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Development of a Highly Sensitive Whole-Cell Biosensor for Arsenite Detection through Engineered Promoter Modifications

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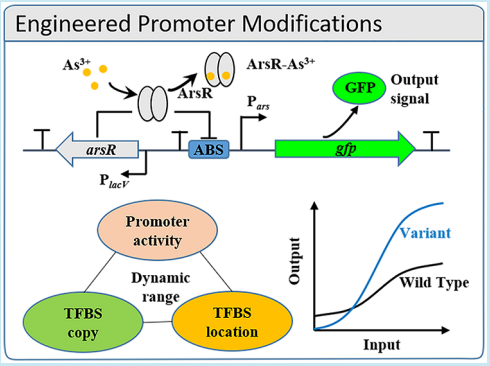
Supporting Information

ABSTRACT: Whole-cell biosensors have attracted considerable interests because they are robust, eco-friendly, and cost-effective. However, most of the biosensors harness the naturally occurring wild-type promoter, which often suffers from high background noise and low sensitivity. In this study, we demonstrate how to design the core elements (i.e., RNA polymerase binding site and transcription factor binding site) of the promoters to obtain a significant gain in the signal-to-noise output ratio of the whole-cell biosensor circuits. As a proof of concept, we modified the arsenite-regulated promoter from *Escherichia coli* K-12 genome, such that it has a lower background and higher expression. This was achieved by balancing the relationship between the number of ArsR binding sites (ABS) and the activity of the promoter and adjusting the location of the auxiliary ABS. A promoter variant ParsD-ABS-8 was obtained with an induction ratio of 179 (11-fold increase over the wild-type promoter) when induced with 1 μM arsenite. Importantly, the developed biosensor exhibited good dose−response in the range of 0.1 to 4 μM (R^2 = 0.9928) of arsenite with a detection limit of ca. 10 nM. These results indicated that the engineered promoter modification approach could be used to improve the performance of whole-cell biosensors, thereby facilitating their practical application.



KEYWORDS: *arsenite detection, whole-cell biosensor, promoter modification, ArsR binding site, regulatory circuit*

Arsenic is a common heavy metal contaminant that occurs naturally in the Earth's crust and is widely distributed through the air, water, and even food, causing serious threats to the environment and human health.[1] Prolonged exposure to arsenic at concentrations above the World Health Organization (WHO) prescribed safety limit level (10 ppb, ca. 0.133 μM) can lead to serious medical conditions, such as peripheral neuropathy, renal systemic effects, cardiovascular disease, and skin lesions.[2] Moreover, arsenic is also a dangerous carcinogen. It has been reported that chronic ingestion or inhalation of arsenic increases the risk of cancer in the liver, bladder, lungs, and skin.[3−6] As a result, arsenic has topped the priority list of hazardous substances in the Agency for Toxic Substances and Disease Registry (ATSDR) in the United States over the years.

With the increasing threat of arsenic exposure to the environment, development of accurate, cost-effective, and eco-friendly field analytical techniques for arsenic detection would be beneficial in assessing arsenic pollution. Currently, arsenic levels are determined primarily using high-end analytical techniques, such as atomic absorption spectroscopy (AAS), gas chromatography−mass spectrometry (GC-MS), and induced coupled plasma-mass spectrometry (ICP-MS).[7−9] While these traditional high-end chemical analytics methods for arsenic detection are reliable and highly sensitive, the equipment required are expensive, nonportable, and can only be operated by trained professionals, thereby limiting the on-site application of the methods.

In the past decade, whole-cell biosensors have been developed for the detection of environmental contaminants because they are robust, eco-friendly, and cost-effective.[10−13] However, the majority of the reported biosensors based on native promoters are often limited in their sensitivity as well as dynamic range.[14−17] As a promising analytical tool, arsenite-responsive whole-cell biosensors were also developed on the basis of the arsenic-resistance operon *arsRBC* of the *Escherichia coli* (*E. coli*) K-12 genome or the *arsRDABC* operon on the R773 plasmid as a sensing element.[18−22] High-throughput screening and directed evolution have been used to improve the sensitivity of biosensors,[20,23] but these methods are time-

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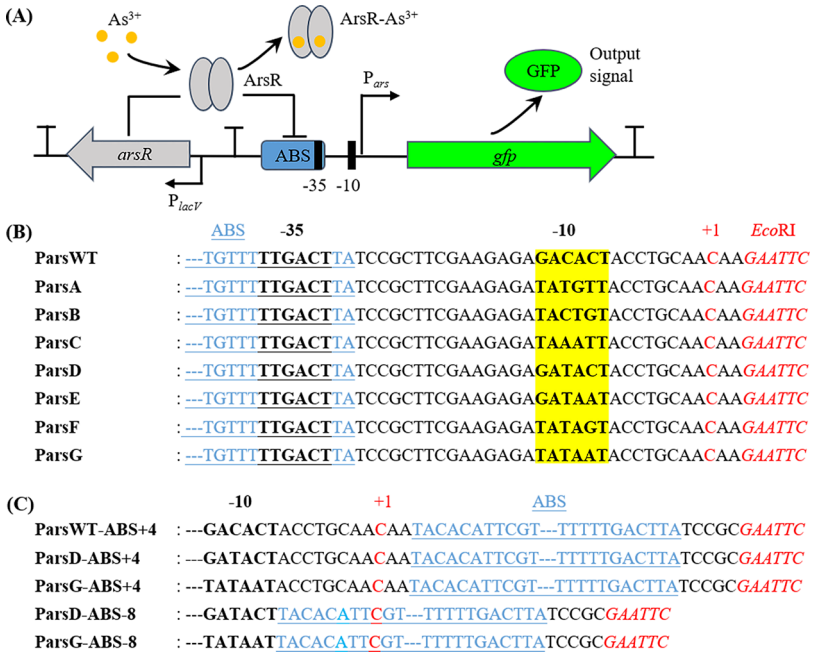

Figure 1. Design and improvement of arsenic biosensors. (A) Construction of arsenic responsive biosensor genetic circuit. (B) Increasing the promoter activity by mutating −10 sites (shaded in yellow) in the wild-type promoter. (C) Adding ABS behind the −10 site or transcription initiation site to decrease leaky promoter expression. The bold sequences represent the −10 and −35 sites, the ArsR binding site (a proximal ABS) is colored blue and underlined, while the *Eco*RI restriction site and transcription initiation site are colored red.

consuming. To reduce leakage during the expression of arsenite-regulated promoters, researchers have tried adding ArsR binding sites (ABS). However, this also reduces the signal output unless a higher concentration of arsenite is added.[15,24,25] Recently, various types of genetic circuits, including feedback circuits, uncoupled circuits, and genetic amplifiers, have been designed to optimize cellular responses and to improve signal output.[26−28] Nevertheless, there are considerably few ways to accurately quantify the presence of arsenite at low concentrations while maintaining a high signal output and low background noise in a genetic circuit.[15]

Most promoters in *E. coli* are regulated by sigma 70 factor (σ^{70}), a housekeeping transcriptional factor that enables the binding of RNA polymerase to σ^{70} promoter's −10 and −35 sites and subsequent initiation of transcription.[29,30] Using σ^{70} promoters controlled by a repressor (such as LacI/P_{lac}, TetR/P_{tet}, and ArsR/P_{ars}) as research models, it has been shown that the repression efficiency of a promoter is mainly determined by the promoter strength, binding constants of the repressor to its binding motif, concentration of repressor, and the number and position of binding sites.[15,31−34] To date, there is no consensus on how to obtain higher signal output and dynamic range of biosensors by promoter modification.

Here, we describe a method to increase the dynamic range of arsenite-responsive biosensors through promoter engineering. First, we performed a series of mutations in the −10 site of the wild-type promoter ParsWT to tune promoter activity. Next, we added ABS downstream of the −10 site or the transcription initiation site to reduce promoter leakage. Consequently, we obtained an arsenite-inducible whole-cell biosensor variant ParsD-ABS-8, with an 11-fold increase in the induction ratio compared with the wild-type ParsWT when 1 μM arsenite was added. To our knowledge, this improvement in the induction ratio is the highest among all reported studies that use native arsenite-regulated promoters. The ParsG-ABS-8 variant exhibited a visible fluorescence change upon addition of 0.25 μM arsenite, which is a very intuitive warning for arsenite contamination exceeding the WHO limit (0.133 μM). At the same time, the limit of detection (LOD) of the two variants (ParsD-ABS-8, ParsG-ABS-8) was 10 nM, which indicates an excellent performance in arsenite detection for the analysis of real samples.

RESULTS AND DISCUSSION

Site-Directed Mutagenesis of Wild-Type Promoter in arsR Gene. Native bacteria have evolved *arsR*-P_{ars} (a feedback circuit) regulatory expression machinery which endows the bacteria with enhanced resistance to arsenic.[27] Nevertheless, this feedback circuit is not necessarily optimal for the design of higher sensitivity biosensors for arsenic detection. From the perspective of regulating expression mechanisms, feedback circuits tend to have lower reported outputs than uncoupled circuits at higher arsenite concentrations.[26,27] Interestingly, regardless of the circuit system (feedback or uncoupled), using the *arsR*-P_{ars}^{K12} operon from the *E. coli* K-12 genome as a sensing element yields more sensitive fluorescence output than the *arsR*-P_{ars}^{R773} operon from *E. coli* R773 plasmid for induction at the same arsenite concentration.[26] Therefore, we chose *arsR*-P_{ars}^{K12} as a sensing element to construct an uncoupled ArsR-controlled genetic circuit that produces GFP fluorescence as a function of arsenite concentration (Figure 1A). In this genetic circuit, ArsR is expressed by a constitutive promoter (P_{lacV}) to represses the transcription of a reporter gene (*gfp*) under the control of ArsR-repressed promoter (P_{ars}). When trivalent arsenic was present, repression was relieved, and GFP expression was turned on.

As shown in Figure 1A, the ABS contains a −35 site (TTGACT) in the wild-type promoter. It is known that the −35 sites should be kept unchanged to ensure a strong binding affinity of ArsR to the ABS.[35] To enhance promoter activity,

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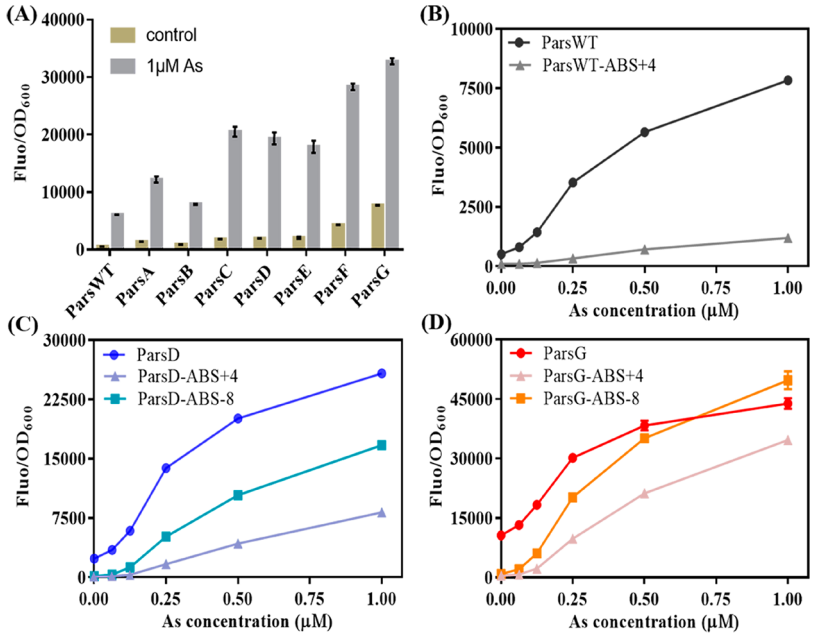

Figure 2. Performance of the improved biosensors in response to arsenite. (A) The fluorescence of biosensors containing wild-type or mutated promoters. (B) The fluorescence of biosensors with ParsWT or ParsWT-ABS+4 promoter in response to arsenite (from 0 to 1 μM). (C) The fluorescence of biosensors with ParsD, ParsD-ABS+4, or ParsD-ABS-8 promoter in response to arsenite. (D) The fluorescence of biosensors with ParsG, ParsG-ABS+4, or ParsG-ABS-8 promoter in response to arsenite. Error bars are smaller than the data symbols (n = 3).

Table 1. Induction Ratio and Background Noise of the Arsenite Biosensors

	Report variant (*E. coli* DH5α)	Basal expression[c]	Induction ratio at conc. (μM) 0.0625	0.125	0.25	0.5	1
Single ABS[a]	ParsWT	514±11	1.6	3.0	7.5	12.1	16.8
	ParsD	2352±47	1.5	2.5	6.0	8.7	11.2
	ParsG	10631±76	1.2	1.7	2.8	3.6	4.1
Dual ABS[b]	ParsWT-ABS+4	103±5	1.1	1.8	5.3	12.6	21.8
	ParsD-ABS+4	117±8	1.5	4.1	24.3	63.2	122.2
	ParsG-ABS+4	469±12	1.8	5.2	23.2	50.5	82.7
	ParsD-ABS-8	142±2	3.0	13.0	54.7	111.2	179.3
	ParsG-ABS-8	880±23	2.4	7.3	24.3	42.3	59.9

[a]There is only one ArsR binding site on the promoter [b]There are two copies of the ArsR binding site on the promoter [c]Mean Fluo/OD_{600} value ± standard deviation (n = 3). Black arrow indicates a gradual increase in promoter activity. Red arrow indicates a decrease in induction ratio. Blue arrow indicates an increase in induction ratio

we performed a series of mutations on the −10 sites in ParsWT promoter (Figure 1B). Seven arsenite-responsive biosensors with the seven promoter variants were thus constructed. These variants and the wild-type promoters were examined and compared for 1 μM arsenite induction. Our results showed that the mutated variants exhibit a higher fluorescence output than the wild-type for 1 μM arsenite induction (Figure 2A). The fold-changes in fluorescence are shown in Figure S1. Some variants (ParsF and ParsG) with high arsenite-induced activities revealed low levels of induced fold-change due to high background fluorescence. We hypothesized that mutations in the −10 sites might increase the efficiency of RNA polymerase binding to the promoter, thereby reducing the efficiency of ArsR repressor binding to ABS, resulting in a high background noise. These results are consistent with previous reports that increasing or decreasing the promoter activity does not effectively improve the promoter dynamic range.[15,29,36]

Addition of ArsR-Binding Site Resulted in Decrease of Background Noise. The activity of the promoter can be increased by site-directed mutagenesis of −10 sites. However, the induced fold-change was decreased for these mutated variants after the addition of arsenite because of the high background noise (Figure 2A and Figure S1). Therefore, it is important to reduce the leaky expression in the variants. Adding an additional copy of ABS is a common and effective means of reducing background noise. To investigate the effect of the second ABS site on the fluorescence of output and background, we selected three promoters, ParsWT with low activity, ParsD with medium activity, and ParsG with high activity, for further study. The second binding site was added downstream of the ParsWT transcription initiate site, yielding ParsWT-ABS+4 (Figure 1C). The ParsWT biosensor and the ParsWT-ABS+4 biosensor were induced by different concentrations (0, 0.0625, 0.125, 0.25, 0.5, and 1 μM) of arsenite. Although we used the same promoter lacV to express the

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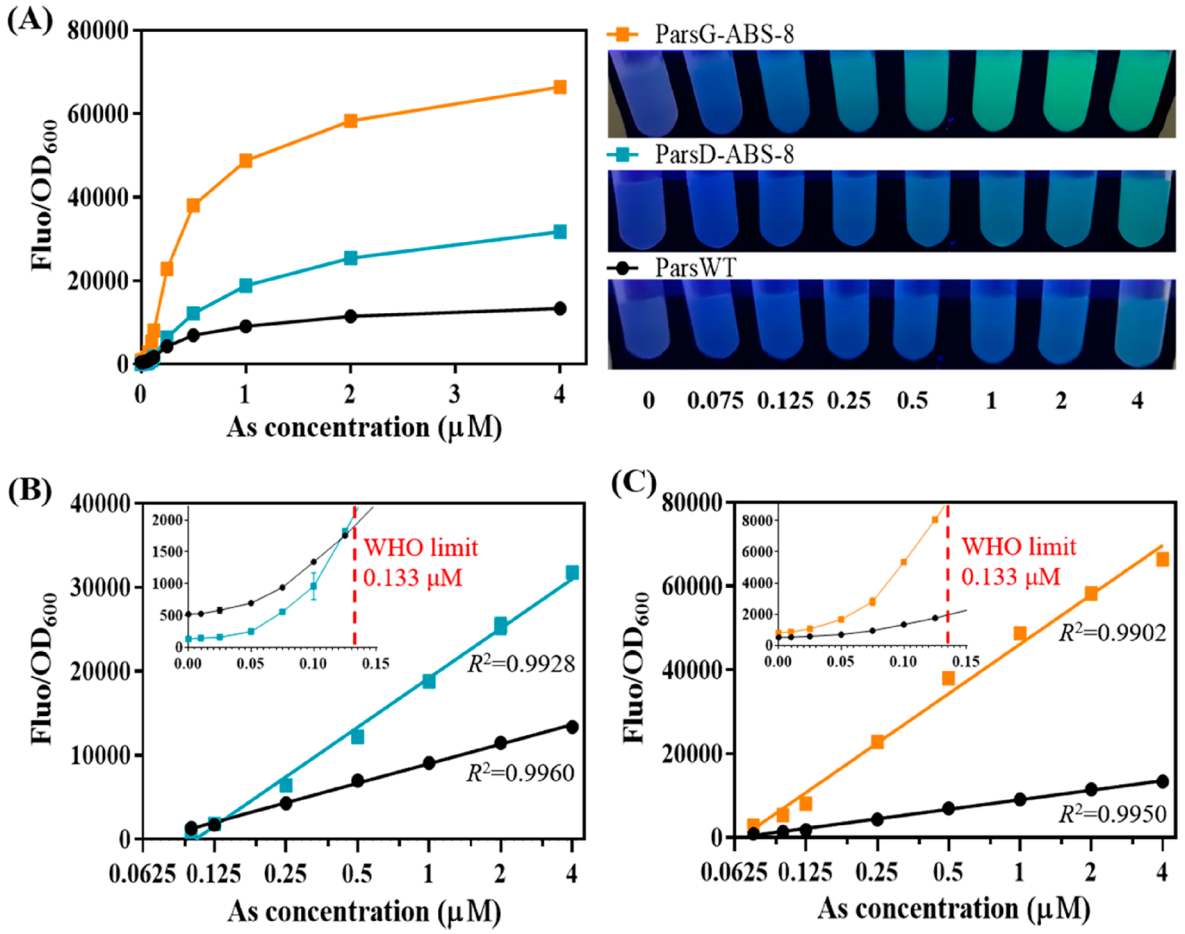

Figure 3. Dose-dependent response to arsenite. (A) Dose−response curves of the biosensors (ParsWT, black circles; ParsD-ABS-8, turquoise squares; and ParsG-ABS-8, orange squares) when exposed to arsenite concentrations of 0 to 4 μM. The color change of liquid cultures of biosensors when exposure to a portable hand-held UV lamp set at 365 nm wavelength. (B) Best-fit parameters for ParsWT and ParsD-ABS-8 biosensors from 0.1 to 4 μM range. (C) Best-fit parameters for ParsWT and ParsG-ABS-8 biosensors from 0.075 to 4 μM range, (B, C) data from (A). Error bars are smaller than the data symbols (n = 3).

regulatory protein ArsR, there were significant differences in background noise, sensitivity, and output between the sensors. The background fluorescence of the dual ABS biosensor was lower than the single ABS in the absence of arsenite (Figure 2B), decreasing from 514 to 103 (Table 1). Unfortunately, while the background noise was reduced, the sensitivity and signal output of the biosensor were also greatly diminished. The signal intensity of ParsWT-ABS+4 biosensor decreased from 8000 to 1200, compared with ParsWT when exposed to 1 μM arsenite. On the other hand, the induction ratio of ParsWT-ABS+4 was lower than that of ParsWT when arsenite was less than 0.5 μM. Particularly when induced with 0.0625 μM arsenite, the induction ratio of ParsWT was 1.6, whereas ParsWT-ABS+4 was almost unresponsive (Table 1). This result indicates that adding a second ABS directly to the wild-type promoter results in a decrease in the sensitivity and signal output of the biosensor.[15,37]

To effectively reduce the background noise of variant promoters (i.e., ParsD, ParsG), we investigated the influence of placement of an additional ABS downstream of either the −10 site or the transcription initiation site in the two constructs (Figure 1C). First, the addition of a second ABS to the promoter at two positions showed a significant difference in background noise, induction ratio, and promoter activity. The second ABS was added in ParsD promoter downstream of the transcription initiation site, and its background was significantly reduced from 2352 to 117, which was slightly higher than ParsWT-ABS+4. Interestingly, the fluorescence output was significantly higher than that of ParsWT-ABS+4 and ParsWT when induced with 1 μM arsenite (Figure 2B,C). The second ABS was directly added downstream of the −10 site, generating ParsD-ABS-8, for which, the background fluorescence was as low as 142 (slightly higher than ParsWT-ABS+4 and ParsD-ABS+4, about 4-fold lower than ParsWT). Surprisingly, the fluorescence output increased to 16 500 when induced with 1 μM arsenite, thus greatly improving the induction ratio of the sensor (Figure 2C). A similar modification was performed for the ParsG promoter. The background fluorescence of ParsG-ABS+4 dropped from 10 631 to 469, which is about 22.7-fold lower than that of ParsG. Meanwhile, when 1 μM of arsenite was used, the reported output still reached 34 500, a value slightly lower than that of ParsG, and the induction ratio reached 82.7-fold. The induction ratio of ParsG-ABS-8 was only 59.9-fold at 1 μM arsenite due to the high background noise in the absence of arsenite. Overall, the addition of a second ABS directly downstream of the −10 site to the arsenic-responsive promoter showed a higher background noise and signal output than downstream of the transcription initiation site (Figure 2C,D). This may be due to a change in the sequence immediately

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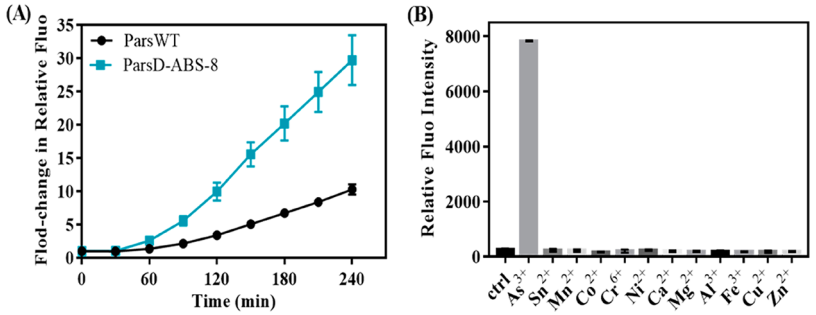

Figure 4. Response dynamics and specificity of arsenite-induced bacterial biosensors. (A) Fold-change in the relative fluorescence of biosensors (ParsWT and ParsD-ABS-8) in response to 0.5 μM arsenite within 0−240 min. (B) The selectivity of the ParsD-ABS-8 biosensor detection on As^{3+} (0.5 μM) and other kinds of metal ions (5 μM for each ions). The average fluorescence for each sample was recorded after induction for 4 h at 30 °C. Error bars represent standard deviation from the mean (n = 3).

downstream of the −10 site, resulting in enhancement of promoter activity. The ParsD-ABS-8 biosensor had the highest induction ratio (1, 3, 13, 54.7, 111.2, and 179.3, respectively) in all tested biosensors, for different concentrations (0, 0.0625, 0.125, 0.25, 0.5, and 1 μM) of arsenite. To our knowledge, none of the currently reported arsenite-regulated promoters has such a high induction ratio. As shown in Table 1, increasing the promoter activity by mutating the RNA polymerase binding site would decrease the induction ratio of biosensors. The sensitivity of the arsenite-responsive biosensor containing the wild-type promoter was severely reduced after the addition of the second ABS. Interestingly, the higher the promoter activity, the lower was the effect of adding a second copy of ABS on the signal output of the biosensor (Table S1).

These results indicated that promoter modification with a single strategy, for example, adding transcription factor binding sites (TFBS) or increasing promoter strength, is not enough to obtain optimal sensing performance. Instead, balancing the relationship between the number of TFBS and the activity of the promoter is crucial to improve the induction ratio and sensitivity of the sensor. This conclusion explains why Zhang et al. obtained a fatty acid biosensor with a high dynamic range by placing two FadR binding sites on both sides of the −10 site of strong phage promoter, PA1.[17] This raises the question of the mechanism behind the improvement in the sensor's induction ratio and sensitivity by the engineered promoter modifications. It is likely that the double repressor binding sites effectively block the binding of RNA polymerase, resulting in transcriptional inhibition.[25] However, for wild-type promoters with low activity, this blocking is so excessive that it can only be alleviated at high concentrations of inducer. For the promoters with high activity, excessive inhibition is attenuated due to the increased affinity of RNA polymerase for the promoter. Thus, the promoters with high activity containing double repressor binding sites can obtain a high induction ratio and ensure sensitivity. Besides, adjusting the relative position of the second repressor binding site at the promoter can also fine-tune the induction ratio and signal output of the sensor (Table 1).[15,37] A systematic test was performed on ParsD promoter (Figure S2A), and combined with previous reports,[15] we inferred that the location of the ArsR binding site further away from the −10 site was not conducive for maintaining a high signal output of the sensor.

Dose-Dependent Response to Arsenite. To examine the dose−response of the two best-performing variants (ParsD-ABS-8, ParsG-ABS-8) to arsenite, the wild-type (ParsWT) and two variant biosensors were induced by a series of arsenite concentrations (0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5, 1, 2, and 4 μM). As shown in Figure 3A, the variants have a higher dynamic range than the wild-type biosensor. When illuminated with a portable hand-held UV lamp set at 365 nm wavelength, ParsG-ABS-8 exhibited visible fluorescence at arsenite concentrations above 0.25 μM, whereas ParsWT showed no visible fluorescence even at the arsenite concentrations of 4 μM (Figure 3A, right). A good dose-dependent induction response of the variant biosensors for arsenite occurred between 0.1 and 4 μM (ParsD-ABS-8, R^2 = 0.9928, Figure 3B) or between 0.075 and 4 μM (ParsG-ABS-8, R^2 = 0.9902, Figure 3C). Obviously, the report output of the biosensor variants increased more sensitively than that of the wild type after induction with arsenite. Moreover, the background fluorescence of the ParsD-ABS-8 biosensor was 4-fold lower than that of ParsWT, achieving a bidirectional improvement in high signal output and low background noise. In addition, although the background noise of the ParsG-ABS-8 biosensor was higher than that of ParsWT, the increase in signal output was also more pronounced after arsenite induction. In particular, as shown in the insets of both panels B and C of Figure 3, with an increase in arsenite concentration from a level below the WHO limit to a high concentration, the increase in fluorescence of the variants was steeper than that of wild-type biosensors. This result indicates that engineered promoter modifications can significantly improve biosensor performance in terms of background noise and sensitivity.

Indeed, there are additional methods to further improve the performance of whole-cell biosensor to meet detection limits and signal output requirements in practical applications. Decreasing the density of intracellular arsenite receptor protein (ArsR) is an effective strategy for improving the signal output and sensitivity of whole-cell biosensor (Figure S2B).[27,32,37] The same outcome could be reproduced by increasing intracellular ligand densities, specifically, by overexpressing importers and knocking out exporters.[26,27,38] More sensitive arsenic sensors should be further explored by constructing cell-free biosensors. Currently, the optimal concentration of transcriptional and translation-related components in *E. coli* has been comprehensively studied,[39,40] making it possible to develop biosensors with high signal outputs and low detection limits. Therefore, cell-free components may become a potential sensing platform independent of the cell wall and genetic regulation.[41]

Dynamics and Specificity of Arsenite Biosensor. For arsenite-induced biosensors, response time and specificity are

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two important factors that should be considered in the context of field applications. The bacteria cultures were exposed to 0.5 μM arsenite for different incubation periods ranging from 0 to 240 min. A time-dependent assay with both wild-type ParsWT and ParsD-ABS-8 biosensors is shown in Figure 4A. For 0.5 μM arsenite concentration, the ParsD-ABS-8 biosensor exhibited more than a 2-fold fluorescence increase within 60 min, whereas the ParsWT biosensor took over 90 min to achieve the same fold-change of the fluorescence intensity. This result indicates that the response of modified ParsD-ABS-8 biosensor to arsenite is quicker than that of the wild-type ParsWT. As shown in Figure 4B, the variant ParsD-ABS-8 biosensor had specific response to arsenite, and no obvious response to other kinds of metal ions was detected. This result indicates that the developed ParsD-ABS-8 biosensor maintained an excellent selectivity, similar to the wild-type.

Real Sample Analysis. Natural river waters with complex ingredients may affect the growth and health of bacterial biosensors, and in some cases, lead to inaccurate results.[20] In addition, the complex components in natural water might affect the occurrence state of the target inducer that made it inaccessible to the bacteria. Therefore, we tested the performance of ParsD-ABS-8 biosensor with a series of artificial arsenite-contaminated river water samples. The arsenite content in the original river water sample was found to be 0.025 μM by ICP-MS. Arsenite calibration curves were established by ParsD-ABS-8 biosensor with final induction concentrations of 0.1, 0.125, 0.25, 0.5, 1, 2, and 4 μM (Figure S3). Arsenic concentrations of spiked arsenite-contaminated river water samples were calculated on the basis of the equation: $Y = 14894 + 4316\log_2(x)$. As shown in Table 2, the recovery of arsenite measured by ParsD-ABS-8 biosensor was in the range of 90.8% to 105.3%, showing exceedingly good agreement with the actual concentration of the samples. These results indicate that the biosensor exhibited strong anti-interference and good stability in determining the arsenite concentration in the river water samples.

Table 2. Accuracy and Reliability of the Constructed Biosensors for Real Samples

samples	ICP-MS (μM)	add As (μM)	Fluo/OD_{600} [a]	estimated (μM)	recovery
river water	0.025	0.125	2707 ± 15	0.141	94.0%
	0.025	0.500	11012 ± 337	0.536	102.1%
	0.025	0.750	12707 ± 74	0.704	90.8%
	0.025	3.000	22107 ± 534	3.185	105.3%
	0.025	4.000	23557 ± 83	4.020	99.8%

[a]Mean Fluo/OD_{600} value ± standard deviation (n = 3).

CONCLUSIONS

In summary, we developed a highly sensitive whole-cell arsenite biosensor through engineered promoter modification, which is an effective method to improve the dynamic range of the sensor without reducing its sensitivity. The induction ratio of the arsenite-induced biosensor was over 200-folds, when induced with 4 μM arsenite. The biosensor showed a detection of limit of ca. 10 nM, which is much lower than the WHO-prescribed permissible level (10 ppb, 133 nM) in drinking water. This study has revisited the whole-cell biosensors based on bacterial transcription regulation, while achieving bidirectional improvements in background decrease and signal output increase. It also shows that coordination between the number of TFBS and promoter activity is important to improve the signal-to-noise ratio of biosensors, which might have profound implications for further practical applications of whole-cell biosensor. In addition, expression of different reporter genes (e.g., *luxCDABE*, *rfp*, or *lacZ*) should be considered for further development of highly sensitive whole-cell biosensors with luminescent, colorimetric, or electrochemical read-out.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and Reagents. Plasmid cloning and biosensor characterization were all performed in *E. coli* DH5α strain. Bacteria were cultured in Luria−Bertani broth (LB) medium under aerobic conditions. Solid media was prepared by supplementation with 15 g/L agar. Tetracycline (50 μg/mL) was used as a selection marker during cloning and incubation. Bacteria inoculated from a single colony on freshly streaked plates were grown overnight in 5 mL of LB in sterile 15 mL universal tubes at 37 °C with shaking (200 rpm). Sodium arsenite (NaAsO2) and other chemicals used were analytical grade and obtained from Sigma-Aldrich.

Construction of Arsenite Reporter Plasmids. The plasmids and primers used in this study are described in detail in Supplementary Table S2 and Table S3. The detailed sequences of target genes are shown in Table S4. All plasmids were constructed by using pPROBE-TT (purchased from Addgene, U.S.A.) as a backbone.[42] Standard molecular manipulation was carried out for polymerase chain reaction (PCR), DNA purification, plasmid enzyme digestion, recombination, and transformation. The *arsR* gene containing the appropriate ribosome binding site (RBS) and the constitutive P_{lacV} (lac variant) promoter were amplified from a wild-type *E. coli* K-12 genome using the primer pairs HA-arsR-F and HR-*Hin*dIII-arsR-R, HR-*Sac*I-lacV-F and HA-lacV-R, respectively. The *arsR* gene was fused downstream of the constitutive P_{lacV} promoter by fusion PCR and then cloned into the *Hin*dIII and *Sac*I sites of the sensor detection platform pPROBE-TT vector to generate p-TT-lacVarsR (Figure S4). The ArsR-regulated wild-type promoter ParsWT (including an *agrB* terminator) was also amplified from a wild-type *E. coli* K-12 genome using the primer pair HR-*Sac*I-Pars-F and HR-*Eco*RI-Pars-R.[43] Following PCR product purification, it was cloned into the *Sac*I and *Eco*RI sites of p-TT-lacVarsR vector to generate p-TT-lacVarsR-ParsWT, named ParsWT (Figure S5). As depicted in Figure 1A, which shows an uncoupled arsenite-induced biosensor circuit, the gfp gene is under control of the ParsWT promoter as the signal output, which is in opposite direction to the *arsR* gene. To enhance promoter activity, seven variants (Figure 1B) were produced, wherein −10 sites of the ParsWT promoter were mutated with primers. To reduce leaky promoter expression, as shown in Table S2, five variants (ParsWT-ABS+4, ParsD-ABS+4, ParsG-ABS+4, ParsD-ABS-8, ParsG-ABS-8) and other variants were produced by primer extension PCR, wherein an extra copy of the ABS was placed downstream of the promoter −10 site. All variants were generated by primer extension PCR and validated by sequencing before their use, and all biosensors had names identical to their respective promoters.

Fluorescence Measurement and Data Analysis. Single colonies of *E. coli* DH5α biosensor strains harboring validated plasmid were grown overnight at 37 °C in 5 mL LB media supplemented with 50 μg/mL tetracycline. The overnight

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cultures were then 100-fold diluted into 200 mL of fresh LB medium supplemented with tetracycline and incubated at 37 °C in a 1000 mL flask with shaking at 200 rpm, until the optical density at 600 nm (OD_{600}) reached between 0.5 and 0.6. Various concentrations (all samples in triplicate) of arsenite were added to 3 mL aliquots of the bacterial culture in 24-well deep well plates (Canvic, China). After incubation for 4 h at 30 °C, culture samples of 1 mL were centrifuged for 1 min at 13 400*g*, and the supernatant was decanted. The bacterial pellet was suspended in 1 mL of phosphate buffer, and 200 μL of this suspension was transferred into white and black 96-well plates (Fluotrac 200; Greiner, Germany). The OD_{600} (absorbance at 600 nm) and the fluorescence (excitation at 480 nm, emission at 500 to 600 nm, sensitivity = 75%) were read by a microplate reader (BioTek Instruments, Winooski, VT, U.S.A.). The signal output of different biosensors for a series of arsenite concentrations was shown in Fluo/OD_{600} (Fluorescence/OD_{600}). Considering the background of the bacteria itself, the induction ratio was calculated as follows:

$$\text{induction ratio} = \frac{\text{RFU (arsenite)} - \text{RFU (bg)}}{\text{RFU (control)} - \text{RFU (bg)}}$$

where RFU (arsenite) and RFU (control) denote the average Fluo/OD_{600} (from triplicates) in wells containing bacteria biosensor with or without arsenite, respectively, and RFU (bg) denotes the average Fluo/OD_{600} of GFP-free *E. coli* DH5α.

The time-dependent and specificity response experiments of biosensors on arsenite were performed directly on 96-well black plates. The backgrounds fluorescence was determined using blank wells loaded with LB media only and was subtracted from the readings of other wells. The fold-change in Relative Fluo was calculated at different times, as follows:

$$\text{fold change in Relative Fluo} = \frac{\text{Fluo (incubation period)}}{\text{Fluo (initial)}}$$

where Fluo (incubation period) and Fluo (initial) denote the fluorescence for specified incubation periods and the fluorescence at 0 min, respectively.

Real Sample Analysis. The original river water samples (taken from the rivers flowing through the campus of East China University of Science and Technology) were filtered by using a 0.22-μm filter to remove impurities and bacteria. Subsequently, the arsenic content was detected by ICP-MS. To determine whether the ions or other complex compounds in the river water affect the biosensor, LB medium was directly prepared with the river water sample and added with a suitable concentration of tetracycline for bacterial culture, until the OD_{600} of the bacterial culture had reached between 0.5 and 0.6. Various concentrations (0.125, 0.5, 0.75, 3, and 4 μM, all samples in triplicate) of arsenite were added to 3 mL aliquots of bacterial culture in 24-well deep-well plates. At the same time, the medium was prepared using ultrapure water for bacterial culture, and a standard curve was obtained by adding a series of concentrations (0.1, 0.125, 0.25, 0.5, 1, 2, and 4 μM) of arsenite. The experiment was performed at room temperature to mimic on-site test conditions. The arsenite concentration in the river water sample was then estimated on the basis of the standard curve equation.

■ ASSOCIATED CONTENT

Supporting Information

. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.9b00093.

Some data about biosensors and vectors are shown in Figures S1−S5; and all information about the gene sequences, plasmids, and primers is listed in Tables S1−S4 (PDF)

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Sheng-Yan Chen and Bang-Ce Ye designed the research; Sheng-Yan Chen performed research with the help of Wenping Wei, Yanbin Tong, and Jianjiang Lu. Bang-Ce Ye contributed new reagents/analytic tools; Sheng-Yan Chen, Bin-Cheng Yin, and Bang-Ce Ye analyzed data and wrote the manuscript.

Notes

The authors declare no competing financial interest.

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