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MINI REVIEW

Microbial biosensors for diagnostics, surveillance and epidemiology: Today's achievements and tomorrow's prospects

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Abstract

Microbial biosensors hold great promise for engineering high-performance, field-deployable and affordable detection devices for medical and environmental applications. This review explores recent advances in the field, highlighting new sensing strategies and modalities for whole-cell biosensors as well as the remarkable expansion of microbial cell-free systems. We also discuss improvements in robustness that have enhanced the ability of biosensors to withstand the challenging conditions found in biological samples. However, limitations remain in expanding the detection repertoire, particularly for proteins. We anticipate that the AI-powered revolution in protein design will streamline the engineering of custom-made sensing modules and unlock the full potential of microbial biosensors.

Microbially derived biosensors can be engineered to detect a wide variety of molecules using diverse sensing modules. These biosensors have numerous applications in human healthcare, including diagnostics, therapy and environmental monitoring (Figure 1).

EXPANDING THE SENSING REPERTOIRE OF WHOLE-CELL BIOSENSORS

Whole-cell bacterial biosensors (WCBs) have tremendous potential in biomedical applications such as diagnostics and health monitoring, enabling biomarker detection in clinical samples or even within the body. Recently engineered WCBs have been optimized by applying new synthetic biology tools to sense molecules within a clinically relevant range of concentrations (McNerney, et al., 2019a; Chang et al., 2021; Courbet et al., 2015; Watstein & Styczynski, 2018). Several labs also demonstrated the possibility of using bacteria for biomarker detection in vivo (Daeffler et al., 2017; Gurbatri et al., 2024; Inda et al., 2019; Riglar et al., 2017).

WCBs also have a large potential for environmental monitoring of hazardous substances, such as heavy metals: mercury, arsenic or copper (Saltepe et al., 2022; Wan et al., 2019).

Nonetheless, the popularization of WCBs has been hindered by the lack of newly discovered small molecule sensors. Experimental screening of natural microbial isolates to discover novel sensing modules has been demonstrated and represents a promising approach (Grazon et al., 2020). In addition, the deluge of sequencing data has generated a large source from which several new transcription factors (TFs) detecting small molecules of interest can be discovered. Recently, searchable databases have been published (Delépine et al., 2016; d'Oelsnitz et al., 2024), as well as bioinformatic pipelines for identifying new small-molecule responsive transcriptional regulators. These

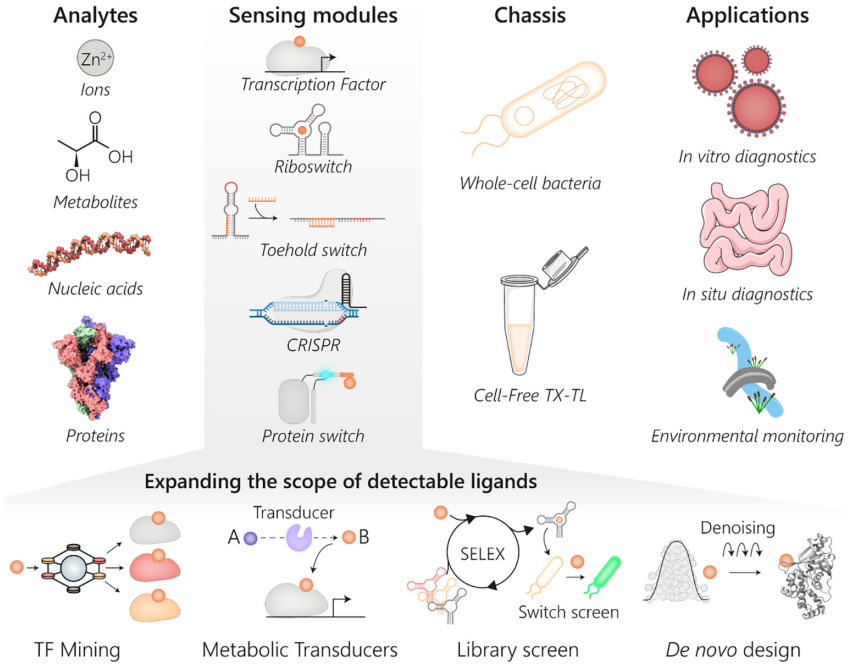

FIGURE 1 Bacterial-based biosensors have been engineered to detect a broad range of molecules through the use of different sensing modules. These modules are generally embedded into a specific chassis that is the most suitable for one particular application. Recently, efforts have been put towards expanding the space of detectable ligands via different approaches including mining of transcription factors, the addition of metabolic transducers to the genetic circuit, toehold switches for nucleic acid detection, aptamers-based riboswitches and de novo designed sensor proteins. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; SELEX, Systematic Evolution of Ligands by Exponential Enrichment; TF, transcription factor; TX-TL, transcription translation.

approaches take advantage of classical features associated with ligand-responsive transcription regulators controlling metabolic cluster activity: (i) self-regulation of TF expression and (ii) localization of their gene in close proximity to the metabolic cluster they regulate (Hanko et al., 2020, 2023). Software tools to identify TF binding sites are also extremely useful for finding natural promoters or building hybrid ones containing TF operators. The Snowprint pipeline has enabled the discovery of previously uncharacterized regulators repurposed for sensing diverse ligands in *Escherichia coli* (*E. coli*), including tetrahydropapaverine, geraniol, olivetolic acid and ursodiol (d'Oelsnitz et al., 2024). Recent advancements in deep learning algorithms have also contributed to the development of accurate prediction tools such as DeepTFactor, PredicTF and DeepReg (Kim et al., 2021; Ledesma-Dominguez et al., 2024; Oliveira Monteiro et al., 2022).

However, using natural TFs requires repurposing existing promoters that sometimes do not work in different species, or designing and screening hybrid promoters containing operator sequences (d'Oelsnitz et al., 2024). Another approach is to decouple sensing from signalling by engineering modular receptors in which the sensing domain can be plugged into a reusable actuation domain. We recently developed such modular receptors in *E. coli* based on the DNA binding domain of CadC, a transmembrane one-component system activated by dimerization (Jung et al., 2018). Synthetic receptors responding to caffeine were built using CadC as a signalling module and a VHH recognizing this molecule as ligand binding domain (LBD) (Chang et al., 2018). Substituting the LBD with sensing domains from *Vibrio cholerae* enabled the detection of bile salts, a biomarker of liver dysfunction, in the serum of patients that had undergone liver transplant (Chang et al., 2021). Similar strategies have been successfully applied to two-component systems (TCS), and further extended to the discovery of ligands activating previously orphan TCS (Schmidl et al., 2019; Wang et al., 2021; Zhao et al., 2024). The intrinsic modularity of these approaches facilitates the expansion of the sensing repertoire of WCBs since they do not require a complete redesign of the biosensing system.

A recent challenge in the field of WCBs has been to unlock new sensing modalities in living bacteria. Biomarkers of interest for medical or environmental diagnostics include small molecules, but also nucleic acids and proteins. Yet, over several decades, WCBs were limited to the detection of small molecules that could cross cellular membranes. For extracellular DNA detection, recent approaches have harnessed naturally competent bacteria. Notably, *Bacillus subtilis* was engineered to detect extracellular DNA from bacterial pathogens using homologous recombination linked to growth and fluorescence (Cheng et al., 2023; Cooper et al., 2023; Nou & Voigt, 2024). *B. subtilis* was also engineered to detect human sequences

with particular single-nucleotide polymorphisms (SNPs) using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) interference (Cheng et al., 2023; Cooper et al., 2023; Nou & Voigt, 2024). The same principle was extended to in vivo applications by Cooper et al. to detect tumour DNA using *Acinetobacter baylyi*, a highly competent bacteria, that uptakes cancer DNA which recombines with the bacterial sensor DNA to express an antibiotic resistance gene (Cheng et al., 2023; Cooper et al., 2023; Nou & Voigt, 2024). The ability to detect specific DNA sequences in the bacterial environment both in vitro and in vivo offers tremendous possibilities for diagnostics and therapeutic applications.

Finally, extracellular protein detection remains a highly challenging task for bacteria biosensors, as proteins cannot easily enter the cell to interact with intracellular sensing elements. One way to circumvent this challenge was proposed by Kylilis et al. that used bacteria displaying nanobodies at their surface and which aggregate in the presence of the target protein (Kylilis et al., 2019), recapitulating well-known agglutination assays. The authors demonstrated the detection of fibrinogen, a biomarker of cardiovascular diseases and inflammation, in human plasma, with a detection limit as low as 10 pM. This agglutination system doesn't require advanced equipment, an important feature for point-of-care testing. Nevertheless, to date, no system linking the detection of extracellular proteins to the activation of gene expression has been reported. This is a critical requirement for reporter-based assays and for in vivo biomarker-triggered therapeutic production. Engineering such signal transduction pathways remains a frontier in the field. Unsolved challenges include engineering membrane proteins leading to an actuation signal inside the cells upon protein binding (e.g. allosteric change or dimerization), managing the tick cell wall in gram-positives or signal transduction across the periplasm for gram-negative. Given the importance of protein biomarkers, we expect this area to be the focus of intense development in the near future.

UNLEASHING THE POTENTIAL OF CELL-FREE BIOSENSORS

Cell-free transcription-translation (TX-TL) systems have undergone remarkable advancements in recent years, enabling the execution of complex genetic circuits in vitro (Garenne et al., 2021). Cell-free systems (CFS) offer several advantages over traditional cell-based assays. They allow the detection of molecules that do not cross cellular membranes and are less susceptible to compounds toxic to living cells. Their composition can be easily tailored to suit specific needs, they are inexpensive to produce, and can be deployed in the field. Importantly, they circumvent regulatory concerns associated with genetically modified organisms (GMOs), simplifying their implementation. Cell-free systems thus hold great promise in delivering robust and affordable point-of-care diagnostics (Voyvodic & Bonnet, 2020). Since the COVID-19 pandemic, significant efforts have been put into the development of sequence-specific nucleic acid detection systems. Amalfitano and colleagues used a toehold switch to detect SARS-CoV-2 RNA isolated from patient nasopharyngeal swabs, and embedded their biosensor into a glucose meter, to provide a user-friendly interface (Amalfitano et al., 2021). Toehold switches were also harnessed to develop paper-based tests to detect Zika and Chikungunya viruses in human serum (Karlikow et al., 2022). Other nucleic acid detection strategies have exploited CRISPR systems, in particular Cas12a and Cas13a, offering sensitivities down to attomolar concentrations of target sequences (Broughton et al., 2020; Gootenberg et al., 2017; Karlikow et al., 2023). Nguyen and co-workers went a step further and integrated their CRISPR-based sensor into a wearable mask, enabling non-invasive SARS-CoV-2 detection within 90 min (Nguyen et al., 2021).

In addition to nucleic acids, CF-based diagnostics have been applied to diverse molecules. Protein-binding riboswitches were leveraged for the detection of human monomeric C-reactive protein, human interleukin-32γ and phage MS2 coat protein (Vezeau et al., 2023), as well as antibodies spiked in human serum samples (Patino Diaz et al., 2022). Transcription factor-based diagnostics were also developed for 3-oxo-C12-HSL, a *Pseudomonas aeruginosa* biomarker in sputum samples from cystic fibrosis (Wen et al., 2017), deoxycholic acid in serum and faeces samples (Beabout et al., 2023), and to monitor zinc levels in blood (McNerney, et al., 2019b).

Although industrial activities represent an essential part of the modern world's economy, they account for the emission of a significant amount of atmospheric, soil and water pollutants, often threatening entire ecosystems. Providing rapid, low-cost, and field-deployable methods to monitor pollutant concentrations in environmental and food samples is one of the key promises of cell-free synthetic biology. Notably, Jung and colleagues developed the ROSALIND (RNA Output Sensors Activated by Ligand Induction) platform and demonstrated the detection of various water contaminants including antibiotics, heavy metals, as well as other classes of molecules used as additives or disinfectants (Jung et al., 2020, 2022). To ensure the compatibility of their approach with the detection of protein synthesis inhibitors, the authors used an output that only required transcription—a fluorescent RNA aptamer. Other examples of CF biosensors for water contaminants monitoring include fluoride (Thavarajah et al., 2020), atrazine (Silverman et al., 2020), mercury and gamma-hydroxybutyrate (Gräwe et al., 2019). A

key feature of cell-free-based sensors is their ability to sustain freeze-drying, which facilitates their storage and distribution to remote areas.

IMPROVING MICROBIAL BIOSENSOR OPERATION IN COMPLEX MATRICES

One of the main challenges for real-world usage of microbial biosensors is ensuring their performance in complex environments. Clinical and environmental samples can present strong matrix effects affecting sensor activity, often not evaluated during sensor development. Several paths are possible to optimize whole-cell and cell-free biosensor operation in complex matrices. Sample preprocessing, including dilution, filtration, treatment with RNAse inhibitors, heat inactivation, together with encapsulation strategies, is crucial for ensuring functionality in clinical samples (Beabout et al., 2023; Boyd et al., 2023; Courbet et al., 2015; Voyvodic et al., 2022; Watstein & Styczynski, 2018; Zúñiga et al., 2022). New output modules such as pigments (McNerney, et al., 2019a) or electrical current produced by engineered electron transport chains have also been used (Atkinson et al., 2022). Operation in complex matrices can also be optimized by applying additional engineering cycles to improve sensor properties such as limit-of-detection, signal output strength and fold-change. For instance, directed evolution of the periplasmic domain of the TcpP receptor in *E. coli* enabled robust performance for endogenous bile acid detection in serum and faecal samples (Chang et al., 2021; Zúñiga et al., 2022). Promoter engineering allowed bypassing interference from non-target molecules and improved biosensor dynamic range for the detection of endogenous thiosulphate, tetrathionate (Daeffler et al., 2017) or L-lactate in 3D tumour spheroids (Zúñiga et al., 2021).

In vivo monitoring of molecules also requires a stable performance over time. This was achieved via chromosomal integration for a tetrathionate-responsive memory device in *E. coli* (Riglar et al., 2017). The sensor device retains the memory of tetrathionate exposure in the gut, analysed by faecal testing, and detected tetrathionate in both infection-induced and genetic mouse models of inflammation over 6 months. Another approach is to couple microbial biosensors with electronic devices as in the work of Mimee and colleagues (Mimee et al., 2018) that combined engineered sensor bacteria with ultra-low-power microelectronics to enable in situ detection of gastrointestinal bleeding in pigs.

Operation in complex environments is a critical issue, and the sensor engineering community should develop standards and good practices enabling us to move away from idealized systems and fully translate new developments into real-world applications (Richards & deMello, 2023).

KEY TECHNOLOGICAL CHALLENGE: ENGINEERING OF NOVEL LIGAND BINDING CAPABILITIES

One of the primary hurdles facing the development of new biosensors is the need to widen the scope of detectable ligands. The diversity of detectable molecules is primarily constrained by the availability of existing sensing modules such as bacterial transcription factors or riboswitches. To circumvent this limitation, some approaches have employed metabolic transducers that convert the target molecule into a different molecule such as benzoic acid or H_2O_2, which can be detected by available transcription factors (Libis et al., 2016; Soudier et al., 2022; Voyvodic et al., 2019). Rational engineering and directed evolution methods have also been applied to switch the specificity of allosteric transcription factors to detect new aromatic compounds such as resorcinol and protocatechuic acid, as well as alkaloids (F M Machado et al., 2019; d'Oelsnitz et al., 2022; Nasr et al., 2023).

Unlike transcription factors, the generation of new riboswitches responding to a ligand of interest can be achieved through the screening of synthetic libraries using Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Tuerk & Gold, 1990), often paired with an in vivo selection step. Consequently, artificial riboswitches sensitive to histamine, naringenin and caprolactam could be selected and embedded into bacterial chassis (Dwidar et al., 2019; Jang et al., 2017, 2019). Nonetheless, the development of novel riboswitches remains limited as they not only require binding of the ligand but also conformational RNA switching. Since SELEX only focuses on finding high-affinity binders, Boussebayle and colleagues developed Capture-SELEX that integrates both high-affinity binding and conformational switching (Boussebayle et al., 2019). They demonstrated the functionality of their screening method by selecting a conformation-switching paromomycin aptamer, which was subsequently engineered into a riboswitch with a KD of 20 nM, operating effectively in vivo. Another approach, de novo rapid in vitro evolution of RNA biosensors (DRIVER) was proposed by Townshend and colleagues (Townshend et al., 2021). The authors used aptamer-coupled ribozyme libraries and an NGS-based assay (CleaveSeq) to identify ligand-responsive riboswitches in a fully automated manner. Using their method, they generated several biosensors operating in living cells. Importantly, both Capture-SELEX and DRIVER enable the selection of RNA switches against soluble ligands without the need for their chemical modifications and immobilization.

Going beyond these approaches, the recent revolutions in protein structure prediction (Abramson et al., 2024; Jumper et al., 2021) and de novo protein design (Watson et al., 2023) have opened the door to

completely synthetic protein sensors. For example, Langan and colleagues designed bioactive protein switches called LOCKR, in which a 'key' peptide can interact with a 'cage' protein inducing a large conformational change that unlocks a defined protein function (Langan et al., 2019). Design principles from the LOCKR architecture were then applied to develop the LucCage system, in which a luminescent signal is produced upon binding of the ligand of interest (Quijano-Rubio et al., 2021). LOCKR-derived biosensors have since been harnessed to detect a variety of targets such as SARS-CoV-2, HER2, BCL-2, cardiac troponin-I, parathyroid hormone (PTH), glucagon and Ras activity (Quijano-Rubio et al., 2021; Vázquez Torres et al., 2024; Zhang et al., 2024). While de novo-designed protein sensors have been primarily applied to protein sensing, emerging tools such as RoseTTAFold All-Atom hold immense promise for broadening the range of detectable chemical entities (Krishna et al., 2024). Integrating these de novo sensing domains into bacterial or cell-free platforms promises to revolutionize and streamline microbial biosensors design. One can also envision complete de novo signal transduction cascades liberated from the constraint of evolutionary history to obtain insulated sensing devices exquisitely tailored to specific needs. The next generation of microbial biosensors, supported by these groundbreaking technologies, will fully realize their potential to deliver critically needed technologies for healthcare and the environment.

AUTHOR CONTRIBUTIONS

Julien Capin: Conceptualization; writing – original draft; writing – review and editing. **Emile Chabert:** Conceptualization; writing – original draft. **Ana Zuñiga:** Conceptualization; writing – original draft. **Jerome Bonnet:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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