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Engineered bacteria-enabled biosensing: integration with artificial intelligence for enhanced diagnostic precision

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Engineered bacteria—microbial strains endowed with bespoke functionalities *via* genetic or protein engineering—represent a cornerstone of synthetic biology. These living diagnostics have shown immense potential across diverse sensing applications, including disease diagnostics, environmental surveillance, and food safety assessment. Recent advances in artificial intelligence (AI) have further propelled the field by enabling the efficient analysis of complex biological datasets, identification of nuanced signal patterns, and rational optimization of genetic circuits. In this review, we first summarize the fundamental design principles underpinning engineered bacterial biosensors, encompassing chassis selection, genetic circuit construction, and signal transduction modules. We then explore emerging roles of AI in component discovery, regulatory circuit refinement, and predictive modeling of system behavior. Representative case studies highlight the translational potential of these intelligent biosensors in real-world monitoring scenarios. Finally, we discuss key challenges—including biosafety, long-term stability, and regulatory hurdles—and propose future directions for the development and clinical implementation of AI-augmented engineered bacterial sensing platforms.

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1 Introduction

Accurate and timely detection is critical for public health protection, environmental preservation, and sustainable development. However, as real-world sensing scenarios become more complex—from multiplexed environmental monitoring to rapid, decentralized disease diagnostics—

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conventional analytical platforms are increasingly constrained by their cost, technical complexity, and limited deployability. For instance, detecting trace pollutants such as heavy metals and volatile organic compounds (VOCs) in environmental matrices remains challenging due to their low abundance and heterogeneous distribution.[1,2] In food safety, the prompt identification of microbial pathogens and chemical residues is essential for preventing outbreaks,[3] while in clinical settings, early diagnosis is directly linked to improved outcomes and reduced healthcare burdens.[4–7] Accurate and timely detection represents a cornerstone of public health protection. Nevertheless, traditional analytical platforms are often hampered by high operational costs, limited throughput, and poor scalability. Detecting trace pollutants is particularly challenging because of their low environmental concentrations. While gold-standard techniques, including chromatography and mass spectrometry, deliver excellent accuracy and sensitivity, their reliance on costly instrumentation and prolonged analytical workflows restricts their applicability for routine, large-scale monitoring. Accordingly, there is a growing interest in developing affordable and scalable alternatives capable of enabling rapid, high-throughput screening, particularly in resource-constrained settings.[8,9]

Engineered bacteria—microorganisms rewired through genetic and protein engineering—offer a promising solution to these limitations. Naturally equipped with diverse signal-sensing and adaptive capabilities, bacteria can be reprogrammed to function as living biosensors, capable of detecting specific chemical or physical cues and generating quantifiable outputs in real time.[10,11] These systems are low-cost, self-replicating, and operable in complex environments, making them ideal for field-deployable applications in environmental monitoring, food safety, and medical diagnostics.[12]

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Despite their promise, engineered bacterial biosensors face persistent challenges, including limited sensitivity, slow response kinetics, and suboptimal performance in complex biological matrices. Recently, artificial intelligence (AI) has emerged as a transformative enabler in biosensor engineering. AI techniques—ranging from deep learning to probabilistic modeling—enable high-throughput data analysis, optimization of genetic circuits, and predictive modeling of sensor performance.[13] Integrating AI with engineered bacterial detection is expected to overcome the limitations of traditional methods in precision, speed, and adaptability. This fusion enables intelligent, rapid, high-throughput, and cost-effective detection, supporting applications in environmental monitoring, food safety, and personalized medicine.[14] The convergence of synthetic biology and AI thus represents a new paradigm for intelligent, adaptive biosensing platforms. In this review, we first discuss core design principles of engineered bacterial biosensors, including chassis strain selection, genetic circuit architecture, and signal transduction mechanisms. We then explore how AI accelerates biosensor development through enhanced component discovery, circuit optimization, and predictive modeling. Representative case studies highlight translational applications in environmental and clinical settings. Finally, we outline current limitations and future opportunities for advancing AI-integrated bacterial biosensing toward clinical and field implementation.

2 Construction of engineered bacteria

Genetically engineered bacteria (GEBs) are microbial strains modified through synthetic biology and genetic engineering to express exogenous proteins, sense environmental cues, or execute programmed functions. The construction of GEBs for biosensing applications involves three key steps: (i) selection of appropriate chassis strains, (ii) integration of sensing and effector modules, and (iii) optimization of signal processing and output systems—including quorum sensing networks and logic circuits—for precise and spatiotemporal control of gene expression.[15,16]

2.1 Chassis strains selection

Chassis strain selection frequently varies across different detection scenarios (Table 1). Chassis strain selection forms the foundation of intelligent bacterial biosensor design. The host strain not only dictates the efficiency and stability of synthetic gene circuits but also determines the organism's adaptability, biosafety profile, and long-term viability in physiological or environmental settings.[17] In AI-integrated biosensing systems, the inherent biological response dynamics of the chassis directly influence data fidelity, interpretability, and system responsiveness. Therefore, tailoring the chassis strain to the intended application scenario is a critical prerequisite for high-performance engineered biosensor platforms.

Table 1 Commonly used chassis strains

Species of strains	Advantages	Disadvantages	Applications	Selection criteria
Escherichia coli	Easy genetic engineering; strong ability to express	LPS; inclusion bodies; low stress tolerance	Host intestinal diagnosis; *in vitro* sensing platform	Select for rapid prototyping and high-yield signal output in sterile IVD or controlled laboratory settings
Lactobacillus spp.	GRAS certification; high safety	Slow growth; limited genetic tools	Inflammatory bowel disease; probiotic delivery systems	Choose when human biocompatibility and safety (GRAS) are the primary requirements for long-term oral administration
Bacillus subtilis	Good stability; easy for dry transportation	Protease secretion; sporulation control	Portable biological detectors; paper-based diagnostic devices	Select if the biosensor requires long-term shelf-life at room temperature or deployment *via* paper-based analytical devices
Acinetobacter baylyi	Strong natural drug resistance; multi-drug resistance; high natural competence	Fewer standardized promoters and inducible systems; strictly aerobic; potential crosstalk between endogenous and synthetic pathways	Tumor DNA	Prefer for detecting horizontal gene transfer or environmental/tumor DNA *via* natural transformation mechanisms
Attenuated *Salmonella* (*e.g.*, *S. typhimurium* strains)	Excellent stability	Risk of virulence reversion; residual endotoxicity (LPS); strict containment regulations	Soil pollution monitoring; detecting pollutants in food (*e.g.*, pesticide residues)	Use for sensing in complex food and soil matrices
Bacteroides spp.	Strong natural colonization ability; respond to endogenous signals	Genetic toolkit underdevelopment; difficult manipulation; slower growth; difficult to scale up in standard industrial fermenters	Long term *in vivo* diagnosis; chronic disease monitoring	Select if the target environment is the anaerobic human colon and long-term stable colonization (>1 week) is required
Vibrio natriegens	Fast growth rate; strong salt/pollution tolerance; non-pathogenic	High metabolic demand; elevated oxygenation/cooling costs; corrosive growth media; bioreactor damage risk; replication-induced instability; accelerated plasmid loss	Detection of organic pollutants; detection of pollutants in high salt wastewater	Choose if sample salinity exceeds 1.5% or if a rapid "time-to-result" (<1 hour) is critical for the diagnostic workflow
Pseudomonas putida	Good stability; strong ability to resist environmental stress	Metabolic rigidity; flux redirection difficulty; the formed biofilm may clog the equipment; fewer orthogonal genetic parts (promoters/ribosome binding sites) available compared to *E. coli*	Heavy metal detection; organic pollutant detection; metabolite detection	Prefer for monitoring industrial effluents or environments with high chemical toxicity and fluctuating osmotic pressures

2.1.1 Commonly used chassis strains

For environmental applications. Several fast-growing and environmentally resilient bacteria have emerged as preferred chassis for environmental biosensing. *Vibrio natriegens*, one of the fastest-growing bacterial species known, exhibits high gene-editing efficiency and robust salt tolerance, making it ideal for marine or high-salinity condition.[18,19] Engineered variants such as VCOD-2 have demonstrated exceptional competence for exogenous DNA integration, with transformation efficiencies surpassing those of most natural microorganisms.[20] *Pseudomonas putida*, a metabolically versatile Gram-negative bacterium, thrives under a wide range of environmental stresses including fluctuations in temperature, pH, organic solvents, and oxidative conditions. Its model strain KT2440, recognized as a safe host organism by the U.S. NIH Recombinant DNA Advisory Committee, has been widely adopted in pollutant detection and metabolic engineering.[21] KT2440 has been engineered to degrade persistent environmental toxins such as aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and pesticides.[22]

For biomedical application. For clinical and diagnostic contexts, *Escherichia coli* and *Lactobacillus* species are the most frequently used chassis due to their well-characterized genomes, high genetic tractability, and generally recognized safety.[23] *E. coli* remains the prototypical host for *in vitro* biosensors, thanks to its rapid growth and extensive synthetic biology toolbox. Among clinical strains, *E. coli* Nissle 1917—a probiotic with a longstanding safety record—is gaining prominence in the development of *in vivo* biosensors, particularly for gastrointestinal diagnostics due to its ability to colonize the human gut.[24] *Bacillus* species also represent promising chassis, especially for applications requiring environmental persistence. Their spore-forming capability provides resilience in extreme or fluctuating conditions, such as those found in the digestive tract. Additionally, non-pathogenic human commensals are being explored as application-specific hosts. For instance, selected skin microbiota strains are under investigation as chassis for dermatological biosensors, owing to their native residence on human skin and their ability to avoid immune clearance.

2.2 Sensing modules

Sensing modules constitute the core functional units of engineered bacterial biosensors, enabling the conversion of pathological or environmental cues—such as heavy

metal ions, pollutants, disease-associated metabolites, inflammatory markers, or pathogen-derived molecules—into quantifiable biological outputs. Recent advancements in synthetic biology and molecular engineering have facilitated the development of modular, programmable, and highly specific sensing architectures.[25]

In engineered microbes, sensing modules are responsible for detecting target analytes and initiating downstream responses. Upon recognition of the target, the sensing element triggers signal transduction *via* mechanisms including ligand-induced conformational changes, allosteric dimerization, conditional protein stabilization, or enzymatic cascade amplification.[26] These mechanisms ensure the fidelity and responsiveness of biosensing under physiological or environmental conditions.

A functional sensing module typically comprises three components: (i) an input recognition element, which determines the specificity and sensitivity of the system; (ii) a signal transduction mechanism, which processes the input into an intracellular cue; and (iii) an output response module, which generates a measurable signal such as fluorescence, color change, or metabolite production (Fig. 1). The performance of the sensing module—especially its sensitivity, dynamic range, and background noise—is critically dependent on the design of the input recognition element. These recognition components can be drawn from natural sensor domains (*e.g.*, transcription factors, two-component systems, or riboswitches) or designed *de novo* using computational protein engineering and machine learning-guided screening.

The modularity of synthetic sensing systems allows for flexible reconfiguration, enabling bacteria to respond to a wide range of targets with high selectivity. Furthermore, AI-driven strategies are increasingly being applied to enhance component selection, model allosteric behavior, and optimize signal transduction cascades, thereby accelerating the development of next-generation biosensors capable of real-time, multiplexed detection in complex environments.

2.2.1 Input recognition elements. Input recognition elements serve as the first point of interaction between engineered bacteria and their surrounding microenvironment. These components initiate biosensing by detecting specific molecular cues and triggering downstream signal transduction. Common input recognition strategies include transcription factors, nucleic acid aptamers, riboswitches, and two-component systems (TCS), each offering distinct advantages in terms of specificity, programmability, and environmental responsiveness (Table 2).

Transcription factors. Transcription factors are endogenous proteins that regulate gene expression in response to small molecules, metabolites, or redox signals.[27,28] They are among the most widely used input modules in engineered bacterial systems due to their modularity and adaptability. Natural transcription factors can be repurposed or engineered to recognize non-native ligands, thereby expanding the sensing repertoire. For instance, the transcription factor LldR specifically binds to lactate and is commonly employed for sensing hypoxic tumor microenvironments characterized by elevated lactate levels.[29] Mimee *et al.* utilized engineered bacteria expressing bile acid-responsive transcription factors to monitor gastrointestinal physiology, enabling noninvasive diagnostics of intestinal disorders.[30] Other classic examples include MerR and ArsR, which recognize mercury (Hg^{2+}) and arsenic (As^{3+}), respectively. Upon metal ion binding, these transcription factors activate promoter regions that drive reporter gene expression, enabling heavy metal quantification in environmental samples. Additionally, the quorum sensing regulator LuxR, responsive to *N*-acyl homoserine lactones, has been leveraged in environmental biosensors for detecting petroleum-based pollutants. When coupled with the LuxI operon, this system enables construction of luminescent bacterial reporters for pollutant detection in aquatic ecosystems.

Nucleic acid aptamers. Aptamers are short, single-stranded DNA or RNA sequences capable of binding target molecules

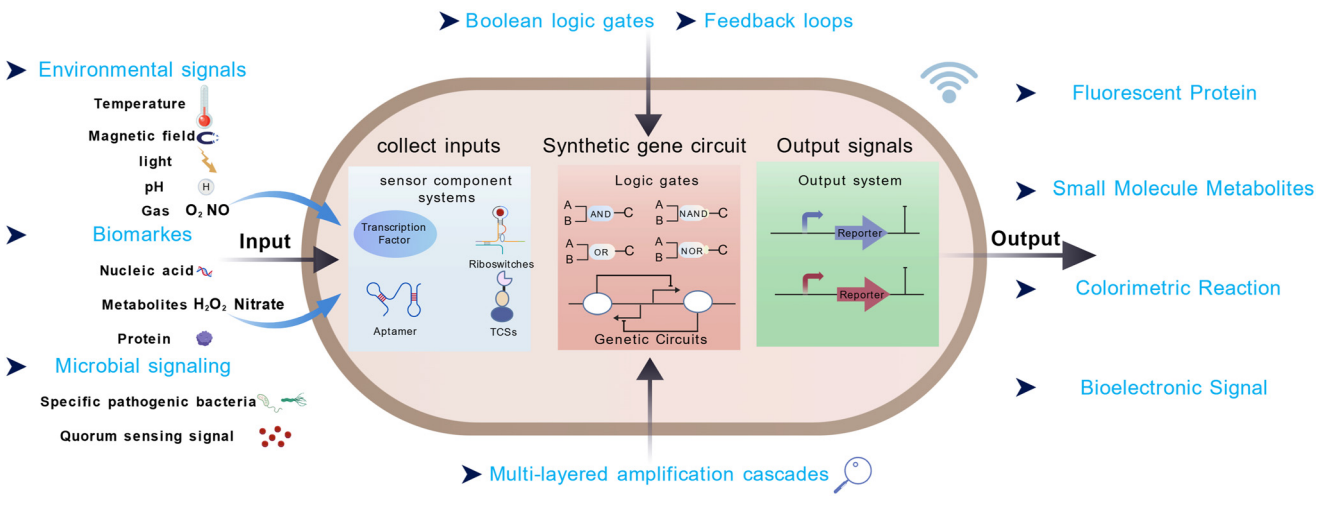

Fig. 1 Bacterial sensing system.

Table 2 Comparison of different sensing elements

Type of sensing element	Typical target	Sensitivity (LOD)	Response time	Advantages	Disadvantages	Application
Transcription factors (TFs)	Heavy metals; antibiotics; intracellular metabolites	High	Medium (30–120 min)	Easy to engineer/modify; tunable dynamic range	Requires cell penetration; leaky expression	General type; intracellular small molecule
Two-component systems (TCSs)	Peptides; environmental factors (pH/temperature); extracellular toxins	Extremely high	Relatively fast (20–60 min)	Extracellular sensing; inherent signal amplification	Difficulties in expressing membrane proteins; and cross-talk is prone to occur between systems	Extracellular macromolecules; environmental monitoring
Riboswitches	Small molecules (purines; coenzymes; amino acids)	Medium	Fast (<30 min)	Fastest response speed; extremely low metabolic burden	Difficult to detect extremely low concentrations; complex structure design	Real-time monitoring; metabolic flux

—including proteins, metabolites, and nucleic acids—with high affinity and specificity.[31] Their synthetic accessibility, biocompatibility, and design flexibility make them attractive components for programmable biosensing. Recent efforts have incorporated aptamers into engineered bacteria to enable the detection of complex biological targets, such as cytokines and tumor biomarkers.[32] For example, engineered microbial platforms equipped with cancer-specific aptamers have demonstrated enhanced sensitivity and selectivity in tumor detection. Moreover, strategies that couple extracellular aptamer-based recognition with intracellular signaling circuits have enabled real-time monitoring of immunological markers. Such systems offer a promising avenue for developing *in vivo* biosensors capable of operating in dynamic disease microenvironments.

Riboswitches. Riboswitches are structured RNA elements that regulate gene expression in response to direct binding of small molecules or ions, typically without requiring intermediary proteins.[33,34] Upon ligand binding, riboswitches undergo conformational changes that affect transcription initiation or translation efficiency. These elements offer rapid signal response and minimal background noise, making them suitable for real-time detection of metabolic or ionic changes. Common applications of riboswitches include detection of magnesium ions (Mg^{2+}) and fluoride (F^-).[35] Recent studies demonstrate that engineered riboswitches can precisely regulate target genes based on exogenous metabolite concentration gradients, revealing substantial application potential in precision medicine and biosensing.[36] Their RNA-based nature also makes them amenable to integration with CRISPR-based gene regulation and RNA-sensing platforms.

Two-component systems (TCS). TCS are widespread signal transduction modules in bacteria that enable adaptive responses to a broad range of extracellular and periplasmic stimuli.[26,37,38] A canonical TCS consists of a sensor histidine kinase that detects the external signal and a response regulator that modulates gene expression in response.

In contrast to their one-component counterparts, two-component systems (TCS) allow microorganisms to monitor a diverse spectrum of environmental cues, ranging from nutrient concentrations (*e.g.*, amino acids, carbon sources) and the presence of chemical stressors (*e.g.*, antibiotics) to changes in physicochemical conditions such as pH and osmotic pressure.[39,40] Engineered TCS circuits have been designed to detect inflammatory cues such as nitric oxide and nitrate, or pathogen-associated molecular patterns such as lipopolysaccharide (LPS).[41] Their robust dynamic range, high signal fidelity, and compatibility with complex biological environments make them ideal candidates for *in vivo* biosensing applications, including inflammation and infection monitoring.

2.2.2 Signal transduction mechanisms. Signal transduction mechanisms are central to enabling engineered bacteria to interpret, process, and respond to complex environmental and pathological cues with high fidelity. Synthetic biology has empowered the design of sophisticated genetic circuits that mimic computational logic, facilitating the integration of diverse signals through modules such as Boolean logic gates, feedback loops, and multi-layered amplification cascades.

In the context of *in vivo* disease diagnostics, the target signals—such as aberrant metabolites, inflammation-associated molecules, or tumor-derived biomarkers—are often present at low concentrations and accompanied by substantial background noise.[42] These characteristics pose considerable challenges for sensitivity, specificity, and false-positive avoidance in microbial biosensing. To address these limitations, engineered bacterial systems increasingly rely on two key strategies: signal amplification and logic-based signal processing.[43]

Signal amplification strategies. Signal amplification plays a pivotal role in enhancing weak or transient stimuli into robust, detectable outputs. Several amplification architectures have been developed: (1) cascaded amplification: by arranging signal transduction elements in a hierarchical manner, weak input signals are progressively strengthened through multiple tiers, culminating in a significant phenotypic or reporter response.[44] (2) Positive feedback loops (PFLs): upon initial activation, the system

amplifies its own input, enabling exponential response propagation. For instance, Sayut *et al.* demonstrated the use of synthetic PFLs to boost low-level promoter activity, significantly increasing output signal strength.[45] (3) Autoinduction systems: these rely on quorum sensing-like strategies, wherein an initially stimulated bacterium produces and releases autoinducers or signal molecules, triggering a synchronized response in neighboring cells.[46] This collective behavior not only enhances detection sensitivity but also enables population-level signal integration. These amplification strategies can be customized or combined based on the specific requirements of target analytes, signal dynamics, and the background complexity of the sensing environment.

Logic gate-based signal processing. In addition to amplification, logic gate designs are frequently integrated into bacterial circuits to enhance decision-making precision and reduce false-positive responses.[47–49] Boolean logic operations such as AND, OR, NOT, and NOR enable bacteria to respond only when signal combinations meet predefined thresholds or contextual constraints: (1) AND gates: trigger outputs only when multiple input conditions are met simultaneously—*e.g.*, co-detection of lactate and hypoxia in tumor microenvironments ensures tumor-specific activation. (2) OR gates: permit response initiation upon recognition of any input signal, offering sensitivity across a broader range of pathophysiological states. (3) NOT/NOR gates: introduce inverse logic, suppressing reporter expression in the presence of inhibitory cues or absence of critical ligands, thereby improving context-selective detection. For example, a two-input AND gate toehold switch design not only enhances signal specificity but also effectively reduces background leakage. This system employs an engineered RNA structure that forms an inhibitory stem-loop, thereby sequestering the ribosome binding site (RBS) and blocking downstream gene translation. Only upon recognition of both specific RNA inputs does the structure unfold, exposing the RBS and initiating protein expression, ensuring that the downstream response is activated only when all input conditions are met[50] (Fig. 2).

2.2.3 Output response modules. Output modules are responsible for translating sensed molecular inputs into interpretable and quantifiable signals. For engineered bacteria functioning as living diagnostics, these modules enable the detection of disease-relevant cues through diverse output modalities, including fluorescence, colorimetric change, small-molecule secretion, and bioelectronic signals. Selection of the appropriate output strategy is application-specific and must balance factors such as sensitivity, stability, compatibility with biological matrices, and field-deployability (Table 3).[51]

Fluorescent and luminescent outputs. Fluorescent proteins, such as green fluorescent protein (GFP) and mCherry, remain among the most widely used reporters for real-time visualization of bacterial activation. These proteins provide robust, non-invasive readouts and are easily monitored *via* fluorescence microscopy or flow cytometry.[52] In advanced applications, engineered probiotic strains equipped with fluorescent reporters have been integrated with photodetector arrays to achieve *in situ* signal monitoring. For instance, intelligent bacterial systems sensitive to pro-inflammatory cytokines (*e.g.*, TNF-α) can activate fluorescent or bioluminescent output upon exposure to disease markers.[53] An illustrative example is the i-ROBOT system developed by Ye *et al.*, in which fluorescent reporter circuits embedded in engineered *E. coli* enable noninvasive diagnosis of inflammatory bowel disease (IBD) in mice. Signal intensity in fecal samples correlates with inflammation severity, enabling real-time disease quantification.[54]

Colorimetric outputs. Colorimetric reactions offer intuitive, equipment-free readouts based on visible color changes triggered by enzymatic activity or chromogenic substrates. One widely adopted example is the expression of β-galactosidase, which catalyzes the conversion of X-gal into a blue precipitate.[55] These outputs are ideal for point-of-care

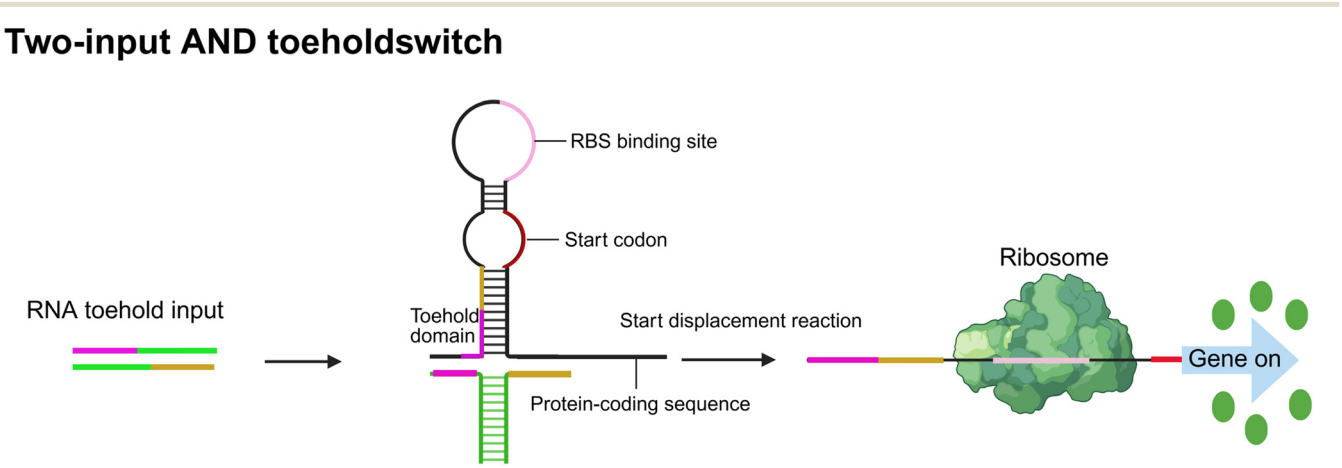

Fig. 2 Synthetic route of double AND gate.

Table 3 Comparative analysis of bacterial output modules

Output module	Mechanism	Sensitivity	Kinetics	Robustness	Application scenarios	Limitations
Fluorescent/luminescent	Expression of reporter proteins (GFP; mCherry; bioluminescence)	High	Real-time monitoring possible	Proteins are generally stable; but signal requires external excitation	Laboratory settings; flow cytometry; and *in situ* imaging	Typically requires external equipment; less suitable for naked-eye field diagnosis
Colorimetric	Enzymatic conversion of substrate to visible color (β-galactosidase)	Medium	Dependent on enzymatic turnover rates	Needs optimization	Point-of-care (POC) and low-resource settings	Non-quantitative (often binary yes/no)
Small-molecule secretion	Metabolic production of diffusive compounds (indoles; short-chain fatty acids; aldehydes)	High	Faster kinetics than protein-based reporters	Excellent stability and diffusivity	Complex biological samples (*e.g.*, wound exudates, stool)	Requires specific sensor integration
Bioelectronic	Extracellular electron transfer (EET) to electrodes	High	Real-time response	Long-term stability	Continuous, remote, and non-invasive monitoring (digital health)	Complex fabrication; requires managing the bio-material interface

and low-resource settings due to their low cost, ease of use, and instrument-free operation. Ongoing efforts aim to enhance the stability and signal-to-noise ratio of colorimetric reporters, enabling broader deployment in portable diagnostic formats.

Small-molecule secretion. Engineered bacteria can also be programmed to produce small-molecule metabolites (*e.g.*, indoles, short-chain fatty acids, or aldehydes) in response to specific stimuli. These compounds exhibit excellent stability and diffusivity, and can be detected using portable analytical devices such as electrochemical sensors.[56] Compared to protein-based reporters, small-molecule outputs allow faster kinetics and reduced background interference, making them suitable for real-time detection in complex matrices such as stool or wound exudates.

Bioelectronic readouts. The convergence of synthetic biology and bioelectronics has enabled the development of electrogenic bacterial biosensors, which transduce molecular recognition events into measurable electrical currents. This is typically achieved by engineering extracellular electron transfer (EET) pathways that connect metabolic responses to electrodes.[57,58] For example, *E. coli* engineered to sense nitrate can generate detectable current changes upon target recognition. This principle was applied in an implantable bioelectronic system developed by Mimee *et al.*, which wirelessly transmitted diagnostic signals from the intestinal lumen to external receivers, enabling continuous and non-invasive monitoring of IBD.[59] Compared with traditional fluorescent or colorimetric reporters, bioelectronic outputs offer higher temporal resolution, real-time response, remote readout capability, and seamless integration with digital health platforms.[60]

Future work in this domain is focused on improving long-term stability, minimizing host immune interference, and optimizing the interface between microbial circuits and conductive materials (*e.g.*, carbon nanotubes, flexible electrodes). AI-driven signal processing will further refine noise filtering, pattern recognition, and early anomaly detection in bioelectronic systems.

2.3 AI-driven intelligent design and optimization

AI is emerging as a transformative force in synthetic biology, enabling the rational design, optimization, and deployment of engineered microbial systems across diverse applications, including disease diagnostics, environmental monitoring, and biosensing. Traditional trial-and-error approaches are increasingly being supplanted by AI-driven methodologies, which can accelerate workflows, improve predictive accuracy, and support adaptive decision-making. Recent advances in deep learning, graph neural networks, and evolutionary algorithms have equipped researchers with powerful computational tools that enable the systematic exploration and optimization of microbial chassis, genetic circuits, and sensing systems (Table 4).

2.3.1 Virtual screening: AI-assisted selection of microbial chassis and genetic components. Traditional microbial engineering workflows rely heavily on laborious experimental screening, often constrained by throughput and cost. AI-powered virtual screening tools facilitate the prioritization of optimal genetic parts and microbial hosts by analyzing large-scale omics datasets and genotype–phenotype associations.

Crucially, multi-omics integration has transitioned from descriptive profiling to the predictive discovery of novel biological entities that were previously obscured by data complexity. For instance, in the context of chassis selection, machine learning frameworks applied to metagenomic data have successfully pinpointed specific probiotic strains, such as distinct *Lactobacillus reuteri* isolates associated with healthy gut metrics, validating their potential as robust vectors for gastrointestinal biosensing.[61] Regarding genetic components, explainable AI models (*e.g.*, XGBoost) have mined diverse fungal genomes to identify four previously uncharacterized xylose transporter genes, demonstrating the

Table 4 Comparison of different AI models

Model	Application domain	Model architecture	Prediction accuracy	Data requirements	Computational cost	Strengths	Limitations
DeepPromoter[65]	Promoter strength prediction	CNN + LSTM	Accuracy up to ~98–99% on human and mouse promoter datasets	~60 000 labeled promoter sequences (EPDnew); fixed-length (~300 bp) DNA sequences	Moderate training cost; single-GPU training feasible; fast inference	High accuracy with relatively small datasets; captures hierarchical sequence features	Limited generalization beyond trained species; relies on labeled promoter datasets
AlphaFold2 (ref. 80)	Protein structure prediction	Transformer-based attention networks with Evoformer	Near-experimental accuracy (median backbone RMSD ~0.96 Å in CASP14)	>100 000 PDB structures; millions of protein sequences from UniRef; BFD; MGnify	Extremely high; TPU-based training (~1 week); GPU inference minutes–hours per protein	Unprecedented structural accuracy; broad proteome coverage	High computational and data demands; inference cost scales cubically with sequence length
RoseTTAFold[81]	Protein structure prediction	3-Track neural network (sequence; distance; coordinate)	High but lower than AlphaFold2	PDB + multiple sequence alignments (MSAs)	High but lower than AlphaFold2	Faster inference; lower hardware requirements	Slightly reduced accuracy
ESMFold[82]	Protein structure prediction	Large language model (protein LLM)	Moderate–high; lower than AlphaFold2	Hundreds of millions of protein sequences (no MSAs required)	Moderate; faster inference than AlphaFold2	MSA-free; scalable to large datasets	Reduced accuracy for complex folds
DeepCRISPR[67,83]	gRNA design and efficiency prediction	Hybrid CNN-autoencoder	High in human/mouse contexts; moderate in non-mammalian systems	Limited experimentally validated gRNA datasets	High training cost; moderate inference cost (GPU-dependent)	Rapid design and screening	Limited species generality; requires epigenetic data; high data dependency

capacity to expand the library of functional parts available for sensing and metabolic engineering.[62] Furthermore, deep learning has revealed characteristic tRNA sequence motifs that accurately distinguish probiotic from pathogenic bacteria, providing a novel, sequence-based metric for evaluating the biosafety of potential biosensor chassis.[63] These findings illustrate how AI-driven screening can uncover previously overlooked chassis candidates and functional parts beyond conventional choices. These approaches enable rational chassis selection based on ecological compatibility, safety, and engineering flexibility.

2.3.2 Intelligent design: optimization of genetic elements and circuit logic. AI-based sequence-to-function prediction models are increasingly used to inform the design of regulatory elements, such as promoters, ribosome binding sites, and inducible systems. Deep learning frameworks—including convolutional neural networks (CNNs), long short-term memory (LSTM) models, and transformer-based architectures—have demonstrated efficacy in predicting promoter strength and tuning regulatory dynamics.[64] DeepPromoter combines CNN and LSTM layers to extract hierarchical sequence features, enabling quantitative prediction of promoter activity. The model processes promoter sequences, transforms the input data, and extracts hierarchical features, enabling the quantitative prediction of promoter activity. DeepPromoter-designed sequences demonstrated up to a 10-fold increase in transcriptional activity compared to wild-type controls, closely aligning with the predicted strength profiles and validating the model's capability to capture complex regulatory motifs.[65] Xiong *et al.* proposed an integrated framework for promoter synthesis and strength prediction based on diffusion models and transformer architectures. This approach leverages deep learning to capture the multidimensional features of regulatory elements from vast genomic datasets, thereby optimizing the design of synthetic promoters and demonstrating outstanding performance in predicting promoter strength[66] (Fig. 3). Unlike CNN-based approaches, the transformer model is capable of capturing hierarchical sequence features, including nucleotide composition, dinucleotide patterns, and positional information, thereby facilitating more accurate quantitative prediction of promoter activity. For model evaluation, the transformer-based approach achieved a parson correlation coefficient of 0.295 between predicted and experimentally annotated promoter strengths, outperforming the baseline CNN model, which reached a parson correlation coefficient of 0.25. Other tools, such as gRNA designer, also facilitate the computational optimization of genetic elements. Validation studies have demonstrated that high-scoring guide RNAs

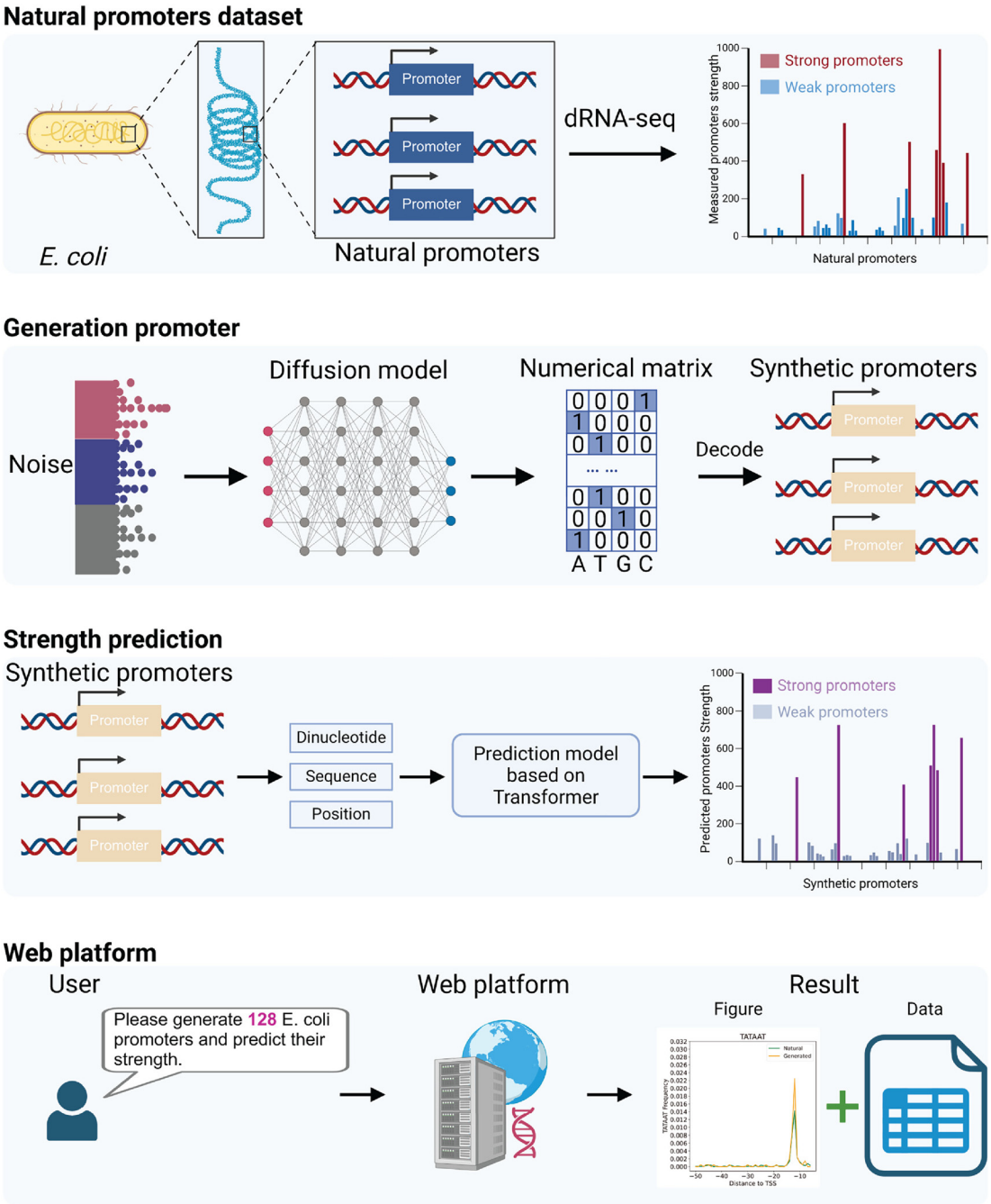

Fig. 3 The computational framework for promoter generation and strength prediction.[66]

predicted by these models yield significantly higher editing efficiencies and indel frequencies *in vivo* compared to unoptimized controls.[67]

Structure-based protein engineering has also been revolutionized by deep learning models such as AlphaFold, which accurately predict the three-dimensional conformations of sensor proteins, including their ligand-binding domains, conformational stability, and membrane integration.[68,69] This structural insight guides the engineering of input modules with improved sensitivity and selectivity. Beyond component-level design, generative adversarial networks (GANs) and reinforcement learning algorithms have enabled the construction of synthetic circuits capable of executing Boolean logic operations (*e.g.*, AND, NOR, NAND), supporting multi-signal integration and programmable microbial behaviours.[70] Microfluidic testing of these AI-designed circuits verified that the engineered bacterial populations executed logic operations with digital-like precision, matching the theoretical truth tables generated by the computational models.

2.3.3 Performance prediction: modeling dynamic gene circuit behaviours. AI is uniquely suited to predict the

emergent behaviour of synthetic gene circuits in complex biological contexts. Reinforcement learning (RL) offers a powerful framework for exploring the dynamic response of engineered systems to fluctuating inputs and environmental noise.[71] For instance, a novel lactate-responsive regulatory element and sensor, designed through bioinformatics screening, exhibited resistance to glucose-mediated repression in the tumor microenvironment, enabling engineered bacteria to accurately sense lactate concentrations in tumor tissues.[72]

AI-guided pathway design tools have also facilitated the optimization of metabolic flux, as exemplified by an *E. coli* strain engineered for 3-phenylpropanol production with significantly improved yield.[73] Genome-scale kinetic models integrated with multi-agent reinforcement learning can iteratively refine enzyme expression profiles without predefined stoichiometric constraints.[74] These capabilities support predictive modeling of complex, multi-input genetic systems and enable real-time control of biosynthetic processes.

2.3.4 High-throughput simulation: accelerating the design–build–test–learn (DBTL) cycle. The DBTL cycle forms the cornerstone of synthetic biology, but its manual implementation limits throughput. Automated platforms integrating microfluidics, robotic liquid handling, and real-time sensors are now being coupled with AI-driven design and analysis pipelines. These systems allow parallelized experimentation on thousands of genetic constructs, with AI algorithms such as Bayesian optimization and active learning guiding experimental prioritization and model refinement.[75,76] Real-time feedback from fluorescence and growth rate measurements feeds into continuously updated models, substantially reducing development timelines while increasing screening efficiency and success rates.

2.3.5 AI-powered analytics: real-time sensing, control, and decision support. Beyond design, AI platforms are increasingly being deployed for real-time monitoring and adaptive control of engineered microbial systems. By integrating multimodal data streams—including optical imaging, gas chromatography-mass spectrometry (GC-MS), and environmental sensing—these systems employ CNNs, LSTMs, and hybrid models to extract key features and detect aberrant behaviours.[77] For instance, in inflammation-responsive biosensors, LSTM-based models can identify abnormal fluctuations in fluorescence intensity and automatically adjust inducer concentrations or sampling conditions. Reinforcement learning algorithms support adaptive optimization of experimental parameters, enabling closed-loop control of biosensor performance.

A major strength of AI-powered platforms lies in their ability to detect subtle phenotypic signatures and complex interactions within high-dimensional datasets. These insights support robust predictions of disease markers, microbial activity states, or biosensor performance. In oncology, AI systems have been used to integrate microbial sensing outputs with clinical imaging data to predict tumour invasiveness and therapeutic response.[78] Importantly, these systems exhibit continuous learning capabilities. As additional data are acquired, model parameters are dynamically refined, enhancing diagnostic precision and adaptability. Integration with expert knowledge systems further improves interpretability and user trust, facilitating deployment in clinical or environmental settings.[79]

By fusing data-driven inference, real-time feedback, and predictive modeling, AI-enabled analytics platforms represent a critical infrastructure for the deployment of intelligent microbial biosensors at scale. This convergence of synthetic biology and artificial intelligence is poised to redefine diagnostic and therapeutic paradigms.

2.3.6 AI as a multilayer enabler in engineered bacterial biosensing. The integration of AI into bacterial biosensing is not merely incremental but fundamentally transformative, as it operates across three clearly delineated layers of the engineering lifecycle: design optimization, performance prediction, and real-time data analytics (Table 5).

At the design optimization level, AI-driven generative and structure-prediction models shift biosensor development from empirical screening toward rational and proactive engineering. For example, generative adversarial networks (GANs) and other generative frameworks can propose novel genetic parts with predefined performance constraints, while structure-based deep learning tools such as AlphaFold enable the atomic-level prediction of receptor–ligand interactions, guiding the optimization of sensing proteins and orthogonal regulatory elements before experimental validation.[84]

At the performance prediction stage, sequence-to-function deep learning models, including convolutional neural networks (CNNs) and long short-term memory (LSTM) architectures, are employed to quantitatively map genetic sequence features to functional outputs. These models enable *in silico* evaluation of circuit dynamics, signal-to-noise characteristics, and metabolic burden, thereby eliminating

Table 5 The role of artificial intelligence in biological sensors

AI role	Functional objective	AI/ML algorithms	Representative application & outcome
Design optimization	Generation of high-affinity receptors and orthogonal genetic parts	GANs; transformer models (*e.g.*, AlphaFold)	*In silico* design of specific promoters resulted in a 10-fold increase in sensor sensitivity
Performance prediction	Simulation of circuit behavior and host burden before wet-lab assembly	LSTM; CNN; kinetic modeling	Prediction of leakage rates in genetic circuits with >90% accuracy; reducing screening workload
Real-time analytics	Decoding complex/noisy output signals into binary diagnostic decisions	SVM, random forest; ANN	Smartphone app processing of colorimetric bacterial signals to classify patient samples with clinical-grade precision

low-performing or unstable designs prior to costly build–test cycles and improving overall engineering efficiency.[85]

Finally, in the real-time data analytics phase, AI serves as the computational engine that translates complex biological signals into actionable diagnostic outcomes. Machine learning classifiers such as support vector machines (SVMs) and random forest algorithms, when coupled with portable or point-of-care readout devices, can robustly decode multiplexed fluorescence, luminescence, or colorimetric outputs under noisy conditions and convert them into accurate, binary diagnostic decisions in real time.[86]

Collectively, these three functional layers clarify the concrete role of AI in engineered bacterial biosensing, demonstrating how AI systematically enhances design rationality, predictive accuracy, and diagnostic robustness across the entire biosensor lifecycle.

3 Applications of engineered bacteria in multi-scenario detection

3.1 Environmental monitoring

Amidst escalating concerns over environmental pollution, the demand for rapid, sensitive, and cost-effective monitoring tools continues to rise. Engineered bacteria, empowered by synthetic biology, have emerged as promising "living biosensors" for real-time, *in situ* environmental surveillance. By integrating pollutant-responsive genetic circuits, signal amplification modules, and robust microbial chassis, these systems offer high specificity and sensitivity toward diverse environmental contaminants—including heavy metals, organic compounds, and nutrient overloads. Of particular interest is the recent development of microbial bioelectronic sensors, which transduce biochemical recognition events into electrical outputs, enabling precise and continuous monitoring of target analytes (Table 6).[11,87–89] Recent advances have further integrated AI-driven analysis with these living sensors, allowing automated interpretation of complex biosensor outputs and predictive environmental surveillance.[90]

3.1.1 Detection of heavy metal contaminants. The detection of heavy metals represents a fundamental diagnostic challenge shared across water, soil, and food safety. Heavy metals such as mercury (Hg^{2+}), lead (Pb^{2+}), cadmium (Cd^{2+}), and copper (Cu^{2+}) are persistent environmental toxins with high bioaccumulation potential. Conventional detection methods, while sensitive, often entail expensive instrumentation and complex sample preparation. In contrast, engineered bacterial biosensors utilize metal-responsive transcriptional regulators—such as MerR, PbrR, and ZntR—to drive reporter gene expression in response to specific ions.[91] However, a major challenge in environmental sensing is the "crosstalk" between different metal ions. To address this, recent studies have employed artificial neural networks to analyze the fluorescence patterns from whole-cell sensor arrays. For example, a MerR-based *E. coli* biosensor developed by Che *et al.* demonstrated nanomolar sensitivity to Hg^{2+} with rapid response times *via* fluorescent or electrochemical outputs. Addressing the challenge of multi-pollutant coexistence, synthetic gene circuits have been designed to integrate multiple sensing modules and orthogonal reporters, generating complex composite signals (Fig. 4).[92] By training machine learning classifiers on these signal outputs, the system can decouple interference and accurately identify specific heavy metals within mixed-solution samples, acting as an intelligent biological logic gate.

To improve environmental robustness and field deployability, bacterial sensors have been immobilized in hydrogels, cellulose membranes, and embedded into microfluidic platforms—enabling the development of portable or wearable biosensing devices for real-time monitoring of heavy metals in aquatic environments.[93]

3.1.2 Monitoring of organic pollutants. Volatile and semi-volatile organic pollutants—including aromatic hydrocarbons, phenols, and diesel-derived metabolites—are prevalent in industrial effluents and oil-contaminated marine environments, where they pose serious threats to aquatic ecosystems.[94–96] To enable field-deployable monitoring, researchers are developing "electronic noses" (e-noses) utilizing engineered bacteria. These systems combine microbial sensing elements with pattern recognition algorithms. In the context of offshore oil spills—an increasingly frequent and ecologically damaging phenomenon—engineered strains of *Pseudomonas*,

Table 6 Benchmarking of representative engineered bacterial biosensors across application domains

Application domain	Target analyte	Biosensing mechanism	Operating matrix	Limit of detection (LOD)/range	Response time	Validation level
Environmental[92]	Mercury (Hg^{2+})	*E. coli* (MerR + fluorescence/electrochem)	Water/buffer	Nanomolar sensitivity	Rapid (<1 h)	*In vitro*/lab
Environmental[136]	Phenol	*Pseudomonas* (conductance-based)	Marine water	2 μM	Real-time	Field (simulated)
Food safety[106]	Methyl parathion	*S. paucimobilis* (enzyme inhibition)	Buffer/food	0.1–1 μg mL^{-1} (linear range)	N/A	*In vitro*
Food safety[107]	Histamine	*P. aeruginosa* (transcription factor + fluor.)	Seafood/wine	0.39 ppm	90 min	Real food samples
Disease (cancer)[121,122]	Tumor volatiles/lactate	*E. coli* Nissle 1917 (metabolic conversion)	Urine	Qualitative presence/100% specificity	N/A	*In vivo* (mouse)
Disease (IBD)[137,138]	Thiosulfate	*E. coli* Nissle 1917 (memory/base editing)	Gut lumen/feces	Qualitative event recording	Real-time (recording)	*In vivo* (mouse)

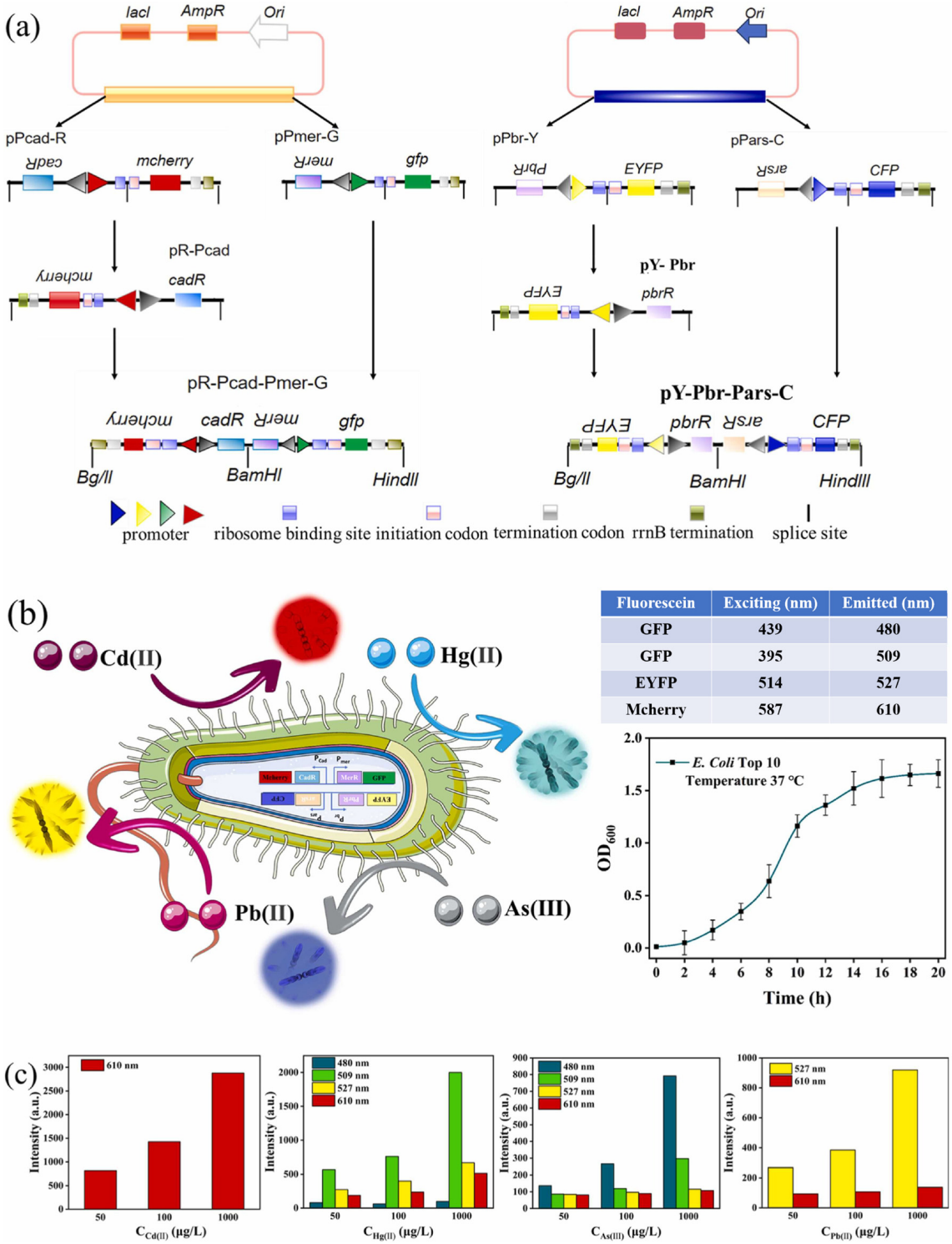

Fig. 4 (a) Schematic diagram of the construction process of carriers pR-Pcad-Pmer-G (left) and pY-Pbr-Pars-C (right), respectively. (b) The TOP10/pR-Pcad-Pmer-G+pY-Pbr-Pars-C, which integrates multiple promoters and reporter genes, can simultaneously sense stimuli from Cd(II), Hg(II), As(III), and Pb(II) and emit red, green, blue, and yellow fluorescence under corresponding wavelength excitation stimuli. (c) The fluorescence intensity bar graph of TOP10/pR-Pcad-Pmer-G+pY-Pbr-Pars-C cultured in different concentrations (50, 100, 1000 μg L^{-1}) of Cd(II), Hg(II), As(III), and Pb(II) solutions for 6 h, respectively.[92]

such as GSN23, have been employed in conductance-based biosensors for the direct detection of phenol.[97] Such systems offer a scalable and cost-effective solution for the continuous monitoring of marine environments, supporting early warning and mitigation of pollution events. AI algorithms assist in monitoring the temporal dynamics of these sensors, predicting

the dispersion trajectory of pollutants based on real-time biological feedback. Similarly, for eutrophication monitoring, dual-strain biosensing systems quantifying nitrate and nitrite are now being integrated with IoT (internet of things) platforms, where AI models analyze long-term nutrient trends to predict and prevent harmful algal blooms.[98,99]

3.2 Applications of engineered bacteria in food safety monitoring

Building upon the sensing principles established for environmental monitoring, food safety applications face the unique diagnostic challenge of functioning within complex organic matrices. Biosensing platforms based on engineered bacteria integrate molecular recognition modules with genetic circuits to transduce contaminant detection into visible colorimetric, fluorescent, or electrochemical signals. These systems offer operational simplicity, scalability, and adaptability for on-site applications.

3.2.1 Detection of pesticide residues. Pesticide residue monitoring in agricultural and food systems represents a representative application scenario within the broader framework of engineered bacterial sensing, sharing core design principles with environmental and food safety biosensors. Although pesticides comprise chemically diverse classes—including organophosphates, pyrethroids, organochlorines, carbamates, and inorganic compounds—their biological detection commonly relies on conserved mechanisms such as enzyme inhibition, metabolic perturbation, or toxin-induced stress responses.[98,100] In contrast to conventional analytical methods that require extensive sample preparation and centralized instrumentation, bacterial biosensors enable direct signal transduction at the point of sampling.[101]

Consistent with strategies employed in environmental toxicity monitoring, luminescent bacterial systems have been adapted for pesticide detection through matrix immobilization and optical readout.[102,103] For example, *Photobacterium leiognathi* immobilized in alginate beads enables quantitative assessment of pesticide-induced luciferase inhibition, as demonstrated for 2,4-dichlorophenoxyacetic acid by Ranjan *et al.*[104] Similarly, biohybrid platforms integrating pesticide-degrading bacteria with nanomaterial scaffolds parallel developments in foodborne contaminant sensing. Mishra *et al.* reported a microplate-based optical sensor combining *Sphingomonas paucimobilis* with polyethyleneimine-functionalized silica nanoparticles, achieving a linear detection range of 0.1–1 μg mL^{-1} for methyl parathion and long-term operational stability exceeding six months.[105,106]

Notably, recent pesticide biosensing systems increasingly adopt AI-enabled signal interpretation pipelines that mirror those described in other application domains. Smartphone-based platforms coupled with deep learning-assisted image analysis standardize optical signal acquisition across heterogeneous sample matrices, while machine learning classifiers such as support vector machines facilitate discrimination between structurally similar pesticide compounds (*e.g.*, methyl parathion *versus* chlorpyrifos).[102] Rather than introducing fundamentally new sensing mechanisms, these AI-driven approaches enhance robustness, specificity, and deployability, reinforcing a convergent trend toward unified, intelligent bacterial biosensing architectures across environmental, food and agricultural monitoring contexts.

3.2.2 Assessment of food spoilage and shelf life. Microbial activity and chemical degradation are primary drivers of food spoilage during storage and distribution. Engineered bacteria designed to detect spoilage-associated volatiles or metabolites offer real-time monitoring capabilities through reporter genes encoding fluorescent proteins, luciferases, or electroactive enzymes. The intensity of the output signal correlates with the concentration of spoilage markers—such as volatile amines or thiols—enabling quantitative assessment of food freshness. Bhatt, Ankita *et al.* developed a *Pseudomonas aeruginosa*-based biosensor carrying a histamine-responsive regulatory element coupled to a fluorescent reporter, allowing rapid detection of histamine in seafood and wine with a detection limit of 0.39 ppm and a response time of 90 minutes.[107] Moving beyond reactive detection, AI-enhanced biosensors enable the predictive modeling of shelf life. Engineered bacteria designed to detect spoilage-associated volatiles (amines, thiols) provide real-time data that can be processed by time-series analysis algorithms to forecast spoilage events before they occur. When integrated with smart packaging and AI logistics systems, this biological data optimizes supply chain decisions, reducing food waste.

3.2.3 Detection of fungal spoilage and food additives. Grain-based food products are highly susceptible to fungal contamination during storage, leading to spoilage and the accumulation of mycotoxins. To address this, researchers at the Institute of Agro-Products Processing Science and Technology (Chinese Academy of Agricultural Sciences) developed a whole-cell biosensor array responsive to early-stage fungal volatiles.[108] Crucially, this system relies on a back-propagation neural network. Using a library of 14 stress-responsive *E. coli* promoters, the back-propagation neural network analyzes the combinatorial bioluminescence patterns to fingerprint specific fungal pathogens. The system accurately predicted fungal spoilage in peanuts and corn two days before visible signs emerged, achieving up to 98% prediction accuracy demonstrating the power of AI in decoding complex biological languages.[109]

3.3 Assessment of soil and crop environments

Crop infections during both cultivation and post-harvest storage pose a major threat to global food security. Diseases such as potato soft rot—caused primarily by *Dickeya* and *Pectobacterium* species—can progress rapidly under storage conditions due to the secretion of cell wall-degrading enzymes. Early-stage infections are often asymptomatic and therefore difficult to

detect, resulting in missed opportunities for timely intervention and leading to substantial postharvest losses.[110,111] Conventional pathogen detection approaches—including morphological characterization, PCR, and ELISA—offer high specificity but are hindered by long processing times, complex sample preparation, and destructive analysis, rendering them impractical for real-time or on-site diagnostics.[112–114] Furthermore, these methods generally lack the sensitivity required to detect pathogens during the latent infection phase.

Pathogens emit specific VOC profiles as metabolic byproducts during infection, reflecting microbial activity even before visible symptoms appear. Continuous monitoring of VOC fluctuations in the crop microenvironment offers the potential for non-destructive, real-time detection of latent infections. Recent advances in synthetic biology have enabled the engineering of living biosensors—typically bioluminescent bacterial strains—that respond selectively to pathogen-associated VOCs.[115] These bacterial systems are programmed with high-affinity olfactory modules and transcriptional circuits that activate luciferase or other reporters upon exposure to trace amounts of infection-related volatiles.

To facilitate practical deployment, engineered bacteria can be immobilized on microfluidic chips or embedded within porous matrices placed near stored crops. Upon detecting VOC levels exceeding predefined thresholds, the bacteria emit bioluminescent signals that can be recorded using portable imaging systems, enabling early diagnosis and rapid mitigation.

Collectively, these emerging biosensing platforms offer a scalable, low-cost, and highly adaptable solution for mitigating postharvest losses and improving crop protection strategies.

3.4 Engineered bacteria for disease detection

Recent advances in synthetic biology have ushered in a new generation of programmable bacterial diagnostics capable of sensing, processing, and responding to disease-associated biomarkers. Engineered bacteria now serve as living diagnostics in diverse clinical contexts, ranging from infectious disease and cancer to gastrointestinal and metabolic disorders. These biosensors leverage modular genetic circuits, quorum-sensing systems, CRISPR-based logic

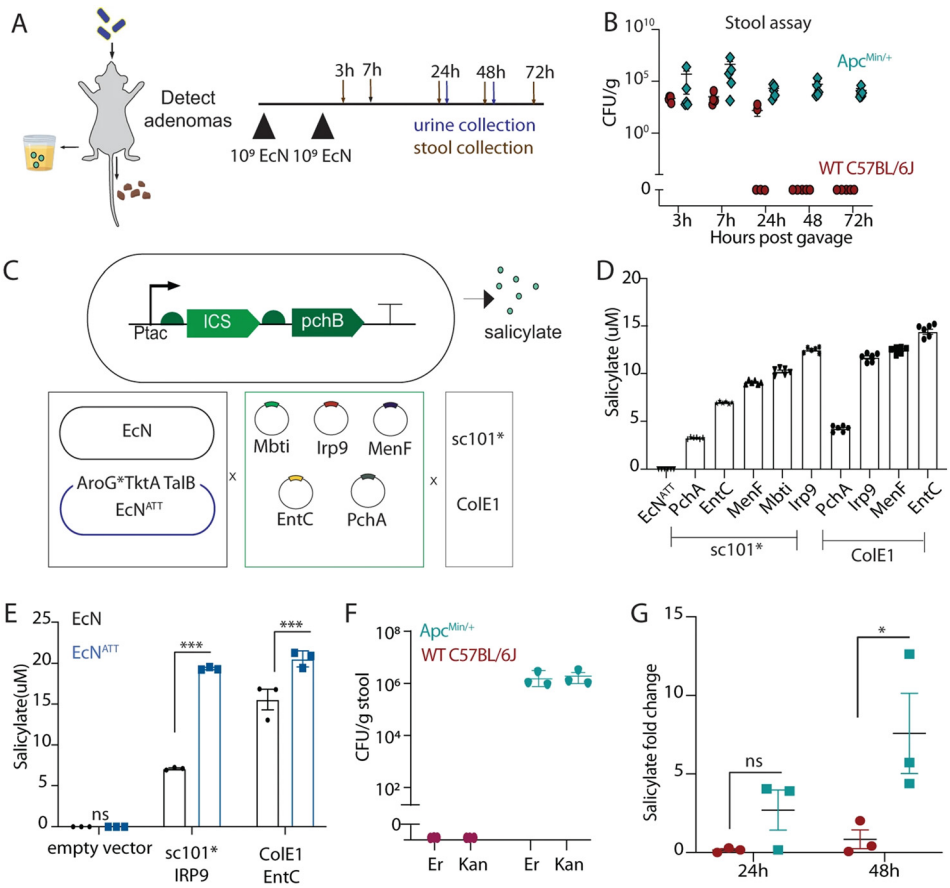

Fig. 5 Engineering tumor-colonizing *E. coli* Nissle 1917 for detection and treatment of colorectal neoplasia. A. Oral delivery and fecal persistence. Schematic of EcN probiotic detection *via* fecal CFU or urinary metabolite quantification. B. Time-course of EcN recovery from stool pellets in 15-week-old $Apc^{Min/+}$ mice following oral dosing (n = 5). C. Design of the ICS-pchB salicylate biosynthesis pathway. D and E. LC-MS quantification of salicylate from EcN^{ATT} variants, showing enhanced production compared to wild-type EcN (n = 3–6 ; ****$p < 0.0001$, Two-way ANOVA). Values normalized to D4-salicylate. F. Recovery of total and plasmid-retaining EcN^{ATT} from stool 48 h post-dosing (n = 3). G. Urinary salicylate quantification *via* LC-MS in WT *vs.* $Apc^{Min/+}$ mice at 24 and 48 h, demonstrating differential diagnostic potential (n = 3; *p = 0.0228).[122]

gates, and integration with wearable or ingestible devices to enable real-time, minimally invasive, and context-aware diagnostics *in vivo*.[116]

3.4.1 Detection of infectious diseases. Rapid and accurate diagnosis of infectious diseases is critical for timely intervention and prevention of transmission.[117] Traditional methods such as culture, serology, and PCR offer high specificity but are limited by prolonged turnaround times and equipment requirements. Engineered bacterial biosensors, by contrast, provide fast, field-deployable solutions by detecting pathogen-associated metabolites or microenvironmental cues. For example, engineered *Escherichia coli* strains have been designed to detect *Vibrio cholerae* by incorporating toxin-regulated promoters (*e.g.*, CTX-responsive elements) that drive reporter expression, enabling visual detection of cholera in fecal samples within hours—faster than PCR and ideal for resource-limited settings.[118–120] Furthermore, CRISPRi-based genetic inverter circuits have been coupled with the *V. cholerae*-specific quorum-sensing molecule CAI-1. In this system, CAI-1 binding relieves repression by dCas9, activating GFP expression and enabling highly specific detection within two hours—approximately 100-fold faster than ELISA.

3.4.2 Cancer diagnosis *via* tumor-sensing bacteria. Engineered microbes are increasingly being applied for early cancer detection by exploiting the unique metabolic and

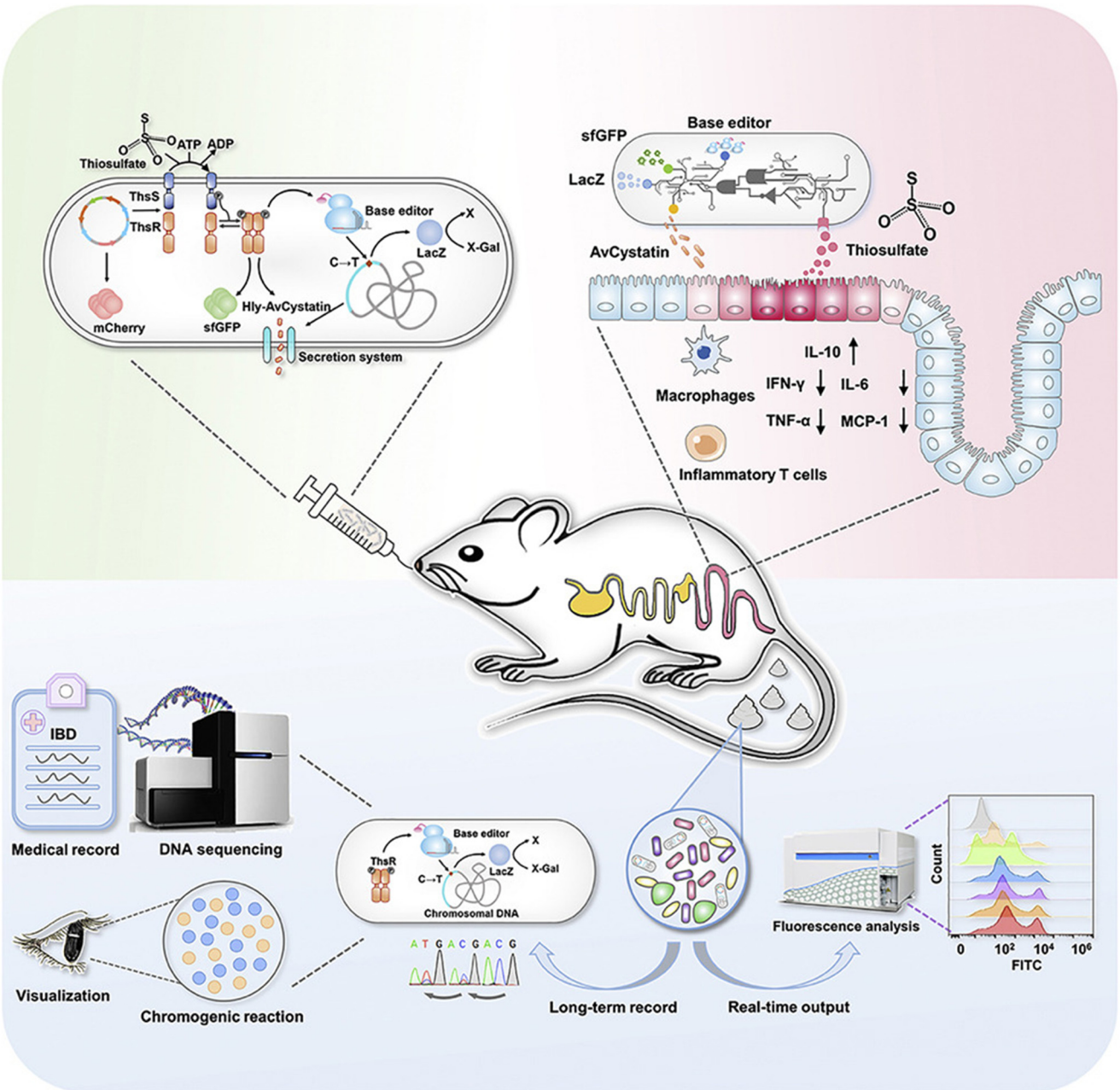

Fig. 6 Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice.[129]

physiological characteristics of the tumor microenvironment. Tumors typically exhibit hypoxia, low pH, and elevated lactate levels due to aerobic glycolysis (Warburg effect). Danino and colleagues developed a lactate-responsive system in *E. coli*, enabling non-invasive detection of tumors *via* metabolic readouts in urine samples.[121] Integration of this metabolic data with patient clinical history *via* random forest classifiers enhances the diagnostic specificity. Gurbatri *et al.* integrated salicylate biosynthesis genes, including irp9, mbtI, menF, entC, and pchA, into the genome or plasmids of *Escherichia coli* Nissle 1917 (EcN). These genes encode enzymes capable of converting native bacterial metabolites into salicylate *via* engineered metabolic pathways. Upon colonization of tumor tissue, the engineered EcN strains can sense characteristic features of the tumor microenvironment, such as hypoxia or specific metabolic intermediates, thereby activating expression of the salicylate production circuit. The salicylate generated *in situ* is subsequently excreted into the host urine, where it can be quantitatively measured using liquid chromatography-mass spectrometry (LC-MS), enabling non-invasive detection of tumor presence[122] (Fig. 5).

Beyond metabolic markers, bacteria have been engineered to detect oncogenic mutations. The CRISPR-based CATCH system, for instance, utilizes *Acinetobacter baylyi* to acquire circulating mutant DNA (*e.g.* KRAS G12D) and activates kanamycin resistance only upon successful homologous recombination. This allows highly specific mutation detection *in vitro* (cell lines, organoids) and *in vivo* (mouse rectal models).[5]

To address the limitations of conventional imaging, which often fails to detect micrometastases, tumor-tropic *Salmonella* strains expressing ZsGreen fluorescent protein have been developed. These bacteria preferentially colonize microlesions as small as 0.043 mm^3—offering detection sensitivity ~2600-fold higher than current radiographic methods.[123] Additionally, dual-responsive biosensors targeting tumor-specific lactate and hypoxia have been designed to provide spatiotemporal maps of tumor progression *via* fluorescence imaging.[124,125]

3.4.3 Monitoring intestinal diseases. Gastrointestinal disorders, including inflammatory bowel disease (IBD), are commonly diagnosed *via* invasive procedures such as colonoscopy and biopsy, which are costly and burdensome. Engineered probiotics—particularly strains based on *E. coli* Nissle 1917 (EcN)—now serve as living biosensors for gut inflammation, with the potential for both diagnosis and therapy.[126,127]

These systems exploit inflammatory biomarkers such as nitrate, thiosulfate, and hydrogen peroxide. Riglar *et al.* engineered *E. coli* with the NarX/NarL system to detect nitrate, enabling real-time *in vivo* inflammation monitoring.[128] Building on this, the i-ROBOT platform developed by Ye and Zhou integrates molecular sensing, logic gating, and therapeutic response in a single EcN chassis. Upon sensing thiosulfate, i-ROBOT initiates base editing to create heritable genomic records and activates colorimetric output while releasing the anti-inflammatory protein AvCystatin. This platform demonstrated diagnostic and therapeutic efficacy in colitis mouse models[129] (Fig. 6). By coupling these biological recorders with external AI analysis of fecal biomarkers, clinicians can reconstruct a temporal map of gut inflammation, enabling personalized treatment adjustments.

To further enhance diagnostic capability, researchers have developed ingestible biosensor devices incorporating engineered bacteria and microelectronics. For instance, commensal *E. coli* expressing hemoglobin-responsive luciferase were encapsulated in a swallowable device capable of detecting gastrointestinal bleeding in large animal models.[30] These "bacterio-electronic" pills transmit real-time luminescence data to external receivers, where machine learning algorithms filter gastrointestinal noise to accurately identify bleeding events. Similarly, Liu *et al.* embedded bioluminescent heme-responsive bacteria into magnetic hydrogels to enable spatially controllable intestinal diagnostics using external magnets and real-time imaging.[130]

3.4.4 Diagnostics for metabolic diseases. Engineered bacterial biosensors are also being explored for metabolic diseases such as diabetes and phenylketonuria (PKU), where dynamic metabolite monitoring is essential. In diabetes, synthetic circuits in *E. coli* enable high-sensitivity glucose detection in urine and blood samples, providing a low-cost, non-invasive diagnostic alternative PKU, caused by phenylalanine hydroxylase deficiency, requires long-term monitoring of phenylalanine levels.[131] Engineered bacterial systems capable of detecting elevated phenylalanine concentrations are under investigation for continuous or dietary compliance monitoring.[132]

3.4.5 Additional applications in inflammatory and neurological disorders. Beyond common diseases, engineered bacteria have been used to monitor a variety of localized and systemic conditions. Biosensors targeting inflammatory markers in gingival crevices have shown promise for the early detection of periodontal disease.[133] Using commensal bacteria such as *Staphylococcus epidermidis*, Shi *et al.* engineered systems that respond to cutaneous inflammatory cues, offering potential for dermatological disease monitoring and treatment.[134] Neurological diseases such as Parkinson's and depression are associated with changes in specific neurotransmitters including dopamine and γ-aminobutyric acid (GABA). Engineered bacteria capable of sensing these neurometabolites in cerebrospinal fluid or serum may facilitate the development of non-invasive screening tools for neurodegenerative disorders.[135] While such applications remain in early developmental stages, they represent a frontier for microbial diagnostics in personalized medicine.

3.5 Artificial intelligence-driven engineering of bacterial biosensors

The integration of AI into whole-cell bacterial biosensing marks a transition from passive signal readout to data-driven and adaptive perception. In this framework, AI

functions as the computational layer linking biological signal generation with quantitative interpretation and rational design. Machine learning algorithms, including artificial neural networks and support vector machines, enable the decoding of complex, non-linear outputs from multiplexed bacterial sensors and effectively alleviate signal crosstalk caused by analyte cross-reactivity, a persistent limitation in whole-cell diagnostics.[139] Beyond signal analysis, AI increasingly supports biosensor engineering through data-driven optimization of genetic circuits.

Embedded within design–build–test–learn workflows, predictive and generative models facilitate the selection of promoter architectures and regulatory logic, reducing experimental search space and accelerating strain development, particularly for multi-input and high-complexity circuits.[140] AI-empowered bacterial biosensors offer several functional advantages. Deep learning-based denoising and feature extraction improve diagnostic precision in heterogeneous matrices such as soil, food, and the gastrointestinal tract, thereby lowering false-positive rates. Moreover, AI enables a shift from binary detection toward quantitative and predictive diagnostics by integrating temporal signal dynamics to infer exposure risk, concentration trends, or system trajectories, including food spoilage and disease progression.[141] AI-driven automation further enhances high-throughput screening and evolutionary optimization of large mutant libraries, supporting rapid adaptation to emerging analytes.

Despite these advances, AI integration introduces new challenges. Model robustness remains dependent on training data quality and contextual diversity, limiting generalizability across environments and hosts. In addition, the limited interpretability of complex models—particularly deep neural networks—poses obstacles for regulatory approval and clinical adoption, highlighting the need for explainable AI, standardized datasets, and rigorous validation pipelines tailored to living diagnostics.

4 Current challenges and mitigation strategies for engineered bacteria in detection

Despite significant advances and promising applications across environmental, agricultural, and clinical domains, the practical implementation of engineered bacterial diagnostics continues to face multiple challenges. Key barriers include biosafety concerns, genetic and functional instability, regulatory ambiguity, and context-dependent sensitivity. Importantly, these challenges become more pronounced when engineered bacteria are coupled with AI-enabled diagnostic pipelines, as real-world deployment requires not only biological safety but also algorithmic robustness, interpretability, and regulatory compliance. Addressing these hurdles is therefore essential for enabling the safe, reliable, and scalable translation of living, AI-assisted biosensor technologies.

4.1 Biosafety and biosecurity concerns

The deliberate release or clinical use of genetically engineered microorganisms raises critical questions regarding ecological safety and human health. Even in well-characterized systems, the potential for horizontal gene transfer (HGT), unintended ecological impacts, and evolution of harmful traits poses considerable risks.[142]

Of particular concern is the possibility that engineered strains may acquire undesirable traits, such as increased persistence, virulence, or antibiotic resistance, through spontaneous mutation or genetic exchange. For instance, biosensors incorporating antibiotic- or metal-responsive regulators could inadvertently accelerate the environmental spread of resistance determinants if insufficiently contained.[143] Such risks are further amplified in complex and dynamic settings, including the human microbiome or open environmental systems, where selective pressures are difficult to predict or control.

From a translational perspective, biosafety risks are not only theoretical but have been experimentally observed to vary across deployment contexts. Engineered bacteria may disseminate synthetic constructs—such as reporter genes or resistance elements—to native microbial communities *via* HGT, perturbing environmental gene pools or conferring fitness advantages to opportunistic strains. If improperly controlled, engineered bacteria may acquire harmful functions or undesirable antibiotic resistance, potentially leading to microbiome disruption or pathogenicity.[143] For example, strains engineered with antibiotic or metal-resistance regulators could inadvertently accelerate the spread of resistance genes in the environment, threatening public health. Additionally, spontaneous mutations under selective pressure may result in the acquisition of undesirable phenotypes, such as increased virulence or persistence. *In vivo* studies have demonstrated that selective pressures in the gut, including bile acids, immune stress, and microbial competition, can accelerate genetic rearrangements, underscoring the need for context-aware containment strategies prior to clinical translation.

To mitigate these risks, synthetic biologists have developed advanced containment strategies. These include the engineering of auxotrophic strains that depend on synthetic nutrients absent in natural environments, and programmable self-destruction circuits that trigger lysis upon task completion.[144] CRISPR-based systems have also been employed to achieve precise, task-dependent genome editing or to facilitate post-delivery clearance by host immunity.[145] A particularly promising class of containment tools involves genetic "kill switches", which activate bacterial suicide pathways in response to predefined stimuli—such as temperature shifts, nutrient depletion, or host-derived signals.[146] These kill switches provide multilayered biosafety assurance and can be tuned to environmental or clinical conditions.[147] In parallel, efforts are underway to establish standardized frameworks for biosafety evaluation, encompassing immune compatibility, genetic mobility, and long-term ecological impact.

4.2 Genetic stability and functional robustness

For a living diagnostic to be a viable product, it must survive not just the host environment, but also the supply chain. Maintaining genetic stability and functional performance over time is arguably the most significant barrier to commercialization (retained integration/encapsulation points, added manufacturing/storage context). Evolutionary instability: *in vivo* environments exert dynamic selective pressures (*e.g.*, inflammation, competition) that accelerate evolutionary drift. "Genetic breaking" of sensor circuits leads to signal degradation (loss-of-function) or constitutive activation (false positives), causing "data drift" that renders pre-trained AI models obsolete. Strategies such as bi-stable toggle switches and redundant genetic encoding are being employed to stabilize memory and output.[148–150]

These challenges are especially critical for AI-enabled diagnostics, as algorithmic prediction accuracy is highly sensitive to signal drift and longitudinal inconsistency. To counteract these issues, researchers are exploring several complementary strategies: (1) genomic integration of synthetic circuits reduces reliance on plasmids and enhances heritability. (2) Redundant circuit architectures (*e.g.*, multi-copy integration) improve resilience to mutation and loss. (3) Resource-aware feedback loops modulate gene expression to balance performance and metabolic load. (4) Microencapsulation using hydrogels or biocompatible polymers protects engineered cells from host immunity, gastric acid, and shear stress while preserving viability and function. (5) Ecological support strategies, including co-culture with synergistic microbiota or expression of surface adhesion proteins, help establish stable niches within host or environmental contexts.[142] (6) Manufacturing and shelf-life: a critical, often overlooked aspect of translation is the physical form of the diagnostic. Liquid cultures are impractical for widespread distribution. Recent efforts have focused on lyophilization (freeze-drying) and sporulation technologies to create "dormant-to-active" sensors that can be stored at room temperature and reactivated upon use. Ensuring that engineered gene circuits retain functionality after rehydration is a key engineering objective. Microencapsulation serves a dual purpose here: protecting the cells from gastric acid or environmental shear during deployment, and providing a defined micro-niche that stabilizes the population against competitive exclusion by native microbes. Recent *in vivo* studies demonstrate that encapsulated or chromosomally integrated bacterial sensors can maintain stable signal outputs over weeks, enabling reliable time-series data acquisition required for AI-based trend analysis and predictive modeling. When applied in combination, these strategies significantly improve the robustness, lifespan, and translational feasibility of engineered bacterial diagnostics in both *ex vivo* and *in vivo* settings.

4.3 Regulatory and ethical considerations

The transition of engineered bacterial diagnostics from laboratory prototypes to clinical and field-ready platforms is hindered by regulatory uncertainty and unresolved ethical questions. Despite their potential as precision medicine tools, these systems operate at the intersection of synthetic biology, data science, and healthcare—domains where existing regulatory frameworks remain fragmented or insufficient.[143] From a regulatory readiness standpoint, living diagnostics are increasingly evaluated under frameworks developed for live biotherapeutic products. In the United States, the FDA currently classifies engineered bacterial systems intended for *in vivo* use as biological products, requiring Investigational New Drug submissions, Good Manufacturing Practice compliance, and validated assays for identity, purity, potency, and genetic stability. However, explicit guidance for diagnostic-only living organisms—particularly those integrated with AI-based data interpretation—remains underdeveloped. Similar regulatory gaps exist within European Medicines Agency and National Medical Products Administration frameworks, complicating international translation.

In vivo diagnostic platforms that collect host-derived physiological or molecular data raise pressing concerns regarding data privacy, security, and ownership. These concerns are amplified in AI-enabled systems that rely on cloud-based computation or continuous data aggregation. Current protocols often lack robust mechanisms for anonymization, secure storage, or responsible data sharing—particularly in systems linked to cloud computing or AI-enabled analysis. Moreover, there is no global consensus on clinical trial design, quality control benchmarks, or approval pathways for microbial diagnostic tools.

To navigate these issues, several actions are essential: (1) establish standardized clinical guidelines for engineered bacterial diagnostics, including preclinical models, trial endpoints, and safety evaluation criteria. (2) Develop regulatory frameworks tailored to living biosensors, informed by precedents from gene and cell therapy. (3) Implement data governance protocols, such as tiered access control, cryptographic encryption, and real-time consent management. (4) Explore privacy-preserving technologies, including federated learning and blockchain, to enable secure, cross-institutional data sharing.

Proactive engagement with regulatory agencies, coupled with transparent risk–benefit assessment and explainable AI design, will be critical for the ethical and equitable deployment of AI-enabled living diagnostics in real-world healthcare and environmental monitoring.

Outlook

The convergence of synthetic biology, microbial engineering, and artificial intelligence is redefining the landscape of biosensing technologies. Engineered bacteria—rationally programmed to detect specific biomarkers, respond to dynamic microenvironments, and produce quantifiable outputs—are emerging as powerful tools for next-generation diagnostics. By integrating modular genetic circuits with adaptive sensing mechanisms, these living systems offer

spatiotemporal resolution, real-time analysis, and seamless compatibility with both *in vitro* and *in vivo* platforms.

The incorporation of artificial intelligence further elevates the capabilities of bacterial biosensors. Machine learning and neural network-based models are increasingly employed to decode complex biological signals, optimize circuit performance, and predict system behavior under multifactorial conditions. Looking ahead, AI-assisted microbial systems may acquire context-aware "biological intelligence"—enabling them to autonomously sense, interpret, and respond to intricate physiological and pathological cues. Such developments will support the realization of fully autonomous sense–analyze–respond closed-loop platforms.

Future iterations of these systems are expected to be tightly integrated with wearable devices, ingestible or implantable diagnostics, and cloud-connected healthcare infrastructures, giving rise to intelligent "living-data" networks. These networks will enable dynamic, continuous, and personalized monitoring of chronic diseases, environmental stressors, and health trajectories. Coordinated bacterial consortia, supported by AI-driven control architectures, may also facilitate distributed sensing and adaptive therapeutic interventions across tissue compartments or ecological niches.

As the field transitions from conceptual demonstrations to translational development, challenges remain—including biosafety, genetic stability, standardization of production pipelines, and alignment with regulatory frameworks. However, sustained advances in chassis design, synthetic circuit optimization, and biocontainment strategies, coupled with increased cross-disciplinary collaboration, are accelerating the path toward clinical and industrial deployment. Ultimately, engineered bacterial diagnostics are poised to become a cornerstone of next-generation biotechnologies—bridging synthetic biology, smart medicine, and digital health. Their broad applicability across environmental monitoring, food safety, agriculture, and precision healthcare holds promise for significant societal and economic impact, ushering in a new era of intelligent, responsive, and personalized biosensing.

Author contributions

Decheng Wu: writing – review & editing, supervision. Hongmei Liu: writing – review & editing, supervision. Qiuke Jing: investigation, writing – original draft.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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References

1 Y. Li, *et al.*, Advances in environmental pollutant detection techniques: Enhancing public health monitoring and risk assessment, *Environ. Int.*, 2025, **197**, 109365, DOI: 10.1016/j.envint.2025.109365.
2 J. Chang, G. Zhou, E. R. Christensen, R. Heideman and J. Chen, Graphene-based sensors for detection of heavy metals in water: a review, *Anal. Bioanal. Chem.*, 2014, **406**, 3957–3975, DOI: 10.1007/s00216-014-7804-x.
3 P. Kumar, D. K. Mahato, M. Kamle, T. K. Mohanta and S. G. Kang, Aflatoxins: A global concern for food safety, human health and their management, *Front. Microbiol.*, 2016, **7**, 2170, DOI: 10.3389/fmicb.2016.02170.
4 M. B. Amrofell, A. G. Rottinghaus and T. S. Moon, Engineering microbial diagnostics and therapeutics with smart control, *Curr. Opin. Biotechnol.*, 2020, **66**, 11–17, DOI: 10.1016/j.copbio.2020.05.006.
5 R. M. Cooper, *et al.*, Engineered bacteria detect tumor DNA, *Science*, 2023, **381**, 682–686, DOI: 10.1126/science.adf3974.
6 I. Tanniche and B. Behkam, Engineered live bacteria as disease detection and diagnosis tools, *J. Biol. Eng.*, 2023, **17**, 65, DOI: 10.1186/s13036-023-00379-z.
7 K. Jin, Y. Huang, H. L. Che and Y. H. Wu, Engineered bacteria for disease diagnosis and treatment using synthetic biology, *Microb. Biotechnol.*, 2025, **18**, 1, DOI: 10.1111/1751-7915.70080.
8 X. Tan, J. H. Letendre, J. J. Collins and W. W. Wong, Synthetic biology in the clinic: engineering vaccines, diagnostics, and therapeutics, *Cell*, 2021, **184**, 881–898, DOI: 10.1016/j.cell.2021.01.017.
9 P. H. Tsou, *et al.*, Exploring volatile organic compounds in breath for high-accuracy prediction of lung cancer, *Cancers*, 2021, **13**, 1431, DOI: 10.3390/cancers13061431.
10 I. Del Valle, *et al.*, Translating new synthetic biology advances for biosensingeinto the earth and environmental sciences, *Front. Microbiol.*, 2020, **11**, 618373, DOI: 10.3389/fmicb.2020.618373.
11 S. A. Ali, D. Mittal and G. Kaur, In-situ monitoring of xenobiotics using genetically engineered whole-cell-based microbial biosensors: recent advances and outlook, *World J. Microbiol. Biotechnol.*, 2021, **37**, 81, DOI: 10.1007/s11274-021-03024-3.
12 T. J. Ford and P. A. Silver, Synthetic biology expands chemical control of microorganisms, *Curr. Opin. Chem. Biol.*, 2015, **28**, 20–28, DOI: 10.1016/j.cbpa.2015.05.012.

13 J. Zhou, J. Dong, H. Hou, L. Huang and J. Li, High-throughput microfluidic systems accelerated by artificial intelligence for biomedical applications, *Lab Chip*, 2024, **24**, 1307–1326, DOI: 10.1039/d3lc01012k.
14 Y. P. Shelke, A. K. Badge and N. J. Bankar, Applications of artificial intelligence in microbial diagnosis, *Cureus*, 2023, **15**, e49366, DOI: 10.7759/cureus.49366.
15 S. Fooladi, N. Rabiee and S. Iravani, Genetically engineered bacteria: a new frontier in targeted drug delivery, *J. Mater. Chem. B*, 2023, **11**, 10072–10087, DOI: 10.1039/d3tb01805a.
16 R. Xie, *et al.*, Living therapeutics: Precision diagnosis and therapy with engineered bacteria, *Biomaterials*, 2025, **321**, 123342, DOI: 10.1016/j.biomaterials.2025.123342.
17 S. Sridhar, C. M. Ajo-Franklin and C. A. Masiello, A Framework for the systematic selection of biosensor chassis for environmental synthetic biology, *ACS Synth. Biol.*, 2022, **11**, 2909–2916, DOI: 10.1021/acssynbio.2c00079.
18 F. Wu, Y. Liang, Y. Zhang, Y. Huo and Q. Wang, Construction of seamless genome editing system for fast-growing vibrio natriegens, *Shengwu Gongcheng Xuebao*, 2020, **36**, 2387–2397, DOI: 10.13345/j.cjb.200157.
19 J. Xu, S. Yang and L. Yang, Vibrio natriegens as a host for rapid biotechnology, *Trends Biotechnol.*, 2022, **40**, 381–384, DOI: 10.1016/j.tibtech.2021.10.007.
20 M. T. Weinstock, E. D. Hesek, C. M. Wilson and D. G. Gibson, Vibrio natriegens as a fast-growing host for molecular biology, *Nat. Methods*, 2016, **13**, 849–851, DOI: 10.1038/nmeth.3970.
21 M. Fan, S. Tan, W. Wang and X. Zhang, Improvement in salt tolerance ability of pseudomonas putida KT2440, *Biology*, 2024, **13**(6), 404, DOI: 10.3390/biology13060404.
22 E. T. Mohamed, *et al.*, Adaptive laboratory evolution of pseudomonas putida KT2440 improves p-coumaric and ferulic acid catabolism and tolerance, *Metab. Eng. Commun.*, 2020, **11**, e00143, DOI: 10.1016/j.mec.2020.e00143.
23 Y. T. Liu, J. Feng, H. C. Pan, X. W. Zhang and Y. L. Zhang, Genetically engineered bacterium: Principles, practices, and prospects, *Front. Microbiol.*, 2022, **13**, 997587, DOI: 10.3389/fmicb.2022.997587.
24 C. Beimfohr, A Review of research Conducted with probiotic E. coli marketed as symbioflor, *Int. J. Bacteriol.*, 2016, **2016**, 3535621, DOI: 10.1155/2016/3535621.
25 Y. Lai, N. Hayashi and T. K. Lu, Engineering the human gut commensal Bacteroides thetaiotaomicron with synthetic biology, *Curr. Opin. Chem. Biol.*, 2022, **70**, 102178, DOI: 10.1016/j.cbpa.2022.102178.
26 C. A. Miller, J. M. L. Ho and M. R. Bennett, Strategies for improving small-molecule biosensors in bacteria, *Biosensors*, 2022, **12**, 64, DOI: 10.3390/bios12020064.
27 D. Roncarati, V. Scarlato and A. Vannini, Targeting of regulators as a promising approach in the search for novel antimicrobial agents, *Microorganisms*, 2022, **10**, 185, DOI: 10.3390/microorganisms10010185.
28 C. Mejía-Almonte, *et al.*, Redefining fundamental concepts of transcription initiation in bacteria, *Nat. Rev. Genet.*, 2020, **21**, 699–714, DOI: 10.1038/s41576-020-0254-8.
29 D. Xiao, *et al.*, A D,L-lactate biosensor based on allosteric transcription factor LldR and amplified luminescent proximity homogeneous assay, *Biosens. Bioelectron.*, 2022, **211**, 114378, DOI: 10.1016/j.bios.2022.114378.
30 M. Mimee, *et al.*, An ingestible bacterial-electronic system to monitor gastrointestinal health, *Science*, 2018, **360**, 915–918, DOI: 10.1126/science.aas9315.
31 W. J. Wang, *et al.*, A brief review of aptamer-based biosensors in recent years, *Biosensors*, 2025, **15**, 120, DOI: 10.3390/bios15020120.
32 T. Mo, *et al.*, Aptamer-based biosensors and application in tumor theranostics, *Cancer Sci.*, 2022, **113**, 7–16, DOI: 10.1111/cas.15194.
33 M. McKeague, R. S. Wong and C. D. Smolke, Opportunities in the design and application of RNA for gene expression control, *Nucleic Acids Res.*, 2016, **44**, 2987–2999, DOI: 10.1093/nar/gkw151.
34 C. Berens, F. Groher and B. Suess, RNA aptamers as genetic control devices: the potential of riboswitches as synthetic elements for regulating gene expression, *Biotechnol. J.*, 2015, **10**, 246–257, DOI: 10.1002/biot.201300498.
35 R. R. Breaker, Riboswitches and the RNA world, *Cold Spring Harbor Perspect. Biol.*, 2012, **4**(2), a003566, DOI: 10.1101/cshperspect.a003566.
36 Y. F. Wu, *et al.*, High content design of riboswitch biosensors: All-around rational module-by-module design, *Biosens. Bioelectron.*, 2023, **220**, 114887, DOI: 10.1016/j.bios.2022.114887.
37 R. B. Bourret and R. E. Silversmith, Two-component signal transduction, *Curr. Opin. Microbiol.*, 2010, **13**, 113–115, DOI: 10.1016/j.mib.2010.02.003.
38 J. T. Lazar and J. J. Tabor, Bacterial two-component systems as sensors for synthetic biology applications, *Curr. Opin. Syst. Biol.*, 2021, **28**, 100398, DOI: 10.1016/j.coisb.2021.100398.
39 S. Ravikumar, M. G. Baylon, S. J. Park and J. I. Choi, Engineered microbial biosensors based on bacterial two-component systems as synthetic biotechnology platforms in bioremediation and biorefinery, *Microb. Cell Fact.*, 2017, **16**, 62, DOI: 10.1186/s12934-017-0675-z.
40 M. T. Laub and M. Goulian, Specificity in two-component signal transduction pathways, *Annu. Rev. Genet.*, 2007, **41**, 121–145, DOI: 10.1146/annurev.genet.41.042007.170548.
41 E. A. Groisman, A. Duprey and J. Choi, How the PhoP/PhoQ system controls virulence and Mg(2+) homeostasis: lessons in signal transduction, pathogenesis, physiology, and evolution, *Microbiol. Mol. Biol. Rev.*, 2021, **85**, e0017620, DOI: 10.1128/mmbr.00176-20.
42 M. Lebovich, M. A. Lora, J. Gracia-David and L. B. Andrews, Genetic circuits for feedback control of gamma-aminobutyric acid biosynthesis in probiotic *escherichia coli* Nissle 1917, *Metabolites*, 2024, **14**, 44, DOI: 10.3390/metabo14010044.
43 C. M. Robinson, N. E. Short and D. T. Riglar, Achieving spatially precise diagnosis and therapy in the mammalian gut using synthetic microbial gene circuits,

Front. Bioeng. Biotechnol., 2022, **10**, 959441, DOI: 10.3389/fbioe.2022.959441.

44 X. Y. Wan, *et al.*, Cascaded amplifying circuits enable ultrasensitive cellular sensors for toxic metals, *Nat. Chem. Biol.*, 2019, **15**, 540–548, DOI: 10.1038/s41589-019-0244-3.

45 D. J. Sayut, Y. Niu and L. H. Sun, Construction and engineering of positive feedback loops, *ACS Chem. Biol.*, 2006, **1**, 692–696, DOI: 10.1021/cb6004245.

46 S. K. Kim, *et al.*, A genetically encoded biosensor for monitoring isoprene production in engineered *escherichia coli*, *ACS Synth. Biol.*, 2018, **7**, 2379–2390, DOI: 10.1021/acssynbio.8b00164.

47 B. C. Stanton, *et al.*, Genomic mining of prokaryotic repressors for orthogonal logic gates, *Nat. Chem. Biol.*, 2014, **10**, 99–105, DOI: 10.1038/nchembio.1411.

48 H. S. Kang, D. Park and J. Kim, Logical regulation of endogenous gene expression using programmable, multi-input processing CRISPR guide RNAs, *Nucleic Acids Res.*, 2024, **52**, 8595–8608, DOI: 10.1093/nar/gkae549.

49 S. J. Chen, X. L. Chen, H. F. Su, M. Z. Guo and H. L. Liu, Advances in synthetic-biology-based whole-cell biosensors: principles, genetic modules, and applications in food safety, *Int. J. Mol. Sci.*, 2023, **24**, 7989, DOI: 10.3390/ijms24097989.

50 D. T. Riglar and P. A. Silver, Engineering bacteria for diagnostic and therapeutic applications, *Nat. Rev. Microbiol.*, 2018, **16**, 214–225, DOI: 10.1038/nrmicro.2017.172.

51 T. H. T. Chau, D. H. A. Mai, D. N. Pham, H. T. Q. Le and E. Y. Lee, Developments of riboswitches and toehold switches for molecular detection-biosensing and molecular diagnostics, *Int. J. Mol. Sci.*, 2020, **21**, 3192, DOI: 10.3390/ijms21093192.

52 L. Liu, W. Huang and J. D. Huang, Synthetic circuits that process multiple light and chemical signal inputs, *BMC Syst. Biol.*, 2017, **11**, 5, DOI: 10.1186/s12918-016-0384-y.

53 M. E. Inda-Webb, *et al.*, Sub-1.4 cm3 capsule for detecting labile inflammatory biomarkers in situ, *Nature*, 2023, **620**, 386–392, DOI: 10.1038/s41586-023-06369-x.

54 Z. P. Zou, Y. Du, T. T. Fang, Y. Zhou and B. C. Ye, Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice, *Cell Host Microbe*, 2023, **31**(2), 199-212.e5, DOI: 10.1016/j.chom.2022.12.004.

55 D. H. Juers, B. W. Matthews and R. E. Huber, LacZ β-galactosidase: structure and function of an enzyme of historical and molecular biological importance, *Protein Sci.*, 2012, **21**, 1792–1807, DOI: 10.1002/pro.2165.

56 Q. Z. Wang, *et al.*, Developing clostridia as cell factories for short- and medium-chain ester production, *Front. Bioeng. Biotechnol.*, 2021, **9**, 661694, DOI: 10.3389/fbioe.2021.661694.

57 J. Zhu, B. Wang, Y. Zhang, T. Wei and T. Gao, Living electrochemical biosensing: Engineered electroactive bacteria for biosensor development and the emerging trends, *Biosens. Bioelectron.*, 2023, **237**, 115480, DOI: 10.1016/j.bios.2023.115480.

58 F. Simonte, G. Sturm, J. Gescher and K. Sturm-Richter, Extracellular electron transfer and biosensors, *Adv. Biochem. Eng./Biotechnol.*, 2019, **167**, 15–38, DOI: 10.1007/10_2017_34.

59 M. Mimee, *et al.*, An ingestible bacterial-electronic system to monitor gastrointestinal health, *Science*, 2018, **360**, 915–918, DOI: 10.1126/science.aas9315.

60 V. A. Pavlov, S. S. Chavan and K. J. Tracey, Bioelectronic medicine: From preclinical studies on the inflammatory reflex to new approaches in disease diagnosis and treatment, *Cold Spring Harbor Perspect. Med.*, 2020, **10**, a034140, DOI: 10.1101/cshperspect.a034140.

61 S. Wu, *et al.*, metaProbiotics: a tool for mining probiotic from metagenomic binning data based on a language model, *Briefings Bioinf.*, 2024, **25**(2), bbae085, DOI: 10.1093/bib/bbae085.

62 M. B. Fiamenghi, *et al.*, Machine learning and comparative genomics approaches for the discovery of xylose transporters in yeast, *Biotechnol. Biofuels Bioprod.*, 2022, **15**, 57, DOI: 10.1186/s13068-022-02153-7.

63 C. M. Bergamini, *et al.*, Machine learning algorithms highlight tRNA information content and chargaff's second parity rule score as important features in discriminating probiotics from non-probiotics, *Biology*, 2022, **11**(7), 1024, DOI: 10.3390/biology11071024.

64 P. Zhang, *et al.*, Deep flanking sequence engineering for efficient promoter design using DeepSEED, *Nat. Commun.*, 2023, **14**, 6309, DOI: 10.1038/s41467-023-41899-y.

65 M. Oubounyt, Z. Louadi, H. Tayara and K. T. Chong, DeePromoter: Robust promoter predictor using deep learning, *Front. Genet.*, 2019, **10**, 286, DOI: 10.3389/fgene.2019.00286.

66 X. Lei, X. Wang, G. Chen, C. Liang, Q. Li, H. Jiang and W. Xiong, Combining diffusion and transformer models for enhanced promoter synthesis and strength prediction in deep learning, *mSystems*, 2025, **10**, e0018325, DOI: 10.1128/msystems.00183-25.

67 J. G. Doench, *et al.*, Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9, *Nat. Biotechnol.*, 2016, **34**, 184–191, DOI: 10.1038/nbt.3437.

68 A. G. Genc and L. J. McGuffin, Beyond AlphaFold2: The impact of AI for the further improvement of protein structure prediction, *Methods Mol. Biol.*, 2025, **2867**, 121–139, DOI: 10.1007/978-1-0716-4196-5_7.

69 G. W. Wei, Protein structure prediction beyond AlphaFold, *Nat. Mach. Intell.*, 2019, **1**, 336–337, DOI: 10.1038/s42256-019-0086-4.

70 A. Racovita and A. Jaramillo, Reinforcement learning in synthetic gene circuits, *Biochem. Soc. Trans.*, 2020, **48**, 1637–1643, DOI: 10.1042/bst20200008.

71 Y. Matsuo, *et al.*, Deep learning, reinforcement learning, and world models, *Neural Netw.*, 2022, **152**, 267–275, DOI: 10.1016/j.neunet.2022.03.037.

72 Z. P. Zou, *et al.*, Self-Adjusting engineered probiotic for targeted tumor colonization and local therapeutics delivery, *Adv. Sci.*, 2025, e06486, DOI: 10.1002/advs.202406486.

73 Z. Liu, X. Zhang, D. Lei, B. Qiao and G. R. Zhao, Metabolic engineering of *escherichia coli* for de novo production of 3-phenylpropanol via retrobiosynthesis approach, *Microb. Cell Fact.*, 2021, **20**, 121, DOI: 10.1186/s12934-021-01615-1.
74 M. Sabzevari, S. Szedmak, M. Penttilä, P. Jouhten and J. Rousu, Strain design optimization using reinforcement learning, *PLoS Comput. Biol.*, 2022, **18**, e1010177, DOI: 10.1371/journal.pcbi.1010177.
75 Y. Cheng, *et al.*, Machine learning for metabolic pathway optimization: A review, *Comput. Struct. Biotechnol. J.*, 2023, **21**, 2381–2393, DOI: 10.1016/j.csbj.2023.03.045.
76 K. Rai, Y. Wang, R. W. O'Connell, A. B. Patel and C. J. Bashor, Using machine learning to enhance and accelerate synthetic biology, *Curr. Opin. Biomed. Eng.*, 2024, **31**, 100553, DOI: 10.1016/j.cobme.2024.100553.
77 D. Zhang, F. Xu, F. Wang, L. Le and L. Pu, Synthetic biology and artificial intelligence in crop improvement, *Plant Commun.*, 2025, **6**, 101220, DOI: 10.1016/j.xplc.2024.101220.
78 M. P. McRae, K. S. Rajsri, T. M. Alcorn and J. T. McDevitt, Smart diagnostics: combining artificial intelligence and in vitro diagnostics, *Sensors*, 2022, **22**, 6355, DOI: 10.3390/s22176355.
79 M. Mirbabaie, S. Stieglitz and N. R. J. Frick, Artificial intelligence in disease diagnostics: A critical review and classification on the current state of research guiding future direction, *Health Technol.*, 2021, **11**, 693–731, DOI: 10.1007/s12553-021-00555-5.
80 J. Jumper, *et al.*, Highly accurate protein structure prediction with AlphaFold, *Nature*, 2021, **596**, 583–589, DOI: 10.1038/s41586-021-03819-2.
81 M. Baek, *et al.*, Accurate prediction of protein structures and interactions using a three-track neural network, *Science*, 2021, **373**, 871–876, DOI: 10.1126/science.abj8754.
82 Z. Lin, *et al.*, Evolutionary-scale prediction of atomic-level protein structure with a language model, *Science*, 2023, **379**, 1123–1130, DOI: 10.1126/science.ade2574.
83 G. Chuai, *et al.*, DeepCRISPR: optimized CRISPR guide RNA design by deep learning, *Genome Biol.*, 2018, **19**, 80, DOI: 10.1186/s13059-018-1459-4.
84 Y. Wang, *et al.*, Synthetic promoter design in Escherichia coli based on a deep generative network, *Nucleic Acids Res.*, 2020, **48**, 6403–6412, DOI: 10.1093/nar/gkaa325.
85 J. A. Valeri, *et al.*, Sequence-to-function deep learning frameworks for engineered riboregulators, *Nat. Commun.*, 2020, **11**, 5058, DOI: 10.1038/s41467-020-18676-2.
86 N. Hashemi Goradel, *et al.*, Biosensors for the detection of environmental and urban pollutions, *J. Cell. Biochem.*, 2018, **119**, 207–212, DOI: 10.1002/jcb.26030.
87 N. Hashemi Goradel, *et al.*, Biosensors for the detection of environmental and urban pollutions, *J. Cell. Biochem.*, 2018, **119**, 207–212, DOI: 10.1002/jcb.26030.
88 B. Wang, M. Barahona and M. Buck, A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals, *Biosens. Bioelectron.*, 2013, **40**, 368–376, DOI: 10.1016/j.bios.2012.08.011.
89 Q. Gui, T. Lawson, S. Shan, L. Yan and Y. Liu, The application of whole cell-based biosensors for use in environmental analysis and in medical diagnostics, *Sensors*, 2017, **17**(7), 1623, DOI: 10.3390/s17071623.
90 A. Doshi, *et al.*, Engineered bacterial swarm patterns as spatial records of environmental inputs, *Nat. Chem. Biol.*, 2023, **19**, 878–886, DOI: 10.1038/s41589-023-01325-2.
91 C. Liu, *et al.*, Engineering whole-cell microbial biosensors: Design principles and applications in monitoring and treatment of heavy metals and organic pollutants, *Biotechnol. Adv.*, 2022, **60**, 108019, DOI: 10.1016/j.biotechadv.2022.108019.
92 H. Che, *et al.*, Multi-targets detection via combination of multi-stimulus-response engineered bacteria and hydrogel-based separation platform, *J. Hazard. Mater.*, 2024, **478**, 135578, DOI: 10.1016/j.jhazmat.2024.135578.
93 C. Liu, *et al.*, Engineering whole-cell microbial biosensors: Design principles and applications in monitoring and treatment of heavy metals and organic pollutants, *Biotechnol. Adv.*, 2022, **60**, 108019, DOI: 10.1016/j.biotechadv.2022.108019.
94 C. M. Reddy, *et al.*, Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 20229–20234, DOI: 10.1073/pnas.1101242108.
95 B. Q. Huynh, *et al.*, Public health impacts of an imminent Red Sea oil spill, *Nat. Sustain.*, 2021, **4**, 1084–1091, DOI: 10.1038/s41893-021-00774-8.
96 F. Dubocq, B. B. Bæringsdóttir, T. Wang and A. Kärrman, Comparison of extraction and clean-up methods for comprehensive screening of organic micropollutants in fish using gas chromatography coupled to high-resolution mass spectrometry, *Chemosphere*, 2022, **286**, 131743, DOI: 10.1016/j.chemosphere.2021.131743.
97 N. Kolahchi, *et al.*, Direct detection of phenol using a new bacterial strain-based conductometric biosensor, *J. Environ. Chem. Eng.*, 2018, **6**, 478–484, DOI: 10.1016/j.jece.2017.12.023.
98 C. I. L. Justino, A. C. Duarte and T. A. P. Rocha-Santos, Recent progress in riosensors for environmental monitoring: A review, *Sensors*, 2017, **17**, 2918, DOI: 10.3390/s17122918.
99 T. T. Wang, *et al.*, Enhanced assessment of water quality for both nitrate and nitrite using engineered E. coli with para-aminobenzoic acid biosynthesis, *Anal. Chim. Acta*, 2025, **1338**, 343580, DOI: 10.1016/j.aca.2024.343580.
100 N. Verma and A. Bhardwaj, Biosensor technology for pesticides–a review, *Appl. Biochem. Biotechnol.*, 2015, **175**, 3093–3119, DOI: 10.1007/s12010-015-1489-2.
101 M. Kim, *et al.*, Chemostat-like microfluidic platform for highly sensitive detection of heavy metal ions using microbial biosensors, *Biosens. Bioelectron.*, 2015, **65**, 257–264, DOI: 10.1016/j.bios.2014.10.028.
102 Q. Li, P. Wang, R. Chen, W. Li and Y. J. Wu, Construction of genetically engineered bacteria that degrades organophosphorus pesticide residues and can be easily

detected by the fluorescence, *Environ. Technol.*, 2014, **35**, 556–561, DOI: 10.1080/09593330.2013.837936.

103 S. Parvez, C. Venkataraman and S. Mukherji, A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals, *Environ. Int.*, 2006, **32**, 265–268, DOI: 10.1016/j.envint.2005.08.022.

104 R. Ranjan, N. K. Rastogi and M. S. Thakur, Development of immobilized biophotonic beads consisting of Photobacterium leiognathi for the detection of heavy metals and pesticide, *J. Hazard. Mater.*, 2012, **225–226**, 114–123, DOI: 10.1016/j.jhazmat.2012.04.076.

105 J. Kumar and S. F. D'Souza, An optical microbial biosensor for detection of methyl parathion using Sphingomonas sp. immobilized on microplate as a reusable biocomponent, *Biosens. Bioelectron.*, 2010, **26**, 1292–1296, DOI: 10.1016/j.bios.2010.07.016.

106 A. Mishra, J. Kumar and J. S. Melo, An optical microplate biosensor for the detection of methyl parathion pesticide using a biohybrid of Sphingomonas sp. cells-silica nanoparticles, *Biosens. Bioelectron.*, 2017, **87**, 332–338, DOI: 10.1016/j.bios.2016.08.048.

107 A. Bhatt, S. Jain and N. K. Navani, Rapid, sensitive, and specific microbial whole-cell biosensor for the detection of histamine: A potential food toxin, *J. Agric. Food Chem.*, 2024, **72**, 27466–27478, DOI: 10.1021/acs.jafc.4c06315.

108 J. Ma, *et al.*, Accurate and non-destructive monitoring of mold contamination in foodstuffs based on whole-cell biosensor array coupling with machine-learning prediction models, *J. Hazard. Mater.*, 2023, **449**, 131030, DOI: 10.1016/j.jhazmat.2023.131030.

109 J. Ma, *et al.*, Accurate and non-destructive monitoring of mold contamination in foodstuffs based on whole-cell biosensor array coupling with machine-learning prediction models, *J. Hazard. Mater.*, 2023, **449**, 131030, DOI: 10.1016/j.jhazmat.2023.131030.

110 V. Felipe, *et al.*, First report of *Pectobacterium brasiliense* causing blackleg and soft rot of potato in Argentina, *Plant Dis.*, 2024, DOI: 10.1094/pdis-03-24-0558-pdn.

111 X. Ma, A. Schloop, B. Swingle and K. L. Perry, *Pectobacterium* and *dickeya* responsible for potato blackleg disease in new york state in 2016, *Plant Dis.*, 2018, **102**, 1834–1840, DOI: 10.1094/pdis-10-17-1595-re.

112 M. B. Suárez, *et al.*, New pCR-based assay for the identification of pectobacterium carotovorum causing potato soft rot, *Plant Dis.*, 2022, **106**, 676–684, DOI: 10.1094/pdis-08-21-1676-re.

113 R. Czajkowski, *et al.*, Detection, identification and differentiation of pectobacterium and dickeya species causing potato blackleg and tuber soft rot: a review, *Ann. Appl. Biol.*, 2015, **166**, 18–38, DOI: 10.1111/aab.12166.

114 Y. Fang and R. P. Ramasamy, Current and prospective methods for plant disease detection, *Biosensors*, 2015, **5**, 537–561, DOI: 10.3390/bios5030537.

115 N. Zanger and E. Eltzov, Optimizing whole-cell biosensors for the early detection of crop infections: A proof-of-concept study, *Biosensors*, 2025, **15**(5), 300, DOI: 10.3390/bios15050300.

116 M. Liu, W. Yang, W. Zhu and D. Yu, Innovative applications and research advances of bacterial biosensors in medicine, *Front. Microbiol.*, 2025, **16**, 1507491, DOI: 10.3389/fmicb.2025.1507491.

117 S. Tuljapurkar, Infectious diseases of humans—Dynamics and control, *Science*, 1991, **254**, 591–592, DOI: 10.1126/science.254.5031.591.

118 R. Rafique, *et al.*, Transmission of infectious vibrio cholerae through drinking water among the household contacts of cholera patients (CHoBI7 trial), *Front. Microbiol.*, 2016, **7**, 1635, DOI: 10.3389/fmicb.2016.01635.

119 Z. Zhou, *et al.*, Engineering probiotics as living diagnostics and therapeutics for improving human health, *Microb. Cell Fact.*, 2020, **19**, 56, DOI: 10.1186/s12934-020-01318-z.

120 M. B. Holowko, H. J. Wang, P. Jayaraman and C. L. Poh, Biosensing vibrio cholerae with genetically engineered *escherichia coli*, *ACS Synth. Biol.*, 2016, **5**, 1275–1283, DOI: 10.1021/acssynbio.6b00079.

121 T. Danino, *et al.*, Programmable probiotics for detection of cancer in urine, *Sci. Transl. Med.*, 2015, **7**, 289ra84, DOI: 10.1126/scitranslmed.aaa3519.

122 C. R. Gurbatri, *et al.*, Engineering tumor-colonizing E. coli Nissle 1917 for detection and treatment of colorectal neoplasia, *Nat. Commun.*, 2024, **15**, 646, DOI: 10.1038/s41467-024-44776-4.

123 J. T. Panteli, B. A. Forkus, N. Van Dessel and N. S. Forbes, Genetically modified bacteria as a tool to detect microscopic solid tumor masses with triggered release of a recombinant biomarker, *Integr. Biol.*, 2015, **7**, 423–434, DOI: 10.1039/c5ib00047e.

124 T. V. Plavec, *et al.*, Targeting of fluorescent lactococcus lactis to colorectal cancer cells through surface display of tumour-antigen binding proteins, *Microb. Biotechnol.*, 2021, **14**, 2227–2240, DOI: 10.1111/1751-7915.13907.

125 Z. M. Geng, *et al.*, Aptamer-assisted tumor localization of bacteria for enhanced biotherapy, *Nat. Commun.*, 2021, **12**, 6584, DOI: 10.1038/s41467-021-26956-8.

126 S. W. de Melo and J. A. Di Palma, The role of capsule endoscopy in evaluating inflammatory bowel disease, *Gastroenterol. Clin. N. Am.*, 2012, **41**, 315–323, DOI: 10.1016/j.gtc.2012.01.005.

127 Z. P. Zou, *et al.*, Genetically engineered bacteria as inflammatory bowel disease therapeutics, *Eng. Microbiol.*, 2024, **4**, 100167, DOI: 10.1016/j.engmic.2024.100167.

128 D. T. Riglar, *et al.*, Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation, *Nat. Biotechnol.*, 2017, **35**, 653–658, DOI: 10.1038/nbt.3879.

129 Z. P. Zou, Y. Du, T. T. Fang, Y. Zhou and B. C. Ye, Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice, *Cell Host Microbe*, 2023, **31**, 199–211, DOI: 10.1016/j.chom.2022.12.004.

130 X. Liu, *et al.*, Magnetic living hydrogels for intestinal localization, retention, and diagnosis, *Adv. Funct. Mater.*, 2021, **31**, 2010918, DOI: 10.1002/adfm.202010918.
131 A. Courbet, D. Endy, E. Renard, F. Molina and J. Bonnet, Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates, *Sci. Transl. Med.*, 2015, **7**, 289ra283, DOI: 10.1126/scitranslmed.aaa3601.
132 F. J. van Spronsen, *et al.*, Phenylketonuria, *Nat. Rev. Dis. Primers*, 2021, **7**, 36, DOI: 10.1038/s41572-021-00267-0.
133 P. Dongiovanni, *et al.*, Salivary biomarkers: novel noninvasive tools to diagnose chronic inflammation, *Int. J. Oral Sci.*, 2023, **15**, 27, DOI: 10.1038/s41368-023-00231-6.
134 J. Shi, *et al.*, Active biointegrated living electronics for managing inflammation, *Science*, 2024, **384**, 1023–1030, DOI: 10.1126/science.adl1102.
135 J. L. Cummings, *et al.*, Biomarker-guided decision making in clinical drug development for neurodegenerative disorders, *Nat. Rev. Drug Discovery*, 2025, **24**(8), 589–609, DOI: 10.1038/s41573-025-01165-w.
136 N. Kolahchi, M. Braiek, G. Ebrahimipour, S. O. Ranaei-Siadat, F. Lagarde and N. Jaffrezic-Renault, Direct detection of phenol using a new bacterial strain-based conductometric biosensor, *J. Environ. Chem. Eng.*, 2018, **6**, 478–484, DOI: 10.1016/j.jece.2017.12.023.
137 D. Zhu, *et al.*, Microbial biosensor for sensing and treatment of intestinal inflammation, *Adv. Sci.*, 2025, **12**, e2504364, DOI: 10.1002/advs.202504364.
138 M. T. Buss, L. Zhu, J. H. Kwon, J. J. Tabor and M. G. Shapiro, Probiotic acoustic biosensors for noninvasive imaging of gut inflammation, *Nat. Commun.*, 2025, **16**, 7931, DOI: 10.1038/s41467-025-62569-1.
139 R. J. S. Banicod, *et al.*, Integration of Artificial Intelligence in Biosensors for Enhanced Detection of Foodborne Pathogens, *Biosensors*, 2025, **15**(10), 690, DOI: 10.3390/bios15100690.
140 C. E. Lawson, *et al.*, Machine learning for metabolic engineering: A review, *Metab. Eng.*, 2021, **63**, 34–60, DOI: 10.1016/j.ymben.2020.10.005.
141 A. Coutant, *et al.*, Closed-loop cycles of experiment design, execution, and learning accelerate systems biology model development in yeast, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 18142–18147, DOI: 10.1073/pnas.1900548116.
142 J. Li, H. Zhao, L. Zheng and W. An, Advances in synthetic biology and biosafety governance, *Front. Bioeng. Biotechnol.*, 2021, **9**, 598087, DOI: 10.3389/fbioe.2021.598087.
143 A. Lerner, C. Benzvi and A. Vojdani, The potential harmful effects of genetically engineered microorganisms (GEMs) on the intestinal microbiome and public health, *Microorganisms*, 2024, **12**, 238, DOI: 10.3390/microorganisms12020238.
144 C. M. Whitford, *et al.*, Auxotrophy to xeno-DNA: an exploration of combinatorial mechanisms for a high-fidelity biosafety system for synthetic biology applications, *J. Biol. Eng.*, 2018, **12**, 13, DOI: 10.1186/s13036-018-0105-8.
145 D. R. George, *et al.*, A bumpy road ahead for genetic biocontainment, *Nat. Commun.*, 2024, **15**, 650, DOI: 10.1038/s41467-023-44531-1.
146 C. T. Chan, J. W. Lee, D. E. Cameron, C. J. Bashor and J. J. Collins, 'Deadman' and 'Passcode' microbial kill switches for bacterial containment, *Nat. Chem. Biol.*, 2016, **12**, 82–86, DOI: 10.1038/nchembio.1979.
147 F. Stirling, *et al.*, Rational design of evolutionarily stable microbial kill switches, *Mol. Cell*, 2017, **68**, 686–697.e3, DOI: 10.1016/j.molcel.2017.10.033.
148 F. Du, *et al.*, Optimizing multicopy chromosomal integration for stable high-performing strains, *Nat. Chem. Biol.*, 2024, **20**, 1670–1679, DOI: 10.1038/s41589-024-01650-0.
149 A. Chen, *et al.*, Application of plasmid stabilization systems for heterologous protein expression in *Escherichia coli*, *Mol. Biol. Rep.*, 2024, **51**, 939, DOI: 10.1007/s11033-024-09881-z.
150 A. Egli, J. Schrenzel and G. Greub, Digital microbiology, *Clin. Microbiol. Infect.*, 2020, **26**, 1324–1331, DOI: 10.1016/j.cmi.2020.06.023.