perform functions novel in nature or even to generate fully synthetic networks [14]. Bacterial bioreporter platforms consist basically of three modules (Figure 1): the sensitive module that recognizes the input signal, the transducer that transmits the detected signal to the reporter module, which in turn provides the output. This modularity allows for a successful application of a synthetic biology strategy towards the development of novel gene networks able to respond to different signals, and with customised read-outs and output functionalities [15]. Briefly, each of these modules can be manipulated at the molecular level to

Figure 1



Whole-cell bacterial biosensor platforms. **(a)** Bacteria have been engineered to couple the detection of a physical or chemical perturbation to the production of a quantifiable output reporter. Examples of input signals and outputs discussed in the review are shown. **(b)** Representation of the two bacterial signal-transduction pathways commonly used to design regulatory-based biosensor platforms. One-component signal-transduction pathways (left) integrate the sensing and DNA-binding function in a single protein, the transcription factor. This protein detects the signal at the cytoplasm and allosterically modifies the DNA-binding domain to activate or represses the expression of the target genes that generate the response (output). Two-component signal-transduction systems (right) frequently involve a membrane-bound sensor histidine kinase that detects the signal at the bacterial envelope and transduces the information by phosphorylation to the cognate transcriptional regulator. This in turn, regulates the expression of the target genes. The signal-binding protein/domain is indicated in blue and the DNA-binding domain or the cognate transcription factor that acts as transducer is shown in red. The target operator/genes are depicted in orange and purple, respectively.

conduct quicker, more sensitive and selective assays. In addition, signal detection can be artificially coupled to a variety of naturally available or engineered gene networks to generate novel outputs for a wider range of biotechnological applications. The main difficulty in assembling different genetic parts can arise from unintended interference among native and synthetic modules, that is, a key to the generation of robust systems is the design of orthogonal parts [16]. Therefore, it is useful to include in the modular strategy preliminary experimental layouts that would allow verifying the correct design and functioning of the different modules separately previous to their assembly into the final arrangement.

In this review, we describe recent advances in biosensor technology improvement by applying synthetic biology approaches. Post-transcriptional (involving modification of the availability, structure or the stability of mRNA molecules) and post-translational (chemical modification of proteins) biosensor platforms were covered in recent reviews [7,8]. Here, we focus on how the different genetic parts or modules in biosensors based on transcriptional circuits can be tailored to improve signal-recognition and detection thresholds, as well as to include novel outputs that could broaden the range of application of biosensing technologies in different fields.

The signal-detection module

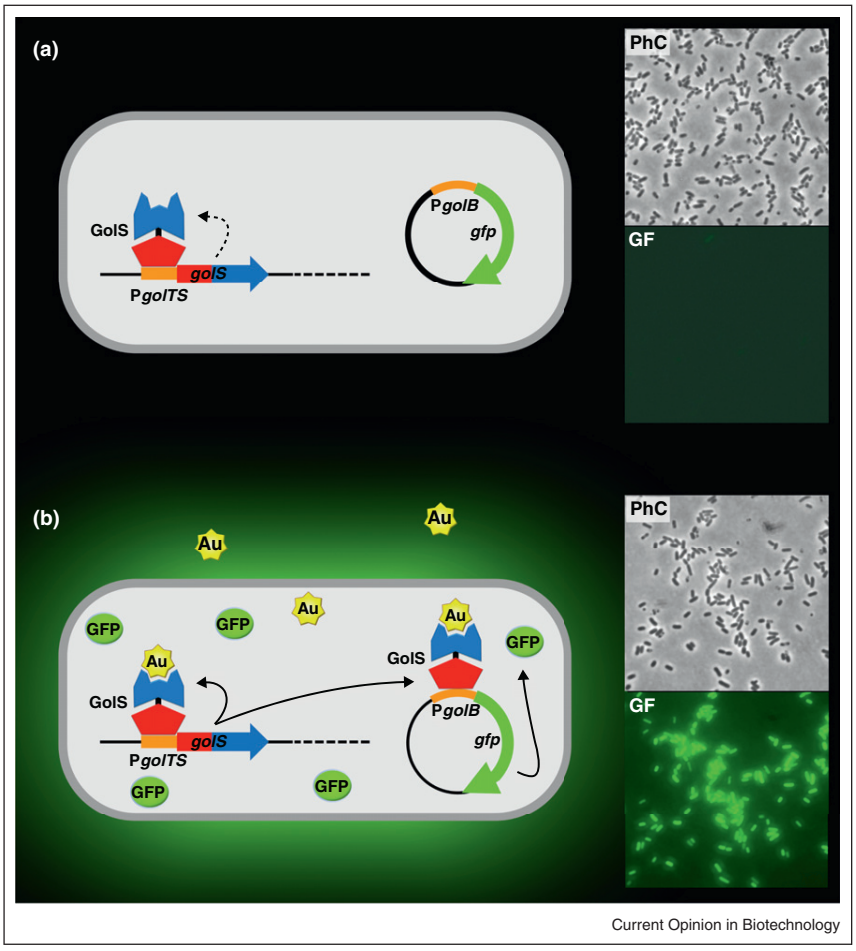
The sensor module is the main responsible for determining selectivity (the ability of detecting a specific cue) and sensitivity (the minimal level of a specific cue that generates a significant signal) in the bioreporter device [4,8]. In biosensors tailored to monitor environmental stimuli, this module usually derives from bacterial sensory proteins that induce or repress natural stress responses or degradation pathways (for toxic compounds such as naphthalene, toluene or phenol) [3,4]. The main disadvantage of these devices is that they usually lack the required selectivity. Thus, for some applications a major challenge has been the development of selective sensory modules for more selective determinations. The increasing number of characterized signal transduction pathways in the bacterial and archaeal kingdoms is providing an amazingly growing repertoire of components for the generation of new biological parts amenable to the modular design and construction of novel and improved biosensors.

Regulators that respond to heavy metal ions and metalloids are widely used in the development of biosensing tools. Most of them are based on two families of metalloregulators, MerR and SmtB/ArsR. These transcription factors integrate the sensing and DNA-binding domains in a single protein, which upon recognition of the signal in the cytoplasm modulates the transcription of its target genes [3]. Most characterized metal-responsive MerR proteins respond to different metal ions with the same charge and similar coordination chemistry [2,17]. For example, the monovalent metal sensor CueR recognizes copper (Cu(I)), silver (Ag(I)) or gold (Au(I)) ions with

similar affinity; and the same occurs with ZntR that responds to zinc (Zn(II)), cadmium (Cd(II)) or lead (Pb(II)) [18,19]. On the contrary, the mercury (Hg(II)) sensor MerR from the Tn21 transposon, the archetype of this family, shows a high selectivity for Hg(II) and in fact has been used in the design of efficient Hg-biosensors [20–23]. The *Salmonella enterica* GolS sensor, another member of this family, is highly selective for Au(I) ions. In spite of its similarities with CueR, GolS is not responsive to Cu(I) or Ag(I) [24]. Based on this property, we used the *Salmonella* gold-sensor to generate fluorescent bioreporters in both the native host as well as in *Escherichia coli* (Figure 2 and Table 1). These devices exhibit minimal interference by chemically related metals such as Cu(I) or Ag(I) and detect the precious metal in a nanomolar range [25•], values that are well below the concentrations typically found nearby gold-ores [26]. The presence of a GolS-homolog CupR and its Au-inducible cluster in *Cupriavidus metallidurans*, a multiresistant β-proteobacterium found to form biofilms on Au grains [27,28], is promising for the design of inexpensive, yet effective biosensors for the evaluation of gold placers, alternative to the currently used analytical tests.

Both site-directed mutagenesis and motif swapping were employed to change metal preference on a number of transcriptional regulators, including MerR metal sensors, allowing for the generation of biosensors sharing some structural/functional modules but with diverse specificities. For instance, modification of the very C-terminus of the *E. coli* CueR sensor led to a mutant protein that do not respond to Ag(I), shows an increased sensitivity to Au(I),

Figure 2



The Au-biosensor. This regulatory circuit is based on the *Salmonella* Au-sensor GolS and its target genes [24,25•]. The gene encoding the transcriptional regulator (*golS*) under the control of its native GolS-regulated promoter (P*golTS*) was introduced into the *E. coli* chromosome. The reporter gene (*gfp*) under the control of the GolS-controlled promoter (P*golB*) is plasmid encoded. **(a)** In the absence of metal, bacterial cells express minimal amounts of GolS. No fluorescence is detected either by fluorometry or by fluorescence microscopy (right). **(b)** In the presence of Au ions, GolS activates its own expression and induces transcription of the reporter gene. The cellular accumulated GFP can be detected by fluorescence microscopy (right) and quantified by fluorometry. PhC, phase contrast microscopy; GF, green fluorescence.

Table 1

Selected engineered biosensors

Input	Sensor module	Transducer module	Output	Design strategy	Ref.
Au(I)	GolS	GolS/P*golB*::*gfp*	GFP	Sensor/transducer modules from *S. enterica* coupled to reporter	[25•]
Cd(II)	MerR	MerR/P*mer*::*lucFF*	Firefly luciferase	Directed evolution on the sensor module	[29••]
Cd(II)	CadR	CadR/P*cadR*::*lacI*^{q}-*gfp* LacI^{q}/P*tac*::*cadR*	GFP	Toggle switch design of the gene circuit	[36•]
As(III)-AHL[a]-H_2O_2	ArsR-LuxR-ArcAB	ArsR/P*arsR*::*luxI or* P*arsR*::*luxR* LuxR/P*luxI*::*luxI* LuxR/P*luxI*::*aiiA* LuxR/P*luxI*::*sfgfp* ArcAB/P*luxI*::*luxI* ArcAB/P*luxI*::*aiiA* ArcAB/P*luxI*::*sfGFP*	sfGFP[b]	Oscillatory switch design of the gene circuit	[38••]
BTEX[c]	XylR	XylR/P_u::*phzMS*	pyocyanin	Novel output-redox active compound	[42••]
DNT[d]/salycilate	DntR	DntR/P_{DNT}::*phzMS*			
AHL	LasR	LasR/P*luxR*::S5 LasR/P*luxR*::E7	pyocin	Novel output-bacteriocin	[50••]

[a] AHL, acyl-homoserine lactone.
[b] sfGFP, super folder variant of GFP.
[c] BTEX, benzene, toluene, ethylbenzene and xylene.
[d] DNT, dinitrotoluene.

but still wild-type responsiveness to Cu(I) [19]. Switched metal responsiveness was achieved with chimaeric CueR and GolS proteins by surgical exchange of their metal binding loops ([24] and MM Ibañez *et al.*, unpublished). In addition, the metal selectivity of the Hg(II) sensor MerR was modified by introducing random mutations into key metal-binding regions [29••]. The evolved Cd(II) selective sensors generated using this approach were successfully employed in the construction of efficient whole-cell biosensors (Table 1).

Domain swapping has also been used to improve the design and construction of environmental biosensors based on two component regulatory systems [15,30]. These sensor/response systems are composed by a histidine kinase that detect the signal (light, pH, temperature, etc.) and phosphorylates a cognate response regulator, which activates or represses transcription of its target genes [31].

The transducer module

The interaction of the signal with the sensor module affects the ability of the DNA-binding module (or the cognate transcriptional regulator) to promote or repress transcription of the reporter module (Figure 1). The efficiency of the regulator/promoter interaction, the intrinsic promoter strength and the presence of competing regulators will influence biosensor performance [4,8]. For example, the cellular concentration of the Au-sensor GolS must be precisely controlled to avoid undesirable interference with Cu homeostasis that depends on the paralog CueR regulator, as GolS can also interact with CueR target operators [25•,32]. Re-engineering the existing promoter by introducing, removing or modifying activator or repressor sites, can also contribute to tune the promoter's sensitivity to a signal. Promoter rewiring was applied to arsenic-responsive bioreporters controlled by the transcriptional repressor ArsR, an archetype of the SmtB/ArsR family, which is autoregulated [33]. Expression of ArsR from an inducible promoter, introduction of additional copies of the ArsR-regulated promoter upstream of the reporter gene or modification of the ribosome-binding site proved to be very effective in improving sensitivity of As-biosensors [33–35].

The performance of a Cd(II) whole-cell biosensor was tailored to display a faster response to increasing Cd concentrations and lower background comparing to previously designed biosensors by applying the basic toggle switch concept to gene circuit design [36•]. In this circuit (Table 1), the expression of the reporter protein was double controlled by imposing an artificial transcriptional control to the Cd-responsive MerR-type regulator gene (CadR) [37]. The *cadR* gene was transcribed from a *tac* promoter induced by isopropyl-β-D-thiogalactopyranoside and negatively regulated by the LacI^{q} repressor expressed from a divergent CadR-dependent *cadR* promoter, which also controls GFP production [36•]. Thus, in the presence of Cd(II), CadR induced the expression of LacI and GFP. LacI represses further expression of the sensor protein while GFP reports the presence of metal.

Recently, synchronized oscillating biosensors were constructed in order to reduce the heterogeneity of the response between individual reporter cells within a

population after sensing the input signal [7]. These frequency-modulated bacteria express a reporter fluorescent protein in unison in response to the signal yielding synchronized pulses of light that were detected in a liquid crystal display (LCD)-like macroscopic clock [38••]. In this design, a two-level regulatory gene expression control was employed to synchronize the reporter expression among cells over long distances (Table 1). The first level uses a quorum-sensing system, which upon detection of acyl-homoserine lactone (AHL) activates and synchronizes GFP expression within an individual colony [39]. The second level involves a gas-phase diffusible signal, that is, H_2O_2, generated upon exposure of GFP-containing cells to bursts of high-intensity blue light [40]. This reactive oxygen species inactivates ArcAB, the native aerobic response control system [41], which relieves repression from an engineered target site at the AHL-responsive promoter region, generating a positive feedback that rapidly synchronizes the bacterial population. The basic platform was set-up to sense arsenic, rewiring the regulatory network to produce LuxR, the AHL-sensor (or the AHL-synthase, LuxI) from an arsenite-responsive ArsR-controlled promoter [38••]. The response to the presence of arsenic is visualized as an alteration in the rate at which they produce synchronized pulses of fluorescence.

The output module

Besides the used of classical reporter proteins (i.e. GFP or luciferase), bacterial sensing/response pathways were engineering in *E. coli* to couple the detection of a harmful pollutant with an electrochemical output [42••]. Regulatory circuits that respond to BTEX chemicals (benzene, toluene, ethylbenzene and xylene), or to the industrial pollutant dinitrotoluene or salycilate, were engineered to induce the production of pyocyanin, a redox-active substrate [43–45] (Table 1). The authors proposed that this product could be converted directly into electricity in an associated microbial fuel cell (a device in which microorganisms oxidize compounds and transfer the electrons to an electrode) [42••]. This technology that allows performing continuous monitoring of these pollutants could be the first step in the development of a new generation of self-powered biosensors.

Native as well as engineered gene networks comprising innovative outputs have been embedded into environment-responsive regulatory circuits to broaden the application range of biosensor devices [4,9,10,46]. For example, cells can be programmed to integrate multiple environmental signals into a single genetic circuit, communicating the state of a specific microenvironment to activate a natural behavior, like biofilm formation, or to commit cell suicide after detecting a deleterious input signal [47–49]. These devices can also be programmed to synthesize a toxic protein to control cell population or to kill other cells [8,9,47]. For instance, Saeidi *et al.* [50••] developed a synthetic genetic system that enables *E. coli* to sense and eradicate pathogenic *Pseudomonas aeruginosa*. In this design, the narrow-spectrum bacteriocin pyocin [51] and the pyocin-releasing system [52] were cloned under the control of an AHL-responding promoter (Table 1). Therefore, when the quorum sensing signal produced by *P. aeruginosa* is detected by the AHL-sensor in the *E. coli* cells, the accumulated pyocin is released, diffusing towards the target pathogen to kill it [50••]. Given the stalled development of new antibiotics and the increasing emergence of multidrug resistant pathogens, this novel design may constitute a twist in the future development of strategies against infectious diseases. Moreover, diverse genetic circuits could be designed to perform a temporal control of gene expression by timing the supply of an extracellular signal or to balance metabolism, thereby increasing product biosynthesis [10,30]. These sensor bacteria can be particularly useful in metabolic engineering, for example to coordinate the metabolic activities of different strains when more than one microbial strain is needed to produce a desired compound.

Bacterial signal transduction pathways were also used in the development of synthetic reporters for the detection of environmental contaminants by plants. Taking advantage of the conservation of two-component signal/transduction pathways between bacteria and plants, Antunes *et al.* [53••] used re-designed periplasmic binding proteins normally involved in bacterial chemotaxis, to produce specific pollutant sensors coupled to the *E. coli* PhoR/PhoB system to function in *Arabidopsis thaliana* and *Nicotiana tabacum* plants. Transgenic plants expressing the fully synthetic signal transduction pathway are able to link the detection of contaminants to a phenotype that can be conveniently monitored. This basic modular platform can be further re-designed to include different sensing capabilities and outputs that contribute to extend the range of applications of these biodetectors.

Conclusions

Advances in understanding and manipulating bacterial signal transduction pathways, combining transcriptional, post-transcriptional and post-translational modulation of gene expression, offer new opportunities for the application of synthetic biology approaches and tools to the design of new whole-cell biosensors. The improvement of existing sensors by fine-tuning signal recognition or the generation of new sensors with specificity for natural or synthetic chemicals by performing domain-swapping or directed evolution will upgrade determinations. Also, the possibility of introducing innovative outputs and to apply microengineering to biosensor design, in addition to rewiring bacterial signaling systems to function in other organisms may also widen the use of bacterial reporter assays in online and *in situ* environmental monitoring and remediation as well as in controlling the state of a specific

microenvironment during an industrial process. New applications, such as in chemotherapy and biopharmacy are expected to expand by the use of new generations of biosensory devises.

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