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REVIEW

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Detection of environmental pollutants using transcription factor-based whole-cell biosensors

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Abstract

The widespread presence of heavy metals and aromatic compounds in environmental systems has emerged as a critical global concern. While conventional analytical methods such as spectroscopy and chromatography provide high precision, their substantial costs and labor-intensive procedures significantly limit their practical application in routine environmental monitoring. Transcription factor-based whole-cell biosensors represent a promising alternative technology, providing real-time, highly sensitive, and cost-effective detection capabilities for environmental pollutants. This review is a comprehensive examination of the recent advances in transcription factor-based biosensors for detecting heavy metals and aromatic compounds. We also emphasize the molecular mechanisms of diverse TF families and their real-world applications in environmental monitoring. Finally, we summarize the current engineering strategies that are employed to enhance biosensor performance and discuss future directions for improving transcription factor-based biosensors for environmental detection.

Keywords Transcription factors, Whole-cell biosensors, Heavy metal ions, Aromatic compounds, Environmental pollutant detection

Introduction

Environmental pollution has become one of the most formidable challenges for humanity in the twenty-first century, threatening the ecosystem balance and compromising human health on a global scale [1, 2]. Among various environmental contaminants, heavy metals are considered the most threatening to human health due to their high bioavailability. They can be absorbed through dietary intake, drinking water, inhalation, and even dermal contact [3]. Heavy metal contamination originates from diverse anthropogenic sources, including mining, industrial processes, vehicular emissions, electronic waste, and agricultural practices. The negative impacts of heavy metal exposure on human health have been found, such as neurological disorders, developmental abnormalities, cardiovascular diseases, renal dysfunction, hepatic damage, reproductive disorders, immunosuppression, and various forms of cancer [4–7]. For example, mercury exposure is associated with severe neurological effects, particularly in developing fetuses and young children, leading to cognitive impairment, motor dysfunction, and sensory deficits [8]. Lead exposure, even at low levels, can cause reduced IQ, attention deficit disorders, behavioral problems, and increased risk of hypertension and cardiovascular disease in adults [9].

Aromatic hydrocarbons are organic chemicals characterized by benzene ring structures, which are another extensive group of globally dispersed environmental contaminants with significant health implications. BTEX (benzene, toluene, ethylbenzene, and xylene) compounds are common, volatile, organic compounds that are mostly emitted from petroleum refining processes, industrial emissions, and vehicular exhaust [10, 11]. Among the BTEX components, benzene poses the greatest health risk and is identified as a carcinogenic substance. Severe health complications, including leukemia,

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myelodysplastic syndrome, and hematological disorders, can occur with chronic inhalation of benzene [12]. Prolonged exposure to toluene and ethylbenzene has been shown to exert a deleterious effect on the central nervous system, manifesting in symptoms such as headaches, dizziness, and cognitive impairment [13]. Xylene has also been proven to induce respiratory tract irritation and dermal sensitization [14]. Low-volatility aromatics, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and certain nitroaromatic compounds, present substantially greater analytical challenges. Unlike volatile compounds, these substances tend to partition into particulate matter and environmental matrices, complicating their detection and quantification. The incomplete burning of organic material forms PAHs, which are long-lasting environmental pollutants with carcinogenic and mutagenic properties [15, 16]. Despite being banned decades ago, PCBs persist in the environment and are associated with cardiovascular disease, neurotoxicity, endocrine disruption, and immunosuppression [17, 18]. Nitroaromatic compounds such as TNT and RDX are ubiquitously present in the environment because of military activities and sea-dumped munitions [19]. Due to their carcinogenicity and mutagenicity, constant leakage and accumulation of these compounds risk potential long-term threats to human health [20]. The widespread distribution and persistence of these contaminants make effective environmental monitoring critically important.

Many analytical methods have been employed in environmental monitoring, including atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC). However, these methods face many practical restraints such as high costs, the need for skilled personnel, complex and time-consuming sample pretreatment procedures, and extended analysis times [21]. These limitations have prompted the search for better cost-effective alternative methods that demand less technical expertise and exhibit high specificity. Numerous biosensors have been developed with various technologies, including electrochemical, biological, chemical, and nanomaterials [22, 23]. Among these, whole-cell biosensors based on TFs have attracted significant attention due to their ease of operation, cost-effectiveness, and suitability for rapid on-site detection [24]. Whole-cell biosensors still have some limitations, such as restricted dynamic range, sensitivity, and specificity compared to traditional methods. Nonetheless, advances in synthetic biology are progressively addressing these limitations and enhancing their performance for environmental monitoring [25, 26].

The construction principle of TF-based biosensors involves integrating an analyte-responsive TF and a regulated promoter with the reporter gene to construct a genetic circuit that functions as a unit [25]. As shown in Fig. 1, first, the TFs serve as the effector sensing and recognition element. These TFs are usually derived from bacterial regulatory elements that respond to specific chemicals. The second component is the promoter sequence containing specific binding sites where TFs interact upon analyte recognition, thereby influencing the transcriptional activity of the promoter. The third component is a reporter gene that encodes a quantifiable output signal. Commonly used reporters include fluorescent protein, luciferase, and β-galactosidase (lacZ). The reporter gene products generate signals that can be readily detected, such as fluorescence, luminescence, or colorimetric signals. Upon entry of the target analytes into the bacterial cell, they interact with the TF, inducing a conformational change that activates or inhibits the reporter gene expression [27]. These TF-based biosensors mainly utilize three fundamental regulatory mechanisms, including activation, repression, and derepression. In

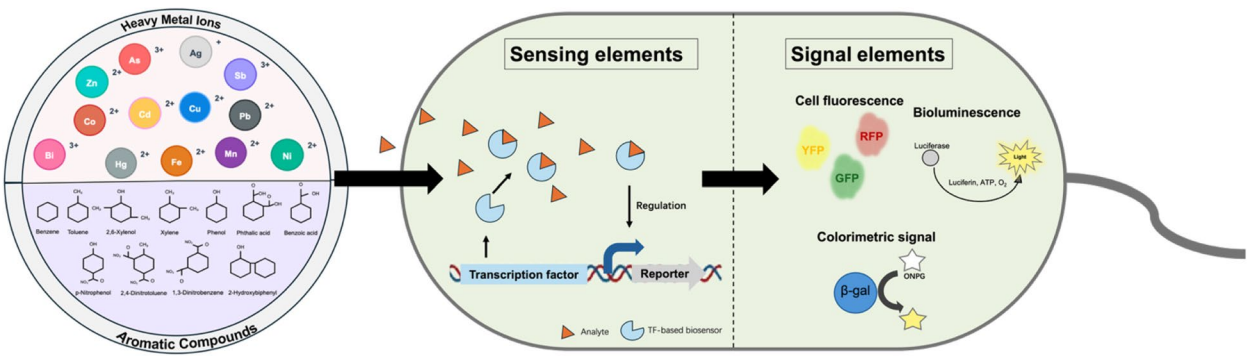

Fig. 1 Schematic diagram representing a typical whole-cell biosensor for environmental pollutant detection

the activation mechanism, the effector promotes the binding of TF to promoter regions and facilitates the recruitment of RNA polymerase to activate transcription. In repression, the effector helps TF bind to promoter regions, blocking RNA polymerase access and inhibiting transcription. In derepression, the effector binds to the TF, inducing the conformational changes that release the TF from promoter regions, thereby allowing RNA polymerase to initiate transcription (Fig. 2).

This review focuses on TF-based whole-cell biosensors, due to their notable advances in environmental detection. The diversity of TF families offers a broad range of molecular tools for biosensor development. Heavy metal-responsive TFs include the ArsR-SmtB, MerR, Fur, NikR, DtxR, CopY, and CsoR-RcnR families, while regulatory proteins such as the AraC/XylS, NtrC, LysR, and IclR families show multifunctional recognition capabilities for aromatic compounds. We outline the main TF families involved in detecting heavy metals and aromatic compounds, describing their molecular mechanisms and recent progress. Finally, we emphasize current trends in their applications and prospects for the future of TF-based biosensors.

Heavy metal-responsive transcription factors

ArsR/SmtB family

The ArsR/SmtB family of TFs plays a critical role in enabling bacteria to adapt to fluctuating metal concentrations in their environment. These proteins can interact with a broad spectrum of metal ions, including As(III), Sb(III), Bi(III), Cd(II), Pb(II), Zn(II), Co(II), Ni(II), Cu(II), and Ag(I) [28–34]. Structurally, the ArsR/SmtB family proteins are winged-helix homodimers that contain specialized metal-sensing motifs. Upon binding metal ions, they undergo allosteric conformational changes that reduce their DNA-binding affinity. This conformational shift leads to the dissociation of the TFs from the operator/promoter region, thereby derepressing the transcription of genes involved in metal resistance [35]. The ArsR/SmtB family of TFs has been extensively engineered into biosensors and is widely applied for monitoring ionic pollutants in environmental detection.

ArsR-based biosensors

ArsR is the prototypical member of the family and has been widely adapted for As(III) biosensing. Chen et al. engineered a whole-cell arsenic biosensor by refactoring the ArsR-regulated P_{ars} promoter from *E. coli* [34]. They designed synthetic promoters by embedding ArsR

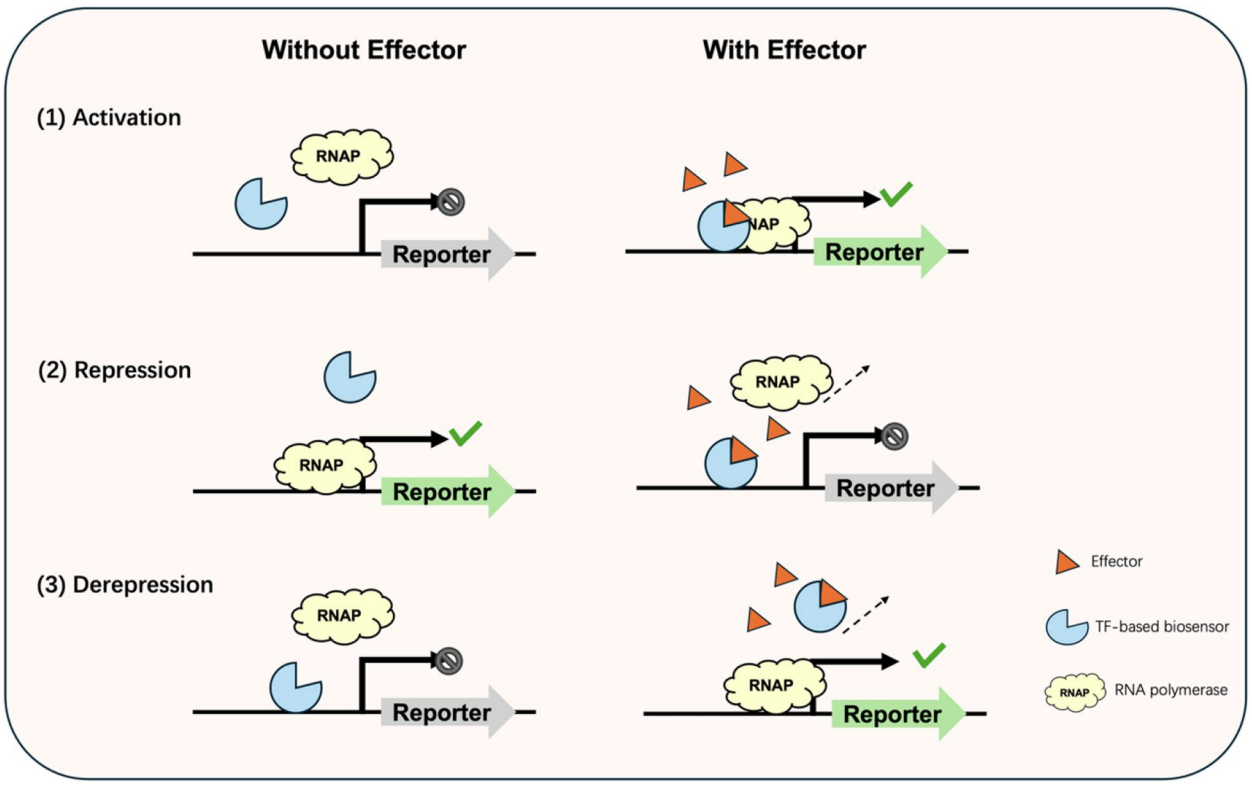

Fig. 2 The regulation mechanisms of three TF-based biosensor types. **1** Activation, **2** Repression, **3** derepression

binding sites into the core promoter region to maximize repression and reduce leaky expression. Among variants, P_{arsOC2} showed the best performance, enabling a highly sensitive biosensor with up to 183.5-fold change and a detection limit as low as 0.24 ppb. The biosensor demonstrated high specificity for arsenic and was further developed into a colorimetric platform compatible with a smartphone application for arsenic testing of groundwater samples [34]. Additionally, Xue et al. investigated a novel ArsR biosensor (ArsRRC06630), which was discovered from wastewater and integrated into *Pseudomonas putida* KT2440 to enable arsenic-responsive gene expression [36]. The engineered strain featured a plasmid-free, antibiotic-free, and inducer-free design with a growth-regulated suicide circuit, ensuring biocontainment under low arsenic conditions. ArsRRC06630 exhibited significantly high As(III) adsorption of up to 16 mg/g. Notably, Biochar was introduced as both a functional adsorbent and a biosafety tool, enhancing arsenic removal and capturing of *P. putida* for easy separation. The system achieved 92.08% arsenic removal, offering a sensitive, safe, and effective strategy for arsenic bioremediation [36]. In addition to arsenic, ArsR proteins can be tailored to detect antimony [29, 37].

AntR-based biosensors

AntR is a Sb(III)-specific TF within the ArsR/SmtB family. Viswanathan et al. characterized AntR from *Comamonas testosteroni* JL40 that specifically senses toxic antimonite Sb(III). AntR regulates the *antRCA* operon which is responsible for Sb(III) resistance [28]. This biosensor was successfully applied in a two-plasmid GFP reporter system in *E. coli* to selectively respond to Sb(III) and methylarsenite MAs(III). Using AntR from the same species for biosensor development, Zhang et al. presented a novel whole-cell biosensor for detecting bioavailable Sb(III) [38]. By inserting a second copy of the Sb(III)-responsive antR gene downstream of its inducible promoter (P_{ant}), they significantly reduced promoter leakage and improved the detection limit to 0.009 mM, well below the WHO standard. Additionally, the biosensor was integrated into a tea-bag-style membrane device for on-site application, showing good compatibility with chemical methods [38].

CadC-based biosensors

Cadmium is another prevalent heavy metal pollutant targeted by ArsR-SmtB family TFs. Shen et al. developed a highly sensitive whole-cell biosensor for cadmium detection by sequentially engineering the TF CadC from *Staphylococcus aureus* and its DNA-binding site CadO [39]. The optimized biosensor, CadO66, exhibited a 19-fold lower detection limit (0.034 μg/L) and a 2.9-fold higher specificity compared to the wild type. To enable field detection, the GFP reporter was replaced with lacZ, and a visual detection platform using SDBS detergent and smartphone imaging was established, reducing assay time to 1 h [39]. This system accurately detected cadmium in real wastewater samples, demonstrating strong agreement with standard atomic absorption methods and showing promise for environmental applications.

MerR family

MerR-family proteins are homodimeric regulators with a conserved architecture: an N-terminal DNA-binding domain, a long α-helical linker (coiled coil), and a C-terminal metal-binding domain (MBD) [40, 41]. In the absence of an effector, the regulator protein forms a stable but inactive pre-initiation complex with RNA polymerase. Upon binding metal ions, the protein undergoes a conformational change that bends and twists the DNA, realigning the promoter elements to enable efficient transcription initiation (Fig. 3). Importantly, MerR remains DNA-bound regardless of the inducer, and activation is achieved through DNA structural remodeling rather than changes in binding affinity [40, 41]. For metal response, MerR family proteins show recognition of multiple metal ions such as Hg(II), Pb(II), Cu(II), Zn(II), and Ag(I) [42–46].

MerR-based biosensors

Recent studies have leveraged the Hg(II)-responsive property of MerR to engineer highly sensitive and specific whole-cell biosensors. Guo et al. presented a rational design strategy for tuning whole-cell heavy metal biosensors using MerR family TFs [44]. The authors discovered and applied the "Parabola Principle" to optimize the detection sensitivity of the MerR-based mercury biosensor. They tested different promoter strengths for merR and found that with a fluorescence output at 0.25 μM, Hg(II) followed a parabolic trend. A predicted optimal promoter (P429-1) was used to construct biosensor P429-merR, which showed strong, specific fluorescence in response to Hg(II) with minimal interference from other metals. It enabled visible detection of Hg(II) in ultrapure, lake, and industrial water at the threshold concentration of 0.25 μM [44]. To further improve the sensitivity of the biosensor, Zhu et al. engineered an ultrasensitive whole-cell biosensor with the MerR from *Shigella flexneri* R100 for detecting ultratrace mercury using directed evolution and 5′ UTR optimization [47]. The evolved mutant (m4-1) showed a detection limit of 0.313 ng/L for inorganic mercury and 98 ng/L for methylmercury. To enhance visual detection, dual GFP reporters with different 5′ UTRs were introduced, with the 2GC variant enabling naked-eye detection at 2.5 ng/L of Hg(II) [47].

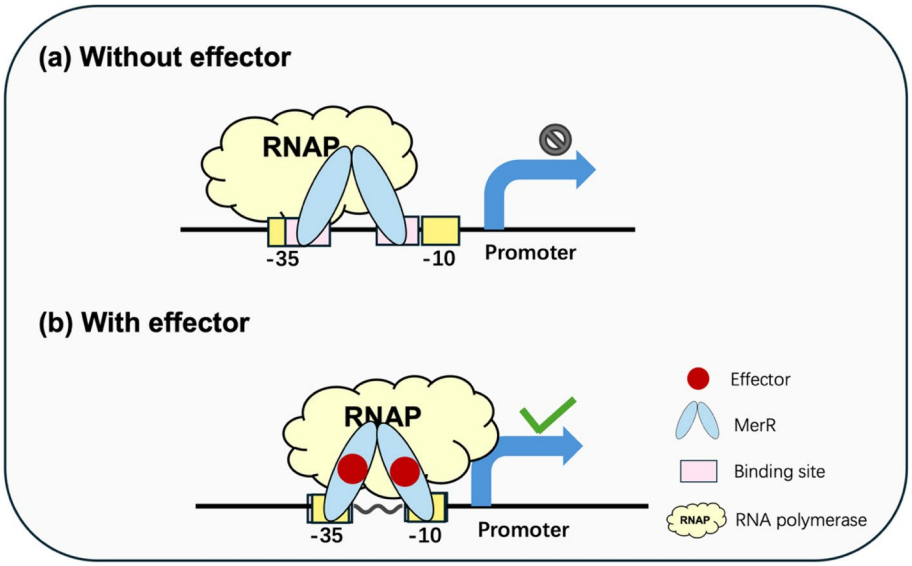

Fig. 3 The activation mechanism of MerR. **a** Without effector, MerR forms an inactive pre-initiation complex with RNA polymerase. **b** With effector, metal binding causes DNA bending/twisting, enabling transcription initiation

PbrR-based biosensor

The TF PbrR belongs to the MerR family and activates transcription after binding to lead ions. Shen et al. developed a highly sensitive whole-cell biosensor for Pb(II) detection by evolving the Pb-responsive TF PbrR through directed evolution and fluorescence-activated cell sorting (FACS) [45]. After three rounds of screening, the optimized mutant PbrR-E3 exhibited an 11-fold increase in fluorescence and a detection limit of 0.045 μg/L, which is 88 times lower than that of the wild type. The evolved biosensor exhibited improved specificity, robust performance in environmental water, and accurate detection of Pb^{2+} in tea infusions [45].

CueR-based biosensor

Recent studies have highlighted the significant potential of CueR-based biosensing systems for copper detection and speciation analysis. Costello et al. developed both whole-cell and cell-free biosensors using the bacterial copper-responsive CueR TF to detect and differentiate organic and inorganic copper forms [46]. The whole-cell biosensor measured copper uptake, and a cell-free system was used to eliminate cellular uptake factors. The study revealed that organic copper chelates were more readily absorbed by cells compared to inorganic copper forms. Furthermore, the oxidation state of copper has been proved to be critical, with Cu_2O demonstrating significantly higher uptake than CuO [46]. This provides valuable insights into the bioavailability of different copper forms in environmental contexts.

Other metal-responsive transcription factor

In addition to the ArsR/SmtB and MerR families, there are several other families that contain metal-responsive TF members (Table 1). The CsoR-RcnR family includes CsoR, which responds to Cu(I) ions, and RcnR, which exhibits specificity for Ni(II) and Co(II) ions [59, 60]. The DtxR family comprises DtxR for Fe(II) sensing, MntR for Mn(II) regulation, and other members [49, 50]. The Fur family includes Fur for Fe(II) regulation, Zur for Zn(II) homeostasis, Mur for Mn(II) sensing, and Nur for Ni(II) response [52–54, 83]. The NikR family consists of nickel-specific regulators that respond exclusively to Ni(II) [55, 56]. The CopY family contributes to Cu(II)-responsive regulation [84].

The TF families mentioned above are increasingly being exploited for the development of heavy metal biosensors. For example, Jeon et al. developed the first Mn(II)-specific bacterial cell-based biosensor that employed the MntR regulator controlled by the mntS promoter. The optimized biosensor achieved a detection range of 0.01–10 μM Mn(II) with high specificity and greater than 90% accuracy in environmental samples by deleting the Mn(II) exporter gene mntP [51]. Cayron et al. also developed a similar, highly sensitive nickel detection biosensor by systematically engineering the RcnR/rcnA regulatory system. While performing a C35A mutation in RcnR to confer nickel specificity, knocking out the nickel efflux pump gene (rcnA), and introducing a gene involved in nickel uptake, the optimized biosensor achieved a detection limit of 80 nM (4.7 μg/L) [61].

Table 1 Transcription factors of the different families that respond to heavy metal ions and aromatic compounds

Transcription factors Families	Transcription factors	Metal-responsive	References
ArsR-SmtB family	ArsR, AntR, CadC, BxmR, SmtB, CzrA, NmtR	As(III), Sb(III), Bi(III), Cd(II), Pb(II), Zn(II), Co(II), Ni(II), Cu(II), Ag(I)	[28, 29, 31–34, 37–39]
MerR family	MerR, PbrR, CueR, ZntR	Hg(II), Pb(II), Cu(II), Zn (II), Ag(I)	[42–48]
DtxR family	DtxR, MntR	Fe(II), Mn(II)	[49–51]
Fur family	Fur, Zur, Mur, Nur	Fe(II), Mn(II), Zn(II), Ni(II)	[52–54]
NikR family	NikR	Ni(II)	[55, 56]
CopY family	CopY	Cu(I)	[57, 58]
CsoR-RcnR family	CsoR, RcnR	Ni(II), Co(II), Cu(I)	[59–61]
AraC/XylS family	XylS, BenR	benzoic acid, p-toluic acid, m-toluic acid, Phthalic acid PA) and terephthalic acid (TPA), 2, 6-xylenol, Chlorobenzoic acids (Cba)	[62–66]
NtrC/XylR family	XylR, MopR, DmpR, HbpR	benzene, toluene, xylenes, 3-methylbenzylalcohol, aromatic, 2-hydroxybiphenyl, 2-aminobiphenyl, benzopyrene (Bap), tetracycline (Tet)	[67–74]
LysR family	BenM, DntR, MexT, NahR, PhnR	polycyclic aromatic hydrocarbons (PAHs), cis,cis-succinate, adipic acid, salicylate, 2,4-dinitrotoluene (DNT), 1,3-dinitrobenzene (1,3-DNB)	[75–79]
IclR family	TphR, PobR, PcaR	terephthalic acid (TPA), para-hydroxybenzoate (PHB), p-nitrophenol, dicarboxylic acids (DCAs)	[80–82]

The Fur, NikR, and DtxR families are metal-dependent repressors that play crucial roles in bacterial metal homeostasis. These transcriptional regulators employ specialized metal-binding pockets to precisely sense metal concentration and adjust gene expression accordingly. The Fur protein operates as a dimer, containing three metal-binding sites. Specifically, S1 maintains structural stability through tetrahedral ZnS_4 coordination, S2 serves as the regulatory site that triggers conformational changes upon binding metals, and S3 enhances DNA-binding affinity. Under conditions of iron abundance, the Fur protein binds to Fe(II); then the Fe-Fur complex binds to binding sites in target promoters. This blocks RNA polymerase access and represses transcription. Although Fur can function as an activator in certain contexts, its primary role is to repress transcription of the iron uptake system [85, 86]. NikR is a Ni(II) regulatory protein with a graded response system. This regulator has a high-affinity Ni(II) site, a low-affinity Ni(II) site, and a K(I) site. At moderate nickel levels, Ni(II) binds to the high-affinity site, providing basal repression on the nik operon. While excess nickel is present, the binding of Ni(II) to the low-affinity site further enhances DNA affinity, achieving complete repression. K(I) helps maintain protein structural stability, allowing the DNA-binding domain to be correctly positioned [87, 88]. MntR, from the DtxR family, primarily responds to Mn(II) and functions as a repressor. Upon Mn(II) binding, the Mn(II)-MntR complex represses the mntH gene, which is also subject to repression by Fe(II)-Fur as a dual repression system [89]. Waters et al. further revealed that MntR represses mntS expression while positively regulating mntP transcription. This dual-mode regulation allows MntR to simultaneously control manganese uptake and efflux systems in response to cellular manganese levels [90].

CopY employs a derepression mechanism similar to that of the ArsR-SmtB family proteins, but it specifically demonstrates a dual-metal sensing mechanism. Under low copper conditions, CopY binds Zn(II) and associates with the cop operator sequence, repressing transcription of copper response genes. When copper concentration increases, Cu(I) binding displaces Zn(II) and causes allosteric conformational changes of CopY. This can prompt CopY dissociation from DNA, which then activates transcription [57, 58]. CsoR is also a Cu(I)-sensing transcriptional repressor that inhibits gene transcription when Cu(I) is absent, and releases repression upon Cu(I) binding. Interestingly, Igbaria-Jaber et al. employed site-directed spin labeling (SDSL) combined with EPR spectroscopy to characterize *Mycobacterium tuberculosis* CsoR, revealing that only minor conformational changes (1–2 Å) happened upon DNA binding. This differs from other copper metalloregulators (CueR, CopY) that exhibit large structural rearrangements upon DNA binding. This suggests that CsoR metal-sensing employs a more subtle and efficient allosteric mechanism for transcriptional regulation [91]. Recent research has significantly advanced our understanding of metal-sensing mechanisms at the molecular level. These insights have enabled the development of biosensors with improved sensitivity and specificity, expanding their potential applications in environmental monitoring and assessment.

Hybrid biosensors

Combining different TFs from diverse families can enrich their individual functions and enable the construction of multifunctional biosensors. For instance, Hui et al. engineered a dual-sensing whole-cell biosensor by integrating two cadmium-responsive transcriptional regulators from different protein families [92]. They applied both the ArsR/SmtB family protein CadC from *Staphylococcus aureus* and the MerR family protein CadR from *P. putida*. In this biosensor system, CadC controlled the expression of the green fluorescent protein eGFP, while CadR regulated the red fluorescent protein mCherry. Although both respond to cadmium, CadC also detects lead, while CadR strongly responds to mercury [92]. This integration enhanced the biosensor's ability to distinguish cadmium from other interfering metals. Furthermore, Wang et al. developed a triple-input biosensor system using two cell consortia connected via quorum-sensing communication to simultaneously detect arsenic, mercury, and copper ions [93]. The system utilized TFs ArsR for As(III) and MerR for Hg(II) in the first consortium, which produced the quorum-sensing molecule 3OC6HSL upon activation. The second consortium integrated this signal with Cu(II) detection via CusR, employing AND logic gates derived from *Pseudomonas syringae* to produce a fluorescent output only when all three metal contaminants exceeded their threshold concentrations [93]. This approach significantly enhanced detection specificity, thereby improving accuracy and enabling monitoring in complex environmental conditions where multiple contaminants coexist. In another example, Guo et al. developed a dual-color whole-cell biosensor for the sensitive and selective detection of toxic Pb(II) and Hg(II) in environmental waters [94]. The PbrR-regulated vioABE genes and the MerR-regulated vioC gene to produce distinct pigments within a dual-sensing construct (pPb-vioABE-Hg-vioC) were integrated in engineered *E. coli.* The brown-green prodeoxyviolacein correlated with Pb(II) concentration and purple deoxyviolacein indicated Hg(II) concentration. The biosensor enabled visual, equipment-free detection with low detection limits, namely 0.732 nM for Pb(II) and 0.183 nM for Hg(II) [94]. This biosensor showed reliability in freshwater and seawater and remained stable over multiple passages.

Aromatic compound-responsive transcription factors

AraC/XylS family

The AraC/XylS family TFs exist especially in prokaryotic gram-positive organisms and cyanobacteria. Regulators in this family have been proven to serve diverse functions, such as carbon metabolism, stress response, and pathogenesis. Most AraC/XylS family proteins play the roles of transcriptional activators to the cognate promoters, while several members serve as repressors or dual-function regulators [95]. Multiple AraC/XylS family regulators have been identified to respond to certain aromatic compounds and have been engineered and utilized as biosensor tools.

XylS-based biosensor

XylS and its corresponding promoter P_m originate from *P. putida* and control the degradation of aromatic hydrocarbons. The Pm promoter contains two binding sites, a proximal site (PS) and a distal site (DS). When accepting effectors, the first XylS monomer binds to the PS and facilitates the binding of a second monomer to the DS, forming a dimer that causes DNA bending and then activates transcription (Fig. 4) [96–98]. This regulator can recognize benzoic acid and its derivatives and has been widely engineered as biosensors for various aromatic compounds [62]. Although the substrate promiscuity of XylS allows the wide-range detection of aromatic compounds, a strict substrate specificity is required for the accurate monitoring of specific compounds. To engineer XylS to respond to p-toluic acid rather than m-toluic acid more effectively, Ogawa et al. developed a dual selection system by employing the tetR regulation system and two antibiotics, figuring out that three residues N7, T74, and I205, are critical for *p*-toluic acid recognition, and finally switching the substrate specificity to *p*-toluic acid [63]. Phthalic acid (PA) and terephthalic acid (TPA) are hydrolysis products of poly (PET) and phthalate esters (PAEs). To support the screening of effective PET- and PAE-degrading enzymes, Li et al. acquired XylS mutants that are able to accept PA or TPA as effectors by directed evolution. With the engineered biosensor, a PAE hydrolase mutant with 2.0- and 2.5-fold activity increase toward dibutyl phthalate (DBP) and p-nitrophenyl butyrate was screened [64]. Recently, with the development of machine learning technology, the XylS biosensor has been used to generate a high-throughput dataset as training data, serving for the efficient evolution of the toluene-degrading enzyme XylM to catalyze the non-natural substrate, 2, 6-xylenol. The final mutant they acquired with two cycles of evolution showed 15 times higher productivity [65].

BenR-based biosensor

The AraC/XylS family TF from *P. putida* BenR is able to respond to benzoic acid as an effector as well. When interacting with benzoate, BenR binds and activates the P_{Ben} promoter. Voyvodic et al. employed BenR coupling with synthetic metabolic cascades to build a cell-free biosensor system, demonstrating a workflow to expand the range of molecule detection. To conduct the

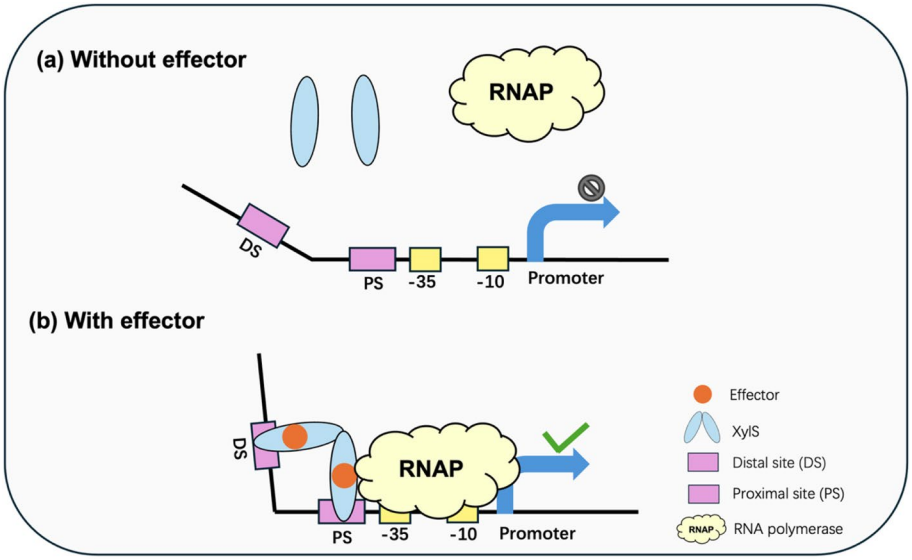

Fig. 4 Activation mechanism of XylS. **a** Without effector, XylS cannot bind to the two promoter sites (PS and DS). **b** With effector, XylS binds to both PS and DS sites, forming a dimer that bends DNA and activates transcription

plug-and-play modular workflow, the TF BenR and two metabolic modules, HipO and CocE, which served for the catalyzing of enzymatic reactions, were cloned into cell-free vectors individually. This design also enabled the calibration by plasmid titration. Finally, the optimized cell-free BenR biosensor was proven to be able to detect benzoate in different commercial beverages with fold changes of up to ~ 180, as well as hippuric acid and cocaine in human urine samples [66]. As the next generation of biosensors, cell-free transcription/translation systems offer several advantages, including rapid response, elimination of GMO release risks, and long-term storage stability [99, 100]. However, compared to whole-cell platforms, they face significant limitations in multiple aspects. The production cost of cell-free systems is higher due to the need for extracting and purifying complex cellular machinery. Additionally, cell-free systems gradually lose activity over time, limiting their operational lifetime to hours or days and requiring frequent replacement [101]. Although cell-free biosensors have been developed to detect chemicals in certain samples, the development of this kind of biosensor still has a long way to go.

NtrC/XylR family

TFs from the NtrC family are typically involved in regulating aromatic catabolism pathways. When the N-terminal domain binds to the alkylbenzene effectors, the C-terminal domain will be triggered to bind to the DNA region and activate transcription depending on the alternative sigma factor σ^{54} [102]. Due to the capability of sensing aromatic compounds, many TFs in the NtrC family present potential to be engineered as aromatic biosensors.

XylR-based biosensor

XylR is a widely studied NtrC family TF identified in *P. putida*. XylR was proven to bind to several aromatic compounds such as benzene, toluene and xylenes, and activate the transcription of the corresponding promoter P_u. XylR has been utilized as biosensors for the detection of aromatic pollutants in several studies. Willardson et al. developed biosensors based on the *Pseudomonas putida* mt-2 XylR regulator from the TOL plasmid. They characterized the substrate specificity and sensitivity of XylR-based biosensors, which showed a detection range of up to 39 ± 3.8 μM for 3-xylene and 2.690 ± 160 μM for 3-methylbenzylalcohol. They further demonstrated the detection of toluene-based contaminants in both water and soil samples [67]. Beyond this, XylR has been subjected to extensive engineering and optimization in several other studies [68–70].

MopR-based biosensor

The NtrC family TF MopR is able to respond to the aromatic pollutants, phenol and its derivatives. Ray et al. revealed the crystal structure of *Acinetobacter calcoaceticus* MopR in complex with phenol and derivatives, analyzed the binding mechanism from the perspective of protein structure [71]. Roy et al. established a whole-cell biosensor system based on MopR by adopting rational design. The engineered MopR biosensor *MopR*^{HY}*Luc* was able to detect phenol down

to ~ 1 ppb. Based on this biosensor, the MopR mutants were further engineered to detect novel compounds like benzene and xylene, and the presented biosensors were further applied for the pollutant detection of water samples. The MopR-based biosensors in this study showed outstanding performance, including high sensitivity, high selectivity, high operational range from ~0.01 to > ~100 μM and enhanced luciferase emission signal (LEF ~ 14–18) [72].

DmpR-based biosensor

Xue et al. comprehensively characterized the substrate specificity and dynamic performance of several aromatic biosensors, including the NtrC family regulators, HbpR and DmpR. According to the results of this study, HbpR showed responsiveness to 2-hydroxybiphenyl and 2-aminobiphenyl, while DmpR is able to respond to phenol and its derivatives [73]. Based on DmpR, Sun et al. constructed modular genetic circuits in *E. coli*, achieved the amplification of the detection signal of six phenolic contaminants with high sensitivity, enabling the detection of benzopyrene (Bap) and tetracycline (Tet) down to 2.5 and 2.2 ppb. Meanwhile, the engineered DmpR biosensor enabled the screening of enzymes with ester-hydrolysis activity, aiding the isolation and characterization of several novel enzymes. Especially, the screened new serine peptidase SP260 and SP759 are reported to hydrolyze both amide bonds and ester bonds, showing great potential for application [74].

LysR family

LysR-Type Transcriptional Regulators (LTTRs) represent the largest family of prokaryotic transcriptional regulators, and those proteins typically function as homotetramers [97]. The target promoters of LTTRs contain a regulatory site (RS) and two activation sites (AS1 and AS2). In the absence of effector molecules, LTTRs bind to the RS and AS1, inducing DNA bending that blocks RNA polymerase access and represses transcription. Upon binding a specific inducer (often a metabolic intermediate or small molecule), conformational changes are triggered within the protein, forcing the dimer bound at AS1 to move to AS2. This transition typically relieves DNA bending, promotes a relaxed DNA configuration, and facilitates the recruitment of RNA polymerase to the promoter, activating transcription (Fig. 5) [96, 103]. LTTRs are capable of binding a wide range of metabolites, including organic acids, metal ions, flavonoids, sugar phosphates, and nucleotides [104]. Recently, Demeester et. al summarized the interesting molecules sensed by members of the LysR family [103].

BenM-based biosensor

LTTR-based biosensors have been engineered to detect a wide range of environmental contaminants, including aromatic hydrocarbons, heavy metals, and explosives, with high specificity and sensitivity [75]. Notably, LTTRs such as NahR and BenM have been utilized to construct biosensors for detecting PAHs and other industrial byproducts in soil and water. For instance, Pham et al. introduced a computational workflow leveraging

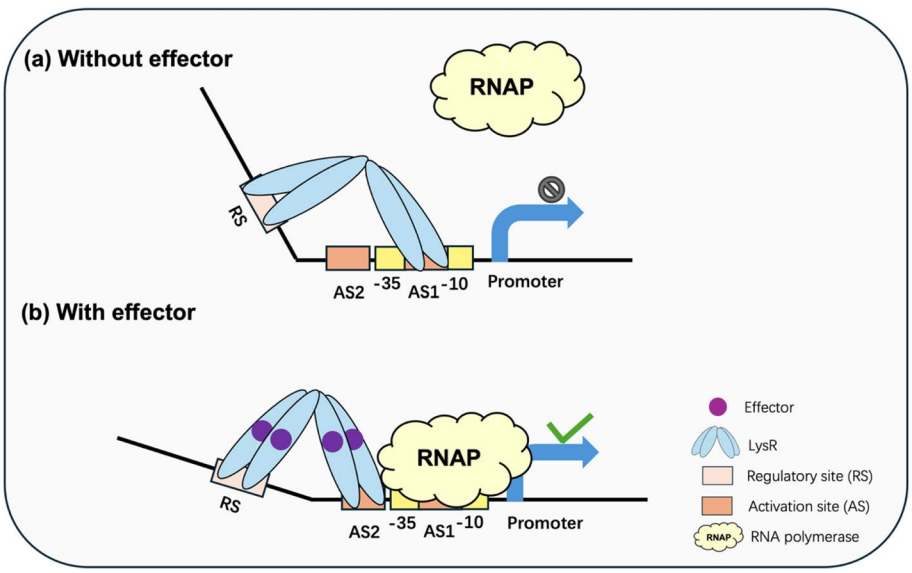

Fig. 5 Derepression mechanism of LysR. **a** Without effector, LysR binds to RS and AS1 sites, causing DNA bending that blocks RNA polymerase access. **b** With effector, effector binding causes LysR to shift from AS1 to AS2, relieving DNA bending and allowing transcription

molecular docking to engineer TF specificity. Using this approach, they modified the LysR-type TF BenM to shift its ligand preference from cis, cis-succinate to adipic acid through targeted single amino acid substitutions. The engineered biosensor, when implemented in a cell-free system, demonstrated enhanced sensitivity to adipic acid. Molecular dynamics simulations further revealed how individual mutations affect BenM's ligand-binding mechanism and structural dynamics. This study represents the first application of biomolecular modeling to alter BenM specificity and underscores the potential of computational tools for rational TF design. Moreover, the adipic acid biosensor developed could facilitate the screening and engineering of enzymes for adipic acid biosynthesis [76]. Molecular dynamics simulations of another LTTR, LysG, highlighted the critical role of the short linker helix (LH) connecting the effector- and DNA-binding domains in mediating conformational changes. Directed evolution of the LH yielded LysG variants with an extended operational range while retaining effector specificity. The optimized variant LysG-E58V outperformed the wild-type in enzyme high-throughput screening and dynamic regulation of the L-lysine biosynthetic pathway. LH mutations appear to enhance DNA binding and promote transcriptional activation upon effector binding. This LH engineering approach was also successfully applied to improve BenM biosensing, demonstrating its potential as a universal strategy for developing and optimizing LTTR-based whole-cell biosensors [105].

DntR-based biosensor

These advances highlight the potential of LTTRs in addressing environmental monitoring needs. Among the most critical applications is the detection of explosive residues, which pose severe and long-lasting risks to both human health and environmental integrity in post-conflict regions. To address this, several LysR-type regulators have been repurposed or engineered for explosive detection. For example, DntR, originally isolated from bacterial strains capable of degrading 2,4-dinitrotoluene (DNT), responds to salicylate rather than DNT itself. This observation underscores an important consideration in biosensor design: regulators associated with catabolic pathways may not always respond directly to the target substrate or its intermediates, especially in the case of synthetic or recently introduced xenobiotics. Building on this principle, Li et al. developed a lycopene-based whole-cell biosensor in *Escherichia coli* for the visual detection of DNT, aiding in the identification of buried explosives. This system employed the DNT-responsive *yqjF* promoter as the sensing module and the *crtEBI* gene cassette for lycopene biosynthesis as the reporter. To enhance performance, the metabolic flux toward lycopene production was optimized, and a terminator was added to reduce background noise. The engineered strain, LSZ05, achieved a detection limit as low as 1 mg/L of DNT and effectively detected DNT in two different soil samples. Beyond explosives, this lycopene-based biosensor also holds promise for detecting heavy metals [77].

MexT-based biosensor

In parallel, Wang et al. developed a biosensor system capable of safely and efficiently detecting the explosive compound 1,3-dinitrobenzene (1,3-DNB), leveraging the MexT TF from *P. putida*. *MexT* binds to the bidirectional promoter region between the *mexT* and *PP_2827* genes, positively regulating *PP_2827* transcription and significantly enhancing downstream gene expression in the presence of 1,3-DNB. By engineering various configurations of the *mexT* gene and promoter, a highly sensitive MexT-based biosensor was created, capable of detecting 0.1 μg/mL of 1,3-DNB in liquid and 0.5 mg/kg in sandy soil. This system demonstrated excellent specificity, long-term stability, and suitability for large-scale environmental monitoring [75]. Together, these examples illustrate the versatility and practical potential of LTTR-based biosensors in environmental detection, particularly for pollutants with high societal impact, such as explosives and heavy metals.

PhnR-based biosensor

An innovative flux-sensing approach was pioneered by Tecon et al., utilizing the LysR-family transcription factor PhnR for analyzing phenanthrene fluxes. This biosensor was constructed using the phenanthrene-degrading strain Burkholderia sp. RP007 with a plasmid containing the phnS promoter region and the GFP gene. The biosensor demonstrated a unique ability to sense phenanthrene bioavailability by responding to its flux (release rate) rather than its aqueous-phase concentration. Although this biosensor requires longer incubation times (2–3 days), it offers a valuable tool for a biologically relevant assessment of poorly soluble PAHs in real-world settings [78]. Expanding to multiple aromatic compound detection, Patel et al. developed a dual-strain fluorescent biosensor system for parallel detection of monoaromatic hydrocarbons (MAHs) and PAHs. The system used the TbuT promoter–operator with GFP for BTEX detection and the PhnR promoter–operator with CFP for PAH sensing, generating two separate *E. coli* strains. Both strains achieved 0.1–1 μM detection limits with a 30-min response time, representing a 10–100 fold improvement in sensitivity over traditional luxAB systems [79].

IclR family

IclR family, while structurally and functionally distinct from LTTRs, also modulates gene expression through signal-responsive DNA binding. Named after the *E. coli* IclR protein, which represses the *aceBAK* operon involved in the glyoxylate shunt, this family typically consists of proteins around 250 amino acids in length. Unlike LTTRs, IclR-type regulators often function as dimers or higher-order oligomers and commonly recognize asymmetric operator sequences. A distinctive sequence profile, located outside the HTH motif and spanning amino acids 151–229 in *E. coli* IclR, has been identified as a more specific signature for this family. Structural studies, including the full-length 3D structure of *Thermotoga maritima* TM0065, have confirmed the modular architecture of IclR proteins, which comprise a compact DNA-binding domain and a larger effector-binding domain. Functionally, IclR-type regulators act as repressors, activators, or dual regulators depending on promoter architecture and effector availability. Repression can occur through RNA polymerase occlusion, destabilization of the transcriptional open complex, or ligand-induced dissociation from DNA. In contrast, activation may involve effector-independent DNA binding and promotion of RNA polymerase recruitment or access, though the exact mechanisms and binding sites for activation remain incompletely defined [106].

TphR-based biosensor

The IclR family of transcriptional regulators, including TphR and PobR, plays a key role in bacterial responses to environmental pollutants, especially aromatic compounds. Among them, TphR has been employed in biosensors for detecting breakdown products of polyethylene terephthalate (PET), a major environmental pollutant. A TphR-based biosensor, incorporating the TF and a fluorescent reporter, was engineered to assess terephthalic acid (TPA) transport in evolved strains. Notably, in *C. thiooxidans* S23, expression of sfGFP under the control of the *tphC* promoter enabled TPA detection down to 10 μM. Furthermore, a mutant strain lacking the TPA degradation genes (*tphA2A3BA1*) exhibited a 10,000-fold increase in sensitivity, allowing detection at nanomolar levels. These systems offer promising tools for environmental screening and point-of-care applications [80].

PobR-based biosensor

Similarly, PobR, another IclR-type regulator, has proven valuable for biosensor development targeting aromatic pollutants. It specifically responds to para-hydroxybenzoate (PHB) in various bacterial species and negatively regulates *pobA*, encoding the enzyme that converts PHB into protocatechuic acid (PCA). Building on this regulatory mechanism, Ma et al. engineered and characterized pobR1-3 elements and developed five whole-cell biosensors in *E. coli* (BL21/pPNP-mRFP, -CFP, -YFP, -GFP, and -amilCP) for direct detection of p-nitrophenol in soil. These biosensors achieved detection limits of 6.21–25.2 μg/kg across four soil types, with a linear detection range of 10–10,000 μg/kg, demonstrating high sensitivity and robust performance for soil contaminant analysis [81].

PcaR-based biosensor

Expanding beyond aromatic compounds, PcaR, another member of the IclR family, is known to bind dicarboxylic acids (DCAs), which are organic compounds containing two carboxylic acid (-COOH) groups. As DCAs are among the most abundant organic compounds in atmospheric aerosols, their detection is crucial for assessing air quality. PcaR shows specificity toward C4-C7 DCAs, especially adipic acid, though biosensor selectivity can be challenged by structurally similar compounds. Pham et al. characterized both the functional and structural properties of PcaR, performed an in vitro ligand spectrum analysis, and constructed a genetic circuit in *Saccharomyces cerevisiae* to evaluate its utility as an in vivo biosensor. Their work contributes to the development of robust, reliable biosensors for real-time, on-site environmental monitoring aligned with global sustainability goals [82].

Conclusion and outlook

The development of TF-based biosensors has advanced considerably in monitoring environmental pollutants. This paper summarizes the recognition mechanisms of various TF families and their applications in detecting heavy metals and aromatic compounds, highlighting their crucial role in improving environmental monitoring. Notably, various members in the ArsR-SmtB and MerR families have been extensively studied for various heavy metal detection. Many members of the AraC/XylS family exhibit high specificity for certain aromatic compounds, serving for engineering aromatic compounds-responsive biosensors. The largest TF family in bacteria, LysR, responds to a wide range of metabolic intermediates and environmental pollutants, providing significant diversity for environmental detection. Other TF families show promise for detecting specific pollutants but still require further optimization to achieve adequate specificity and sensitivity in complex environmental conditions. Through engineering, some TFs have transitioned from laboratory research to field sample tests, showing potential for practical field deployment.

To improve biosensor performance, a variety of engineering strategies have been developed and utilized.

Protein engineering is the most direct optimization method, altering substrate specificity and response sensitivity of TFs through directed evolution and rational design [29, 45, 47, 61]. Although this approach is labor-intensive, time-consuming, and requires multiple rounds of screening, it is highly effective in achieving dramatic improvements in detection limits. Metabolic engineering strategies like efflux pump gene knockouts can also boost biosensor sensitivity by increasing intracellular analyte accumulation [48, 51, 61]. However, under prolonged operation or stress conditions, this strategy may compromise cellular fitness and biosensor robustness. Furthermore, Genetic circuit optimization via promoter engineering and regulatory element modification can improve signal output and reduce background noise [34, 44, 47]. Promoter engineering provides a balanced approach, enabling fine-tuning of expression levels to optimize the dynamic range without extensive structural modifications. Beyond single-TF optimization, hybrid biosensors are particularly valuable for complex environmental monitoring scenarios. By integrating regulators from different TF families, these systems can detect multiple contaminants at the same time [92–94]. Therefore, an optimal engineering strategy or combinatorial optimization can be selected based on specific application requirements, maintaining biosensor stability while achieving moderate sensitivity improvements.

Meanwhile, the integration of biosensors with smartphone applications and field-deployable formats further enhances their portability, enabling real-time, on-site pollutant detection [39]. However, the real-world use of whole-cell biosensors requires addressing critical biosafety concerns. Multiple strategies have been developed to address concerns regarding the potential environmental release of GMOs and associated regulatory challenges for whole-cell biosensors. These biocontainment measures, including toxin expression systems, auxotrophic strains, and regulation of essential genes, can effectively prevent the escape of cells into the environment [26, 36, 107]. Alternatively, physical containment systems using some hydrogel materials can be implemented to isolate biosensors from direct environmental contact [108, 109]. Established regulatory frameworks governing biosafety have become increasingly well-defined, with continued discussions on adaptive governance as technologies advance [110]. Addressing these regulatory requirements is essential for translating laboratory innovations into practical environmental monitoring tools.

Additionally, computational modeling and artificial intelligence are increasingly used to accelerate the mining, design and engineering of more precise and efficient biosensors [65, 76, 105]. Benefiting from its strong inductive and predictive capabilities, machine learning enables efficient, large-scale analysis of transcriptomic data for the targeted discovery of desired transcription factors. Through the training with the gene expression profiles acquired from RNA-seq, the unsupervised algorithm, independent component analysis (ICA), was proven to uncover the TF regulation network on a genome scale, and the TF inference results provided valuable information for novel TF mining [111, 112]. In addition to exploiting new TF elements, machine learning also serves for highly efficient biosensor development. Computational workflows leveraging molecular docking and structural modeling enable rational design of mutations to shift ligand preferences and improve binding characteristics [76]. Notably, AlphaFold 3 achieved breakthrough progression in protein-ligand interaction prediction, with a success rate of 76.4% on the PoseBusters benchmark. This significantly outperformed traditional docking tools and provided powerful support for biosensor design [113]. Molecular dynamics simulations provide critical insights into how specific modifications affect ligand-binding processes and structural dynamics of regulatory proteins [105]. Looking ahead, machine learning can be a powerful tool for designing and optimizing biosensors. Through appropriate training datasets and deep learning methods, it has the potential to facilitate rational design strategies that could significantly reduce development time and costs. While these computational approaches are still in the early stages of integration into biosensor research, continued advances in this area show great potential. As the field matures, combining synthetic biology with advanced computational methods will enable faster and more precise development of powerful environmental monitoring tools.

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Authors' contributions
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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
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References

1. Fuller R, Landrigan PJ, Balakrishnan K, Bathan G, Bose-O'Reilly S, Brauer M, et al. Pollution and health: a progress update. Lancet Planet Health. 2022;6(6):e535–47.
2. Landrigan PJ, Fuller R, Acosta NJR, Adeyi O, Arnold R, Basu NN, et al. The Lancet Commission on pollution and health. Lancet. 2018;391(10119):462–512.
3. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68(1):167–82.
4. Chowdhury R, Ramond A, O'Keeffe LM, Shahzad S, Kunutsor SK, Muka T, et al. Environmental toxic metal contaminants and risk of cardiovascular disease: systematic review and meta-analysis. BMJ. 2018;362:k3310.
5. Xu X, Nie S, Ding H, Hou FF. Environmental pollution and kidney diseases. Nat Rev Nephrol. 2018;14(5):313–24.
6. Munzel T, Hahad O, Lelieveld J, Aschner M, Nieuwenhuijsen MJ, Landrigan PJ, et al. Soil and water pollution and cardiovascular disease. Nat Rev Cardiol. 2025;22(2):71–89.
7. Filippini T, Wise LA, Vinceti M. Cadmium exposure and risk of diabetes and prediabetes: a systematic review and dose-response meta-analysis. Environ Int. 2022;158:106920.
8. Qin C, Yu C, Li L, China Kadoorie Biobank Collaborative G. Regarding associations of egg consumption with cardiovascular disease in a cohort study of 0.5 million Chinese adults. Heart. 2018;104(21):1803.
9. Xie Y, Xiao H, Zheng D, Mahai G, Li Y, Xia W, et al. Associations of prenatal metal exposure with child neurodevelopment and mediation by perturbation of metabolic pathways. Nat Commun. 2025;16(1):2089.
10. Liu X, Zhang X, Dufresne M, Wang T, Wu L, Lara R, et al. Measurement report: exploring the variations in ambient BTEX in urban Europe and their environmental health implications. Atmos Chem Phys. 2025;25(1):625–38.
11. Khoshakhlagh AH, Yazdanirad S, Mousavi M, Gruszecka-Kosowska A, Shahriyari M, Rajabi-Vardanjani H. Summer and winter variations of BTEX concentrations in an oil refinery complex and health risk assessment based on Monte-Carlo simulations. Sci Rep. 2023;13(1):10670.
12. Nakhjirgan P, Fanaei F, Jonidi Jafari A, Gholami M, Shahsavani A, Kermani M. Extensive investigation of seasonal and spatial fluctuations of BTEX in an industrial city with a health risk assessment. Sci Rep. 2024;14(1):23662.
13. Tehrani AM, Hajiketabi S, Berijani N, Samadi M. Investigating the respiratory and systemic effects of exposure to BTEX among municipal solid waste workers. Environ Pollut. 2025;366:125525.
14. Mohebbi M, Jafari AJ, Gholami M, Baghani AN, Shahsavani A, Kermani M. Measurement and health risks assessment of BTEX compounds exposure in beauty Lahijan City salons. Sci Rep. 2024;14(1):23515.
15. Montano L, Baldini GM, Piscopo M, Liguori G, Lombardi R, Ricciardi M, et al. Polycyclic aromatic hydrocarbons (PAHs) in the environment: occupational exposure, health risks and fertility implications. Toxics. 2025;13(3):151.
16. Mojiri A, Zhou JL, Ohashi A, Ozaki N, Kindaichi T. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. Sci Total Environ. 2019;696:133971.
17. Montano L, Pironti C, Pinto G, Ricciardi M, Buono A, Brogna C, et al. Polychlorinated biphenyls (PCBs) in the environment: occupational and exposure events, effects on human health and fertility. Toxics. 2022;10(7):365.
18. Gupta P, Thompson BL, Wahlang B, Jordan CT, Zach Hilt J, Hennig B, et al. The environmental pollutant, polychlorinated biphenyls, and cardiovascular disease: a potential target for antioxidant nanotherapeutics. Drug Deliv Transl Res. 2018;8(3):740–59.
19. Maser E, Strehse JS. Can seafood from marine sites of dumped world war relicts be eaten? Arch Toxicol. 2021;95(7):2255–61.
20. Adomako-Bonsu AG, Jacobsen J, Maser E. Metabolic activation of 2,4,6-trinitrotoluene; a case for ROS-induced cell damage. Redox Biol. 2024;72:103082.
21. Zhang Y, Zhu Y, Zeng Z, Zeng G, Xiao R, Wang Y, et al. Sensors for the environmental pollutant detection: are we already there? Coord Chem Rev. 2021;431:213681.
22. Vikesland PJ, Wigginton KR. Nanomaterial enabled biosensors for pathogen monitoring-a review. Environ Sci Technol. 2010;44(10):3656–69.
23. Zamani M, Klapperich CM, Furst AL. Recent advances in gold electrode fabrication for low-resource setting biosensing. Lab Chip. 2023;23(5):1410–9.
24. Song K, Ji H, Lee J, Yoon Y. Microbial transcription factor-based biosensors: innovations from design to applications in synthetic biology. Biosensors (Basel). 2025;15(4):221.
25. Kim HJ, Jeong H, Lee SJ. Synthetic biology for microbial heavy metal biosensors. Anal Bioanal Chem. 2018;410(4):1191–203.
26. Hicks M, Bachmann TT, Wang B. Synthetic biology enables programmable cell-based biosensors. ChemPhysChem. 2020;21(2):132–44.
27. Zhang J, Gong X, Gan Q, Yan Y. Application of metabolite-responsive biosensors for plant natural products biosynthesis. Biosensors (Basel). 2023;13(6):633.
28. Viswanathan T, Chen J, Wu M, An L, Kandavelu P, Sankaran B, et al. Functional and structural characterization of AntR, an Sb(III) responsive transcriptional repressor. Mol Microbiol. 2021;116(2):427–37.
29. Lee W, Kim H, Jang G, Kim BG, Yoon Y. Antimony sensing whole-cell bioreporters derived from ArsR genetic engineering. Appl Microbiol Biotechnol. 2020;104(6):2691–9.
30. Osman D, Cavet JS. Bacterial metal-sensing proteins exemplified by ArsR-SmtB family repressors. Nat Prod Rep. 2010;27(5):668–80.
31. Lee S-Y, De La Torre A, Yan D, Kustu S, Nixon BT, Wemmer DE. Regulation of the transcriptional activator NtrC1: structural studies of the regulatory and AAA+ ATPase domains. Genes Dev. 2003;17(20):2552–63.
32. Eicken C, Pennella MA, Chen X, Koshlap KM, VanZile ML, Sacchettini JC, et al. A metal–ligand-mediated intersubunit allosteric switch in related SmtB/ArsR zinc sensor proteins. J Mol Biol. 2003;333(4):683–95.
33. Liu T, Chen X, Ma Z, Shokes J, Hemmingsen L, Scott RA, et al. A Cu(I)-sensing ArsR family metal sensor protein with a relaxed metal selectivity profile. Biochemistry. 2008;47(40):10564–75.
34. Chen SY, Zhang Y, Li R, Wang B, Ye BC. De novo design of the ArsR regulated P(ars) promoter enables a highly sensitive whole-cell biosensor for arsenic contamination. Anal Chem. 2022;94(20):7210–8.
35. Saha RP, Samanta S, Patra S, Sarkar D, Saha A, Singh MK. Metal homeostasis in bacteria: the role of ArsR-SmtB family of transcriptional repressors in combating varying metal concentrations in the environment. Biometals. 2017;30(4):459–503.
36. Xue Y, Li Y, Li X, Zheng J, Hua D, Jiang C, et al. Arsenic bioremediation in mining wastewater by controllable genetically modified bacteria with biochar. Environ Technol Innov. 2024;33:103514.
37. Chen X, Yao H, Song D, Lin J, Zhou H, Yuan W, et al. A novel antimony-selective ArsR transcriptional repressor and its specific detection of antimony trioxide in environmental samples via bacterial biosensor. Biosens Bioelectron. 2023;220:114838.
38. Zhang L, Ye L, Jing C. A novel whole-cell biosensor for bioavailable antimonite in water and sediments. Appl Environ Microbiol. 2023;89(1):e0181722.
39. Shen L, Chen Y, Hu L, Zhang C, Liu L, Bao L, et al. Development of a highly sensitive, visual platform for the detection of cadmium in actual wastewater based on evolved whole-cell biosensors. ACS Sens. 2024;9(2):654–61.
40. Tulin G, Figueroa NR, Checa SK, Soncini FC. The multifarious MerR family of transcriptional regulators. Mol Microbiol. 2024;121(2):230–42.
41. Hobman JL. MerR family transcription activators: similar designs, different specificities. Mol Microbiol. 2007;63(5):1275–8.
42. Changela A, Chen K, Xue Y, Holschen J, Outten CE, O'Halloran TV, et al. Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR. Science. 2003;301(5638):1383–7.
43. Binet MR, Poole RK. Cd(II), Pb(II) and Zn(II) ions regulate expression of the metal-transporting P-type ATPase ZntA in *Escherichia coli*. FEBS Lett. 2000;473(1):67–70.
44. Guo M, Du R, Xie Z, He X, Huang K, Luo Y, et al. Using the promoters of MerR family proteins as "rheostats" to engineer whole-cell heavy metal biosensors with adjustable sensitivity. J Biol Eng. 2019;13:70.

45. Shen L, Chen Y, Pan J, Yu X, Zhang Y, Guo B, et al. Development of a highly sensitive PbrR-based biosensor via directed evolution and its application for lead detection. J Hazard Mater. 2025;488:137489.
46. Costello A, Parker J, Clynes M, Murphy R. Development of whole-cell and cell-free biosensors for the detection and differentiation of organic and inorganic forms of copper. Metallomics. 2020;12(11):1729–34.
47. Zhu K, Chen D, Cai Y, Zhang T, Ma J, Bao L, et al. Engineering the ultrasensitive visual whole-cell biosensors by evolved MerR and 5′UTR for detection of ultratrace mercury. Environ Sci Technol. 2023;57(44):16964–73.
48. Fu Y, Li J, Wang J, Wang E, Fang X. Development of a two component system based biosensor with high sensitivity for the detection of copper ions. Commun Biol. 2024;7(1):1407.
49. Merchant AT, Spatafora GA. A role for the DtxR family of metalloregulators in gram-positive pathogenesis. Mol Oral Microbiol. 2014;29(1):1–10.
50. Brune I, Werner H, Hüser AT, Kalinowski J, Pühler A, Tauch A. The DtxR protein acting as dual transcriptional regulator directs a global regulatory network involved in iron metabolism of *Corynebacterium glutamicum*. BMC Genomics. 2006;7:21.
51. Jeon Y, Lee Y, Kim Y, Park C, Choi H, Jang G, et al. Development of novel *Escherichia coli* cell-based biosensors to monitor Mn(II) in environmental systems. Front Microbiol. 2022;13:1051926.
52. Bagg A, Neilands JB. Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. Biochemistry. 1987;26(17):5471–7.
53. Díaz-Mireles E, Wexler M, Sawers G, Bellini D, Todd JD, Johnston AWB. The fur-like protein Mur of *Rhizobium leguminosarum* is a Mn(2+)-responsive transcriptional regulator. Microbiology (Reading). 2004;150(Pt 5):1447–56.
54. Kandari D, Joshi H, Bhatnagar R. Zur: zinc-sensing transcriptional regulator in a diverse set of bacterial species. Pathogens. 2021;10(3):344.
55. Li Y, Zamble DB. Nickel homeostasis and nickel regulation: an overview. Chem Rev. 2009;109(10):4617–43.
56. Leitch S, Bradley MJ, Rowe JL, Chivers PT, Maroney MJ. Nickel-specific response in the transcriptional regulator, *Escherichia coli* NikR. J Am Chem Soc. 2007;129(16):5085–95.
57. Hirsch M, Hofmann L, Shenberger Y, Gevorkyan-Airapetov L, Ruthstein S. Conformations and local dynamics of the CopY metal sensor revealed by EPR spectroscopy. Biochemistry. 2023;62(3):797–807.
58. Cobine PA, George GN, Jones CE, Wickramasinghe WA, Solioz M, Dameron CT. Copper transfer from the Cu(I) chaperone, CopZ, to the repressor, Zn(II)CopY: metal coordination environments and protein interactions. Biochemistry. 2002;41(18):5822–9.
59. Liu T, Ramesh A, Ma Z, Ward SK, Zhang L, George GN, et al. CsoR is a novel *mycobacterium tuberculosis* copper-sensing transcriptional regulator. Nat Chem Biol. 2007;3(1):60–8.
60. Higgins KA, Giedroc D. Insights into protein allostery in the CsoR/RcnR family of transcriptional repressors. Chem Lett. 2013;43(1):20–5.
61. Cayron J, Prudent E, Escoffier C, Gueguen E, Mandrand-Berthelot MA, Pignol D, et al. Pushing the limits of nickel detection to nanomolar range using a set of engineered bioluminescent *Escherichia coli*. Environ Sci Pollut Res Int. 2017;24(1):4–14.
62. Xian Y, Zhang F, Wang M, Zhao X, Sun X, Lu Z, et al. Whole-cell biosensor engineering based on the transcription factor XylS/Pm for sensitive detection of PCB intermediate chlorobenzoic acid. Biochem Eng J. 2024;202:109153.
63. Ogawa Y, Katsuyama Y, Ueno K, Ohnishi Y. Switching the ligand specificity of the biosensor XylS from meta to para-toluic acid through directed evolution exploiting a dual selection system. ACS Synth Biol. 2019;8(12):2679–89.
64. Li J, Nina MRH, Zhang X, Bai Y. Engineering transcription factor XylS for sensing phthalic acid and terephthalic acid: an application for enzyme evolution. ACS Synth Biol. 2022;11(3):1106–13.
65. Ogawa Y, Saito Y, Yamaguchi H, Katsuyama Y, Ohnishi Y. Engineering the substrate specificity of toluene degrading enzyme XylM using biosensor XylS and machine learning. ACS Synth Biol. 2023;12(2):572–82.
66. Voyvodic PL, Pandi A, Koch M, Conejero I, Valjent E, Courtet P, et al. Plug-and-play metabolic transducers expand the chemical detection space of cell-free biosensors. Nat Commun. 2019;10(1):1697.
67. Willardson BM, Wilkins JF, Rand TA, Schupp JM, Hill KK, Keim P, et al. Development and testing of a bacterial biosensor for toluene-based environmental contaminants. Appl Environ Microbiol. 1998;64(3):1006–12.
68. de Las Heras A, de Lorenzo V. Cooperative amino acid changes shift the response of the σ^{54}-dependent regulator XylR from natural m-xylene towards xenobiotic 2,4-dinitrotoluene. Mol Microbiol. 2011;79(5):1248–59.
69. Kim MN, Park HH, Lim WK, Shin HJ. Construction and comparison of Escherichia coli whole-cell biosensors capable of detecting aromatic compounds. J Microbiol Methods. 2005;60(2):235–45.
70. Wang GH, Tsai TH, Kui CC, Cheng CY, Huang TL, Chung YC. Analysis of bioavailable toluene by using recombinant luminescent bacterial biosensors with different promoters. J Biol Eng. 2021;15(1):2.
71. Ray S, Gunzburg MJ, Wilce M, Panjikar S, Anand R. Structural basis of selective aromatic pollutant sensing by the effector binding domain of MopR, an NtrC family transcriptional regulator. ACS Chem Biol. 2016;11(8):2357–65.
72. Roy R, Ray S, Chowdhury A, Anand R. Tunable multiplexed whole-cell biosensors as environmental diagnostics for ppb-level detection of aromatic pollutants. ACS Sens. 2021;6(5):1933–9.
73. Xue H, Shi H, Yu Z, He S, Liu S, Hou Y, et al. Design, construction, and characterization of a set of biosensors for aromatic compounds. ACS Synth Biol. 2014;3(12):1011–4.
74. Sun S, Peng K, Sun S, Wang M, Shao Y, Li L, et al. Engineering modular and highly sensitive cell-based biosensors for aromatic contaminant monitoring and high-throughput enzyme screening. ACS Synth Biol. 2023;12(3):877–91.
75. Wang Z, Ma R, Chen B, Yu X, Wang X, Zuo X, et al. A transcription factor-based bacterial biosensor system and its application for on-site detection of explosives. Biosens Bioelectron. 2024;244:115805.
76. Pham C, Stogios PJ, Savchenko A, Mahadevan R. Computation-guided transcription factor biosensor specificity engineering for adipic acid detection. Comput Struct Biotechnol J. 2024;23:2211–9.
77. Li M, Lv S, Yang R, Chu X, Wang X, Wang Z, et al. Development of lycopene-based whole-cell biosensors for the visual detection of trace explosives and heavy metals. Anal Chim Acta. 2023;1283:341934.
78. Tecon R, Wells M, van der Meer JR. A new green fluorescent protein-based bacterial biosensor for analysing phenanthrene fluxes. Environ Microbiol. 2006;8(4):697–708.
79. Patel R, Zaveri P, Mukherjee A, Agarwal PK, More P, Munshi NS. Development of fluorescent protein-based biosensing strains: a new tool for the detection of aromatic hydrocarbon pollutants in the environment. Ecotoxicol Environ Saf. 2019;182:109450.
80. Dierkes RF, Wypych A, Perez-Garcia P, Danso D, Chow J, Streit WR. An ultra-sensitive *Comamonas thiooxidans* biosensor for the rapid detection of enzymatic polyethylene terephthalate (PET) degradation. Appl Environ Microbiol. 2023;89(1):e0160322.
81. Ma Z, Li Y, Lu Z, Pan J, Li M. A novel biosensor-based method for the detection of p-nitrophenol in agricultural soil. Chemosphere. 2023;313:137306.
82. Pham C, Nasr MA, Skarina T, Di Leo R, Kwan DH, Martin VJJ, et al. Functional and structural characterization of an IclR family transcription factor for the development of dicarboxylic acid biosensors. FEBS J. 2024;291(15):3481–98.
83. Steingard CH, Helmann JD. Meddling with metal sensors: fur-family proteins as signaling hubs. J Bacteriol. 2023;205(4):e0002223.
84. Strausak D, Solioz M. CopY is a copper-inducible repressor of the Enterococcus hirae copper ATPases. J Biol Chem. 1997;272(14):8932–6.
85. Kang SM, Kang HS, Chung WH, Kang KT, Kim DH. Structural perspectives on metal dependent roles of ferric uptake regulator (Fur). Biomolecules. 2024;14(8):981.
86. Dian C, Vitale S, Leonard GA, Bahlawane C, Fauquant C, Leduc D, et al. The structure of the Helicobacter pylori ferric uptake regulator Fur reveals three functional metal binding sites. Mol Microbiol. 2011;79(5):1260–75.
87. Wang SC, Li Y, Robinson CV, Zamble DB. Potassium is critical for the Ni(II)-responsive DNA-binding activity of *Escherichia coli* NikR. J Am Chem Soc. 2010;132(5):1506–7.

88. Wang SC, Dias AV, Zamble DB. The "metallo-specific" response of proteins: a perspective based on the Escherichia coli transcriptional regulator NikR. Dalton Trans. 2009;14:2459–66.
89. Patzer SI, Hantke K. Dual repression by Fe(2+)-Fur and Mn(2+)-MntR of the *mntH* gene, encoding an NRAMP-like Mn(2+) transporter in *Escherichia coli*. J Bacteriol. 2001;183(16):4806–13.
90. Waters LS, Sandoval M, Storz G. The *Escherichia coli* MntR miniregulon includes genes encoding a small protein and an efflux pump required for manganese homeostasis. J Bacteriol. 2011;193(21):5887–97.
91. Igbaria-Jaber Y, Hofmann L, Gevorkyan-Airapetov L, Shenberger Y, Ruthstein S. Revealing the DNA binding modes of CsoR by EPR spectroscopy. ACS Omega. 2023;8(42):39886–95.
92. Hui CY, Guo Y, Wu J, Liu L, Yang XQ, Guo X, et al. Detection of bioavailable cadmium by double-color fluorescence based on a dual-sensing bioreporter system. Front Microbiol. 2021;12:696195.
93. Wang B, Barahona M, Buck M. A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals. Biosens Bioelectron. 2013;40(1):368–76.
94. Guo Y, Zhu DL, Liu MQ, Chen YT, Hu SY, Hui CY. Metabolic engineering-enabled dual-color biosensor for discriminative and sensitive detection of toxic lead and mercury in environmental waters. J Environ Chem Eng. 2024;12(5):114178.
95. Gallegos MT, Schleif R, Bairoch A, Hofmann K, Ramos JL. Arac/XylS family of transcriptional regulators. Microbiol Mol Biol Rev. 1997;61(4):393–410.
96. Gawin A, Valla S, Brautaset T. The XylS/Pm regulator/promoter system and its use in fundamental studies of bacterial gene expression, recombinant protein production and metabolic engineering. Microb Biotechnol. 2017;10(4):702–18.
97. Baugh AC, Momany C, Neidle EL. Versatility and complexity: common and uncommon facets of LysR-type transcriptional regulators. Annu Rev Microbiol. 2023;77:317–39.
98. Kotoky R, Ogawa N, Pandey P. The structure-function relationship of bacterial transcriptional regulators as a target for enhanced biodegradation of aromatic hydrocarbons. Microbiol Res. 2022;262:127087.
99. Perez JG, Stark JC, Jewett MC. Cell-free synthetic biology: engineering beyond the cell. Cold Spring Harb Perspect Biol. 2016;8(12):a023853.
100. Wang T, Lu Y. Advances, challenges and future trends of cell-free transcription-translation biosensors. Biosensors. 2022;12(5):318.
101. Zhang L, Guo W, Lu Y. Advances in cell-free biosensors: principle, mechanism, and applications. Biotechnol J. 2020;15(9):2000187.
102. Galvão TC, de Lorenzo V. Transcriptional regulators à la carte: engineering new effector specificities in bacterial regulatory proteins. Curr Opin Biotechnol. 2006;17(1):34–42.
103. Demeester W, De Paepe B, De Mey M. Fundamentals and exceptions of the LysR-type transcriptional regulators. ACS Synth Biol. 2024;13(10):3069–92.
104. Matilla MA, Velando F, Martin-Mora D, Monteagudo-Cascales E, Krell T. A catalogue of signal molecules that interact with sensor kinases, chemoreceptors and transcriptional regulators. FEMS Microbiol Rev. 2022;46(1):fuab043.
105. Pu W, Chen J, Liu P, Shen J, Cai N, Liu B, et al. Directed evolution of linker helix as an efficient strategy for engineering LysR-type transcriptional regulators as whole-cell biosensors. Biosens Bioelectron. 2023;222:115004.
106. Suvorova IA, Gelfand MS. Comparative analysis of the IclR-family of bacterial transcription factors and their DNA-binding motifs: structure, positioning, co-evolution, regulon content. Front Microbiol. 2021;12:675815.
107. Lee JW, Chan CTY, Slomovic S, Collins JJ. Next-generation biocontainment systems for engineered organisms. Nat Chem Biol. 2018;14(6):530–7.
108. Tang TC, Tham E, Liu X, Yehl K, Rovner AJ, Yuk H, et al. Hydrogel-based biocontainment of bacteria for continuous sensing and computation. Nat Chem Biol. 2021;17(6):724–31.
109. Luisi B, Hegab R, Person C, Seo K, Gleason J. Engineered biosensors in an encapsulated and deployable system for environmental chemical detection. ACS Sens. 2022;7(9):2589–96.
110. Wang F, Zhang W. Synthetic biology: recent progress, biosafety and biosecurity concerns, and possible solutions. J Biosaf Biosecur. 2019;1(1):22–30.
111. Rychel K, Sastry AV, Palsson BO. Machine learning uncovers independently regulated modules in the *Bacillus subtilis* transcriptome. Nat Commun. 2020;11(1):6338.
112. Lim HG, Rychel K, Sastry AV, Bentley GJ, Mueller J, Schindel HS, et al. Machine-learning from *Pseudomonas putida* KT2440 transcriptomes reveals its transcriptional regulatory network. Metab Eng. 2022;72:297–310.
113. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature. 2024;630(8016):493–500.

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