Engineering Microbial Sensor Cell Arrays to Visualize Environmental Contaminants

Xinyi Wan\textsuperscript{1,2} and Baojun Wang\textsuperscript{1,2*}

\textsuperscript{1}School of Biological Sciences, University of Edinburgh, Edinburgh, UK
\textsuperscript{2}Centre for Synthetic and Systems Biology, University of Edinburgh, Edinburgh, UK

*Correspondence can be addressed to BW (baojun.wang@ed.ac.uk)

Running Head: Microbial Sensor Cell Arrays

Summary

Whole-cell biosensors based on bacterial responses to analytes as measured by microelectronics, imaging, or other methods have recently become attractive alternatives to conventional detection methods for detecting various toxic and pathogenic contaminants in aqueous environments. However, frequently they cannot meet practical detection requirements due to biosafety issues and inadequate sensing platforms for field test. To this end, we have developed an innovative sensing platform, a microbial sensor cell array, which integrates whole-cell biosensors and produces an intuitive readout upon sensing. In this sensor array, the sensor cell variants are immobilized in hydrogel and distributed on a microplate in an easy-to-interpret volume bar pattern. By simply mixing with a medium, contaminated water samples can be added to the whole sensor array, and different patterns will form to give an easy-to-interpret visual indication of contamination levels. The sensing platform can also be combined with readily available technology such as cell phone cameras to aid visualization without the need for sophisticated equipment. To demonstrate its field application potential, an arsenic sensor cell array is developed and tested using real environmental water samples. This easy-to-use sensor cell array can be applied to many other sensing applications, including cell-free biosensors, and will provide portable, low-cost environmental monitoring in the field.

Key Words: Whole-cell biosensor, Bacterial bioreporter, Sensor cell array, Hydrogel, Visualization, Arsenic contamination, Environmental monitoring.
1. Introduction

Whole-cell biosensors are cells that can report targets or conditions of interest. They have drawn increasing attention, especially in the rising era of synthetic biology, as alternatives to traditional methods due to being renewable, environmentally friendly and cost-effective (1, 2). Synthetic biology has accelerated new biosensor developments, as well as improving sensors’ behavior for real-life applications (2–4). A growing number of whole-cell biosensors have been engineered for various applications, including environmental assessment, clinical diagnosis, biotherapy, controlled bioprocessing, mineral surveying and landmine clearing (2, 5, 6).

Although many biosensors have been demonstrated successfully in the laboratory, very few have reached the market. Besides biosafety concerns and unsatisfactory sensing performance in the natural environment, there is also a shortage of inexpensive, field-deployable and user-friendly platforms for cellular biosensor storage and large-scale sample screening (7, 8). The majority of biosensor-based detection methods use a single biosensor to report its target with a single reporter. The sensor can report its target quantitatively by fitting its output level to a calibration curve; alternatively, the sensor can be designed to only report a small range of target concentrations (9). However, the former always needs calibration as the cell number and cellular activity may vary under different conditions, while the latter requires serial dilution of samples. Simplified read-outs such as comparing output results with standardized color tables are still difficult and inaccurate.

Wackwitz et al. (2008) proposed a ‘traffic light’ arsenic assay that could function independently of external calibration (1, 10). The idea is to use a set of biosensors with different detection limits to generate ‘traffic light’ reporting patterns upon sensing their target (Fig. 1a). Due to restricted tuning methods, they generated only a limited set of biosensors, resulting in finite detection range. Moreover, the assay still relied on bacterial liquid culture or agar plates, which are not convenient for field testing.

However, the recent development of synthetic biology tools has allowed the creation of a number of whole-cell biosensors with programmed selectivity and sensitivity, including logic gate programming, transcriptional promoter engineering, translational efficiency regulation and post-translational protein
degradation control (3, 11–14). Inspired by the ‘traffic light’ display system and hydrogel entrapment techniques (15), we designed and validated an innovative microbial sensor array that can generate graded volume-bar-like patterns in response to varying arsenic pollutant levels in drinking water (Fig. 1b). Higher than 10 ppb (10 µg/L, ~ 0.133 µM) of arsenic in drinking water is considered unsafe as suggested by WHO, and the new sensor array can detect and distinguish 1 – 500 ppb arsenic from groundwater. In this method, three major steps are involved: (1) immobilizing each sensor variant into hydrogel (agarose gel is used here) and spotting the hydrogel-sensor cell mixture into a multi-well microplate following a sensor array pattern (384-well microplates with clear bottom are used here), (2) incubating the whole sensor cell array with water sample-medium mixture, and (3) visualizing the volume-bar patterns generated by the sensor array with an appropriate light source and filters if fluorescent outputs are used as sensor reporters (green fluorescent protein is used here). Real environmental water samples and antimicrobial conditions are also tested here, allowing us to determine the likely specificity and sensitivity of the sensor in the field. Based on the generated patterns, quantitative analysis of the contaminants can be easily achieved. Moreover, using hydrogel to immobilize engineered cells can mitigate biosafety and practical concerns. Such easy-to-interpret patterns from the sensor array can be simply visualized by a cell phone camera without the need for sophisticated equipment, facilitating its use for portable, low-cost environmental monitoring in the field.

2. Materials

2.1. Strains, media and chemicals

1. Arsenic sensor variants As0 – 7 (As0–5 can be requested from Addgene https://www.addgene.org/plasmids/articles/28197470/, while the others can be requested from the author) developed by Wan et al. (2019) are selected for the sensor cell array test (3). All the sensor variants are hosted by Escherichia coli TOP10 strain (Invitrogen). A strain containing empty plasmids (pSB3K3, http://parts.igem.org) without the reporter gene is used as a negative control.
2. Lysogeny broth (LB) medium: 10 g/L peptone (1072131000, Millipore), 5 g/L NaCl (S/3160/63, Fisher Chemical), 5 g/L yeast extract (1037530500, Millipore) in ddH₂O, sterilized by autoclaving.

3. LB agar: 1.6% (w/v) agar (AGA03, Formedium) in LB medium, sterilized by autoclaving.

4. M9 medium: 11.28 g/L M9 salts (M6030, Sigma-Aldrich), 1 mM thiamine hydrochloride (T4625-25G, Sigma-Aldrich), 0.2% (w/v) casamino acids (BP1424-100, Fisher Chemical), 2 mM MgSO₄ (M/1050/53, Fisher Chemical), 0.1 mM CaCl₂ (C/1400/60, Fisher Chemical), 0.4% (v/v) glycerol (G/0600/17, Fisher Chemical).

5. MOPS EZ Rich Defined Medium (M2105, TEKnova).

6. Glycerol stock solution: 20% (v/v) glycerol in ddH₂O, sterilized by autoclaving.

7. Antibiotic stock solutions: 100 mg/mL ampicillin (A9518, Sigma-Aldrich) in ddH₂O, 50 mg/mL kanamycin (K4000, Sigma-Aldrich) in ddH₂O. Both antibiotics are sterilized by filtration through 0.22 µm syringe filters (SLGP033RS, Millipore). The antibiotic concentration used in the final cell culture is 50 µg/mL for both kanamycin and ampicillin.

8. Arsenite (NaAsO₂) and arsenate (Na₂HAsO₄) stock solutions: 50 mM NaAsO₂ (35000-1L-R, Sigma-Aldrich) and Na₂HAsO₄ (A6756, Sigma-Aldrich) in ddH₂O, sterilized by filtration through 0.22 µm syringe filters. Can be stored at room temperature.

9. Phosphate buffered saline (PBS) solution: 10 × concentrated PBS (K813-500ML, VWR) diluted 10-fold in ddH₂O, sterilized by filtration through 0.22 µm syringe filters.

10. NaCl solution: 0.9% (w/v) NaCl in ddH₂O, sterilized by filtration through 0.22 µm syringe filters.

11. Agarose (BIO-41025, Bioline) solution: 1.25% (w/v) agarose in sterilized 0.9% (w/v) NaCl solution or PBS solution.

### 2.2. Solutions for false negative test

1. Chloramphenicol stock solution: 25 mg/mL chloramphenicol (C0378, Sigma-Aldrich) in absolute ethanol.
2. Acetic acid (338826, Honeywell Research Chemicals).

3. Sodium hydroxide (71690, Sigma-Aldrich).

4. CuSO₄ solution: 1 M CuSO₄ (CHE1714S, SLS) in ddH₂O, sterilized by filtration through 0.22 μm syringe filters.

2.3. Equipment and software

1. 384-well microplate (781906, Greiner Bio-One). Sealing membranes (Z380059-1PAK, Sigma-Aldrich) and plate lids (656171, Greiner Bio-One) are used on the microplate to avoid evaporation (see Note 1).

2. 37°C incubator shaker. 160 rpm is used here. (Other temperatures and shaking speed can be used based on the application).

3. A spectrophotometer (Jenway Genova Plus) and associated 1 mL cuvettes (FB55147, Fisherbrand) for cell density measurement.

4. Centrifuge with 3,000 × g speed.

5. Two heat blocks or water baths, or one for each, set at 42°C and 55°C (see Note 2).

6. 37°C incubator without shaking (other temperatures can be used based on the application).

7. A multichannel pipette (3122000060, Eppendorf) for fast loading sample inducers and media into 16 x 24 = 384-well plates. A twelve-channel pipette with 50 μl capacity is used here.

8. Plate reader such as BMG Labtech FLUOstar fluorometer for green fluorescence measurements (485 nm for excitation, 520 ± 10 nm for emission, Gain = 1,000, bottom reading) (see Note 3).

9. Omega MARS Data Analysis Software for acquiring the plate reading data from the BMG Labtech FLUOstar fluorometer. Microsoft Excel and GraphPad can be used for further data analysis (e.g., response curves, model fitting and heat map generation).

10. Bio-Rad Gel Doc XR+ system (filter 1, SYBR Green mode) for imaging the 384-well microplates.

11. Image Lab software for acquiring high resolution images (600 dpi) from the Bio-Rad Gel Doc XR+ system.
12. Safe Imager™ blue-light transilluminator with an amber filter (S37102, Invitrogen) for cell phone imaging. A dark environment will be needed for fluorescence imaging, for example a dark room or a dark box that can cover transilluminator but allow the cell phone imaging.

13. Cell phone. Any cell phone with camera can be used. OPPO X9000 with built-in high dynamic range (HDR) setting is used here.

14. Photoshop (or other graphics software) can be used to process the images if required.

3. Methods

3.1. Sensor variants test and preparation

1. A set of whole-cell biosensor variants with a range of sensitivities are required for generating the sensor cell array. The sensor variants should detect the same target but with different limits of detection (LOD) (see Note 4). Here, we choose As0 – 7 as examples to start the sensor selection for an arsenic sensor cell array (Fig. 2) (3).

2. Sensor layout preparation for the second-round selection: The sensor variants need to be first tested and compared in a final sensor array application condition with known inducer induction for the second-round selection. Here, the sensors As0 – 7 are used as examples with arsenite NaAsO2 as induction. A layout of a partial 384-well microplate for testing the sensors is shown in Fig. 3a. Then the sensor cells are prepared and tested in the following steps.

3. Day 1: Re-streak the different sensor cell strains and negative control strain(s) on fresh LB agar plates containing the appropriate antibiotic(s) (see Note 5).

4. Day 2: Inoculate each sensor bacteria taken from a single colony on a freshly streaked solid LB agar plate into 2 mL LB medium with appropriate antibiotics individually, and culture the sensor cells overnight at 37°C with shaking (160 rpm). Do this for all sensors concurrently using the same batch of medium. This will minimize variations caused by different media and different growth conditions.

5. Prepare NaAsO2 solutions by diluting the 50 mM stock solution into ddH2O. 40 × concentrated induction solutions are usually made. For example, 40, 400, 2000 and 20,000 ppb NaAsO2
solution are prepared to make the final 1, 10, 50 and 500 ppb NaAsO₂ induction levels. NaAsO₂ solutions can be stored at room temperature.

6. Day 3: Dilute the overnight culture from Day 2 200-fold into 10 ml fresh LB medium with appropriate antibiotics in 50 mL Falcon centrifuge tubes, and culture the cells at 37°C with shaking until the cell density $A_{600}$ (absorbance measured by the spectrophotometer with a wavelength of 600 nm) reaches 1.5 (approximately 4 h 40 min, but may vary based on the shaking speed and amplitude) (see Note 6 and Note 7).

7. While waiting for culture growth, prepare 30 ml fresh 1.25% (w/v) agarose solution in PBS (without antibiotics) and incubate at 55°C to prevent solidification before use (see Note 6). Pre-incubate two 1.5 mL microtubes for each sensor at 55°C (this is easier in a heat block than a water bath). Label the tubes with the sensors’ names. Dispense 800 µL of the agarose solution into each 1.5 mL microtube while keeping all the tubes at 55°C (see Note 6).

8. Prepare fresh medium-inducer mixture: Dilute with ddH₂O for various NaAsO₂ solutions (from step 5) 40-fold into the same batch of M9 medium. As 50 µL of the mixture will be loaded into each well, only 2.4 mL of each medium-inducer mixture is needed, however a larger volume should be made to ease the loading procedure. If the mixtures are prepared in advance, they can be kept at 4°C before using on the same day.

9. Concentrate the cells 5-fold in PBS by centrifugation at 3,000 $\times$ g for 5 min and then resuspend in PBS solution with 250 µg/mL kanamycin or ampicillin. From this step onwards, cells should be kept on ice.

10. Move one microtube labeled As0 from the 55°C heat block (from step 7) to a 42°C heat block and incubate for 5 min before mixing it with 200 µL re-suspended sensor As0 cells. This results in a cell density of $OD_{600} \approx 1.5$, a final agarose concentration of 1%, and an antibiotic concentration of 50 µg/mL. The cells should be mixed in the agarose by pipetting down and up three times and by inverting the tubes three times.

11. Immediately and quickly load the agarose-cell mixture into a 384-well microplate with 15 µL in each well, following the layout designed in step 2 (Fig. 3a) (see Note 8).
12. Repeat step 11 for the rest of sensors (i.e., As1 – As7).

13. Once the loaded agarose-cell mixtures are solidified in the 384-well microplate, add 50 µL of the medium-inducer mixture (see step 8) to each well. A twelve-channel pipette can be used to rapidly complete this step.

14. Seal the microplate with a sealing membrane and cover it with a plate lid to prevent evaporation. Incubate the plate at 37°C for 40 h without shaking. Different temperature and incubation time can be used based on the sensors’ application.

3.2. Sensor variant measurement and visualization

1. Monitoring the fluorescent output from the plate over time in a plate reader will be preferred for initial testing. The plate can be incubated at desired temperature in the plate reader, and the measurement can be carried out every 20 min. This will help to identify the response time of the immobilized sensors, and also to identify a visible threshold of the sensors’ output (see step 3).

2. At appropriate time points (e.g., 16 h, 20 h, 24 h and 40 h) visualize the green fluorescent output from the plate using either the Bio-Rad Gel Doc XR+ system or the Safe Imager™ blue-light transilluminator with a cell phone. If visualizing with a cell phone camera, an amber filter should be placed over the plate or in front of the cell phone camera. Other methods can be used, but methods similar to those predicted to be used in the final application case are preferred. The Bio-Rad Gel Doc XR+ system is used here after 24 h induction and incubation (Fig. 3b). The images are acquired using Image Lab software at 600 dpi resolution. Each image is first adjusted using Photoshop CS3 software to reduce background by subtracting the brightness of negative controls, and then is adjusted to increase brightness and contrast.

3. A visible threshold can be determined by combining step 1 and step 2 in this section. As shown in Fig. 3c, 1,800 fluorescence units (after negative control correction) is identified as a visible threshold for the green fluorescent output, which is visible to the naked eye and is distinguishable from the negative controls.
4. The sensor variants can be re-evaluated here. For example, although the immobilized arsenic sensors As0 – 7 have similar relative LOD and output expression levels compared to previous tests (Fig. 2b,3c), the output levels for As4, As6 and As7 are too low for visualization even at high arsenite induction. Therefore, As4, As6 and As7 can be discarded and As0 – 3 with As5 are selected to proceed to the final sensor cell array test.

3.3. Sensor cell array design and test

1. Design a sensor array layout using the sensors selected from the previous step. Here, we use ‘As’ and volume bar patterns where the ‘As’ represents arsenic and the volume bars indicates arsenic contamination levels (Fig. 4a).

2. Prepare the sensor variants and inducers as indicated in section 3.1. steps 3 – 14 but load the agarose-cell mixtures following the new layout from step 1 (Fig. 4a). The quantity of both agarose-cell mixtures and the medium-inducer mixtures need to be adjusted accordingly.

3. The same arsenite induction levels can be tested on the sensor cell array, but only one induction level can be applied to one sensor array. Here, six sensor arrays are made from the same batch of cell cultures, and each sensor array is induced with 0, 1, 10, 50 or 500 ppb NaAsO2 (Fig. 4b).

4. Measure the sensor cell arrays using the plate reader and visualize the fluorescent output using a cell phone as described previously (section 3.2. steps 1 and 2). Continuous measurement is not required here, as the optimal incubation time should have been determined in section 3.2. The arsenic sensor array 24 h post induction and incubation time is shown as an example, and a cell phone is used to visualize the fluorescent output (Fig. 4b). The cell phone images are adjusted to increase brightness and contrast using Photoshop CS3 software.

3.4. Groundwater samples test

1. Prepare fresh MOPS EZ Rich Defined Medium following the manufacture’s protocol, but with 0.2% (v/v) glycerol as the carbon source instead of 0.2% (w/v) glucose, and 0.33 mM K_2HPO_4 instead of 1.32 mM K_2HPO_4 (see Note 9). The following list shows the formula of 100 mL
MOPS medium (enough for 8 – 10 sensor cell arrays, all items except glycerol and ddH₂O are from the MOPS EZ Rich Defined Medium kit, and all items are sterilized):

- 10X MOPS Buffer: 10 mL
- 0.132 M K₂HPO₄: 0.25 mL
- 10X ACGU Solution: 10 mL
- 5X Supplement EZ: 20 mL
- Sterile ddH₂O: 58.75 mL
- 20% Glycerol: 1 mL

2. Test and compare the sensors’ response to arsenite (NaAsO₂) and arsenate (Na₂HAsO₄) using MOPS medium following the procedures in section 3.1 and section 3.2 before testing the groundwater samples. Use 0.9% (w/v) NaCl to resuspend cells and to make agarose solution (see Note 9). This shows the sensors’ responses to arsenite and arsenate are similar in MOPS medium (Fig. 5).

3. Prepare fresh 50 mL 2× concentrated MOPS EZ Rich Defined Medium following the list above but only with 8.75 mL of ddH₂O.

4. Groundwater samples are collected from Khulna, Bangladesh. The samples can be stored at –20°C (the samples tested here have been stored for 18 months). They are filtered through 0.22 μm syringe filters and their arsenic contamination has been quantified previously (3) (see Note 10).

5. Prepare the sensor variants and the sensor cell array as described in section 3.3 but use 0.9% (w/v) NaCl to resuspend cells and to make agarose solution (see Note 9).

6. Mix each quantified groundwater sample with 2× optimized MOPS medium (from step 3) at the ratio of 1:1, and incubate the medium-sample mixture with the sensor array at 37°C for 24 h (see Note 11).

7. Measure and visualize the sensor array as described in section 3.3 (Fig. 6).

3.5. False negative test

1. Prepare the sensor cell array as described in section 3.3.
2. Prepare medium-inducer mixtures and add antimicrobial reagents to the medium as an addition to the inducer to provide the following antimicrobial conditions: (1) $25 \mu g/mL$ chloramphenicol, (2) pH = 3.5 (via addition of acetic acid), (3) pH = 11.4 (via addition of sodium hydroxide), (4) 1 mM or 3 mM CuSO$_4$.

3. Compare the sensor arrays’ output with ddH$_2$O and 50 ppb NaAsO$_2$ induction as described in section 3.3 (Fig. 7). ‘As’ pattern is expected to be invisible under the antimicrobial conditions as a result of false negative (see Note 12).

4. Notes

1. Different plates may be used based on the sensor’s reporters. For fluorescence measurement, black plates will be preferred. Plates with a clear bottom are preferred as bottom measurement will be more convenient due to no need to remove sealing membranes or lids.

2. The temperatures will depend on the melting temperature of agarose. For agarose (BIO-41025, Bioline), 55°C is sufficient to prevent solidification for long-term after melting the agarose, while 42°C is safe for sensor cells and can delay solidification for a short time.

3. The plate reader setting can be adjusted according to sensors’ reporters. For example, red fluorescent reporters can use the following settings for measurement: 584 nm for excitation, 620 $\pm$ 10 nm for emission, Gain = 2,000. Gain of fluorescence readings can also be adjusted based on the reporters, the sensors and the experimental conditions. Use of a high gain setting will amplify the background noise and can also saturate the reading, while setting gain too low may lead to an inaccurate estimation of the sensors’ performance. The highest gain of the BMG Labtech FLUOStar plate reader used in this work is 4,095 and it has been suggested by the manufacturer that gain beyond 2,800 may significantly amplify the background electronic noise (17).

4. A sensor set with a larger range of limits of detection (LOD) can provide a sensor array with larger detection range, and more sensor variants with different LOD can provide a more accurate detection range. Design, construction, characterization and optimization of sensor variants with different LOD properties are described in previous studies (3, 17). To determine
the LOD of each sensor, the set of sensor variants need to be characterized and compared under the same culture and induction conditions. The sensors with separate LOD should be chosen for further selection.

5. Always re-streak sensor strains from glycerol stocks on fresh media plates to obtain robust reproducible results. Inoculation directly from old plates or glycerol stocks should be avoided.

6. This step can be either scaled down or up based on the number of sensors and induction levels needed. Only 15 μL of cell culture is required for each well in the 384-well microplate. As shown in Fig. 3a, 5 induction levels and 20 wells of each type of sensor cell are required, therefore only 1.5 mL of cells are needed. However, always prepare an excess of cells in case some are lost during sample loading. It is also useful to work with an excess of the agarose solution, as smaller volumes can easily solidify during sample loading. Moreover, more negative controls will be needed as shown in Fig. 3a. Additionally, consistent volume and culture conditions (tubes type and culturing position, temperature and shaking) should be used to minimize variations in culture growth.

7. Some sensor constructs may cause more metabolic burden to the host cells, resulting in slower growth than the other strains. Therefore, these strains will not reach $A_{600} \approx 1.5$ when all the others have grown enough. Strains that fail to reach $A_{600} \approx 1.5$ can be concentrated to the average $A_{600}$ value of the other strains at this step. In addition, lower dilution factor can be used for the day culture dilution to increase the growth rate. For example, instead of inoculating 50 μL in to 10 mL LB, 150 μL of As5 is used to inoculate the 10 mL LB.

8. The agarose-cell mixture should be loaded into wells as fast as possible after mixing as it will rapidly solidify at 42°C. It will solidify more rapidly at room temperature, therefore keeping the tubes in the heat block while loading is preferred. If premature solidification happens, the mixture should be discarded, and new mixture should be made to continue the loading.

9. For groundwater sample testing, MOPS medium is used instead of M9 medium, and 0.9% (w/v) NaCl is used to resuspend cell culture and to make agarose solution. The $K_2HPO_4$ is further reduced to 1/4 of the original concentration. These changes are made because groundwater
samples can contain both arsenate and arsenite. High levels of phosphates, which share the same uptake systems as arsenate, will inhibit the sensors’ responses to arsenate (3, 18). With the optimized MOPS medium, we can achieve similar LOD of the sensors responding to arsenate and arsenite, and the LOD are also similar to the sensors responding to arsenite in M9 medium. Other media such as LB can also be used to test for arsenate with good LOD, but the high background fluorescence of LB will reduce the visibility of the sensors’ fluorescent output (3). M9 medium with reduced phosphates can improve the sensors’ response to arsenate, but will also reduce the general output levels and the visibility (3). 0.2% (v/v) glycerol is used instead of 0.2% (w/v) glucose in MOPS medium. This is because the agarose-entrapped cells cannot grow and express fluorescent output with glucose as the carbon source. Other hydrogels may solve this issue. MOPS medium can also be used for routine lab testing instead of using M9 medium, but needs to be freshly made each time as precipitation can occur even after overnight storage at 4°C. As with the other contaminants test, if an optimized medium is known, it can be used consistently across all the sensor variant testing. Otherwise, an appropriate media can be optimized accordingly after testing real water samples, and the sensors’ relative responses should be retained.

10. Here, the arsenic is quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and the quantified amount is the total level of both arsenite and arsenate. Other appropriate quantification methods can be used. It is useful to also quantify levels of other elements with which the sensors may have cross talk. These elements can be identified by literature research or a specificity test (3).

11. If needed, further optimization can be done to reduce the incubation time for visualization. For example, cell densities can be optimized to increase fluorescence levels so that the output can reach the visual detection threshold rapidly, and fast enzymatic reaction-based outputs (e.g., NanoLuc (16, 19) or LacZα peptide (20)) can also speed up the detection process.

12. Alternative antimicrobial conditions can be tested here. The antimicrobial conditions may cause false negative results due to their elimination of cell growth and other metabolic activities,
preventing sensor activation. The main success of distinguishing the false negative from true negative is to have a sensor or a cell strain that is continuously active, but will be inhibited under a given antimicrobial condition. For example, the As0 sensor can continuously express green fluorescent protein at a low level, which will increase with high arsenic induction, but this constitutive expression is reduced by the addition of an antimicrobial reagent. Therefore, the ‘As’ pattern will not appear under such conditions, which alerts a false negative result. Similarly, any stains that constitutively express a visible output can be added into the sensor array as a false negative identifier.

References


**Figure Captions**

(a) A ‘traffic light’ sensing system proposed previously (10). A–D represents arsenic sensors with limits of detection (LOD) from low to high descending, where grey circles represent sensors with output state ON while the white circles represent sensors with output state OFF (10). (b) Sensors with varying LOD can be selected from previous studies or be generated by varying the sensing circuits (e.g., tuning the arsenic receptor densities and employing an amplifier or an amplifier cascade in the arsenic sensor) (3).

For example, in the bottom left graph where the sensor variants are characterized in liquid culture, the sensor cell in black has the lowest LOD while the sensor cell in dark blue has the highest LOD. Utilizing agarose entrapment, the sensor cells are immobilized in half of a 384-well microplate (8 × 24 = 192 wells, bottom middle graph) to generate a microbial sensor array that displays an easy-to-read volume bar-like pattern for mobile phone-based easy-to-use and accurate field monitoring of target environmental contaminants such as arsenic in drinking water. The bottom right graph illustrates the use of a mobile showing two arsenic sensor arrays with low arsenic (top) and high arsenic (bottom) detection.
Fig. 2. Response of selected arsenic sensor variants to arsenic in liquid culture.

(a) Response curves showing the selected eight arsenic sensors with varying limits of detection (LOD) in liquid culture (3). Error bars, s.d. (n = 3). (b) LOD and maximum output expression fluorescence (k) of the eight arsenic sensors in a (3). The LOD of the eight arsenic sensors are in the following order: As0 < As1 < As2 < As3 < As4 < As5 < As6 < As7. The As0 has the lowest LOD, making it the most sensitive arsenic sensor among the eight sensors. k value is also an important parameter for the sensors’ performance as the sensors’ output fluorescence intensity is correlated with their visualization in the final sensor array. As shown the in the bar chart, the sensors maximum output expression is in the following order: As0 > As1 > As2 > As3 > As5 > As4 > As6 > As7. Notably, the less sensitive sensor (e.g., As5) does not necessarily has lower output expression, and this may have significant impact on the final sensor selection for the sensor array as shown in Figs. 3 and 4. Error bars, 95% confidence intervals. a.u. = arbitrary units of single cell fluorescence intensity calculated by fluorescence/A600.
Fig. 3. Characterization of immobilized arsenic sensor variants.

(a) Microplate well layout for testing eight arsenic sensors entrapped in agarose gel. Results are shown in b and c. (b) Graph showing the response and the visibility of the agarose gel-entrapped sensors under various arsenic induction levels. Data are collected after 24 h incubation at 37°C. Left panel: fluorometry data. Right panel: photos taken by the Bio-Rad Gel Doc XR+ system. a.u. = arbitrary units. (c) Quantified fluorometry data of the arsenic sensors in b. Data are collected after 24 h incubation at 37°C, and calculated after subtraction of the fluorescent readouts of negative controls. By visually inspecting the agarose gel-entrapped sensors in b and comparing this with the fluorometry data, we determined an effective visible threshold to be 1,800 fluorescence units. Error bars, s.d. (n = 4).
Fig. 4. Microbial sensor array display for easy-to-use monitoring of arsenic contamination.

(a) Design of the sensor array in half of a 384-well microplate. ‘As’ symbol represents arsenic for identifying the type of contamination, and the number of volume bars indicates the relevant arsenic concentration. (b) Agarose gel encapsulation-enabled microbial sensor array for monitoring arsenic contamination, showing different output response patterns upon various arsenic induction levels 24 h post incubation. Left panel: fluorometry data. Right panel: images taken by a cell phone. a.u. = arbitrary units.
Fig. 5. Comparison of arsenic sensors’ responses to arsenite and arsenate in an optimized MOPS medium.

Characterization of arsenic sensors As0–3 and As5 in response to the same concentration of arsenite (NaAsO₂) or arsenate (Na₂HAsO₄), both tested in the optimized MOPS rich medium (with 1/4 PO₄³⁻ and 0.2% (v/v) glycerol). Left panel: fluorometry data. Right panel: images taken by a cell phone. Scale bar, 1 cm. a.u. = arbitrary units.

Fig. 6. Microbial sensor array for monitoring arsenic contamination from groundwater samples.
The water samples are mixed with the optimized MOPS medium (with 1/4 \( \text{PO}_4^{3-} \) and 0.2% (v/v) glycerol) at 1 : 1 ratio. The arsenic concentrations after two times dilution are shown in brackets (quantified by ICP-MS). Compared to Fig. 5, cell phone detection of Sample 22 shows “As” pattern and one strong volume bar but not the second bar, indicating its arsenic concentration is between 1 ppb and 10 ppb, which is consistent with the ICP-MS measurement (1.6 ppb). Similarly, Sample 20 and Sample 5 show “As” pattern with a strong first bar and a weak second bar but no third bar, indicating their concentrations are also between 1 ppb and 10 ppb, which are consistent with the ICP-MS measurement. By this analogy, the cell phone determined patterns for Sample 1 shows arsenic concentration between 10 – 50 ppb, Sample 11 shows 50 – 100 ppb of arsenic, Sample 21 shows 50 – 500 ppb of arsenic, and Sample 15 shows 100 – 500 ppb of arsenic. Left panel: fluorometry data. Right panel: images taken by a cell phone. Scale bar, 1 cm. a.u. = arbitrary units.

![Fig. 7. Characterization of microbial sensor array under lethal antimicrobial conditions.](image)

Cells are incubated in M9 medium with 25 \( \mu \text{g/mL} \) chloramphenicol (Cm), under low pH (pH = 3.5), high pH (pH = 11.4), with 1 mM or 3 mM CuSO\(_4\). The sensor arrays are induced with 0 or 50 ppb As\(^{3+}\) of NaAsO\(_2\). Left panel: fluorometry data. Right panel: images taken by a cell phone. Scale bar, 1 cm. a.u. = arbitrary units.