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Design and fabrication of field-deployable microbial biosensing devices

Hoang Long Pham [1,2,3,4], Hua Ling [1,2,3,4,5] and Matthew Wook Chang [1,2,3,4]

Biosensors could enable a wide range of applications in environmental monitoring, pathogen detection, and biomarker diagnostics. While conventional diagnostic platforms like chemical sensors and PCR-based assays are capable of highly sensitive detection in laboratory conditions, their configurations, production cost, and operational requirements are not suitable for applications beyond the laboratory. Recent advances in synthetic biology, bioelectronics, and materials sciences have paved the way for the creation of novel microbial biosensing devices, which integrate core synthetic biosensors with state-of-the-art deployment platforms to create unique biosensor products. Here, we review the latest developments in microbial biosensing devices and discuss challenges and perspectives towards realizing their broad applications in real-world settings.

Addresses
[1] NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), National University of Singapore, Singapore 117456, Singapore
[2] Synthetic Biology Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117456, Singapore
[3] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[4] Wilmar-NUS Corporate Laboratory (WIL@NUS), National University of Singapore, Singapore 117599, Singapore
[5] HH@NUS SINERGY Laboratory, National University of Singapore, Singapore 117544, Singapore

Corresponding authors: Hua Ling (hua.ling@nus.edu.sg), Matthew W Chang (bchcmw@nus.edu.sg)

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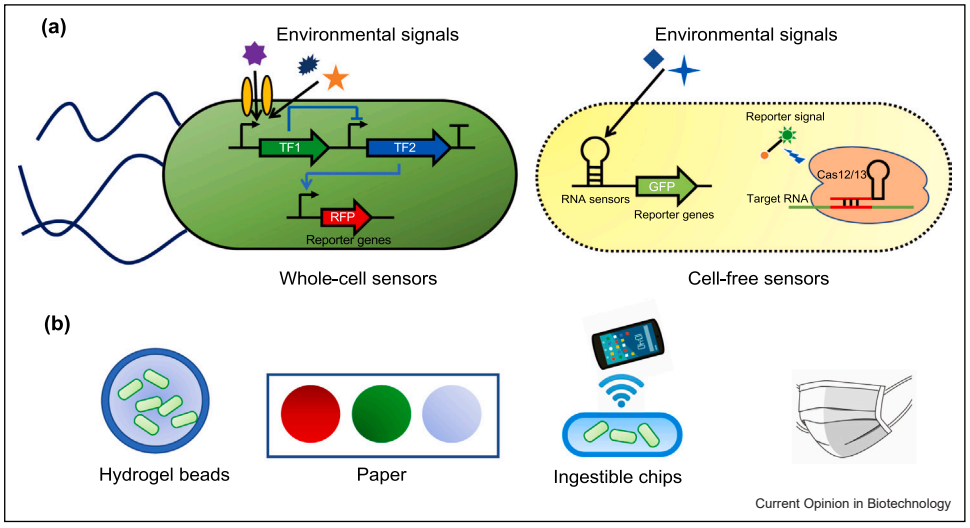
Introduction

Microbes possess a rich repertoire of genetic elements responsible for sensing in diverse ecological niches, making them attractive resources for biosensor development. In the last two decades, significant knowledge has been established in employing microbial cells and their biological components to design and construct high-performance whole-cell sensors (WCSs) and cell-free sensors (CFSs) that can detect a wide range of chemicals [1,2]. WCSs operate as living cells that generate detectable outputs upon their exposure to target chemicals. WCSs are amenable to large-scale production at a low cost by taking advantage of rapid growth and existing cultivation technologies [3,4]. However, challenges associated with their biohazard risks and requirement for viability maintenance need to be resolved to expedite the use of WCSs for practical applications. Meanwhile, CFSs are nonliving mixtures containing bioactive components that enable target detection and output generation. By removing the physical constraints of cell membranes, CFSs could facilitate the detection of cytotoxic targets, while reducing detection time and bypassing the need for live-cell maintenance and containment. Current efforts for CFSs development focus on expanding sensing targets, improving batch-to-batch consistency, and optimizing reaction costs to enable large-scale manufacturing [5,6]. Most WCSs and CFSs reported in recent years have shown promising performance in the laboratory but have yet to be broadly adapted in real-world diagnostic applications. One key challenge is to fabricate these sensors into shelf-stable products for containment, distribution, storage, transport, and utilization. There has been a strong emphasis on integrating high-performance synthetic biosensors with various deployment technologies to mitigate biohazards, facilitate rapid sampling, and enable data analysis beyond the laboratory. In this review, we discuss recent development, challenges, and perspectives of these innovative biosensing devices for applications even outside typical laboratory settings, specifically in environmental monitoring, pathogen detection, and biomarker diagnostics (Figure 1).

Design and fabrication of biosensing devices for environmental monitoring

The capacity to continuously monitor hazardous chemicals such as heavy metals, toxins, and antibiotics in food and water supply is essential for public healthcare. Accordingly, there has been steady growth in the number of WCSs-based biosensing devices developed for environmental monitoring. For example, Wan et al.

Figure 1



Design and fabrication of microbial biosensing devices for applications beyond the laboratory. **(a)** Strategies for microbial biosensor design. Whole-cell sensors can be designed from engineered intracellular transcription factors, two-component systems, RNA-based regulatory elements, or a combination of these biological tools to enable the biosensing of a wide range of chemical targets. Cell-free sensors can be designed from RNA parts or CRISPR-Cas complexes to enable the biosensing of chemicals or nucleic acids from viral targets. **(b)** Platform technologies for deploying whole-cell and cell-free sensors in various applications. Hydrogel beads are stringent biocontainment platforms that deploy live cells for environmental sensing applications. Paper is used to provide a lightweight, flexible, and stable platform for freeze-dried sensors that can be re-activated at the point of use. Ingestible chips provide versatile electronic interfaces for transmitting biological signals detected by live bacterial sensors to handheld devices, enabling their deployment in biological or environmental niches inaccessible via traditional sensing. Wearable biosensors provide real-time monitoring of biomarkers excreted by human users. RFP, red fluorescent protein; GFP, green fluorescent protein; TF, transcription factor.

successfully engineered *Escherichia coli* (*E. coli*) cells into highly sensitive WCSs capable of reporting the presence of arsenic (0.3 part-per-billion (ppb)) and mercury (2 ppb) in groundwater samples [7•]. Notably, their genetic design featured a core modular signal-amplification cascade based on an intracellular receptor (hrpR) and an activator (hrpS), which could be used to fine-tune similar transcription factor-based WCSs. They further fabricated a microbial sensor array based on the agarose hydrogel entrapment and microfluidic encapsulation of sensing cells, which could generate fluorescence signals detectable by a smartphone camera after 24-hour exposure to groundwater samples containing arsenic. However, the array's response time is relatively long, and its reproducibility after long-term storage is uncertain. Therefore, further characterization and optimization are required for such a prototype device before field deployment. In another study, Lopreside et al. engineered and deployed mercury-sensing *E. coli* strains, alongside other components as controls, on disposable paper to fabricate a three-leaf biosensor—enabling the smartphone-based analysis of mercury in water samples within 2 hours [8]. On the other hand, Liu et al. focused on detecting mercury from soil samples. They engineered mercury-sensing *E. coli* cells to produce ethylene (C_2H_4) gas as an output, which in turn, can be analyzed by a handheld C_2H_4 reader [9]. The use of a gas reporter overcame the limitations associated with using conventional fluorescence reporters in opaque samples, such as soil-containing samples. Besides monitoring hazards in the water supply, the Belkin group developed WCSs-based sensing devices for the remote monitoring of explosives in landmines to mitigate humanitarian risks. They first engineered *E. coli* cells to detect and emit fluorescent signals in response to explosives such as 2,4,6-trinitrotoluene and its degradation product, 2,4-dinitrotoluene [10,11]. These sensing strains were then encased into alginate beads to enable trace amounts of such explosives to diffuse while maintaining cell viability within a short operational period. By combining bead-encapsulated cells with a laser-based optoelectronic system, they successfully created a system that allowed for the remote monitoring of antipersonnel mines in a field trial [12••]. Recently, they further optimized the system to make it more amenable for use beyond the laboratory, such as changing the fluorescence-based reporter to one based on bioluminescence, modifying the encapsulation matrix, and redesigning the optoelectronic circuit [13,14]. While WCSs have shown promising sensing performance, concerns regarding the escape risk of these genetically modified organisms into the environment still hinder their applications. Tang et al. tackled this issue by developing a deployable physical containment strategy (DEPCOS) that mitigates the escape risk for WCSs-based biosensing devices. The tough core–shell

hydrogel design of DEPCOS provides stringent containment of the engineered cells and their DNA, while still allowing the diffusion of nutrients and chemicals for cell survival and sensing functions [15••]. Regardless, the long-term sensing capacity and viability of the encapsulated cells require further investigation.

The development of CFSs-based biosensing devices for environmental monitoring is also accelerating. Typically, CFSs can be lyophilized on paper or in test tubes, and functionally rehydrated at the point of use, reducing the need for sophisticated fabrication methods. Transcription factors and RNA-based elements that are functionally stable in *in vitro* environment have been extensively exploited to develop CFSs. Such CFSs can detect essential chemical contaminants, such as arsenite, fluoride, cadmium, and cyanuric acid [16–20]. Jung *et al.* recently developed a CFS platform named RNA Output Sensors Activated by Ligand Induction (ROSALIND), using allosteric transcription factors (aTFs) as the key biosensing elements to control the phage polymerase-driven transcription of fluorescence-activating RNA aptamers output in response to the presence of target chemicals. ROSALIND can detect a wide range of water contaminants, including macrolides and heavy metal ions [21••,22]. Given the availability of diverse aTFs in nature, ROSALIND could be employed to develop a wide range of new CFSs for detecting other target compounds in the future.

Design and fabrication of biosensing devices for pathogen detection

Pathogens pose both immediate and long-term risks to human health. Pathogen outbreaks such as the COVID-19 pandemic could significantly impact the global population, reinforcing the need to develop diagnostic devices as potential countermeasures. In the past years, synthetic biology has seen tremendous efforts in the development of sensitive, low-cost, and user-friendly microbial biosensing devices that can rapidly detect pathogens with minimal equipment. For instance, microbial cells could be engineered to detect signature analytes that indicate the presence of target pathogens in clinical samples. These analytes include cholera autoinducer-1 from *Vibrio cholerae*, auto-inducer I from *Staphylococcus aureus*, and hydroxyphenylacetic acid from *Candida albicans* [23–25]. Recently, Wu et al. engineered *E. coli* cells to detect N-acylhomoserine lactone to sense and report the presence of *Pseudomonas aeruginosa* and *Burkholderia pseudomallei*. The sensing cells were immobilized on paper to create a device, enabling the production of lycopene as a visualizable output after exposure to the filtered culture of target pathogens for 8 hours [26]. By detecting analytes, WCSs do not require steps like sample preparation and nucleic acid amplification as compared to PCR assays or nucleic acid-based CFSs for pathogen detection, greatly simplifying the sensing workflow. However, cell growth must be improved, and signal-generating time must be shortened to enhance WCSs' competitiveness for pathogen diagnostics.

CFSs-based pathogen sensors primarily focus on nucleic acid-based detection, taking advantage of the understanding that pathogens and their mutants could be identified and differentiated through their unique genetic sequences. Pardee et al. demonstrated the feasibility of designing toehold switches that recognize mRNA sequences unique to Ebola and Zika viruses at a femtomolar level and correspondingly trigger transcriptional events to produce outputs in paper-embedded cell-free mixtures [27•,28]. Building on this approach, Takahashi et al. developed CFSs capable of recognizing and distinguishing gut bacteria based on their 16S ribosomal RNA signatures, enabling the ultrasensitive identification of high toxin-producing *Clostridium difficile* in complex stool samples [29]. Recently, many studies have exploited the CRISPR-Cas system's programmable DNA recognition and cleavage activities to create a wide range of highly sensitive nucleic acid sensors for pathogen detection [30,31]. Gootenberg et al. created the first Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) platform, which utilizes the Cas13a protein to recognize target RNA sequences. The Cas13a protein exhibits collateral activity that triggers the cleavage of quenched fluorescent RNA reporters to release output signals [32••]. Intriguingly, SHERLOCK allows for the attomole-level detection and differentiation of targets with a single-base-pair resolution. Subsequently, they created SHERLOCK version 2 (SHERLOCKv2), which synergistically combined Cas13 and its associated enzyme Csm6 for signal amplification. SHERLOCK v2 enabled the sensitive detection of single-stranded DNA from dengue or Zika virus and their mutations in patient liquid biopsy samples [33]. During the COVID-19 pandemic, SHERLOCK promoted the rapid development of a series of innovative biosensing devices for SARS-CoV-2 detection. For example, Arizti-Sanz et al. developed the Streamlined Highlighting of Infections to Navigate Epidemics (SHINE), an optimized SHERLOCK-based assay for the rapid detection of SARS-CoV-2 RNA from unextracted samples [34••]. SHINE employed heating unextracted diagnostic samples to obliterate nucleases to rapidly inactivate nucleases and lyse viral particles, followed by single-step RNA amplification and Cas13-based detection. By incorporating a smartphone-friendly in-tube fluorescence readout procedure, SHINE enabled rapid detection (1 hour) without human exposure to pathogenic samples [34••,35]. Furthermore, Welch et al. deployed SHERLOCK-based sensors on a microfluidic device to create mCARMEN, enabling the high-throughput surveillance of a virus panel, including SARS-CoV-2 variants Delta and Omicron [36]. Interestingly, Nguyen et al. creatively fabricated a paper-based facemask embedded with the SHERLOCK sensors to enable the real-time

detection of SARS-CoV-2 particles resulting from the wearer's respiration [37•]. Chen et al. also developed a DNA endonuclease-targeted CRISPR trans reporter (DETECR) to detect viral DNA in human samples at the attomolar level. In contrast to SHERLOCK, DETECR exploits a Cas12a enzyme that collaterally cleaves fluorescent-quenched single-stranded DNA reporter molecules upon the detection of target DNA sequences [38•]. Building on DETECR, Boughton et al. developed an immunoassay to detect SARS-CoV-2 at a clinically relevant level in respiratory swab specimens within 1 hour [39••].

Design and fabrication of biosensing devices for biomarker diagnostics

Microbial sensors could enable a wide range of biomarker sensing applications both *in vitro* and *in vivo*. For instance, Chang et al. developed a unique synthetic receptor platform known as Engineered Modularized Receptors Activated via Ligand-induced Dimerization (EMeRALD), which allows diverse ligand-binding domains to be fused to a synthetic receptor scaffold derived from *E. coli*. The developed sensors can detect pathological bile salts as biomarkers in human serum samples with high sensitivity and specificity within 2 hours [40,41••]. Meanwhile, McNerney et al. harnessed zinc-responsive transcription elements and isopropyl ß-D-1-thiogalactopyranoside-inducible elements in *E. coli* to create two-input zinc-sensing WCSs that could generate colored pigments in response to zinc at different concentrations [42•]. The authors further demonstrated that the sensing cells could maintain robust performance upon lyophilization and rehydrating in human serum samples, enabling the quantification of zinc concentration at the point of use in 12 hours. At the same time, microbial sensors and microbial sensing devices offer the unique capacity to monitor biomarkers in hard-to-access body niches, including the gastrointestinal (GI) tract. Early works in the field have demonstrated how engineered cells could be deployed as live diagnostics that could eventually become a stable member of the human microbiome and report relevant biomarkers present in the GI tract, such as tetrathionate and nitrate [43–45]. Chien et al. recently introduced a multiplex biosensing capacity into probiotic cells, enabling the simultaneous detection of signals like pH, lactate, and oxygen [46•]. Such a platform could someday be useful for programming cells to localize and deliver drugs at desired niches in the GI tract. Another notable work comes from Mimee et al., whose team created a unique miniaturized ingestible micro-bio-electronic device (IMBED) which embedded core bacteria sensors with an ingestible electronic chip. IMBED can transmit wireless signals from the mammalian gut upon exposure to heme at elevated concentrations, allowing the standoff monitoring of insults to internal organs [47••]. By offering a unique solution for both biocontainment and remote detection, IMBED could inspire many novel *in vivo* biosensing applications in the future.

Conclusions, challenges, and perspectives of microbial biosensing devices

As highlighted in this review, microbial biosensing devices have the potential to enable a wide range of novel biosensing applications. Given the increasing number of microbial biosensing devices in development, the emergence of their commercial products is imminent. However, there are several key challenges that should be addressed in future developments to satisfy regulatory and large-scale production requirements.

Whole-cell sensors-based biosensing devices

Synthetic biologists have established a solid foundation for the design and optimization of WCSs performance. Nonetheless, the number of field-deployable WCSs-based devices remains limited due to modest target diversity and biocontainment issues. To expand target diversity, focus should remain on genomic mining to discover novel biosensing elements capable of detecting diverse molecules. In addition, rational redesign and directed evolution approaches could be wielded to alter the biochemistries of existing biosensing scaffolds to detect noncognate molecules, as exemplified by the development of EMeRALD. With regards to biocontainment, continued innovations in new deployment platforms, such as DEPCOS and IMBED, are essential in creating WCSs-based biosensing products that pose minimal biohazard risks. On the other hand, auxotrophic strains can also provide another layer of biocontainment [48]. We opine that the development of these strains into high-performance WCSs, combined with their integration into compatible deployment platforms, would lead to a wide range of novel WCSs-based sensing devices that fulfill stringent regulatory requirements.

Cell-free sensors-based biosensing devices

Compared with WCSs, CFSs are arguably friendlier for regulatory approval and are more amenable for rapid development in response to urgent diagnostic needs. However, it is challenging to maintain performance consistency while reducing manufacturing costs of CFSs. The output of CFSs could vary between production batches, sampling, and detection instruments at point of use, and among human operators [49]. As the field evolves, the establishment of standardized practices would help reduce the variability of these contributing factors. Currently, the estimated cost of cell-free reaction is US$5/mL, which might hinder their large-scale manufacturing. Optimizing active ingredients, such as finding replacements for expensive energy substrates and cofactors, could help eventually bring down the manufacturing cost to less than US$1/mL [6].

Furthermore, new approaches for developing cell-free reactions from the lysates of diverse microbial strains other than *E. coli* could lead to the discovery of novel biosensing mechanisms, which could facilitate the detection of novel chemical targets in the future.

CRediT authorship contribution statement

HLP wrote the manuscript. HL and MWC commented and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

1. Brooks SM, Alper HS: **Applications, challenges, and needs for employing synthetic biology beyond the lab**. *Nat Commun* 2021, **12**:1390.

2. Hossain GS, Saini M, Miyake R, Ling H, Chang MW: **Genetic biosensor design for natural product biosynthesis in microorganisms**. *Trends Biotechnol* 2020, **38**:797-810.

3. Inda ME, Lu TK: **Microbes as biosensors**. *Annu Rev Microbiol* 2020, **74**:337-359.

4. Wehrs M, Tanjore D, Eng T, Lievense J, Pray TR, Mukhopadhyay A: **Engineering robust production microbes for large-scale cultivation**. *Trends Microbiol* 2019, **27**:524-537.

5. Zhang L, Guo W, Lu Y: **Advances in cell-free biosensors: principle, mechanism, and applications**. *Biotechnol J* 2020, **15**:e2000187.

6. Silverman AD, Karim AS, Jewett MC: **Cell-free gene expression: an expanded repertoire of applications**. *Nat Rev Genet* 2020, **21**:151-170.

7. • Wan X, Volpetti F, Petrova E, French C, Maerkl SJ, Wang B: **Cascaded amplifying circuits enable ultrasensitive cellular sensors for toxic metals**. *Nat Chem Biol* 2019, **15**:540-548.
This work demonstrated an effective modular design strategy to amplify the sensitivity and output of a transcriptional sensor in bacteria, which could be applicable for developing new biosensors.

8. Lopreside A, Montali L, Wang B, Tassoni A, Ferri M, Calabretta MM, Michelini E: **Orthogonal paper biosensor for mercury(II) combining bioluminescence and colorimetric smartphone detection**. *Biosens Bioelectron* 2021, **194**:113569.

9. Liu Y, Guo M, Du R, Chi J, He X, Xie Z, Huang K, Luo Y, Xu W: **A gas reporting whole-cell microbial biosensor system for rapid on-site detection of mercury contamination in soils**. *Biosens Bioelectron* 2020, **170**:112660.

10. Yagur-Kroll S, Lalush C, Rosen R, Bachar N, Moskovitz Y, Belkin S: ***Escherichia coli* bioreporters for the detection of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene**. *Appl Microbiol Biotechnol* 2014, **98**:885-895.

11. Yagur-Kroll S, Amiel E, Rosen R, Belkin S: **Detection of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene by an *Escherichia coli* bioreporter: performance enhancement by directed evolution**. *Appl Microbiol Biotechnol* 2015, **99**:7177-7188.

12. •• Belkin S, Yagur-Kroll S, Kabessa Y, Korouma V, Septon T, Anati Y, Zohar-Perez C, Rabinovitz Z, Nussinovitch A, Agranat AJ: **Remote detection of buried landmines using a bacterial sensor**. *Nat Biotechnol* 2017, **35**:308-310.
This work demonstrated the unique application of microbial sensing devices in the remote detection of explosives, highlighting the versatility of these sensors for novel diagnostic applications.

13. Agranat AJ, Kabessa Y, Shemer B, Shpigel E, Schwartsglass O, Atamneh L, Uziel Y, Ejzenberg M, Mizrachi Y, Garcia Y, *et al.*: **An autonomous bioluminescent bacterial biosensor module for outdoor sensor networks, and its application for the detection of buried explosives**. *Biosens Bioelectron* 2021, **185**:113253.

14. Shemer B, Shpigel E, Hazan C, Kabessa Y, Agranat AJ, Belkin S: **Detection of buried explosives with immobilized bacterial bioreporters**. *Microb Biotechnol* 2021, **14**:251-261.

15. •• Tang TC, Tham E, Liu X, Yehl K, Rovner AJ, Yuk H, de la Fuente-Nunez C, Isaacs FJ, Zhao X, Lu TK: **Hydrogel-based biocontainment of bacteria for continuous sensing and computation**. *Nat Chem Biol* 2021, **17**:724-731.
This study developed a broadly applicable biocontainment platform for bacteria sensors, which could address some safety concerns and facilitate their field deployment in the future.

16. Chen SY, Wei W, Yin BC, Tong Y, Lu J, Ye BC: **Development of a highly sensitive whole-cell biosensor for arsenite detection through engineered promoter modifications**. *ACS Synth Biol* 2019, **8**:2295-2302.

17. Beabout K, Bernhards CB, Thakur M, Turner KB, Cole SD, Walper SA, Chavez JL, Lux MW: **Optimization of heavy metal sensors based on transcription factors and cell-free expression systems**. *ACS Synth Biol* 2021, **10**:3040-3054.

18. He MY, Lin YJ, Kao YL, Kuo P, Grauffel C, Lim C, Cheng YS, Chou HD: **Sensitive and specific cadmium biosensor developed by reconfiguring metal transport and leveraging natural gene repositories**. *ACS Sens* 2021, **6**:995-1002.

19. Thavarajah W, Silverman AD, Verosloff MS, Kelley-Loughnane N, Jewett MC, Lucks JB: **Point-of-use detection of environmental fluoride via a cell-free riboswitch-based biosensor**. *ACS Synth Biol* 2020, **9**:10-18.

20. Yuan S-F, Alper HS: **Improving Spinach2-and Broccoli-based biosensors for single and double analytes**. *Biotechnol Notes* 2020, **1**:2-8.

21. •• Jung JK, Alam KK, Verosloff MS, Capdevila DA, Desmau M, Clauer PR, Lee JW, Nguyen PQ, Pasten PA, Matiasek SJ, *et al.*: **Cell-free biosensors for rapid detection of water contaminants**. *Nat Biotechnol* 2020, **38**:1451-1459.
This study developed a powerful modular cell-free platform for sensing a wide range of chemicals.

22. Jung JK, Alam KK, Lucks JB: **ROSALIND: rapid detection of chemical contaminants with in vitro transcription factor-based biosensors**. *Methods Mol Biol* 2022, **2433**:325-342.

23. Holowko MB, Wang H, Jayaraman P, Poh CL: **Biosensing *Vibrio cholerae* with genetically engineered *Escherichia coli***. *ACS Synth Biol* 2016, **5**:1275-1283.

24. Lubkowicz D, Ho CL, Hwang IY, Yew WS, Lee YS, Chang MW: **Reprogramming probiotic *Lactobacillus reuteri* as a biosensor for *Staphylococcus aureus* derived AIP-I detection**. *ACS Synth Biol* 2018, **7**:1229-1237.

25. Tscherner M, Giessen TW, Markey L, Kumamoto CA, Silver PA: **A synthetic system that senses *Candida albicans* and inhibits virulence factors**. *ACS Synth Biol* 2019, **8**:434-444.

26. Wu Y, Wang CW, Wang D, Wei N: **A whole-cell biosensor for point-of-care detection of waterborne bacterial pathogens**. *ACS Synth Biol* 2021, **10**:333-344.

27. • Pardee K, Green AA, Ferrante T, Cameron DE, DaleyKeyser A, Yin P, Collins JJ: **Paper-based synthetic gene networks**. *Cell* 2014, **159**:940-954.
This work pioneered the development of paper-based cell-free sensors. It demonstrated the feasibility of creating and maintaining stable synthetic sensors built from freeze-dried cellular components on a cellulose matrix for long-term usage, transport, and storage.

28. Pardee K, Green AA, Takahashi MK, Braff D, Lambert G, Lee JW, Ferrante T, Ma D, Donghia N, Fan M, *et al*.: **Rapid, low-cost detection of Zika virus using programmable biomolecular components**. *Cell* 2016, **165**:1255-1266.

29. Takahashi MK, Tan X, Dy AJ, Braff D, Akana RT, Furuta Y, Donghia N, Ananthakrishnan A, Collins JJ: **A low-cost paper-based synthetic biology platform for analyzing gut microbiota and host biomarkers**. *Nat Commun* 2018, **9**:3347.

30. Mustafa MI, Makhawi AM: **SHERLOCK and DETECTR: CRISPR-Cas Systems as potential rapid diagnostic tools for emerging infectious diseases**. *J Clin Microbiol* 2021, **59**:e00745-20.

31. Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F, Collins JJ: **CRISPR-based diagnostics**. *Nat Biomed Eng* 2021, **5**:643-656.

32. •• Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freije CA, *et al*.: **Nucleic acid detection with CRISPR-Cas13a/C2c2**. *Science* 2017, **356**:438-442.
This study developed a first-of-its-kind nucleic acid detection system that exploits the nucleic acid recognition and collateral activity of CRISPR-Cas complex. This work paved the way for creating innovative devices for pathogen diagnostics.

33. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F: **Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6**. *Science* 2018, **360**:439-444.

34. •• Arizti-Sanz J, Freije CA, Stanton AC, Petros BA, Boehm CK, Siddiqui S, Shaw BM, Adams G, Kosoko-Thoroddsen TF, Kemball ME, *et al*.: **Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2**. *Nat Commun* 2020, **11**:5921.
This work created a streamlined workflow to enable the rapid sampling of viral targets from raw patient samples by minimizing sample preparations and combining nucleic acid amplification and detection in a single step.

35. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, Kellner MJ, Tan AL, Paul LM, Parham LA, *et al*.: **Field-deployable viral diagnostics using CRISPR-Cas13**. *Science* 2018, **360**:444-448.

36. Welch NL, Zhu M, Hua C, Weller J, Mirhashemi ME, Nguyen TG, Mantena S, Bauer MR, Shaw BM, Ackerman CM, *et al*.: **Multiplexed CRISPR-based microfluidic platform for clinical testing of respiratory viruses and identification of SARS-CoV-2 variants**. *Nat Med* 2022,, https://doi.org/10.1038/s41591-022-01734-1

37. • Nguyen PQ, Soenksen LR, Donghia NM, Angenent-Mari NM, de Puig H, Huang A, Lee R, Slomovic S, Galbersanini T, Lansberry G, *et al*.: **Wearable materials with embedded synthetic biology sensors for biomolecule detection**. *Nat Biotechnol* 2021, **39**:1366-1374.
This study fabricated a proof-of-concept facemask embedded with cell-free sensors to detect SARS-Cov-2 particles from wearer's breath. This work provides a notable reference for future development of wearable sensing devices.

38. • Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, Doudna JA: **CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity**. *Science* 2018, **360**:436-439.
This study demonstrated the exploitation of the target recognition and collateral cleavage of DNA substrates of Cas12a to develop highly sensitive cell-free sensors to enable new diagnostic applications.

39. •• Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, *et al*.: **CRISPR-Cas12-based detection of SARS-CoV-2**. *Nat Biotechnol* 2020, **38**:870-874.
This study demonstrated the capability of rapidly developing CRISPR-Cas12-based SARS-CoV-2 sensors in response to the global COVID-19 pandemic. Their work highlights the advantages of cell-free sensors in providing quick solutions to address real-world diagnostic challenges.

40. Chang HJ, Mayonove P, Zavala A, De Visch A, Minard P, Cohen-Gonsaud M, Bonnet J: **A modular receptor platform to expand the sensing repertoire of bacteria**. *ACS Synth Biol* 2018, **7**:166-175.

41. •• Chang HJ, Zuniga A, Conejero I, Voyvodic PL, Gracy J, Fajardo-Ruiz E, Cohen-Gonsaud M, Cambray G, Pageaux GP, Meszaros M, *et al*.: **Programmable receptors enable bacterial biosensors to detect pathological biomarkers in clinical samples**. *Nat Commun* 2021, **12**:5216.
This work demonstrated a powerful modular design that exploits the plasticity of two-component systems to create a versatile platform that enables *Escherichia coli* to recognize noncognate targets. The developed biosensors were able to detect various pathological biomarkers.

42. • McNerney MP, Michel CL, Kishore K, Standeven J, Styczynski MP: **Dynamic and tunable metabolite control for robust minimal-equipment assessment of serum zinc**. *Nat Commun* 2019, **10**:5514.
The authors developed an innovative circuit that allows for color-pigment production according to different zinc concentration ranges in the samples. They further demonstrated that the sensors could maintain robust performance upon lyophilization and rehydration in human serum samples, serving as a good reference for future whole-cell sensors development.

43. Daeffler KN, Galley JD, Sheth RU, Ortiz-Velez LC, Bibb CO, Shroyer NF, Britton RA, Tabor JJ: **Engineering bacterial thiosulfate and tetrathionate sensors for detecting gut inflammation**. *Mol Syst Biol* 2017, **13**:923.

44. Riglar DT, Giessen TW, Baym M, Kerns SJ, Niederhuber MJ, Bronson RT, Kotula JW, Gerber GK, Way JC, Silver PA: **Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation**. *Nat Biotechnol* 2017, **35**:653-658.

45. Woo SG, Moon SJ, Kim SK, Kim TH, Lim HS, Yeon GH, Sung BH, Lee CH, Lee SG, Hwang JH, *et al*.: **A designed whole-cell biosensor for live diagnosis of gut inflammation through nitrate sensing**. *Biosens Bioelectron* 2020, **168**:112523.

46. • Chien T, Harimoto T, Kepecs B, Gray K, Coker C, Hou N, Pu K, Azad T, Nolasco A, Pavlicova M, *et al*.: **Enhancing the tropism of bacteria via genetically programmed biosensors**. *Nat Biomed Eng* 2022, **6**:94-104.
This work demonstrated a multiplex bacterial-based biosensor that is functional *in vivo*, which may be used in developing niche-specific bacterial-based drug delivery systems in the mammalian gut.

47. •• Mimee M, Nadeau P, Hayward A, Carim S, Flanagan S, Jerger L, Collins J, McDonnell S, Swartwout R, Citorik RJ, *et al*.: **An ingestible bacterial-electronic system to monitor gastrointestinal health**. *Science* 2018, **360**:915-918.
This study developed a hybrid system exploiting bacteria as the core sensor and an electronic chip as the deployment platform to enable the *in vivo* monitoring of clinical markers in the gastrointestinal tract of live animals. This work highlights the versatility of bacteria-based biosensing devices in enabling unprecedented diagnostic applications.

48. Arnolds KL, Dahlin LR, Ding L, Wu C, Yu J, Xiong W, Zuniga C, Suzuki Y, Zengler K, Linger JG, *et al*.: **Biotechnology for secure biocontainment designs in an emerging bioeconomy**. *Curr Opin Biotechnol* 2021, **71**:25-31.

49. Cole SD, Beabout K, Turner KB, Smith ZK, Funk VL, Harbaugh SV, Liem AT, Roth PA, Geier BA, Emanuel PA, *et al*.: **Quantification of interlaboratory cell-free protein synthesis variability**. *ACS Synth Biol* 2019, **8**:2080-2091.