REVIEW

Engineered cells as biosensing systems in biomedical analysis

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Abstract Over the past two decades there have been great
advances in biotechnology, including use of nucleic acids,
proteins, and whole cells to develop a variety of molecular
analytical tools for diagnostic, screening, and pharmaceuti-
cal applications. Through manipulation of bacterial plas-
mids and genomes, bacterial whole-cell sensing systems
have been engineered that can serve as novel methods for
analyte detection and characterization, and as more efficient
and cost-effective alternatives to traditional analytical tech-
niques. Bacterial cell-based sensing systems are typically
sensitive, specific and selective, rapid, easy to use, low-cost,
and amenable to multiplexing, high-throughput, and minia-
turization for incorporation into portable devices. This crit-
ical review is intended to provide an overview of available
bacterial whole-cell sensing systems for assessment of a
variety of clinically relevant analytes. Specifically, we ex-
amine whole-cell sensing systems for detection of bacterial
quorum sensing molecules, organic and inorganic toxic
compounds, and drugs, and for screening of antibacterial
compounds for identification of their mechanisms of action.
Methods used in the design and development of whole-cell
sensing systems are also reviewed.

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Introduction

The need of living organisms to recognize and respond to
changes in their environment exemplifies biosensing in na-
ture. Biosensing involves selective and sensitive molecular
recognition between proteins and a target ligand analyte, even
when present at very low concentrations. Researchers have
tried to mimic the exquisite properties found in nature by
utilizing naturally occurring recognition elements to develop
biosensing systems for analytical applications. To that end, a
biological recognition element capable of reversible binding
to a target ligand analyte is coupled to a transducer element
that converts the recognition event into a readable/measurable
output. Several biological recognition elements, including
proteins, nucleic acids, and intact cells, have been used as
sensing elements in biosensors for applications in environ-
mental, biological, pharmaceutical, and clinical analysis [1, 2].

Proteins with high specificity for analytes of interest have
been extensively used in the development of biosensors.
These proteins include enzymes, antibodies, and binding
proteins, among others. For instance, glucose oxidase cata-
lyzes oxidation of β-D-glucose to D-glucono-1,5-lactone,
which is further hydrolyzed to gluconic acid [3]. A range
of commercially available biosensors for blood glucose
monitoring and diabetes management use glucose oxidase
as the sensing element coupled to appropriate mediators and
transducers [4]. Antibodies have exquisite specificity for
their cognate antigens. In an immunosensor [5], antibodies
are used as recognition elements coupled with a variety of

detection methods, including, electrochemical, piezoelectric, fluorescence, bioluminescence, absorbance, and surface plasmon resonance; labeled secondary antibodies can also be used in a different format for detection of target analytes. A variety of sensing systems have also been developed by using hinge-motion binding proteins (HMBPs), for example periplasmic binding proteins and the messenger protein calmodulin, with an incorporated signal-generating reporter molecule [6]. In general, these proteins have extraordinary selectivity to their corresponding ligand and/or analyte, with affinities, K_D, typically in the sub-micromolar range—in some cases as low as in the nanomolar range—and undergo conformational changes upon binding to their ligands. As representative examples of periplasmic binding proteins [7], the K_D of the sulfate-binding protein is 10 nmol L^{-1} whereas that of the glucose-binding protein is 20 nmol L^{-1}. Specifically, upon ligand binding, two protein domains bend around a "hinge" region of the protein. Such conformational change can be used to quantify a target analyte and/or ligand by measuring the change in signal intensity of the transduction molecule, which can either be an environmentally sensitive fluorescent probe strategically conjugated to the protein or a reporter protein genetically fused to the HMBP. The advantages of this type of protein-based biosensor are their high specificity toward their ligand analyte, thus resulting in high selectivity, low limits of detection, rapid response times, and amenability to incorporation into various analytical devices. Potential disadvantages of protein-based biosensors can be their storage conditions, transport, and shelf life. In most cases, protein biosensors must be stored and transported refrigerated, which limits their utility and compromises their shelf life when used at room temperature.

Intact cells, including bacterial, yeast, and mammalian cells, are used as sensing elements in biosensing systems. Bacterial cell-based biosensing systems use genetically engineered bacteria capable of generating a signal on selective recognition of the analyte or class of analytes of interest. The ability to produce dose-dependent detectable signals in response to the analyte, as described in the next section, enables selective determination of the bioavailable analyte or class of analytes present in a given sample. Cell-based sensing systems are relatively easy and inexpensive to prepare and store, and are robust: they tend to be stable to environmental changes, for example variations in temperature or pH. In addition, these sensing systems can provide physiologically relevant data and evaluate the bioavailability of the analyte of interest, because the target chemical must be ingested by the cells to trigger a response. Moreover, by using different recognition element–reporter protein pairs, multiple analytes can be detected simultaneously in a sample. Cell-based biosensing systems have high-throughput features because they are amenable to miniaturization and incorporation into high-density analytical devices, thus enabling assay of large numbers of samples in a single analytical run. This is a distinct advantage

over conventional physicochemical analytical methods. However, a whole-cell biosensing system is not without limitations. In whole-cell biosensing systems, analytes must enter the bacteria by diffusion, which may, depending on the rate of diffusion, result in a slow sensor response. Additionally, because the cell biochemical machinery must be activated to produce the reporter protein, the response of a whole-cell sensing system is slow compared with that of protein-based biosensors, which is of the order of seconds or minutes. Additional drawbacks include potential interference with the sensor's response by components of the bacterial cell and high background signal when fluorescent proteins are used as reporters, because of the presence of fluorescent molecules in the cell. Cell batch-to-batch variability, which is intrinsic to living organisms, is a further aspect that may have to be taken into consideration [8].

In this critical review, we discuss the design and development of genetically engineered bacterial cell-based biosensing systems and focus on their applications in clinical analysis. Specifically, we describe whole-cell sensing systems for detection of clinically relevant analytes, including, bacterial quorum sensing molecules, inorganic ions, toxic organic compounds, for example hydroxylated polychlorinated biphenyls, mercury ions, methylmercury, and drugs, for example antibiotics and the chemotherapeutic agent, cytarabine. We also describe the use of whole-cell sensing systems for screening of antibacterial compounds.

Bacterial whole-cell-based biosensing systems

Bacterial biosensing systems can be categorized into two different types, depending on the mode of expression of the reporter protein [9]. Expression of the reporter can either be constitutive or inducible. In constitutive expression systems, the reporter is expressed at high basal levels. An increase in the amount of compounds that are toxic to the cell causes its death thus reducing the reporter protein expressed and its generation of signal. Whole-cell biosensing systems based on constitutive expression have been used to measure the general toxicity of a sample or test compound. A well-known example is Microtox toxicity testing [10], a standardized, commercially available toxicity testing system that uses the spontaneously bioluminescent marine bacteria *Vibrio fischeri* as bacterial sensor for detection of toxic compounds in water samples. When *V. fischeri* in the test kit is exposed to a sample containing toxic compounds, a dose-dependent reduction in bioluminescence is observed, indicating the toxicity level of the sample.

The second class of whole-cell bacterial biosensing systems comprises inducible expression systems in which the cells are genetically engineered to contain a plasmid in which an inducible promoter fused to a reporter gene controls its

expression [9]. In the absence of analyte/inducer, the reporter gene is expressed at very low basal levels, while in the presence of analyte/inducer it is expressed in a dose-dependent manner. Inducible expression systems can be further classified as stress inducible or chemically inducible, depending on the mechanism of activation of the response. In stress inducible cell-based biosensing systems, the reporter gene is placed under the control of a promoter that is activated by stressful conditions, for example heat shock and osmotic stress. Several structurally unrelated compounds can activate these response mechanisms; therefore, such sensing systems are not specific to target compounds and are defined as semi-specific. On the other hand, chemically inducible cell-based systems harbor a plasmid that contains a specific promoter and the genes for regulatory and reporter proteins. The presence of an analyte or class of analytes activates the promoter, triggering the expression of the regulatory and the reporter proteins in a specific manner. The mechanism by which this occurs involves binding of the analyte to the recognition/regulatory protein, which then undergoes a change in conformation, subsequently activating expression of the reporter gene. Reporter gene expression can be negatively or positively regulated. In negative regulation (Fig. 1a), the regulatory protein is bound to the operator/promoter region of an operon and inhibits expression of downstream genes, including the reporter gene. When the analyte is present, it binds to the regulatory protein, which is then removed from the operator/promoter region, thus enabling expression of the reporter gene. In positive regulation (Fig. 1b), the analyte first binds to the regulatory protein and the complex then binds to the operator/promoter region, triggering expression of the reporter gene.

The reporters used in whole-cell sensing systems are typically proteins that can be detected by optical, i.e., colorimetry, fluorescence, bioluminescence, and chemiluminescence, or electrochemical methods. Although the recognition component is important in determining the selectivity, the reporter is crucial in determining the sensitivity of the bacterial sensor. A wide variety of reporter genes have been used in several applications, including gene expression, gene transfer, and cell signaling. The reporter proteins encoded by such genes have also been used as signal-transduction elements in bacterial sensors. These proteins include β-galactosidase [11, 12], bacterial luciferase [13], firefly luciferase [14], and the green fluorescent protein and its variants. Table 1 lists reporters that are commonly used in whole-cell sensing systems, with their catalyzed reactions and methods of detection.

Bacterial quorum sensing molecules

Bacteria, although single-cell organisms, have complex behavior. One such behavior relates to the ability of bacterial cells to communicate with their neighboring cells. This
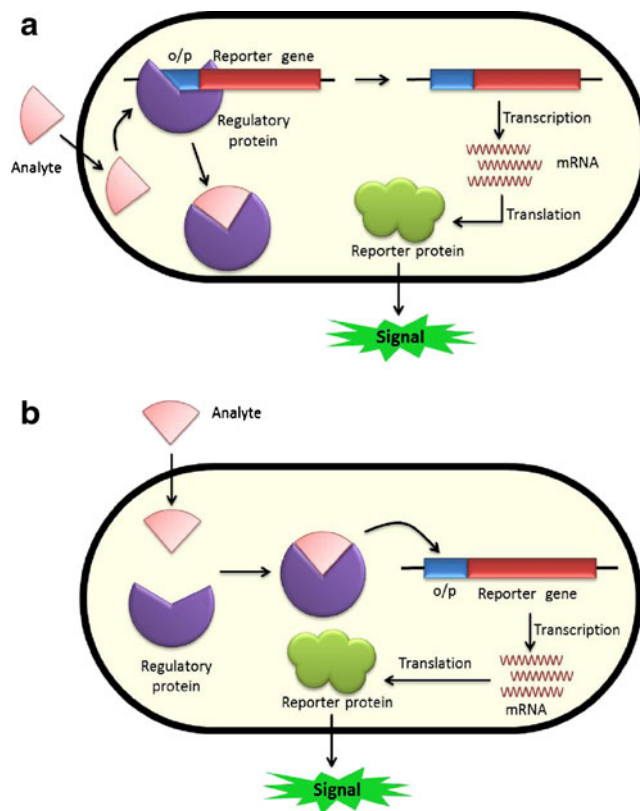

Fig. 1 Chemically inducible whole-cell biosensing systems. (a) Negative regulation of reporter gene expression. (b) Positive regulation of reporter gene expression. Adapted from Gu et al. [9]

phenomenon was first reported in 1965 by Tomasz [15] who described "hormone like activators" that were crucial in the propagation of the Gram-positive bacterium *Streptococcus pneumoniae*, which is one of the organisms responsible for pneumonia pathogenicity in their hosts. Later, in 1970, Hastings et al. observed that the Gram-negative marine bacterium *Photobacterium fischeri* produces bioluminescence only when bacteria reach a certain cellular density in terms of number of cells present, and speculated that light emission was under a bacterial "control mechanism". It is now well understood that bacteria communicate with each other by producing and sensing small chemical molecules in a process termed as quorum sensing (QS). This phenomenon has been observed in a number of bacteria [16], and it is now understood that by means of QS, bacteria are able to control several processes, for example bioluminescence, virulence factor production, biofilm formation, antibiotic production, competence, nodulation, sporulation, clumping, and motility. The small chemical molecules that bacteria utilize for QS are known as quorum sensing molecules (QSMs). By detecting the QSMs, bacteria can modulate the above mentioned processes in a cell density-dependent manner. For example, in a bacterial infection, bacteria do not produce virulence factors until they reach a critical population density and, by doing so,

Table 1 Reporter proteins commonly used in whole-cell sensing systems [8]

| Reporter protein	Catalyzed reaction	Detection method
β-Galactosidase	Hydrolysis of β-galactosides	Chemiluminescence, bioluminescence, fluorescence, colorimetry, electrochemistry
Bacterial luciferase	$FMNH_2 + R\text{-}CHO + O_2 \rightarrow FMN + H_2O + RCOOH + h\nu$ (490 nm)	Bioluminescence
Green fluorescent protein	Post-translational formation of the internal chromophore p-hydroxybenzylideneimidazolinone	Fluorescence
Firefly luciferase	Firefly luciferin $+ O_2 + ATP \rightarrow$ oxyluciferin $+ AMP + P_i + h\nu$ (550–575 nm)	Bioluminescence

they ensure that they can overwhelm the host's immune response [17].

Several groups of QSMs have been identified, which include *N*-acyl homoserine lactones (AHLs) in Gram-negative bacteria and a class of autoinducing peptides (AIPs) in Gram-positive bacteria. Whereas AHLs and AIPs are species-specific and, therefore, used for intra-species communication, a third category of molecules, autoinducer-2 (AI-2), has been found in both Gram-positive and Gram-negative bacteria, suggesting a potential role in inter-species communication within bacteria

[18]. Table 2 lists some of these signaling molecules and the bacteria that use them in cell-to-cell communication. Furthermore, some bacteria have multiple QS circuits and use more than one kind of QSM; for example, the marine bacterium *Vibrio harveyi* uses *N*-(3-hydroxybutanoyl)-L-homoserine lactone, and a furanosyl borate diester form of AI-2 to control bioluminescence [19]. Recently, Sperandio et al. [20] have discovered an additional QSM, autoinducer-3, AI-3 (unknown structure), which binds to the membrane protein QseC and activates virulence in enterohemorrhagic *Escherichia coli*

Table 2 Quorum sensing molecules utilized by various bacteria. (**a**) *N*-Acyl homoserine lactones used by Gram-negative bacteria. (**b**) Autoinducer-2: borate diester form used by *V. harveyi* and hydrated form, not containing boron, used by *S. typhimurium*. (**c**) Linear and cyclic peptides used by Gram-positive bacteria. The underlined tryptophan residue (W) in the ComX pheromone indicates isoprenylation
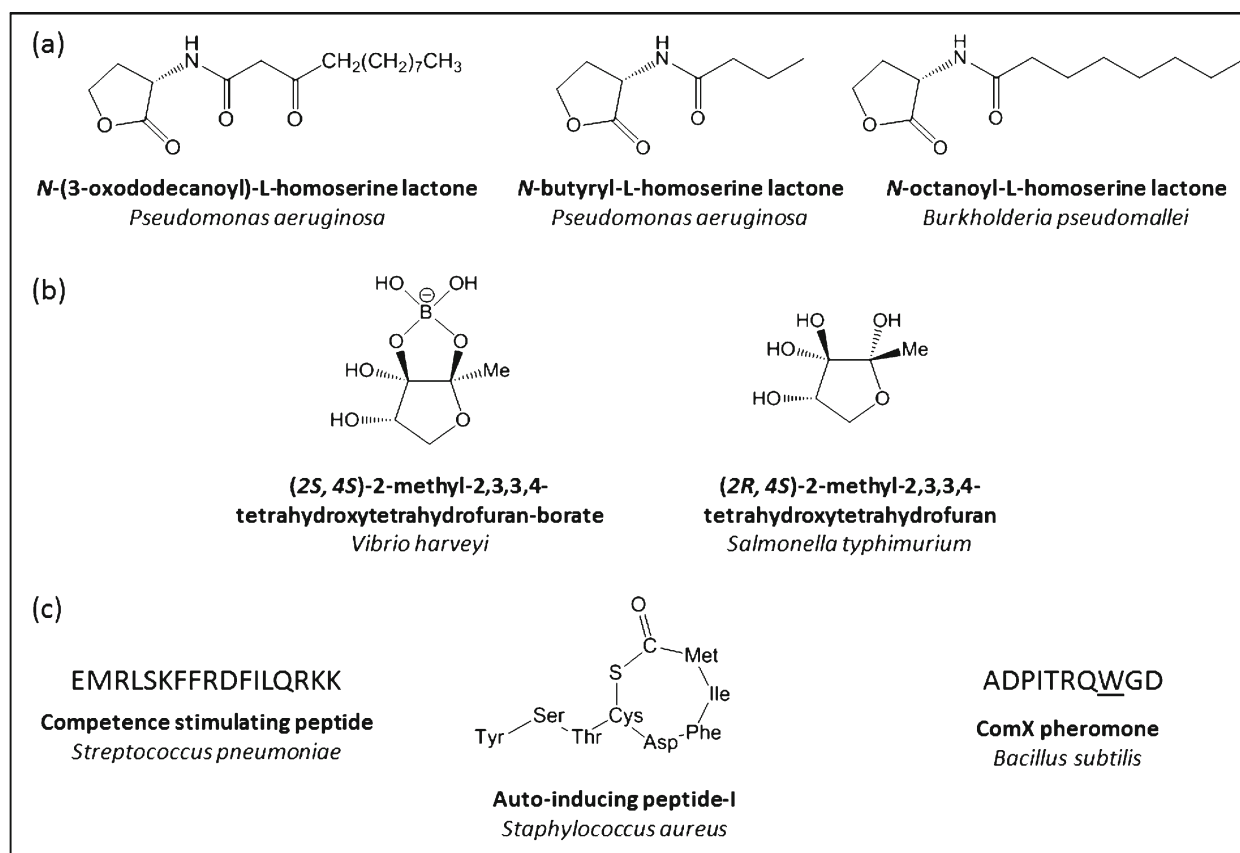
(EHEC). Further, it has been shown that the mammalian hormones, epinephrine and norepinephrine, are recognized by the same protein QseC and activate virulence in EHEC [21, 22], suggesting involvement of the AI-3 QS system in inter-kingdom bacterial–mammalian cells communication.

Numerous in vitro and in vivo studies have demonstrated that expression of bacterial virulence factors responsible for infections in mammalian hosts is regulated by QS. Several such studies concerning gastrointestinal (GI) tract infections have been discussed in a recent review by Kaper and Sperandio [23]. For example, in the case of EHEC, which causes bloody diarrhea and hemolytic-uremic syndrome, involvement of AI-3 in regulating the expression of virulence factors was demonstrated in a HeLa cell infection model using wild-type EHEC and a LuxS mutant-LuxS was shown to be involved in the production of AI-3 [24]. In an animal study [25], burn wounds of mice models were infected with wild-type *Pseudomonas aeruginosa* and variants of the same bacterium carrying mutations in the genes encoding for AHL synthase. The objective of the study was to determine the efficiency of infection by assessing the ability of the bacteria to spread the infection and the time required for the onset of infection. It was observed that in mice infected with *P. aeruginosa* mutants unable to synthesize AHLs, the extent of infection was lower than in those infected with wild type bacterium. The evidence gathered is indicative of the relevance of bacterial communication in diseases of bacterial origin and, thus, the importance of detecting quorum sensing signaling molecules in physiological samples.

Identification of QS regulatory pathways in a number of bacteria [16] has enabled additional whole-cell-based biosensing systems to be engineered to detect QSMs by coupling the genes coding for different QSM recognition and/or regulatory proteins to those of a variety of reporter genes [26]. As an example, Winson et al. designed reporter plasmids by placing the gene cassette *luxCDABE* under the control of the P_{lasI} and P_{RhlI} promoters from the *P. aeruginosa* QS regulatory systems LasI/LasR and RhlI/RhlR, respectively. The plasmids also contained the sequences of the LasR and RhlR proteins, which, upon binding AHLs, bind to the respective promoters, thus activating expression of the reporter protein [27]. In another example, Bassler [28] et al. engineered a strain of *V. harveyi* in such a way that the bacterium's bioluminescence, which depends on expression of the *luxCDABE* gene cassette, was only triggered by AI-2, thus enabling detection of this particular quorum sensing molecule.

Most of the above cell-based systems have been used as bioassays to evaluate the ability of cultured bacteria to produce QSMs. However, only a few have been applied to the detection of QSMs in physiological and clinical samples in order to correlate the presence of pathogenic bacteria with the onset or status of disease. Two independent studies demonstrated the presence of AHLs in sputum samples from patients with cystic fibrosis (CF) [29, 30] by using whole-cell sensing systems. It is known that *P. aeruginosa* and *Burkholderia cepacia* colonize the airway passage and lungs in CF patients, leading to chronic lung infection and finally to destructive lung disease [31]. These two species of bacteria use AHL-dependent QS regulation. In the study conducted by Middleton [29] et al., sputum samples from CF patients colonized by either *P. aeruginosa* or *B. cepacia,* were extracted and then analyzed using an *E. coli* whole-cell-based biosensing system containing plasmids pSB401 and pSB1075 to detect short and long-chain AHLs, respectively. Among *P. aeruginosa*-colonized sputum samples, 71% showed the presence of short-chain AHLs and 61% showed presence of long-chain AHLs. Similarly, among *B. cepacia*-colonized sputum samples, 81% contained short-chain AHLs and 50% contained long-chain AHLs. This AHL production profile was different from that of laboratory cultures of the same microorganisms isolated from sputum samples, in which long-chain AHLs were predominant. The difference in AHL profile was thought to be explained by dissimilar growth conditions, in vitro and in the lung. In that regard, this varied behavior of *P. aeruginosa* from lung infection had previously been observed by Singh et al. [32], who hypothesized that *P. aeruginosa* exists predominantly as a biofilm in CF sputum. Further, LC–MS analysis of the same samples revealed the presence of short-chain AHLs, but not that of long-chain AHLs, indicating that the whole-cell-based biosensing system was more sensitive for detection of AHLs in sputum samples. Another study published the same year by Erickson et al. further corroborated the presence of AHLs in sputum samples from CF patients [30]. *P. aeruginosa*-based whole-cell biosensing systems containing plasmids pKDT17 (to detect long-chain AHLs) and pECP61.5 (to detect short-chain AHLs), both utilizing *lacZ* as a reporter, were used. Although the levels of AHLs detected were low, over 75% of the samples had long-chain AHLs whereas only 26% of the samples had short-chain AHLs. This finding is in contrast with the results of the study by Middleton et al. [29]. However, differences in the groups of patients, along with different sample extraction and/or processing methods and the use of sensing systems based on different microorganisms might explain different results. Along the same lines, Chambers et al. detected a broad range of AHLs in mucopurulent respiratory secretion samples obtained from CF patients [3]. They used *Agrobacterium tumefaciens*-based whole-cell biosensing system A136, containing plasmids pCF218 and pMV26 with *luxCDABE* as a reporter, allowing it to respond to both long and short-chain AHLs (4–12 carbon atoms). AHLs were extracted from the mucopurulent respiratory secretion samples, separated by reversed-phase fast pressure liquid chromatography (FPLC), and each fraction was then assayed with the *A. tumefaciens* A136 sensing system in 96-well microtiter plate format. Further, identities of the AHLs present in positive

fractions were confirmed by comparing their retention times with those of standard AHLs. Using the whole-cell sensing system combined with FPLC, the authors were able to detect low concentrations of AHLs in small volumes of samples from nine (out of thirteen) CF patients and to identify seven different AHLs.

Our research group used whole-cell biosensing systems to evaluate QSMs in physiological samples from individuals affected by bacterial gastrointestinal (GI) disorders, including inflammatory bowel disease (IBD). Two of the major conditions of IBD, ulcerative colitis (UC) and Crohn's disease (CD), involve chronic and relapsing acute inflammation in the large and small intestine, respectively, with CD being able to affect any portion of the GI tract. Current methods of diagnosis and monitoring rely on endoscopic techniques and analysis of mucosal tissue biopsies taken

from the inflamed regions. Alternative tools that can serve as non-invasive biomarkers for such diseases can prove very beneficial. To that end, we have developed and optimized *E. coli*-based bioluminescent whole-cell sensing systems containing plasmids pSB406 and pSB1075, which bear *luxCDABE* as a reporter, for detection of short and long-chain AHLs, respectively [27, 33]. These biosensing systems have then been used to evaluate AHLs in human samples, for example saliva and stool samples, which bear the advantage of being collected non-invasively. The effect of components of the sample matrix was evaluated by generating dose–response curves in spiked pooled samples after minimal processing (no sample extraction nor extensive preparation). Figure 2a shows dose–response curves obtained from buffer and pooled saliva. Importantly, nanomolar limits of detection were obtained in biological matrices, which is relevant
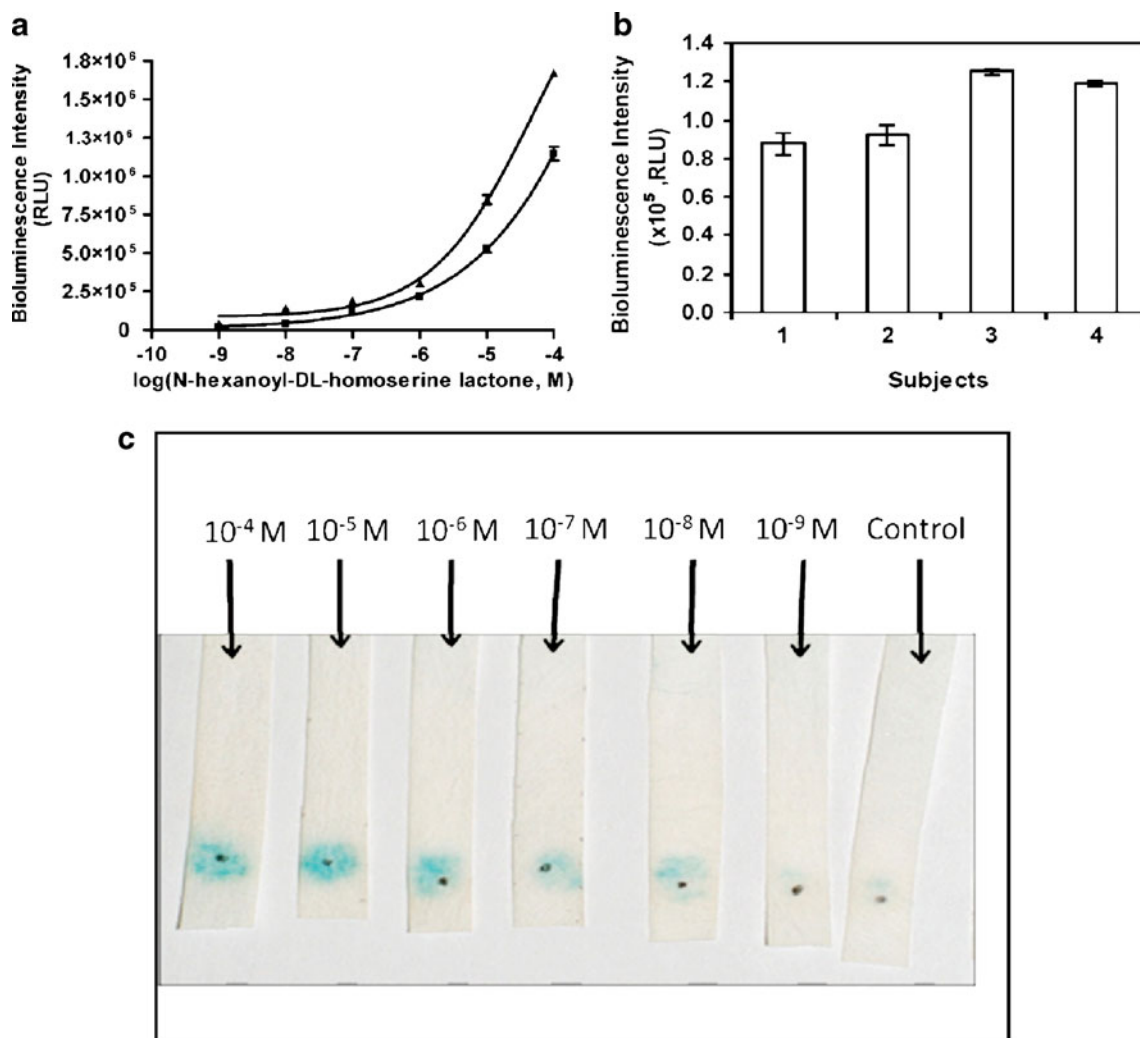

Fig. 2 (a) Evaluation of matrix effect in saliva using the biosensing system containing plasmid pSB406. Dose–response curve achieved by standard addition of analyte to pooled saliva samples from healthy volunteers (*triangles*) and reference dose–response curve (*squares*) obtained during the same analytical run. Reproduced with permission from Kumari et al. [33]. (b) Detection of AHLs in stool samples from infants admitted to NICU. Reproduced with permission from Kumari et al. [26]. (c) Whole-cell paper strips assay for detection of long-chain AHLs. Reproduced with permission from Struss et al. [35]

in that nanomolar concentrations of QSMs are necessary to initiate cell-to-cell communication [34]. Saliva samples from IBD and healthy individuals, and stool samples from newborns (Fig. 2b) admitted to a neonatal intensive care unit (NICU) were then assayed [26]. AHLs were detected at different levels in the specimens tested, showing for the first time the presence of these QSMs in such physiological samples. If a correlation is established between the QSM levels in samples and the health status of a patient, it may be possible to use QSMs as biomarkers of bacteria-related disorders, which should aid in the management of the disease. Studies are currently in progress in our group in which physiological samples from selected sets of GI patients and matched controls are analyzed for their QSM content to evaluate relationships between QSM levels and disease status. One such clinical study involved stool samples that were obtained from infants admitted to the NICU for a variety of illnesses, including bowel inflammation and bacterial sepsis (submitted manuscript). Similarly, we have also characterized and optimized a *V. harveyi*-based whole-cell biosensing system [19] to quantitatively detect AI-2 in saliva, stool, and intestinal fluid samples (manuscript in preparation).

Whole-cell sensing systems using bioluminescent reporters, for example those described above, are advantageous for use in clinical applications because of high sensitivity, high throughput, and multiplexing capabilities. However, they need instrumentation to measure bioluminescent signals, and thus are not ideal for on-site applications. Although portable photodiode-based luminometers exist, they may not be readily available, especially in remote locations and developing countries. To that end, we have developed a whole-cell-based paper-strip assay for AHLs using β-galactosidase as reporter, which can be visualized by the naked eye using a chromogenic substrate [35]. The whole-cell-based sensor consists of *E. coli* cells DH5α-T1 transformed with plasmid pSB908, which includes the genes *lasR* and *lacZ,* encoding LasR and β-galactosidase, respectively, under the control of the promoter P_{lasI}. In brief, the sensing cells were vacuum-dried on precut paper strips. On incubation with a set of standards containing different AHL concentrations and addition of the β-galactosidase chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), a blue precipitate (5,5′-dibromo-4,4′-dichloroindigo) formed in the areas where the cells had been dried. The color intensity was visually relatable to the AHL concentration, as shown in Fig. 2c. The limit of detection was only one order of magnitude higher than that obtained with the bioluminescent *luxCDABE*-based sensing system described above (1×10^{-8} mol L^{-1} vs. 1×10^{-9} mol L^{-1}). Furthermore, the paper strip assay was successfully used to detect AHLs in saliva samples. The paper-strip-based analytical test is portable, easy to use, and inexpensive, and can thus be used in the physician's office and at the patient's bedside, even by untrained personnel. Although simple image processing software

can assist in obtaining quantitative data from analysis of acquired digital images of the paper strips, visual evaluation of the color intensity provides a semi-quantitative estimate of the amount of AHLs present in a sample.

Hydroxylated polychlorinated biphenyls

In the early 20th century, polychlorinated biphenyls (PCBs) were widely used in several industrial applications, including electrical insulating fluid, caulks, adhesives, and printing paper [6]. Their use was banned in the 1970s because of their proven toxicity to humans and animals. Because of resistance to physical, chemical, and biological degradation, PCBs still persist in the environment, contaminating water and soil. Additionally, their lipophilic nature can cause bioaccumulation in living organisms and biomagnification through the food chain. When entering the human body, PCBs are metabolized to hydroxylated-PCBs (OH-PCBs), which have been found in serum and other biological fluids [36]. Therefore, detection of OH-PCBs in physiological samples may serve as a means of evaluating exposure to PCBs. Furthermore, OH-PCBs, although less toxic than PCBs, have significant adverse health effects, given that they can act as endocrine disruptors [37] and increase the formation of reactive oxygen species [1]. This emphasizes the importance of OH-PCB detection in physiological samples. To that end, our group has developed a whole-cell biosensing system for the detection of OH-PCBs based on *E. coli* cells harboring plasmid pHYBP109 in which the *luxAB* genes encoding for bacterial luciferase are under control of the P_{hbpC} promoter. The construct also contains the gene coding for the protein HbpR, which negatively regulates expression of the reporter gene and is activated by 2-hydroxybyphenyl [38]. Given the structural similarities between 2-hydroxybyphenyl and OH-PCBs, we postulated that the system could also be activated by the latter [39]. Indeed, a dose-dependent response to a variety of OH-PCBs was observed, with limits of detection down to 1.0×10^{-9} mol L^{-1}. The sensing system was optimized and standardized in terms of analytical conditions and further used to detect OH-PCBs in spiked human serum samples. The results demonstrated that the optimized whole-cell biosensing system can be used for sensitive, rapid, direct detection of OH-PCBs in human blood serum, thus confirming its potential for use as a tool for screening large numbers of samples.

Mercury(II) ions

Mercury is a heavy metal known to be neurotoxic, embryo-toxic, and able to damage various organs. In addition to occupational exposure, sources of exposure to mercury include use of man-made products, for example disinfectants, batteries, thermometers, and fluorescent bulbs, dental amalgams, and

consumption of mercury-contaminated foods. Mercury can exist in three major forms, elemental, inorganic, and organic. Approximately 80% of mercury released from human activities is in the elemental form [37]. Dental amalgams contain 50% mercury, with silver, tin, and copper [40]. Although mercury is bound within the amalgam, it leaches out in the elemental form Hg(0) as vapor, accounting for 75% of daily mercury exposure. It is then absorbed by the lungs, and oxidized to Hg(II) [41]. Roda et al. have optimized a whole-cell biosensing system based on *E. coli* cells, in which the *luc* reporter gene encoding for firefly luciferase is under the control of a mercury-inducible promoter, for detection of mercury in urine. The whole-cell sensor proved to be selective and sensitive for Hg^{2+} and methylmercury, with a detection limit of 1.67×10^{-13} mol L^{-1} for Hg^{2+}. The analysis of urine samples obtained from individuals with amalgam fillings and control individuals showed slightly significantly higher levels of Hg^{2+} in the former group. Assays were performed in 384-well microtiter plates and required low volumes of samples and reagents, thus proving suitable for high-throughput screening. Therefore, the method could be used for analysis of large numbers of urine samples from individuals with and without amalgam fillings to investigate the relationship between urinary mercury levels and dental amalgam.

Methylmercury

Methylmercury (MeHg) is the most toxic of the organomercury compounds and among the most toxic environmental pollutants worldwide [42]. Clinical investigations have shown that MeHg is the primary form of mercury that accumulates in fish and their consumers. This accumulation leads to mercury poisoning and its associated symptoms, because most species are unable to remove MeHg from the body [43, 44]. Diagnosis of mercury poisoning is performed by correlating the clinical symptoms with the level of MeHg in the patient's blood. Normal levels are typically less than 30 nmol L^{-1} in whole blood, but can vary, depending on dietary and environmental factors [45]. Traditional analytical methods for detection of organomercury compounds focus on chromatographic techniques (gas and liquid chromatography) combined with spectroscopic detection methods (atomic fluorescence spectrometry, atomic absorption spectrometry) [46] or on capillary zone electrophoresis [47]. These methods are impractical for assaying clinical and environmental samples because they require much pretreatment of samples, are highly expensive, and are very time-consuming: they are, furthermore, not amenable to miniaturization and not applicable to on-site analysis. Moreover, these methods only determine the total amount of mercury present in the sample, which makes it hard, if not impossible, to distinguish the amount of bioavailable mercury. To develop more efficient and cost effective methods for

detection of organomercury compounds, many groups have engineered whole-cell biosensing systems (Table 3). Below we highlight two approaches for specific detection of MeHg.

Nagata et al. [48] reported a whole-cell sensing system specific for MeHg capable of rapid detection down to 0.01 nmol L^{-1} levels. The whole-cell sensing system was constructed using two separate plasmids expressed in *E. coli* DH5α; pHY-*mer-lux* and pJM-B (Fig. 3a). The plasmid pHY-*mer-lux* was constructed by fusing the mercury-inducible promoter and its regulatory gene, *merR*, from the *mer*-operon of *Pseudomonas* K-62 with the *luxAB* reporter gene from *V. harveyi* [49]. The second plasmid, pJM-B, was prepared by cloning the *merB* gene from *Psuedomonas* K-62 into the pGEM-T Easy cloning vector. The system functions by constitutively producing the organomercury lyase, MerB, which cleaves the C-Hg bond of MeHg, producing free Hg^{2+}. This free Hg^{2+} can then bind to the Hg^{2+}-selective regulatory protein, MerR, and activate the *mer*-operon, thus producing luciferase in an Hg^{2+}-dose-dependent fashion (Fig. 3b). This system is selective for free Hg^{2+} cleaved from MeHg over free Hg^{2+} that may be present in a sample, although the specific mechanism has not been investigated (Fig. 3c). The selectivity is most likely explained by the high lipid solubility of organic mercury compared with inorganic mercury, which may favor passive diffusion of MeHg into the sensing cells [42]. Nagata et al. postulated that the constructed pHY-*mer-lux* may cause selective uptake of MeHg into the cell because of lack of a native active transport system for inorganic mercury. This type of phenomenon has been reported before for phenylmercury, which is specifically transported into cells via the mercury transporter protein, MerT [50, 51]. As a result, Nagata et al. produced a whole-cell biosensing system that can rapidly and selectively detect bioavailable MeHg with a limit of detection low enough to assess both environmental and physiological samples.

Rantala et al. [13] took a different approach to the detection of MeHg, one that focused on using an already engineered sensor [52], but creating a highly selective, more robust, reproducible, portable assay with detection limits down to 0.3 nmol L^{-1} levels. The whole-cell sensing system was constructed by transforming, in *E. coli* MC1061, a plasmid that fused the mercury inducible promoter and its regulatory gene, *merR*, from the pDU1358 *mer*-operon with reporter gene *luxCDABE*. In addition, the plasmid contains the *merB* gene under the control of the *lac* promoter. The mechanism of the system is similar to that of the Nagata et al. system, except that, because the entire *luxCDABE* cassette is present, there is no need for addition of external substrate to generate the emission of bioluminescence from the luciferase reporter. As constructed, the whole-cell sensing system detects total mercury in the sample. Selectivity for MeHg was achieved by adding ethylenediaminetetraacetic acid (EDTA), which acts as

Table 3 Comparison of whole-cell sensing systems for detection of organomercury. Adapted from Rantala et al. [13]

Host strain	Analyte	Reporter plasmid	Substrate	Limit of detection (nmol L^{-1})	Dynamic range (nmol L^{-1})	Assay time	Ref.
E. coli MC106	Organomercury compounds	pmerBRBSluc	D-Luciferin	0.2	0.2–10	2.5 h	[52]
E. coli DH5α	Organomercury compounds (phenylmercury acetate)	pHYB3LuxAB+pGR1A	10% 1-decanal	50	50–5000	Several hours	[49]
E. coli MC1061	Total mercury	pmerRBPmerluxCDABE	No substrate^c	0.008	8×10^{-12} -5×10^{-8}	120 min^b	[52]
E. coli DH5α	Methylmercury	pHY-mer-luxAB+pJM-B	10% 1-decanal	0.01	0.01–1.0	40 min^b	[48]
E. coli MC1061^a	Total mercury^d	pmerRBluxCDABE	No substrate^c	0.3	0.3–100	60–180 min	[13]

^{a} Freeze-dried sensor cells

^{b} Growing step of the cells not included, only the measurement with the analyte

^{c} Produced by the reporter gene operon

^{d} Chelation of ionic Hg^{2+} makes the sensor specific for organic mercury

a chelating agent, blocking inorganic mercury from entering the cells. Multiple concentrations of EDTA were tested for toxicity and luminescence inhibition, and an optimum

Fig. 3 (a) pHY-*mer-lux* and pJM-B plasmids in *E. coli* DH5α. (b) Simplified reaction mechanism for reporter system. (c) Response of whole-cell sensing system to CH$_3$Hg$^+$ (*triangles*) and Hg^{2+} (*circles*). Adapted from Nagata et al. [48]

concentration was selected (10 mmol L^{-1}). Use of EDTA as a chelating agent also helps to make the sensor more robust, because the cells are protected from the toxic inorganic mercury species that may exist in samples.
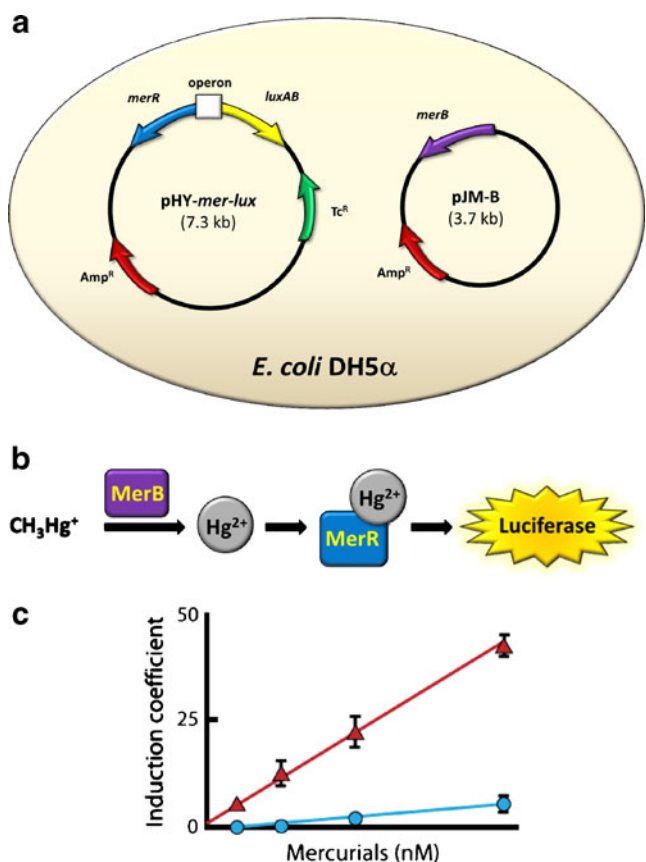
Antibiotics

Since the 1940s, tetracycline has been one of the antibiotics most widely used to treat bacterial infections in humans and animals. With increasing animal farming, animals are routinely administered tetracycline to control infections; a side effect of such practice is the increase in tetracycline-resistant bacterial populations. For example, it has been shown that subinhibitory concentrations of tetracycline promote accumulation of tetracycline-resistance genes in bacteria colonizing the GI tract of mammals [53]. Bahl et al. have investigated the above mentioned effect by determining, in vivo, bioavailable concentrations of the antibiotic in the intestines of tetracycline-treated rats, which continuously received drinking water containing the antibiotic [54]. This was achieved by using a whole-cell biosensing system consisting of *E. coli* MC4100 cells harboring the plasmid pTGFP2, which contains a gene fusion of the tetracycline inducible promoter P_{tet} and *gfp*. Uptake of tetracycline by the sensing cells activates the tetracycline-inducible promoter, subsequently inducing dose-dependent expression of GFP. Fluorescence measurements by flow cytometry demonstrated low bioavailability and proved the potential utility of whole-cell sensing systems as tools to determine antibiotic intestinal bioavailability and therapy optimization. In a similar study, Hansen et al. demonstrated the utility of a whole-cell biosensing system using β-galactosidase as a

reporter for quantitative measurement of chlortetracycline in pig feces. These studies showed the application of whole-cell sensing systems in veterinary clinical medicine, with potential implications in human medicine. Such cell-based assays can further be developed in high-density formats for high-throughput screening of a multitude of samples and for screening natural and synthetic molecules for antibiotic activity [55].

Cytarabine efficacy

Cytarabine (cytosine arabinoside, Ara-C) is the main chemotherapy agent used for treatment of acute myeloid leukemia (AML). However, it is reported that over 30% of patients do not respond to Ara-C, leading to high morbidity and mortality associated with drug cytotoxicity and side effects. Alloush et al. have developed a bioluminescent microbial cell-based biosensor for detection of Ara-C to be used in an in vitro assay to predict response of leukemic cells to Ara-C [56]. In vivo, Ara-C is transported into cells and converted to its active triphosphate form, Ara-CTP. The biosensing *E. coli* HA1 strain is a *cdd* deficient mutant which was created by transposon mutagenesis. The *cdd* mutation is necessary because cytidine/deoxycytidine deaminase, encoded by *cdd*, converts Ara-C into Ara-uracil in *E. coli* cells. This mutant was then transformed with a plasmid enabling expression of human deoxycytidine kinase (dCK), which is needed to start the phosphorylation process leading to active Ara-CTP. A further plasmid carrying the *luxCDABE* gene cassette was transformed into the *E. coli* cells, whose expression and consequent light production in the presence of Ara-C is thought to be because of activation of a bacterial SOS stress response mechanism [57, 58]. A schematic diagram of the assay is presented in Fig. 4. Briefly, monocytic AML cell lines or mononuclear cells from AML bone marrow and peripheral blood samples were exposed to 25 μmol L^{-1} Ara-C (representative of an in vivo dose of 200 mg m^{-2} day^{-1}). If the leukemic cells are responsive, Ara-C is taken up and converted to Ara-CTP. The cells are then lysed and the cell lysate is incubated with the reporter bacteria in the presence or absence of alkaline phosphatase (AP). In the presence of AP, Ara-CTP is converted to Ara-C, which can enter the bacteria and induce high bioluminescence. In the absence of AP, Ara-CTP, which cannot enter the bacterial cells, remains unmodified and low bioluminescence is observed. Thus, the ratio of bioluminescence signals obtained in the presence and absence of AP is indicative of the leukemic cells' ability to convert Ara-C into active Ara-CTP. Because bacterial cells have a faster metabolic rate and shorter cell cycle time, a response from the sensing cells can be obtained in 8 h, compared with several days required when mammalian cells are used in similar studies. Clinical trials are needed to validate this system, which can potentially be used as a predictive tool to determine patient's responsiveness to cytarabine before treatment is started.

Mechanisms of action of antibacterial compounds

Bacterial infections are of worldwide concern, affecting both developing and industrialized nations. To combat such infections, antibacterial compounds are used for sanitation and as pharmaceuticals. Determining the mechanism of action of antibiotics is of critical importance not only to predict potential negative effects but also to identify other therapeutic actions. In that regard, whole-cell biosensing systems have found use in pharmaceutical drug discovery to determine antibacterial mechanisms of action, using *Bacillus subtilis* as the model organism. The analysis of antibiotic triggered mRNA expression profiles has enabled linking of promoter regions with specific antibiotic mechanisms of action [59–62].

Urban et al. generated and validated five whole-cell sensing systems, confirmed the specificity of the mechanism of action, and developed and optimized a 384-well plate assay for identification of the antibacterial mechanism of action [55]. The whole-cell sensing systems were created by fusing the known antibacterial stress response promoter to firefly luciferase (pBest*luc*), inserting this sequence into shuttle vector pHT304, and transforming and expressing these plasmids in *B. subtilis* 1S34 (a non-spore-forming derivative of strain 168). Constructs using different promoter and upstream region lengths were used to generate a system optimized for high-throughput screening ($Z > 0.5$). The Z-factor is a measure of statistical effect size, which can be applied to high-throughput screening to determine whether the response of an assay is large enough to warrant its further use [63]. To determine the specificity of each system, 14,000 pure natural products were tested, including thirty-nine reference antibiotics to confirm the mechanism of induction of action biomarkers, and mechanistically uncharacterized antibiotics and antibiotics with novel structural entities. This product set enabled verification of the specificity of the whole-cell sensing system, because it reduces the risk of finding non-specifically acting antibiotics. Whereas 6% of the compounds had anti-*B. subtilis* activity, only 0.1–0.2% of the compounds per promoter had specific activity. In addition to the advantages of using such a large number of products for screening, natural products have proved to be an invaluable source of chemical diversity for new drug discovery [64, 65], further validating this system for use in pharmaceutical high-throughput screening.

To make these biosensing systems viable for high-throughput screening, an assay was developed and optimized for use in 384-well plates, with luminescence measured by

Fig. 4 Cell lysis assay using *E. coli* HA1 biosensing system for detection of cytarabine. Adapted from Alloush et al. [56]
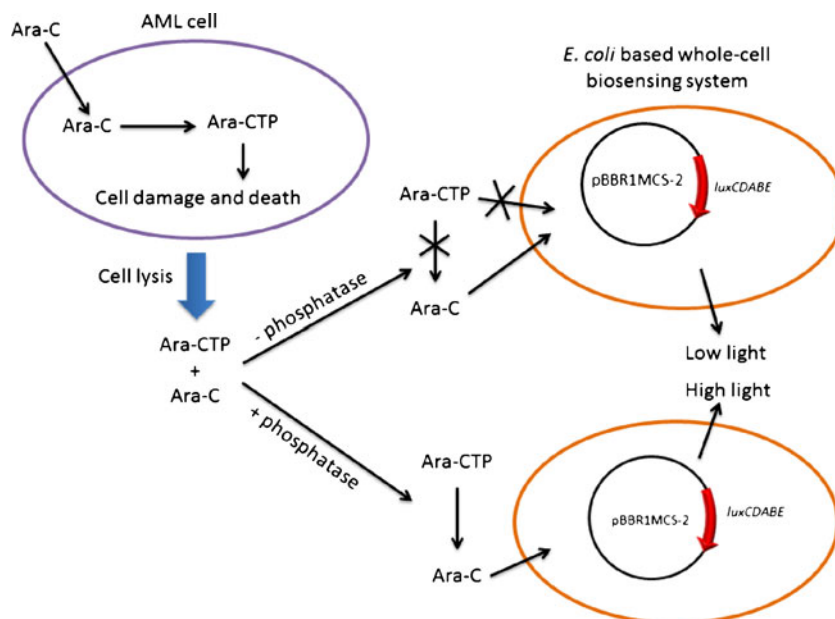
using a CCD camera-based luminescence detector. To validate the developed promoter induction assay, second-line mechanism of action studies, which involved incorporation of radio-labeled metabolic precursors for DNA, RNA, protein, and cell wall biosynthesis in *Staphylococcus aureus* [66], were performed for a representative compound, ferrimycin A1, an antibiotic with a poorly studied mechanism of action. Both the whole-cell sensing systems and the metabolite incorporation studies strongly supported selective protein biosynthesis inhibition as the mechanism of action of this compound. Thus, Urban et al. produced a high-throughput method of screening capable of determining the mechanism of action of antibacterial compounds that is fast, inexpensive, reproducible, and highly specific. However, this system is not without its flaws. The system is unable to assess antibacterial compounds that function at concentrations less than 1.56 μg mL^{-1}, such as fusidic acid and siomycin A. Also, although 14,000 compounds were tested, only thirty-nine were reference antibiotics—testing more reference antibiotics would result in a stronger, more robust comparison matrix.

It is envisaged that continued use of these whole-cell biosensing systems for high-throughput screening may aid in the discovery of novel pharmaceutical compounds, and advance our knowledge of the mechanisms of action of many different types of compound.

Conclusions and future perspectives

Identification of novel biomarkers of disease created a need for analytical tools that enable their detection in physiological fluids for diagnosis and management of disease, and for assessment of therapeutic efficacy. Whole-cell biosensing systems have properties that make them ideal for these purposes. Specifically, they afford very low limits of detection, thus reporting the early presence of a target biomarker, and have sufficient analytical sensitivity to enable discrimination between close biomarker concentrations, which could be crucial in some clinical settings. Their high selectivity also enables direct detection of target analytes in clinical samples with no or minimal sample pre-treatment, a significant advantage over traditional instrumental analytical methods. This feature, with fast response and amenability to incorporation into high-density analytical devices, enable the development of rapid, high-throughput screening assays requiring small sample volumes. Importantly, miniaturization and integration of whole-cell sensing systems into chip, paper, and microfluidics-based devices should enable the development of portable equipment suitable for use on-site, in the physician's office, or at the patient's bedside. Furthermore, paper strips with immobilized whole-cell sensing systems and color visualization of the signal have potential for implementation of easy-to-use dip-stick type devices which could be used by patients for disease management as home-based self-monitoring tests. Such devices could prove especially useful in remote areas and developing countries. Continued work on cell-based biosensors should contribute to further exploiting and enhancing their unique advantages. The identification of additional regulatory proteins and receptors that recognize analytes of biomedical interest should continue to expand the usefulness of whole-cell biosensing systems in all branches of clinical analysis, including, diagnostics, disease monitoring, drug screening, and efficacy testing.

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