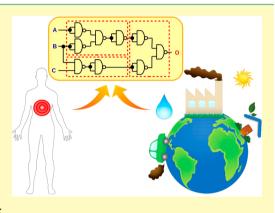# Cellular Biosensors with Engineered Genetic Circuits

Behide Saltepe,[†,‡] Ebru Şahin Kehribar,[†,‡] Side Selin Su Yirmibeşoğlu,[†] and Urartu Özgür Şafak Şeker*,[†]

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ABSTRACT: An increasing interest in building novel biological devices with designed cellular functionalities has triggered the search of innovative tools for biocomputation. Utilizing the tools of synthetic biology, numerous genetic circuits have been implemented such as engineered logic operation in analog and digital circuits. Whole cell biosensors are widely used biological devices that employ several biocomputation tools to program cells for desired functions. Up to the present date, a wide range of whole-cell biosensors have been designed and implemented for disease theranostics, biomedical applications, and environmental monitoring. In this review, we investigated the recent developments in biocomputation tools such as analog, digital, and mix circuits, logic gates, switches, and state machines. Additionally, we stated the novel applications of biological devices with computing functionalities for diagnosis and therapy of various diseases such as infections, cancer, or metabolic diseases, as well as the detection of environmental pollutants such as heavy metals or organic toxic compounds. Current whole-cell biosensors are innovative alternatives to classical biosensors; however, there is still a need to advance decision making capabilities by developing novel biocomputing devices.

KEYWORDS: *synthetic biology, biocomputation, digital circuits, logic gates, analog circuits, whole cell biosensors, biomedical sensors, environmental sensors*

Synthetic biology is an emerging engineering field that aims to build unnatural cellular functionalities, and cellular networks by programming cells using nucleic acids, proteins, and other biomolecules. Generally, synthetic biology considers biological parts as molecular Lego units to build complex synthetic genetic networks. Genetic elements (e.g., promoters, ribosome binding sites, and genes of interest) can be used to tune the simple genetic circuits to design innovative functionalities. In the past decade, using the tools of synthetic biology, biological devices were designed and implemented.[1] Among those, genetic logic gates,[2,3] genetic recorders,[4−8] and RNA based switches,[9] which are summarized in Figure 1, can be counted as milestones of synthetic biology. Also, synthetic biology intends to form more complex structures and circuits to be used in more sophisticated areas. To begin with, logic gate operation with one or many inputs is well characterized systems in electronics which gives the opportunity to create many devices. Mimicking the logic gate operation, synthetic biology has the potential to constitute layered genetic circuits developing a new application in the field, so-called biocomputation.[10] Thus far, a number of logic operation systems have been developed and characterized with varying type of genes, DNA binding elements (i.e., transcription factors), promoters, or ribosome binding sites and a number of host organisms have been utilized from single cell organisms to mammalian cell lines.[2,3,11−13]
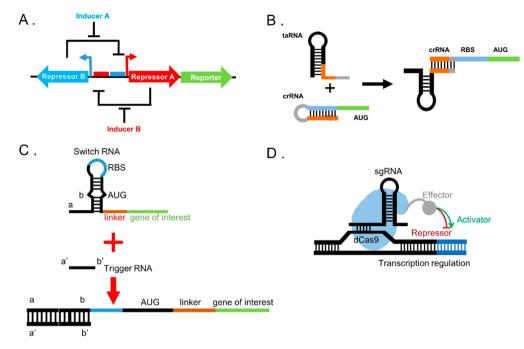
One of the promises of synthetic biology is to program cells to analyze molecular entities in their environment and report their presence in real-time. All of the microorganisms have a highly evolved sensory system to track changes in their environment which is very critical for their surveillance. Compiling these sensors can lead the formation of biocompatible, implantable, cheap, easy to-build sensor systems. At this point synthetic biology can be exploited as a key approach to build and program genetic parts and modules to build desired whole cell sensors.[14]

In this review, we focus on biocomputation tools and their applications in biosensor studies. First, we describe digital and analog computation phenomena with some milestone examples in synthetic biology. Then, we emphasize recent examples of genetic switches and layered logic circuits and their integration to whole cell biosensors. More specifically, we explore a number of whole cell biosensor examples established in biomedical and environmental sensing. Finally, we underline some drawbacks and regulations of whole cell sensors in terms of biomedical and environmental safety concerns.

■ BIOCOMPUTATION IN SYNTHETIC BIOLOGY

Digital Circuits and Logic Operation. A digital circuit uses defined threshold levels to control signal levels.[15] Synthetic biology uses genetic switches to control complex genetic networks. A genetic toggle switch based on bilateral expression inhibition of two repressors was initially proposed (Figure 1A).[16] Gardner et al. demonstrated that two repressor

Figure 1. Some of the tools for digital genetic circuits. A. Toggle switch mechanism is based on the expression of two repressors; Repressor A and Repressor B. In the presence of inducer A, Repressor A is expressed and it represses the expression of Repressor B and vice versa. Reprinted with permission from ref 16. Copyright 2000 Nature Publishing Group. B. Riboregulator-based transcription control is based on the linear–loop interaction of crRNA and taRNA that releases RBS and initiates gene expression. In the absence of taRNA, crRNA forms a stem-loop and blocks RBS. However, taRNA targets the crRNA and release RBS free for ribosome binding. Reprinted with permission from ref 20. Copyright 2004 Nature Publishing Group. C. Unlike conventional riboswitches, Toehold switches keep RBS and start codon unpaired. Linear–linear RNA interaction of toehold and trigger RNA starts between domains a and a′ which releases the rest of the loop. Reprinted with permission from ref 26. Copyright 2014 Elsevier. D. In the CRISPR-based transcription control mechanism, a sgRNA guides dCas9 with an effector domain to a specific DNA sequences on the genome. Effector domain is modified either as a transcription activator or repressor to control the gene expression. Reprinted with permission from ref 27. Copyright 2013 Elsevier.

molecules ("repressor A" and "repressor B") are controlled by two different inducer molecules ("inducer A" and "inducer B"). Each inducer's presence activates the expression of one gene while repressing the expression of another gene. Following this discovery, more complex circuits by using digital paradigm regime have also been achieved with many assistant molecules such as RNA regulators, recombinases (or invertases), or transcription factors (TFs). As an alternative to TF-based circuits, RNA regulators are able to activate,[17] block,[18] or terminate[19] transcription, as well as affect translation at the post-transcriptional level which makes them a group of candidates to advance cellular computation approaches.[20−23] Noncoding RNA (ncRNA) structures have many regulatory purposes not only in prokaryotes but also in eukaryotes.[24,25] For instance, synthetic riboregulators were inspired by this ncRNA regulation in nature. Isaacs et al. designed artificial riboregulators to tightly control the gene expression via pairing of two RNA structures: cis-repressing and trans-activating RNAs (crRNA and taRNA, respectively). Riboregulators are one of the first strategies based on loop–linear interaction between crRNA and taRNA (Figure 1B). crRNA is designed to block the recognition of RBS, found in the stem of the crRNA, by ribosome; thus, gene expression is prevented. Thus, crRNA eliminates leakage caused by promoter activity almost entirely. To initiate gene expression, taRNA is produced under the control of another promoter. The linear part of taRNA targets the loop of crRNA which causes strand displacement that releases RBS free. Since RBS gets free, ribosome recognizes the sequence which initiates the gene expression.[20] Following this mechanism, a new RNA regulator, namely, toehold switch, is

developed by Green et al. Toehold switches (Figure 1C) have some advantages over conventional riboregulators. Unlike conventional riboregulators, toehold switches are based on strand displacement caused by linear–linear interaction between toehold and trigger RNA structures which makes the process thermodynamically more favorable. Trigger RNAs have the potential to trigger more switch RNAs so that expression of the gene of interest increases accordingly, because linear–linear interactions can provide faster kinetics and stronger thermodynamics than loop–linear interactions. Also, conventional riboregulators have sequence restrictions since they need to conserve RBS as paired form in the loop. However, toehold switches keep RBS and start codon unpaired in the loop, so trigger RNA sequence becomes flexible to be designed de novo.[26] Another commonly used RNA regulation mechanism is to use modification of Type II CRISPR and CRISPR-associated (Cas) system (CRISPR/Cas) system for transcriptional control of gene expression (Figure 1D). A nuclease activity absent Cas9, dead Cas9 (dCas9), protein is guided by a small guide RNA (sgRNA) sequence to a target of interest to control the gene expression at DNA level. It is only used to block the transcription via steric hindrance or via modification by effectors such as transcription activators or repressors to activate or terminate the gene expression.[27] In this approach, dCas9 protein has been used to modify the target of interest with TFs.[27−30] Nissim et al. proposed a CRISPR-TF based toolkit for human cells with functional gRNAs. This toolkit is able to activate endogenous RNAP II promoters to control transcription of targeted genetic circuits.[31] Some of the tools of digital genetic regulation are summarized in Figure 1.
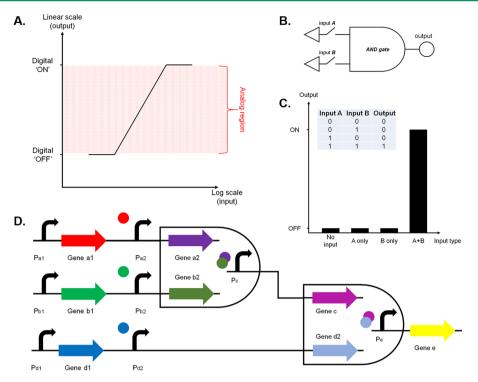
Figure 2. Biocomputation in synthetic biology. A. Digital output is obtained with certain ON and OFF readouts in response to an input with a certain threshold, while analog output is gradual. Reprinted with permission from ref 73. Copyright 2013 Nature Publishing Group. B. Representative AND gate. C. Output response with the truth table of an AND gate which operates only in the presence of two inputs. D. Logic gate multiplexing with three inputs which gives only a single output. Reprinted with permission from ref 3. Copyright 2012 Nature Publishing Group.

Recombinase based genetic logic gates have been very popular recently due to the ease of design.[2,4] Recombinases are mostly identified from viral genomes.[32] They act in unidirectional as well as bidirectional conversion and excision of DNA strands.[33,34] The unidirectional recombination occurs only one time between recognition sites, and this process is not reversible. On the other hand, bidirectional recombinases are used for reversible recombination. The very first discovered recombinase system is Cre (**C**ause **re**combination) enzyme found in bacteriophage P1. This enzyme binds to *loxP* (locus of crossing (x) over of P1) region. Cre recombinase flips DNA strands reversibly. It is shown that some regions on *loxP* is very specific to Cre recombinase that even only a single change may cause a decrease in recombination efficiency.[35,36] FimB/FimE[37] and Hin recombinases represent another group of recombinases modifying DNA sequences irreversibly.[38,39] In the study of Moon et al. a recombinase-based chemosensory toggle mechanism was demonstrated. The synthetic toggle mechanism controls orthogonal CheW adaptor expression. In the FimB/FimE recombinase based system, selected chemotaxis receptors are expressed constitutively. CheW adaptor is expressed via arabinose induction. In the presence of arabinose, fim switches on the expression of preferential binding of CheW to Tar so that the knocked out *Escherichia coli* BW28357 (Δ*tar*, Δ*tsr*, Δ*tap*, Δ*trg*, Δ*aer*, Δ*cheW*) strain moves from serine to aspartate.[40]

Transcription factors (TFs) can be programmed as genetic switches for synthetic biology as well. Many metabolites and environmental factors can trigger expression of their own TFs in cells; those may interact with defined TFs. The interaction of TFs with certain molecules can lead to conformational change in the TFs which alters DNA binding affinity of TFs. Thus, this strategy requires redirecting of TFs upon exposure to a desired

input giving the rewired output.[41] For this purpose, promoter engineering strategies have been developed to increase TFs interaction upon an input signal.[42,43] Transcriptional control of prokaryotes or eukaryotes has been based on using special DNA-binding proteins, namely, activators or repressors,[44] and engineering of these proteins prior to usage.[45−48] For instance, Elowitz et al. proposed a repressilator based genetic oscillator with three repressor proteins, namely, LacI (first repressor), TetR (second repressor), and cI (third repressor). The first repressor inhibits the expression of the second one which represses the third one, and finally the third one represses the first one to complete the whole cycle. The repression of second repressor (TetR) by LacI released the expression of the signal protein (GFP).[49] Other examples of TF-based genetic control systems are the state machines. These are order-based systems for which the output depends on both the current state and the incoming input.[50] These machines allow directed differentiation of cells by controlling the sequential activation of many TFs or other responsible genetic elements in corresponding pathways.[51−53] State machines can be useful tools to understand the state of disease conditions[54] which are based on extra- and intracellular factors and their response to administrated therapeutics.[55−58] Usign these machines a cancer phenotype of a tumor can be ruled by the temporal order of genetic mutations.[54] Oishi et al. proposed a theoretical epigenetic state machine system that utilizes Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi)-based strategy.[59] In another approach, Roquet et al. developed a state encoding recombinase-based state machine (RSM) whose knowledge is stored in DNA strands of living cells.[50]
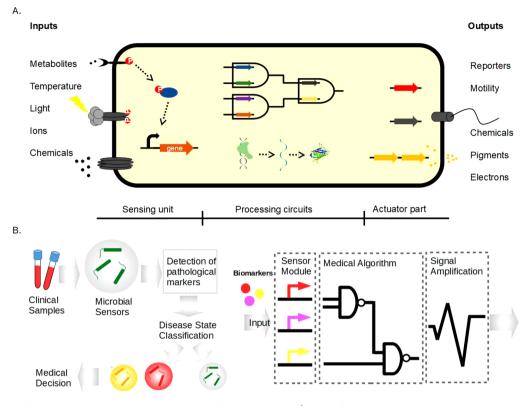
Circuits do not always depend on a single input. For example, the catabolic sugar pathway in natural *E. coli* system depends not only on the presence of an alternative sugar source

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of glucose but also on the absence of glucose itself.[60] A Boolean logic gate integration has many advantages in construction of digital circuits[2] since it provides multiplexing of the system with many gates (i.e., AND, OR, NOR, NAND, XOR, etc.).[2,4,36,61] Integration of electronics into synthetic biology has been accelerated by the usage of Boolean logic gates which trigger an output through multiple inputs. Environmental stimuli response of cells makes them programmable with synthetic genetic logic gates which are able to be used in many areas from biotechnological applications to pharmaceutics.[62−64] By using diffusible signals (i.e., chemical signals) in layered structure of simple logic gates, it is quite possible to create more complex logic gates.[13] The AND gate is one of the very first logic gates; in this very first example, the gate operates in the presence of two inputs only. Figure 2B,C demonstrates a basic AND gate and its operation in the presence of inputs. In more complex systems, 2-input gates are combined with each other to obtain 3- or 4-input AND gates, an example of gate multiplexing is illustrated in Figure 2D.[3] The combination of an AND and a NOT gate has been used to form a NAND gate. In this type of logic gate, output is observed unless both inducers are shown together at the same time.[65] Another combinational logic gate is the NOR gate, which is composed of an OR gate followed by a NOT gate. The NOR gate turns itself ON only when the two inputs are OFF. On the other hand, as a form of OR gate, XOR gates respond when only one of the inputs present.[13]

So far many logic gates have been constructed in different host organisms including bacteria, yeast, and mammalian cells. Especially considering the ease of use of *E. coli* as a host organism, more complex logic gates have been developed.[4,47] On the other hand, yeast cells have many applications in biotechnology research from biofuels to the alcoholic beverage industry.[66,67] Simple synthetic genetic logic gate circuits are applicable to programming yeast cells. Regot et al. demonstrated even more complex logic gate combinations in yeast.[68] Applications of logic gates in mammalian cells were proposed by many researchers including a transcriptional control system by Fusseneger et al. in 2011.[69] Auslander et al. developed a set of two-input based circuits in mammalian cells which are operated in AND, NAND, NOT, and N-IMPLY functions.[70] Further, Gaber et al. enhanced these functions with 16 two-input-based combinations of NOR gates.[71] Recently, CRISPR/Cas based genetic logic gates are becoming popular, where dCas9 protein is used as a synthetic TF to control cellular behaviors.[72] Nielsen et al. constructed NOT and NOR gates based on dCas9 and sgRNAs to control transcriptional regulation by promoter repression. In their circuits, sgRNAs recruit dCas9 on output promoter via logic operation and block transcription initiation. Thereby, this study is an excellent example to prove that logic operations do not require only chemical inputs.

Analog Circuits. Unlike sharp ON and OFF states of digital circuits, analog circuits are based on gradient output in response to input; both phenomena are shown in Figure 2A. Biological systems are the very obvious examples of analog operation, since cells especially are highly dynamic structures which evaluate incoming signals to maintain their surveillance. Even if many processes in cells require digital decisions indicated with 0 and 1, the whole processes depend on gradual analog operation. An especially limited amount of resources guide cells through analog operations. For example, cells have quite low RNA copy numbers which forces cells to make analog computation. Synthetic biology has mimicked digital phenom-

enon for many years to build genetic circuits. Further, synthetic genetic circuits are inspired from analog computation in nature.[74] For example, natural *E. coli* cells use AraC, which is the arabinose regulator, to broaden the dynamic range of arabinose: AraC represses its own production with a negative autoregulation loop; however, binding of arabinose to AraC opens the system up and produces more AraC. Thus, AraC concentration depends on arabinose concentration as an inducer.[75] Likewise, inspiring the natural autoregulation in cells, Nevozhay et al. engineered the response of a promoter (PGAL1-D12) found in *S. cerevisiae* which is repressed by a tetracycline repressor (TetR) and created a negative autoregulation system based on the expression of TetR from the TetR repressing promoter PGAL1-D12.[76] In the sense of biotechnology, analog circuits are commonly used to tune the expression of an engineered gene of interest based on the incoming signals from their surroundings such as in whole-cell biosensor applications. Daniel et al. showed that an analog circuit can be engineered to respond in a very wide dynamic range via a rationally designed positive feedback loop. In this study, two circuits were designed. The first circuit is a positive-feedback loop which is composed of a promoter, a TF, and a reporter. The second circuit is a "shunt" which is composed of a promoter and another reporter. TF produced from the first circuit targets either the expression of itself together with the second circuit or only the expression of the reporter from the second circuit. Thereby, the first circuit alleviates saturation of the TF−inducer interaction while the shunt circuit derivates TFs away from the first circuit and prevents saturation of the promoter with TFs. They characterized their circuits with three different promoter−TF couples, namely, P_{BAD}-AraC, P_{lux}-LuxR, and P_{lacO}-LacI.[73] To increase the dynamic range via analog operation, Wang et al. constructed an amplifier with *hrp* (hypersensitive response and pathogenicity) operon in *Pseudomonas syringae*, which is composed of a sigma54-dependend *hrpL* promoter, two activators (HrpR and HspS) and a repressor (HrpV). In their circuit, *hrpS* and *hrpR* were controlled with arsenic responsive promoter and RBS sequences with different strength, and output was controlled with *hrpL* promoter. Their results show that arsenic sensor itself shows narrow dynamic range while arsenite dynamic range broadens with their signal amplifier. Thus, they propose that signal amplifiers which increase sensitivity and output dynamic range could be used in environmental studies to detect even lower amounts of pollutants.[77] In another study, Wang et al. tuned sensitivity and dynamic range of cellular sensors composed of TetR or LuxR and ArsR TFs. In each case, TFs concentration has changed with constitutively active promoters with different strengths. Afterwards, TFs are subjected with their ligand of interest to target their promoter (i.e., LuxR is subjected with AHL molecules in the range of 0−25000 nM) and GFP output is recorded upon TF−ligand interaction. Hence, the study shows that increased TFs in cells increase the detection limit and the dynamic range of the output.[78]

Mixed Circuits: Digital-To-Analog (D-To-A) and Analog-To-Digital (A-To-D). Cells respond to environmental changes in an analog fashion in nature, which leads to an analog input−output chain. However, it is possible to convert analog response to a digital output with some specific modifications. When an analog signal is modified to differentiate "OFF" and "ON" states, the system gives a digital readout accordingly. A-to-D conversion is mainly dependent on ultrasensitivity which is defined as a nonlinear input−output curve making a large

Figure 3. A whole-cell biosensor's working principle. A. The cell receives signals (i.e., small metabolites, chemicals, ions, temperature shift, or light) from its environment which activates processing circuits inside the cell. Signal process could be via different mechanisms such as transcriptional regulation on the DNA or logic operation introduced artificially to the cell. Based on the process inside, the cell responds to the incoming signal through chemical secretion, motility changes, or reporter expression. Inspired from ref 89 and reprinted with permission from ref 90. Copyright 2017 Springer. B. Synthetic gene circuits for theranostic applications. Architecture of a whole-cell biosensor for medical diagnosis. The sensor recognizes disease biomarkers and generates an output via logic gate operations. Reprinted with permission from ref 91. Copyright 2015 the American Association for the Advancement of Science.
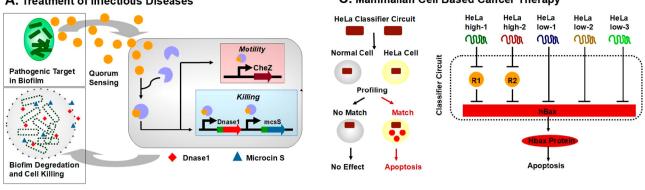
output change with a small input change.[15] This phenomenon can be generated by multimerization or cooperative binding of proteins on a promoter.[79] Toggle switch[16] is the most famous and the very first example of A-to-D converter based on cooperative binding describing ultrasensitivity. The first D-to-A converter was proposed to use multiple recombinases expressed an output via many constitutively active promoters with changing strengths. This combination allows gradual expression of the output.[4] As in previous systems, many others have been developed.[8,80]

The behavior of cells can be recorded to understand cellular and intercellular dynamics of each cell via recording elements. For instance, recombinase-based memory recording on living cells[2,4,39,81] gives rise to binary information written on DNA strands of cells, but it has limitations such that only a bit of information storage needs large amounts (i.e., a few hundred) of DNA strands, recombinase should be overexpressed, and also targeted DNA strands should contain recombinase recognition sites. On the other hand, the Synthetic Cellular Recorders Integrating Biological Events (SCRIBE) strategy is developed to record digital as well as dynamic events occurring in cells, which uses genomic homology containing single-strand DNA (ssDNA) to mutate the genome of *E. coli*.[8] Since the digital switching phenomenon has limitations, such that the orthogonal regulatory elements (i.e., TFs or recombinases) are limited; the analog information could not be recorded by them. Cells are dynamic structures and an analog recording is required to understand the niches in cells. Recently, Perli et al.

demonstrated that a designed self-targeting guide RNAs (stgRNAs) can guide Cas9 to create mutations repeatedly in mammalian cells.[5] This analog recording system is named Mammalian Synthetic Cellular Recorders Integrating Biological Events (mSCRIBE), and unlike conventional small guide RNA (sgRNA) usage, this technique uses stgRNA which is able to direct Cas9 several times to the same target region that records mutation-based analog memory in cells.
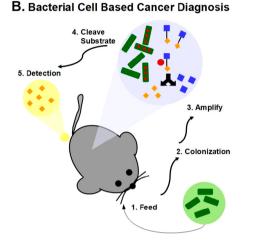
WHOLE-CELL BIOSENSORS

Whole cell biosensors are promising sensing tools as cost-effective and stable biosensors. As a general design strategy, there are three different approaches: (a) designing a reporter circuit based on the cellular systems, (b) designing a system to obtain whole cell as effectors, and (c) wiring the multiple cells via synthetic circuits.[82] Most of the host organisms encoding the sensor circuits are cheap and easy-to-handle compared to high-tech sensing equipment.[83,84] In principle all of the metabolic activities can be programmed as a sensors circuit's output, but optical signals are the most preferred. Other types of output signals can be listed: changes in metabolic activity, gene expression profile, and pH as a response of inducer agent.[85−87] As stated earlier, expression of a reporter protein (i.e., fluorescent proteins, luminescent proteins, or colorimetric enzymes) as an output is a sensitive, simple, rapid, and easy way in these biosensors.[85,88] A whole cell biosensor demonstration is overviewed in Figure 3A. In the case of multiplexing, simultaneous usage of different reporter proteins allows
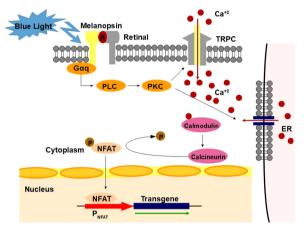
Figure 4. Cellular biosensors with complex genetic logic operations for numerous diseases. A. Schematic of pathogen-seeking *E. coli* that controls motility and activates pathogen killing through quorum-sensing signaling of the pathogenic target. Reprinted with permission from ref 97. Copyright 2014 American Chemical Society. B. Cancer diagnosis platform upon oral delivery of probiotic EcN. The probiotic bacteria specifically grow within the tumors and encodes for enzyme LacZ to cleave systemically injected substrate and release products for detection from urine. Reprinted with permission from ref 103. Copyright 2015 the American Association for the Advancement of Science. C. Circuit operation for HeLa cell type classification. The multi-input classifier circuit controls selective killing of HeLa cells through apoptosis, in response to HeLa-high and HeLa-low miRNA markers. Reprinted with permission from ref 111. Copyright 2011 the American Association for the Advancement of Science. D. Synthetic phototransduction cascade to control transgene expression upon exposure to blue light. Light-controlled expression of glucagon-like peptide 1 is utilized for type II diabetes. Reprinted with permission from ref 113. Copyright 2011 the American Association for the Advancement of Science.

multiple output detection of biosensors. Similar to FPs, an enzyme, *β*-galactosidase (*β*-gal), is a common enzyme reporter providing ultrasensitive results with high dynamic range.[88]

In this review we are focused on two mainstream fields for which the cellular sensors were developed, biomedical applications and environmental applications. In biomedical applications, cellular sensor-device based theranostic approaches for infectious disease conditions, cancer, and metabolic diseases are covered from the latest studies, whereas in environmental applications, only cellular sensors for heavy metal sensing are considered.

Cellular Sensors for Biomedical Applications. *Theranostic Applications for Infectious Diseases.* Infectious diseases are one of the leading causes of death worldwide. The spread of antibiotic resistance among bacterial pathogens and increased mortality rates due to untreatable infections of multi-drug-resistant microorganisms yields to serious worldwide health problems.[92] Besides treatment methodologies, noninvasive, rapid, and accurate diagnosis of infections is of considerable importance for optimization of treatment and survival of patients.[93,94] In response to all those necessities for theranostics of infectious diseases, computation tools of synthetic biology

hold great potentials for easily programmed sensor modules in the field of whole-cell biosensors. The central idea of whole-cell-biosensors for theranostic approaches is depicted in Figure 3B.

To address drug resistant bacteria in persistent biofilms, Lu et al. proposed a phage-based strategy to attack and clear bacterial biofilm matrix effectively.[95] The authors engineered *E. coli* specific bacteriophage T7 to express DspB, an enzyme hydrolyzes the exopolysacchride (EPS) adhesin *β*-1,6-*N*-acetyl-D-glucosamine, using protein expression machinery of host upon bacterial infection. Following the host lysis, released DspB degrades the EPS component of the biofilm and provides a viable option to fight against biofilms in infectious diseases. In order to fight against *Pseudomonas aeruginosa* infection, Saeidi et al. designed a QS based on/off switch in *E. coli*, that opens the killing circuit in response to AHL 3OC12HSL, QS molecule of *P. aeruginosa*.[96] In the killing circuit, the LasR transcription factor is expressed constitutively in addition to Pyocin S5, a strong bactericide against *P. aeruginosa* ln7 strain, and E7 lysis protein which are expressed under the control of pLuxR promoter. pluxR is activated upon binding of LasR-3OC12HSL complex. After a certain threshold, E7 protein lyses

E. coli and releases Pyocin S5 that damages the pathogen and inhibits biofilm formation up to 90% in a mixed culture. Another study from the same group improved the killer bacteria system, turning *E. coli* into a pathogen seeker, reprogramming the chemotaxis of *E. coli* in response to AHL as an on/off switch, as summarized in Figure 4A.[97] The system utilizes the ΔcheZ strain of *E. coli*, deficient in motility, and redesigning the chemotaxis of the bacteria. Recombinant expression of CheZ under the control of LasR-AHL responsive promoter pLasI provides a programmable chemotaxis of *E. coli* toward *P. aeruginosa*, turning it into a pathogen seeker. In addition to the directed motility, the same on/off switch responsive to LasR-AHL complex secretes anti-biofilm peptide DnaseI to degrade *P. aeruginosa* biofilms and antimicrobial peptide microcin S to kill the pathogens released from the biofilm structure as well as the pathogens in the planktonic state.[98]

In the human microbiome, colonizing microbial cells establishes a network based on the interactions among themselves and interactions with the host immune system. Dysbiosis in the microbiome are associated with several diseases, such as ulcerative colitis or obesity. Therefore, microbiome engineering is of major importance for treatment of dysbiosis related complications.[99] Programming of commensal bacteria using synthetic gene circuits for microbiome engineering holds great potential for theranostic applications. Kotula et al. constructed a memory circuit in *E. coli* based on the cI/Cro genetic switch of phage lambda.[6] The system is designed to start in the cI state and transcription of *cro* gene is controlled by a trigger element. Therefore, exposure of the trigger element in the mouse gut causes *E. coli* to switch into Cro state. Mimee et al. introduced genetic parts for the human commensal *Bacteroides thetaiotaomicron* to generate permanent integrase based genetic memory devices.[100] A consecutive array of integrase sites is used to record expression of multiple integrases in response to different stimuli. In addition, they implemented genetic NOT gates based on CRISPRi mediated gene knockdown of a reporter gene upon induction via an external stimulus. Those designs can facilitate surveillance of the biomarkers in the gut microenvironment for diagnosis and long-term therapeutics.

Synthetic Biocomputation for Theranostic Applications for Cancer. Cancer is one of the major causes of death, affecting millions of people each year. Since the new cases are also expected to rise within the next decades, there is a significant effort to find cures for cancer. Current cancer therapies are generally based on conventional approaches such as chemotherapy, that have several major drawbacks such as toxicity to normal tissue, incomplete tumor targeting, inadequate tissue penetration, and limited toxicity to cancer cells. In order to propose enhanced theranostic applications, synthetic biologists developed several approaches for microbial and mammalian whole-cell sensors built on synthetic gene circuitry.[101]

Bacterial cells with synthetic genetic circuits have been widely used as whole cell biosensors for cancer diagnosis. One of the earliest genetic computation tools to sense is an on/off switch. Alloush et al. demonstrated an *E. coli* biosensor as a screening system to predict drug response in leukemia.[102] Since the main drug to treat acute myeloid leukemia, Ara-C (cytosine arabinoside), fails treatment in some patients, screening of the drug response in patients prior to treatment is of critical importance. Alloush et al. designed a microbial sensor where the patient's blood is lysed and exposed to the bacterial sensor after drug treatment. The sensor is designed to switch on the bioluminescence generation when Ara-CTP is converted to Ara-C. Therefore, the ratio of bioluminescence reflects the Ara-CTP concentration in the patient's blood, which represents the patient's ability to respond drug Ara-C before proceeding with chemotherapy.

Early detection of cancer is a critical step for success rates of anticancer therapies. Advances in genetic circuitry and synthetic biology offer practical solutions for rapid and easy diagnosis of cancer utilizing bacterial cells. Danino et al. proposed a simple method for diagnosis of liver cancer from urinary samples, called PROP-Z.[103] In the study, tumor colonizing probiotic *E. coli* Nissle 1917 (EcN), constitutively expressing LacZ, is designed into an AND gate, and then orally administered to mouse tumor models. The tumor colonization of bacteria is collected as an input via LacZ production. After 24 h following bacterial administration, the engineered probiotic colonizes liver tumors and cleared from the healthy organs. At that point, the second input LuGal, a lacZ substrate that produces luciferin upon cleavage, is administered to the animal models via systemic injection. The output of the AND gate can be collected from the urine as luminescence, as abstracted in Figure 4B. The urine signal generated by oral delivery of PROP-Z and systemic injection of LuGal provides a sensitive and easy-to-use early identification tool for liver cancer. In addition, imaging of tumors through methods such as magnetic resonance imaging (MRI) and optoacoustic deep tissue imaging (MSOT) is a powerful diagnostic approach to avoid false positive or false negative results. Kircher et al. reported the Melanogenic Vaccinia Virus based reporter system for MRI based on a doxycycline inducible synthetic gene circuit.[104]

In addition to cancer diagnosis efforts, microbial whole-cell biosensor and synthetic gene circuits have been broadly used for cancer therapy in synthetic biology, by reason of intrinsic tumor colonization ability of several bacterial species that causes natural cytotoxicity to tumors and decrease in tumor size. To increase the effectiveness of microbial cancer therapy, there is an immense effort to integrate synthetic gene circuitry with tumor colonizing bacteria.[101] In this aspect, Swofford et al. utilized tumor colonizing *Salmonella* to design a tumor destroyer that benefits from QS-controlled gene circuitry with the lux-QS system from *Vibrio fischeri* to avoid damage to healthy tissue.[105] In the system, expressions of LuxI, LuxR, reporter protein, and toxic molecules are controlled under pLuxI promoter. LuxI produces the quorum sensing agent 3OC6HSL and at a certain bacterial density, 3OC6HSL level reaches a threshold to switch on expression from pLuxI promoter. Therefore, expression of proteins is controlled via a density-dependent switch to limit the cytotoxicity to tumors densely colonized by *Salmonella*. Constitutive expression of reporter protein GFP revealed that *Salmonella* colonizes not only tumors, but liver as well, only at lower bacterial density. QS-*Salmonella* circuitry remained off in the liver tissue, overcoming the concern of nonspecific toxicity. In a related study using an attenuated *Salmonella typhimurium* strain, Zheng et al. designed an on/off switch secreting *Vibrio vulnificus* flagellin B (FlaB) in response to an external stimulus, L-arabinose.[106] FlaB activates the immune response via Toll-like receptor 5 (TLR5) signaling pathway and, therefore, has an excellent utilization capacity for cancer immunotherapy. Following the initial bacterial colonization of tumors, L-arabinose is introduced into mouse models to switch on FlaB expression. FlaB secreting *Salmonella* demonstrated strong

tumor-suppressive effects, appearing as an effective agent for cancer immunotherapy.

Besides microbial theranostic approaches for cancer, cell based therapies also reveal promising results. One such approach is engineering chimeric antigen receptors (CAR) to direct T-cells to recognize and destroy cancer cells.[107] In order to control toxicity of T-cells with uncontrollable excessive expression, Wu et al. proposed an AND gate based on a split receptor with antigen binding domain and intracellular signaling domains expressed as different polypeptides.[108] In another related study that targets cancer cells by CARs, Roybal et al. considers that tumor specific single antigens are very rare and the availability of targeting antigens on healthy tissues causes severe side effects.[109] In an attempt to solve this problem, an AND gate approach was proposed to activate dual-receptor T-cells in response to two different antigens. In the sequential AND gate design, synNotch receptors specific to one cancer antigen controls expression of CARs responsive to another antigen. In general, antigen binding to synNotch receptors triggers cleavage of a transcriptional activation domain to control gene expression under tetracycline response elements (TREs). TREs are activated by synNotch receptor against mesothelin antigen is expressed by a promoter with TREs. In the proposed sequential AND gate, T cells are only activated in the presence of both cancer antigens, CD19 and mesothelin. Animal studies revealed that the circuitry provides tumor targeting only in the presence of both antigens and is harmless to bystander tissues with only one of the targeted antigens. Such combinatorial antigen targeting designs have great potential to enhance the specificity to therapeutic T cell approaches.

Nissim et al. proposed an AND gate for the accurate discrimination of cancer cells from healthy cells.[110] In their design, a transcription factor is split into two as binding domain (BD) and transcription activation domain (AD) to control expression of output killer protein, TK1. Then, AD and BD expression is controlled via two different native TFs from native promoters. Those promoters are selected from a pool of highly active promoters in cancer cell lines to discriminate and eradicate tumor cells with improved precision. There are several other studies that report different logic gate operations using different cancer biomarkers as inputs. Xie et al. reported a multi-input logic gate design to discriminate between specific cancer cells based on endogenous miRNA level signatures, described in Figure 4C.[111] The classifier gene circuit combines the sensory information from several markers into a specific state to determine the expression state of apoptosis inducing protein hBax. They designed two main circuits as classifier adding repeats of selected miRNA sequences to 3′ UTR region of output mRNA, HeLa low and HeLa high sensors according to the relative levels of specific miRNAs in HeLa cells compared to healthy cells. HeLa high sensors control expression of repressors of hBax, the high level of miRNAs blocks the repressor and initiate apoptosis via expression of hBax. In contrary, HeLa low sensors directly control expression of hBax, where high levels of miRNAs inhibit hBax expression and therefore inhibit apoptosis. They showed that multi-input logic gates based on endogenous miRNA levels of cancer cells performs as effective classifiers to accurately diagnose and kill cancer cells.[112]

Theranostic Applications for Metabolic Diseases. Treatment of metabolic disorders is mainly based on small molecule drugs that require daily uptake, and dosage regulations are mainly empiric. In addition, diagnosis of most metabolic disorders is based on biomarkers such as metabolites or proteins, and their detection is limited by the low concentration of biomarkers in complex biological environment. Cells integrated with genetic circuits that can sense and treat metabolic diseases provide novel theranostic strategies. Kemmer et al. designed a mammalian on/off switch to control urate levels in blood that fluctuates in disorders such as tumor lysis syndrome and gout. HucR is a repressor of hucO operator site in *Deinococcus radiodurans* R1 and increased uric acid levels releases HucR from hucO, initiating transcription in response to uric acid. In this study, expression of urate oxidase is linked to hucO activation, reducing the urate levels to nonpathologic values in the bloodstream. Since the system is shutdown under pathological urate concentrations, it can monitor and provide homeostasis.[114] Another important element of blood homeostasis is glucose levels. Ye et al. designed an optogenetic device to maintain glucose homeostasis in bloodstream, as depicted in Figure 4D. In the design, the signal transduction through melanopsin pathway that results in Ca^{2+} influx, is connected to the nuclear factor of the activated T cells (NFAT) signaling pathway via calmodulin, a calcium sensor protein that activates a conformational change in NFAT to induce gene expression. The blue light induced expression of shGLP-1 resulted in the improvement of blood glucose homeostasis in diabetic animal models.[113]

Many metabolic disorders are linked to each other and develop complexions that are hard to address via a one-disease-one-drug approach. Metabolic syndrome, for example, is a collection of distinct pathologies such as hyperglycemia, obesity, hypertension, and dyslipidemia. In order to provide a coordinated collective treatment for metabolic disease, Ye et al. designed a theranostic strategy built on a synthetic signaling cascade that combines drug and gene based therapies to treat all the main pathologies in metabolic syndrome. Guanabenz is an antihypertension drug that activates alpha-2-selective adrenergic receptors and decreases blood pressure. Guanabenz also interacts with trace amine- associated receptor (TAAR1). In the proposed circuit, Guanabenz sensing with TAAR1 is linked gene activation via pCRE promoter and phosphorylated CREB1 interaction, through a cAMP dependent signaling pathway. In the study, expression of fusion protein GLP-1-Fcm IgG-Leptin is activated upon exposure to Guanabenz. The fusion protein comprises enhancer effect of GLP1 for insulin secretion to provide blood glucose homeostasis and the body weight controlling effect of leptin to lower cholesterol and free fatty acid levels. Treatment with Guanabenz results in a decrease in blood pressure, as well as activating the sensor system that acts as an ON switch for the expression of fusion protein to target hyperglycemia, obesity, and dyslipidemia. This designer cell approach to target several diseases at a time through genetic logic operations and sensors holds great potential to improve theranostic applications for multifactorial diseases.[115]

Cellular Sensors for Environmental Monitoring. Environmental pollution is related with human activities such as industrialization. Inadequate disposal policies of chemical waste or spills can cause release of inorganic, such as heavy metals, or hazardous organic toxic materials to the environment.[116] These pollutants such as heavy metals could be highly stable in the environment.[117] Due to their low biodegradability, heavy metals accumulate in living organisms and the environ-
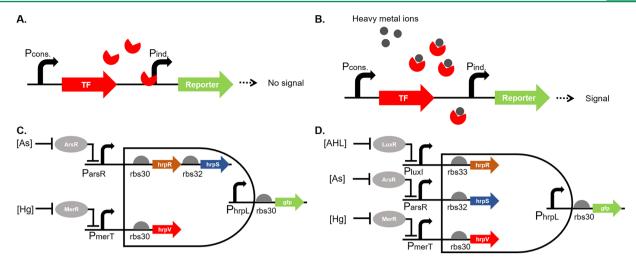
Figure 5. Environmental monitoring with engineered whole cell biosensors. A and B show the general scheme of heavy metal sensor systems. In the absence of heavy metal ions, TF blocks the output (A), while in the presence of heavy metal ions the sensor gets activated accordingly (B). C. 2-input AND gate logic operation based on *hrp* operon based on arsenic and mercury sensing. D. 3-input based AND gate logic operation on *hrp* operon based on QS, arsenic, and mercury sensing. C and D are reprinted with permission from ref 125. Copyright 2014 Creative Commons.

ment; this situation results in a broad range of drawbacks such as ecological toxicity or human diseases.

In conventional environmental monitoring, samples collected from the environment are subjected to chemical and physical analysis to determine the actual composition. However, this approach requires analytical instrumentation as well as an expertise which makes the whole process expensive, time-consuming, and slower. On the other hand, this method does not give all the answers about chemical pollutants in the environment such that bioavailability or effects on organisms are two missing pieces of the whole picture. Thus, small whole-cell biosensors are good candidates in environmental monitoring studies to control bioavailability, toxicity, and genotoxicity of pollutants on living systems.[118]

Cellular biosensors are widely used to detect many environmental heavy metal pollutants. Many environmental biosensors focus on figuring out the toxicity of the sample rather that detecting what the sample is. These biosensors are designed with a promoter–TF couple and a reporter.[118] In Figure 5A and B Tauriainen et al. developed a cadmium sensor via using regulatory protein CadC and cadC promoter couple together with firefly luciferase genes as a reporter in *Bacillus subtilis* cells.[119] This whole cell biosensor responds to cadmium (Cd), lead (Pb), and antimony (Sb) with nanomolar sensitivity. Likewise, Ivask et al. developed a whole cell biosensor in order to respond to zinc (Zn), Pb, and Cd by using ZntR regulatory protein and its promoter couple zatAp in *E. coli* cells.[85,88] There are many other sensors to detect a diverse range of heavy metal ions. One of them is arsenic that is a highly toxic heavy metal and accumulates easily in organisms due to its nonbiodegradable nature. In order to detect arsenic, the arsenic detoxification ars operon from *E. coli* is commonly used. Wu et al. defines the transcriptional regulator of this operon ArsR. The working mechanism of this sensor is based on the binding of ArsR transcriptional protein to its promoter couple arsR promoter.[120] If arsenite is added to the system, the reporter is transcribed upon dissociation of ArsR due to its interaction with arsenite.[121] This sensor system was improved by redesigning promoters of regulatory gene ArsR and reporter protein.[122,123] Wang et al. connected *E. coli* based logic gated cellular biosensors in AND gate fashion in order to sense

arsenic, mercury, copper, zinc ions, and bacterial quorum sensing molecules in an aqueous environment and forming a quantitative fluorescent output. Then they create a triple-input logic gate based on the knowledge they gather from the previous system. In the triple-input logic gate, they create two populations are able to sense As^{3+}, Hg^{2+}, and Cu^{2+} levels and wired by synthetic cell–cell communication module.[124] Later, Wang et al. constructed a new AND gate logic operation based on *hrp* operon in *P. syringae*. In their study, they built 2- and 3-input AND gates whose inputs are As^{3+}, Hg^{2+}, and AHL molecule. In the 2-input AND gate, HrpR and HrpS are produced from a noninverted As^{3+} input as under the control of the arsenic responsive promoter, while HrpV is produced from an inverted Hg^{2+} input as under the control of mercury responsive promoter. Thus, output will be ON only when the system is induced with arsenic, coupling HrpS-HspR activators. However, in the presence of both inducers, HspS will form a complex with HrpV blocking the *hrpL* promoter, which encodes the output. This 2-input AND gate was extended with a 3-input AND gate operation, and HrpS and HrpR expression is divided in two parts such that HrpS was kept under the control of arsenic responsive promoter while HspR was put under the control of QS responsive promoter. Both AND gates are finely tuned by changing RBS in each unit so that the system eliminates almost the entire leakage and operates in low ion concentrations upon induction.[125]

Although microbial sensors are a valuable solution for routine analysis of heavy metals in order to monitor environment, they have some restrictions such as underperformance in sensitivity[126] and selectivity especially in multiplex detection,[127] providing population level data which is directly affected by genotypical and therefore phenotypical heterogeneity of the population[128] and stochastic protein expression.[129] Yet, these limitations could be obviated by a combination of micro/nanotechnologies and microbial biosensors.[130] The most common engagement of whole cell biosensors and micro/nanotechnologies is the application of these biosensor systems into microfluidic systems. Kim et al. developed a microfluidic system which can sense a concentration gradient of heavy metal ions in combination with a whole cell biosensor for Pb and Cd. By comparison of the dynamic range capacity of such a system

with the conventional methods, a 3- or 4-fold change in sensitivity is observed. The reason for this improvement is explained as the combination of many factors, microfluidic chambers supplying fresh growth media and heavy metal ions as inducers, as well as removing the waste that is secreted from the cells. Although there is a drawback such as narrow dynamic range, this system has advantages such as cost-effectiveness, time savings, and suitability for high throughput analysis of multiple samples over conventional methods.[126] Volpetti et al. developed a biodisplay platform based on a microfluidic system with engineered cells. This biodisplay system allows 768 independently programmable cell cultures to screen any analytes. In the study, they scanned the response of engineered bacterial cells cultivated in a biodisplay platform against varying arsenite and arabinose concentration in time. They state that biodisplay shows highly sensitive results. On the other hand, the biodisplay system can also be said to be environmentally friendly since it prevents the escape of genetic materials or other chemicals to environment.[131]

BIOSAFETY AND REGULATIONS FOR APPLICATION OF WHOLE CELL BIOSENSORS TO THE FIELD

Whole cell biosensors give promising results in the field with many benefits, but there are some concerns about using these systems in the field directly because these systems are classified as genetically modified organisms (GMOs). From the biosafety point of view, synthetic biology-enabled whole cell biosensors could be a risk at different levels if they escape their enclosed area such as microfluidic chamber or are released to the environment. Therefore, risk governance is applied to this field. In light of these regulations, researchers develop some strategies to minimize the risks in synthetic biology. They started with two main strategies: inhibition of the spread of recombinant and synthetic DNA to environment and other organisms via Horizontal Gene Transfer (HGT) and preventing the pollution of habitat via overrunning of engineered microorganisms.[132] For this purpose, researchers use plasmids as vectors to carry synthetic circuits rather than introducing the genome. Moreover, designing a vector which does not share high similarity between mobile elements or host genome is another safety precaution during design.[133] Even though these precautions reduce the risk, there is also a chance to take up plasmid via natural transformation processes of other organisms.[134] To overcome to this issue, addition of regulatory elements to the system controls the essential gene expression,[135] developing microbial kill switches[136] and other vector suicide strategies.[137] As a novel strategy, orthogonal biology or xenobiology is used.[138] The main motivation of this strategy is based on the creation of a completely novel genetic code and a system which does not interfere with natural systems of microorganisms. Construction of genetic code with Xeno Nucleic Acids (XNAs) makes this genetic code invisible to natural systems and eliminates the spread risk.

CONCLUSIONS AND FUTURE PERSPECTIVE

Recent advancements in synthetic biology and biocomputation tools have led to the design of novel genetic circuits that can conduct a variety of logic operations. Numerous genetic switches based on TF-promoter pairs, RNA regulators (riboswitches, toehold switches etc.) and recombinases have been generated to construct digital circuits in cells. In addition,

several analog circuits have been created in order to respond to a wide dynamic range of inputs. Furthermore, genetic circuits that can handle analog-to-digital or digital-to-analog conversions as well as circuits that can compare, count, and process multiple inputs have been reported. Cellular biosensors, compared to the conventional biosensors, that use biological elements (e.g., enzymes, antibodies, or nucleic acids) as recognition layers to detect analyte of interest and respond with a measurable output, provide distinct features such as self-replication, ease of reproducibility, flexibility of usage, and low cost. Implementation of biocomputation tools into whole cell biosensors holds great potential for a variety of applications. Various cellular sensors have been developed to fight against infectious diseases, cancer, and metabolic diseases. Additionally, several biosensors with genetic circuits have been designed for environmental monitoring and detection of pollutants such as heavy metals. Despite the advantages of whole cell biosensors in many fields, there are still some concerns on biosafety and regulation issues.

Building complex genetic networks is a difficult task that requires novel parts for the construction of functional biocomputation devices. This is still a challenge to program innovative cellular functionalities. TF-promoter couples are widely employed to build cellular sensors with engineered genetic circuits. Therefore, identification of new orthogonal TF-promoter pairs is of great importance to increase the sensitivity and specificity of circuits. Additionally, many cellular biosensors have been designed with different functions, but most of them are not tested for orthogonality or cross-talk for multiple analytes. Therefore, it is necessary to build orthogonal circuits to improve the efficiency of these systems, especially for multianalyte sensor designs. Selection and screening of orthogonal TF-promoter pairs from nature is a common approach. Furthermore, next generation sequencing based transcriptome analysis has the capacity to reveal new orthogonal TF-promoter sets. Construction and screening of promoter libraries for the selection of the best performing mutants or protein engineering applications to build libraries of mutant TFs for improved specificity are also efficient approaches. Similarly, identifying currently available genetic networks through "omic"technologies such as genomics, transcriptomics, proteomics, and metabolomics can push the limits of the currently available whole cell biosensors. Exploring and understanding the mechanism of new pathways have great potential toward building next generation cellular sensors.

Currently available RNA switch systems can address many challenges, but they are not diverse enough to detect any type of analyte. Discovery of original RNA based switches responsive to the targeted analytes is significant for construction of novel genetic circuits. Therefore, it is necessary to extend the toolbox of RNA aptamers. Exploration of large RNA aptamer libraries with multiplexed screening systems is a powerful tool to address those necessities. Furthermore, modeling the interactions of RNA aptamers with the analytes and constructing the libraries based on these models instead of creating random libraries can be favorable in terms of time and cost. Additionally, random or directed evolution approaches can be implemented to increase the selectivity and sensitivity of the RNA based switch molecules.

Whole cell biosensors are cost-effective, easy-to-use, and stable sensor systems. Implementation of biocomputation tools and aforementioned advancements in genetic circuit design has the potential to reinforce the feasibility of cellular sensors in

terms of recognition systems. However, without efficient monitoring systems, the yield and efficacy of advanced cellular biosensors will be diminished. Consequently, adequate monitoring of the cellular sensory responses requires advanced quantitative technologies. For example, microfluidic systems are effective tools to detect multiple analytes at the same time. Multiplexing can be achieved via creation of large cell arrays by delivering individual cells to the targeted spots on a chip. Such cellular sensor chips will very useful for on-site applications at reasonable prices.

Synthetic biology is a fast-growing field, and with current possibilities in gene synthesis, modeling, and implementation, innovative cellular sensors can be established for *in vitro* and *in vivo* applications. In this regard, cellular sensors can be coupled with electronic chip technologies. In addition, mammalian cell based cellular sensor can be transplanted in tissues for real time monitoring and tissue regeneration applications, as well as in targeted delivery drug delivery approaches. Therefore, cellular biosensors with implemented genetic circuits have the potential to serve as microrobots that can sense and respond to environmental cues for numerous biomedical and environmental applications.

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Notes
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■ VOCABULARY

Genetic Circuit, A cluster of DNA sequences that encodes for functional proteins or regulatory RNAs.; Genetic Logic operation, Any operation event controlled by genetic elements and includes genetic parts these events obey Boolean operators. AND, OR, XOR, NOT, etc. are some representative examples of logic operation phenomenon.; Riboswitch, Small regulatory RNAs which control the gene expression via their unique hairpin structures. Riboswitches hinder ribosome to initiate translation.; Theranostic, A combination of diagnosis and therapeutics for personalized medicine.; Biosensor, An analytical device that used biological elements as recognition layers (i.e., enzyme, antibody, DNA or RNA aptamers) and a detectable signal (i.e., colorimetric, voltamertic, amperometric) is formed upon interaction of the target analytes with recognition layers.

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