ACS SENSORS

pubs.acs.org/acssensors

Article

Tunable Multiplexed Whole-Cell Biosensors as Environmental Diagnostics for ppb-Level Detection of Aromatic Pollutants

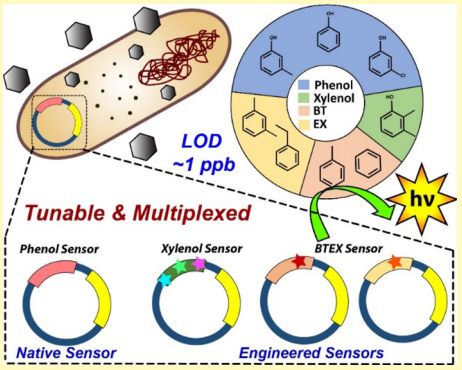
Rohita Roy,[§] Shamayeeta Ray,[§] Arindam Chowdhury,* and Ruchi Anand*

Cite This: https://doi.org/10.1021/acssensors.1c00329

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Aromatics such as phenols, benzene, and toluene are carcinogenic xenobiotics which are known to pollute water resources. By employing synthetic biology approaches combined with a structure-guided design, we created a tunable array of whole-cell biosensors (WCBs). The MopR genetic system that has the natural ability to sense and degrade phenol was adapted to detect phenol down to ~1 ppb, making this sensor capable of directly detecting phenol in permissible limits in drinking water. Importantly, by using a single WCB design, we engineered mutations into the MopR gene that enabled generation of a battery of sensors for a wide array of pollutants. The engineered WCBs were able to sense inert compounds like benzene and xylene which lack active functional groups, without any loss in sensitivity. Overall, this universal programmable biosensor platform can be used to create WCBs that can be deployed on field for rapid testing and screening of suitable drinking water sources.



KEYWORDS: *programmable sensors, structure-guided design, protein engineering, synthetic biology, BTEX, luciferase*

Highly toxic aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) contaminate our water system via industrial activities, and seepage into ground water can result in toxic concentrations of xenobiotics.[1−4] Exposure to these classes of compounds has grave implications to human health,[5] and they are categorized by the Environmental Protection Agency (EPA), USA, as priority pollutants.[6] Hence, there is a dire need to focus on accurate and sensitive detection strategies for these xenobiotics. Chemical methods to selectively sense these aromatic pollutants are limited to generic chromatographic and mass spectrometry analysis, which are both cumbersome and not easily portable.[7,8] Furthermore, owing to the lack of active functional groups, development of selective chemical sensors for these aromatics remains challenging. A judicious alternative is to construct biologically inspired sensors,[9,10] which have been perfected via the selection pressure of evolution.[11]

Biosensors based on enzymes such as tyrosinase and laccase have been used to detect phenol;[12,13] however, these sensors lack both specificity and selectivity as phenol is not their cognate substrate. In contrast, usage of a natural phenol sensor (MopR) has resulted in specific in vitro biosensors for phenol.[14] Furthermore, this MopR-based in vitro design has been exploited to detect other monoaromatics.[15−17] However, in MopR, the difficulty to produce purified proteins compounded with propensity of degradation of sensitive protein solutions results in limited detection sensitivity. To circumvent some of these issues, recent advances in synthetic biology has enabled the use of cell-free in vitro translation for protein synthesis,[18] which can be freeze-dried and used for long periods and further be developed as paper-based dipsticks for on-field testing.[19] However, sensing using living organisms is optimal in accessing the real exposure risk that the contaminant poses to human health.[20,21] Thus, an alternate viable approach is to develop whole-cell biosensors (WCBs), where the bacterial membrane serves as a natural protection for the sensor unit from direct contact with the harsh environment,[22] making these robust as well as opportune systems to learn about bioavailability of a contaminant. For instance, cell-based sensors for mercury and arsenic have been employed as easy-to-use on-field detection units[23−25] and can be integrated into portable devices.

A common strategy for the creation of WCBs is to exploit the natural ability of certain organisms which illicit a response to specific molecules.[21] Certain strains of soil bacteria, such as *Pseudomonas sp.* and *Acinetobacter sp.*, which grow in polluted environments[26,27] under high concentrations of carcinogens such as BTEX, phenol, xylenols, sense these pollutants and eventually degrade them.[28−33] Recently, Xue et al, reported such a sensor array for the detection of a wide range of aromatics. They exploited the natural genetic systems of some of these specialized bacteria and used individual machineries to

Received: February 16, 2021
Accepted: April 27, 2021

make several sensor units with varying sensing capacities.[34] While such multiplexing approach is beneficial, there were two limitations in their approach. First, a naturally occurring bacterial species that harbors the sensor system for a pollutant of choice is necessary. Second, readouts based on green fluorescent proteins (GFPs) exhibit limited detection sensitivity as opposed to an enzymatic reporter such as luciferase which augment signals owing to multiple substrate turnovers.[35] These issues result in limited selectivity and adversely affect the detection sensitivity.

To circumvent some of these problems, here we have developed a universal programmable genetic platform. We chose a single natural sensor system, which is robust, and by deftly bridging structure-guided design with synthetic biology created a plethora of WCBs were created (Figure 1). This

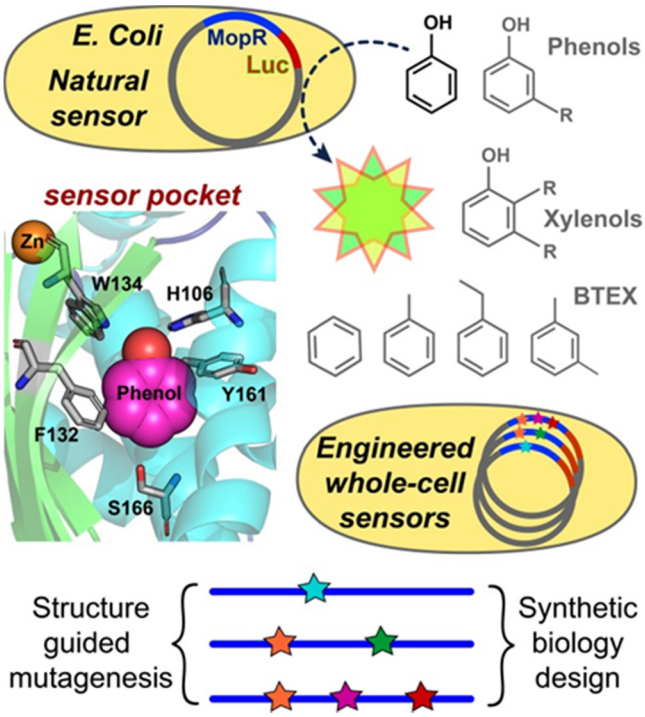


Figure 1. Design strategy of a programmable genetic biosensor array. The concept involves creation of WCBs using a combination of structure-guided mutagenesis with synthetic biology approaches. The natural MopR sensor for phenol was used as a starting scaffold to engineer biosensors for a variety of xenobiotics via introduction of appropriate mutation(s) in the phenol binding pocket. This multiplexing strategy using a single genetic system is extendable to create efficient WCBs for a wide range of aromatic pollutants.

flexible genetic platform poses a significant edge as it opens up new avenues to detect unnatural pollutants for which cellular sensing machinery does not exist in nature. Furthermore, we demonstrate that our approach yields WCBs with a wide operational range and high sensitivity applicable for direct detection of specific xenobiotics in the drinking water range (the EPA has set toxicity limit of ~5 ppb).[6]

RESULTS AND DISCUSSION

The base design of the bacterial sensors used in this study exploits the phenol catabolism pathway from the organism *Acinetobacter calcoaceticus*, NCIB8250.[26,27,36] This pathway operates under the control of the σ54-dependent RNA polymerase which gets triggered by phenol, subsequently inducing its degradation.[36−38] The phenol-sensing protein, MopR, used in this study only switches to an "ON" state when the protein binds phenol, triggering transcription of the downstream gene cluster.[39] We exploited this property of the sensor protein and created the phenol sensor by introducing the biosensory machinery into *Escherichia coli*. Briefly, the sensor was constructed by incorporating the entire MopR gene cassette constituting of the *Pmop* σ70 promoter and *Pm* σ54 into a promoterless plasmid.[40] The gene sequence and genetic circuit design of the whole-cell based biosensor is provided in Figures S1 and S2, Supporting Information, (SI). Furthermore, to enable sensitive detection of phenol with nominal background interference, its downstream catabolism gene cluster was replaced by the luciferase (Luc) reporter gene.

The *MopRLuc* biosensor thus created exhibited the most sensitivity toward phenol (Figure 2a). In addition, we find that this sensor exhibits reasonably high sensitivity to two other structurally analogous aromatics with substitutions at the meta positions, such as 3-chlorophenol (3-CP) and *m*-cresol. This was advantageous as along with phenol, both 3-CP and *m*-

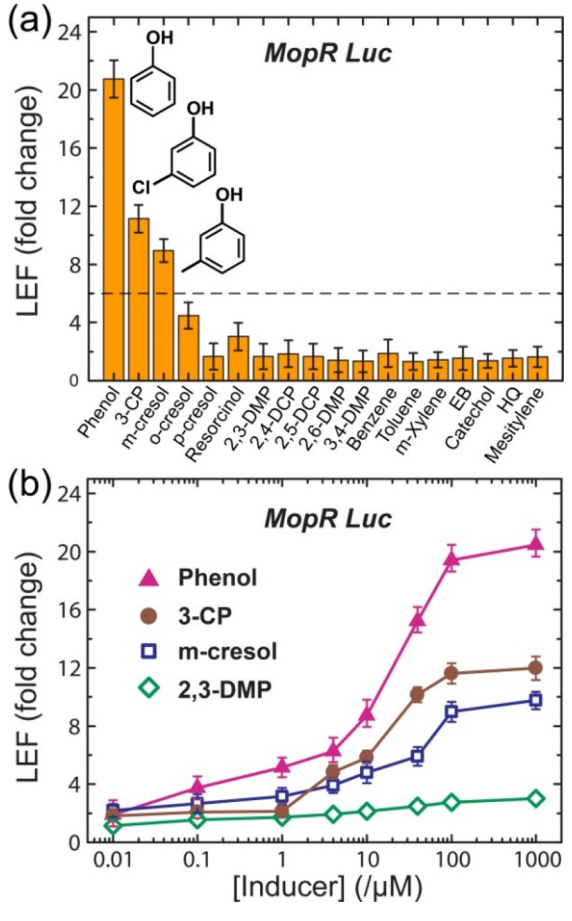


Figure 2. Natural WCBs for phenol. (a) Fold change in the luminescence enhancement factor (LEF) of *MopRLuc* for a wide variety of aromatic phenols and BTEX [1 mM] in an aqueous medium. Structures of all the compounds tested are provided in Figure S3 , SI. The dashed horizontal line represents sixfold enhancement of luciferase luminescence with respect to that in the absence of any inducer (control). (b) Concentration-dependent LEF response of *MopRLuc* for phenol and analogous phenolics with meta substitutions, along with that for a disubstituted bulkier phenol.

https://doi.org/10.1021/acssensors.1c00329
ACS Sens. XXXX, XXX, XXX−XXX

cresol are high priority pollutants that have several toxic effects such as genotoxicity and kidney failure. Sensor profiling also reveals that the *MopRLuc* sensor does not detect bulkier alcohols such as resorcinol and 2,3-dimethyl phenol (2,3-dmp). The structural analysis of the sensor pocket (Figure S4a, SI) depicts that the binding pocket is too small to accept these compounds. Furthermore, it is interesting to note that the sensor can even differentiate between phenols with *meta*-, *para*-, and *ortho*-substitutions, as is evident from our data (Figure 2a).

To obtain the sensitivity, dynamic range, and limit of detection (LOD) of *MopRLuc*, we evaluated the dose-dependent response of the sensor with a gradual increase in the concentration of analytes (Figure 2b) over a wide range, which encompass the drinking water level (~10 nM) to high toxic industrial contamination (~mM). We find that this sensor is able to detect phenol with high sensitivity at both low as well as high concentrations in water. In contrast, although this sensor is unable to detect 3-CP and *m*-cresol in drinking water limits, it is quite effective at industrial safety levels (1–100 ppm). This natural WCB sensor exhibits extremely low LOD (~0.94 ppb) for phenol, lower than prior reported values (Table 1). It is relevant to mention that current commercial

Table 1. Comparison of LODs of In Vitro and In Vivo Biosensors

	detection sensitivity (LOD)		
xenobiotic target	in vitro biosensor (ppb)	WCBs[a] (ppb)	WCBs (this work) (ppb)
phenol	10[b]	47[c]	~0.94
3-chlorophenol	1280[b]		1.28
m-cresol	108[d]		1.08
2,3-dimethyl phenol	12[e]	425[f]	1.22
benzene	~300[g]	150[h]	0.78
toluene	~300[g]	40[h]	0.92
ethylbenzene	~300[g]		1.05
m-xylene	~300[g]	~40,000[h]	1.05

[a]Data for the lowest reported LOD. [b]ref 15. [c]not selective, ref 40. [d]not selective, ref 41. [e]ref 17. [f]not selective, ref 42. [g]ref 16. [h]ref 43.

methods used to detect phenol to ensure safe drinking limits require the usage of concentrators and subsequent LC–MS-based detection.[7] However, in our WCB design, direct detection of phenol in drinking water can be accurately achieved without any prior preconcentration procedures. Furthermore, the possibility of easy integration with portable optical or electrochemical setups[44] allows these biosensors to be employed for on-field testing.

Inspired by the robustness and efficiency of the phenol sensor, we decided to use *MopRLuc* as a model system to genetically engineer an array of WCBs for other xenobiotics. To ensure the appropriate design of engineered WCBs, the X-ray structure of the phenol sensor domain of MopR (PDB ID 5KBE)[14] was used as a starting scaffold (Figure 3a). The challenge in multiplexing this natural genetic setup lies in ensuring that judicious mutations are made in the phenol-binding pocket, such that neither does the selected mutations make the modified unit promiscuous nor does it lead to a loss of sensitivity. Here, knowledge from our previous design for the creation of in vitro protein-based sensor units was taken into consideration.[15−17] The most prudent mutations for

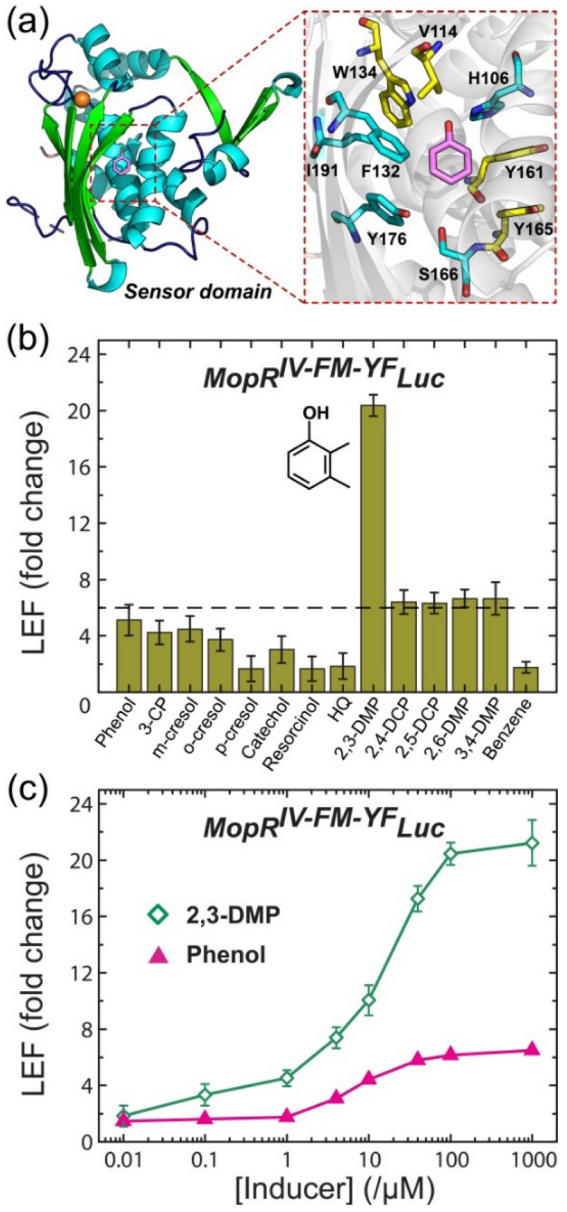


Figure 3. Engineered WCBs for xylenol. (a) Structure of the sensor domain of MopR depicting the conserved (yellow) and variable (cyan) residues in the binding pocket (inset) of the NtrC family of proteins (XylR, MopR, and DmpR). (b) Fold change in LEF of *MopR*$^{IV\text{-}FM\text{-}YF}$*Luc* for various aromatic phenols and benzene [1 mM] in an aqueous medium. The dashed horizontal line represents sixfold enhancement of luciferase luminescence with respect to that in the absence of any inducer. (c) Concentration-dependent LEF response of *MopR*$^{IV\text{-}FM\text{-}YF}$*Luc* for the target, 2,3 dmp, and that for the natural substrate for MopR, phenol.

engineering a new sensor activity were deciphered by aligning protein sequences of several related, natural aromatic biosensors,[37,45,46] and the residues of the binding pocket were categorized as completely conserved or variable (Figure 3a, inset). It was concluded that the mutations in the variable region are responsible for the shift in the sensor profile.[15−17] Hence, we chose to selectively alter these residues to generate an array of in vivo biosensors.

The first WCB chosen to be engineered was that of a bulkier phenol derivative, a xylenol, which is a priority pollutant owing to high toxicity and long residence times in the environment.

https://doi.org/10.1021/acssensors.1c00329
ACS Sens. XXXX, XXX, XXX−XXX

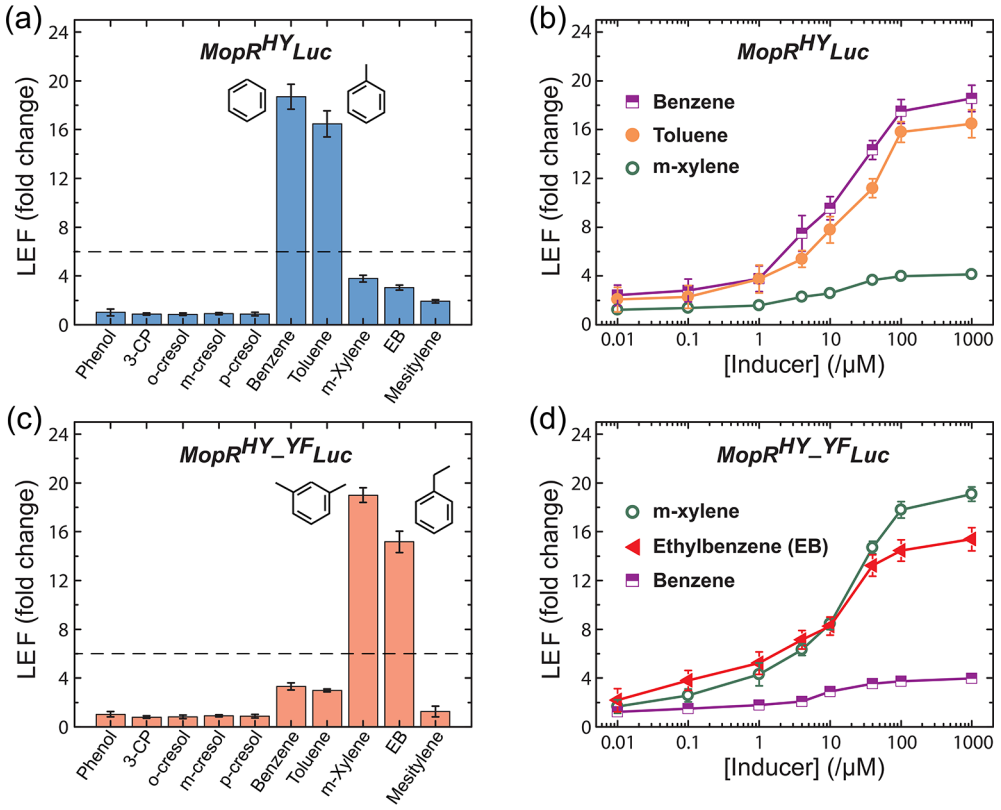


Figure 4. Engineered WCBs for BTEX. (a) Fold change in LEF of the BT sensor, *MopR*^{HY}*Luc*, for various aromatics [1 mM] in an aqueous medium, and (b) concentration-dependent LEF response of *MopR*^{HY}*Luc* for the targets, benzene and toluene, along with that for *m*-xylene. (c) Fold change in LEF of EX sensor, *MopR*$^{HY\_YF}$*Luc*, for various aromatics [1 mM] in an aqueous medium, and (d) concentration-dependent LEF response of *MopR*$^{HY\_YF}$*Luc* for the targets, ethylbenzene and xylene, along with that for benzene. Horizontal dashed lines in (a and c) represent sixfold enhancement of luciferase luminescence with respect to that in the absence of any inducer.

In order to select the appropriate mutations, sequence comparison between the phenol sensor (MopR) and 2,3-dmp sensor (DmpR) proteins was performed. This analysis revealed that conversion of the phenol to a xylenol sensor can be realized by introducing three pocket mutations. Upon visualization of the structure (Figure S4a, SI), it becomes apparent that rewiring the sensor pocket via replacement of (i) a rigid phenylalanine at position 132 (F132) by an aliphatic flexible methionine group, (ii) the bulkier isoleucine (residue 191) with a smaller valine, and (iii) tyrosine by phenylalanine (Y176F) leads to creation of space in the cavity to accommodate bulkier substituted phenols (Figure S4b, SI). Following this design, the three mutations were made in the MopR gene and introduced into the WCB design. We find that the resulting triple mutant *MopR*$^{IV\text{-}FM\text{-}YF}$*Luc* biosensor is remarkably selective toward 2,3-dmp, and moreover, the increase in pocket size renders this an inefficient phenol sensor (Figure 3b,c). Intriguingly, this modified WCB can even discriminate between 2,3-dmp and other (2,5- and 3,4-) xylenols, likely owing to a mismatch with the geometry of the binding pocket. Most importantly, our engineered WCB exhibits a remarkably low LOD of ∼1.2 ppb (Figure 3b,c), a 400 times enhancement compared to that reported for the natural DmpR-based WCB system (Table 1).

This success of the engineered WCB demonstrated that the structure-guided design is indeed adaptable at the transcription level, which prompted us to extend our protein engineering methodologies to generate WCBs for the BTEX group of molecules. An earlier report has revealed that removal of the phenolic anchor in the binding pocket of MopR (Figure S5e, SI) by introducing a tyrosine residue in place of histidine (H106Y) results in transformation of the phenol sensor to a benzene and toluene (BT) sensor.[16] However, the primary limitation of the resulting in vitro sensor was the sharp drop in sensitivity and the inability to detect BT below ∼300 ppb. In contrast, our engineered BT WCB, *MopR*^{HY}*Luc* (Table 1) exhibited an LOD of ∼1 ppb, which was 300-fold better than that of the in vitro counterpart[16] and comparable to the natural BT sensor system. The sensing profile further revealed that the *MopR*^{HY}*Luc* WCB was extremely selective toward BT and shows negligible (8−10-fold lower) detection for other phenolics (Figure 4a,b). The performance of our BT WCB serves as a validation step to ensure that our structure-guided synthetic design is viable to generate new sensor arrays without any compromise in sensitivity.

Next, we focused our attention to engineer a WCB for which a natural genetic system is yet to be discovered, as this would open doors to utilize our strategy for compounds that would otherwise remain undetectable and escape monitoring. As a model system, we chose medium-sized aromatics ethylbenzene and *m*-xylene, common contaminants introduced into the ground-water system through oil spills. For design of this biosensor, we started with the parent BT sensor (*MopR*^{HY}*Luc*) and introduced an additional mutation in the binding pocket for the substituted aromatics ethylbenzene and xylene (EX) (Figure S5f, SI). To achieve shape-complementarity, a tyrosine was replaced by a phenylalanine (Y176F) of *MopR*^{HY}*Luc*, thus enabling the creation of the double mutant, *MopR*$^{HY\_YF}$*Luc*

https://doi.org/10.1021/acssensors.1c00329
ACS Sens. XXXX, XXX, XXX−XXX

(Figure S5e, SI). This engineered WCB was very selective and detected these target compounds (EX) with high accuracy (Figure 4c,d). Thus, by increasing the pocket size judiciously, the EX sensor was unable to efficiently interact with BT and other phenolics, leading to low sensitivity for other aromatics. As there are no known natural genetic systems which sense and degrade these two xenobiotics, our engineered EX WCB is 10^4-fold more sensitive than all other reported sensors for EX(Table 1). Moreover, both sensitivity and LOD for EX remain uncompromised and is at par (∼1 ppb) with that for the original phenol WCB (*MopRLuc*).

To test the robustness and viability of this approach in a realistic setting, such as in drinking water sources, we performed challenge experiments by spiking drinking water with a target pollutant along with high doses of a competing pollutant. Interference tests (Figure S6, SI) establish the selectivity and sensitivity of our WCBs for a target pollutant in the presence of completing xenobiotics, as often encountered in environmental samples. For instance, interference analysis of *MopRLuc* shows that 2,3-dmpR did not significantly affect the phenol sensor's performance (Figure S6a, Supporting Information SI). Similarly, challenge experiments on *MopR*$^{IV\_FM\_YF}$*Luc*, *MopR*^{HY}*Luc*, and *MopR*$^{HY\_YF}$*Luc* revealed that each engineered sensor unit exhibits very similar response for the respective targets in the presence and absence of high concentrations (∼100 μM) of other competing aromatics (Figure S6b−d, SI). These measurements further enhanced our confidence toward the practical viability of our programmable design and assert that WCBs engineered here will be viable in scouting for target pollutant levels in water sources, making them attractive for on-field deployment.

Apart from high detection sensitivity and selectivity, other relevant features that are common to WCBs engineered here are: (i) extremely high operational range (∼0.01 to > ∼100 μM) which allows detection of the target xenobiotics spanning both industrial level contaminants as well as that in drinking water levels, and (ii) high dynamic range in terms of fold enhancement of the luciferase emission signal (LEF ∼14−18) which is beneficial owing to lower errors in detection. Performance analysis with a variety of known sensors for monoaromatics reveal that the sensors presented here exhibit higher specificity as well as considerably enhanced detection sensitivity compared to all prior reports (Figure S7, SI and Table 1). We emphasize that even for EX, for which the natural sensing machinery does not exist, the MopR platform is the most efficient sensing unit as compared to its counterparts (Figure S7, SI). Overall, observations presented in this work highlight that the phenol sensor template of MopR is a versatile system capable of engineering for multiplexing. Our mutation-based design principle of shape-complementarity, where the pocket is adapted to the molecular structure of a small target aromatic, is the key to successful creation of WCBs without any compromise on selectivity and sensitivity.

CONCLUSIONS

To conclude, we demonstrate the construction of a malleable WCB system which can be tuned to select for a particular monoaromatic pollutant. The design combines synthetic biology with a structure-based shape-complementarity design to create a unique genetically rewired biosensor-molecule unit that is exclusive and selective. The added advantage of WCBs is their robustness owing to the sensor unit being embedded inside the bacterial cell, shielded from the outside harsh environment. We emphasize that the performance of our WCBs is significantly better than the current commercial LC−MS systems which require preconcentration of water samples to achieve similar detection limits. The combined effects of a wide operational range, enhanced LOD, quick readout, and bioavailability make these multimodal in vivo sensors ideal for the detection of xenobiotics in industrially polluted water sources, as well as for rapid testing and screening of genotoxic pollutants in search of appropriate drinking water reservoirs. The developments presented here open up uncharted avenues to exploit these sensors as the next generation systems to efficiently detect aromatic toxins.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.1c00329.

All details on materials and methods, data analyses procedures, and supplementary data in the form of Figures S1−S7 (PDF)

AUTHOR INFORMATION

Corresponding Authors

Arindam Chowdhury − *Department of Chemistry, Indian Institute of Technology Bombay Powai, Mumbai 400076, India;* orcid.org/0000-0001-8178-1061; Email: arindam@chem.iitb.ac.in

Ruchi Anand − *Department of Chemistry, Indian Institute of Technology Bombay Powai, Mumbai 400076, India; DBT-Welcome Trust India Alliance Senior Fellow, Mumbai 400076, India;* orcid.org/0000-0002-2045-3758; Email: ruchi@chem.iitb.ac.in

Authors

Rohita Roy − *Department of Chemistry, Indian Institute of Technology Bombay Powai, Mumbai 400076, India;* orcid.org/0000-0001-9868-3505

Shamayeeta Ray − *Department of Chemistry, Indian Institute of Technology Bombay Powai, Mumbai 400076, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssensors.1c00329

Author Contributions

[§]R.R. and S.R. contributed equally to the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

R.A. and A.C. acknowledge financial support from DBT (Govt. of India) grant no. BT/INF/22/SP23026/2017 to help carry out this work. R.A. acknowledges financial support from Wadhwani Research Center for Bioengineering (Gant No. WRCB DGDON446) and DBT (Grant No. BT/PR18927/BCE/8/1376/2016). R.R. thanks IIT Bombay for PhD fellowship. S.R. is the recipient of Prime Ministers Fellowship for Doctoral Research. Authors greatly appreciate critical comments from Pradeepkumar P.I.

REFERENCES

(1) Watthaisong, P.; Pongpamorn, P.; Pimviriyakul, P.; Maenpuen, S.; Ohmiya, Y.; Chaiyen, P. A Chemo-Enzymatic Cascade for the

Smart Detection of Nitro- and Halogenated Phenols. *Angew. Chem. Int. Ed.* **2019**, *58*, 13254−13258.
(2) Bolden, A. L.; Kwiatkowski, C. F.; Colborn, T. New Look at BTEX: Are Ambient Levels a Problem. *Environ. Sci. Technol.* **2015**, *49*, 5261−5276.
(3) Weisel, C. P. Benzene Exposure: An Overview of Monitoring Methods and Their Findings. *Chem. Biol. Interact.* **2010**, *184*, 58−66.
(4) Samanta, S. K.; Singh, O. V.; Jain, R. K. Polycyclic Aromatic Hydrocarbons: Environmental Pollution and Bioremediation. *Trends Biotechnol.* **2002**, *20*, 243−248.
(5) Dehghani, M.; Fazlzadeh, M.; Sorooshian, A.; Tabatabaee, H. R.; Miri, M.; Baghani, A. N.; Delikhoon, M.; Mahvi, A. H.; Rashidi, M. Characteristics and Health Effects of BTEX in a Hot Spot for Urban Pollution. *Ecotoxicol. Environ. Saf.* **2018**, *155*, 133−143.
(6) Keith, L.; Telliard, W. ES&T Special Report: Priority Pollutants: I-a Perspective View. *Environ. Sci. Technol.* **1979**, *13*, 416−423.
(7) Wille, K.; De Brabander, H. F.; Vanhaecke, L.; De Wulf, E.; Van Caeter, P.; Janssen, C. R. Coupled Chromatographic and Mass-Spectrometric Techniques for the Analysis of Emerging Pollutants in the Aquatic Environment. *TrAC - Trends Anal. Chem.* **2012**, *35*, 87−108.
(8) Khoddami, A.; Wilkes, M. A.; Roberts, T. H. Techniques for Analysis of Plant Phenolic Compounds. *Molecules* **2013**, *18*, 2328−2375.
(9) Cammann, K.; Lemke, U.; Rohen, A.; Sander, J.; Wilken, H.; Winter, B. Chemical Sensors and Biosensors—Principles and Applications. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 516−539.
(10) Van Der Meer, J. R.; Belkin, S. Where Microbiology Meets Microengineering: Design and Applications of Reporter Bacteria. *Nat. Rev. Microbiol.* **2010**, *8*, 511−522.
(11) Snoek, T.; Chaberski, E. K.; Ambri, F.; Kol, S.; Bjørn, S. P.; Pang, B.; Barajas, J. F.; Welner, D. H.; Jensen, M. K.; Keasling, J. D. Evolution-Guided Engineering of Small-Molecule Biosensors. *Nucleic Acids Res.* **2020**, *48*, No. e3.
(12) Wee, Y.; Park, S.; Kwon, Y. H.; Ju, Y.; Yeon, K. M.; Kim, J. Tyrosinase-Immobilized CNT Based Biosensor for Highly-Sensitive Detection of Phenolic Compounds. *Biosens. Bioelectron.* **2019**, *132*, 279−285.
(13) Rodríguez-Delgado, M. M.; Alemán-Nava, G. S.; Rodríguez-Delgado, J. M.; Dieck-Assad, G.; Martínez-Chapa, S. O.; Barceló, D.; Parra, R. Laccase-Based Biosensors for Detection of Phenolic Compounds. *TrAC - Trends Anal. Chem.* **2015**, *74*, 21−45.
(14) Ray, S.; Gunzburg, M. J.; 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*, 2357−2365.
(15) Ray, S.; Panjikar, S.; Anand, R. Structure Guided Design of Protein Biosensors for Phenolic Pollutants. *ACS Sensors* **2017**, *2*, 411−418.
(16) Ray, S.; Panjikar, S.; Anand, R. Design of Protein-Based Biosensors for Selective Detection of Benzene Groups of Pollutants. *ACS Sensors* **2018**, *3*, 1632−1638.
(17) Ray, S.; Senapati, T.; Sahu, S.; Bandyopadhyaya, R.; Anand, R. Design of Ultrasensitive Protein Biosensor Strips for Selective Detection of Aromatic Contaminants in Environmental Wastewater. *Anal. Chem.* **2018**, *90*, 8960−8968.
(18) Silverman, A. D.; Karim, A. S.; Jewett, M. C. Cell-Free Gene Expression: An Expanded Repertoire of Applications. *Nat. Rev. Genet.* **2020**, *21*, 151−170.
(19) Singh, A.; Lantigua, D.; Meka, A.; Taing, S.; Pandher, M.; Camci-Unal, G. Paper-Based Sensors: Emerging Themes and Applications. *Sensors* **2018**, *18*, 2838.
(20) Liu, X.; Germaine, K. J.; Ryan, D.; Dowling, D. N. Whole-Cell Fluorescent Biosensors for Bioavailability and Biodegradation of Polychlorinated Biphenyls. *Sensors* **2010**, *10*, 1377−1398.
(21) Gui, Q.; Lawson, T.; Shan, S.; Yan, L.; Liu, Y. The Application of Whole Cell-Based Biosensors for Use in Environmental Analysis and in Medical Diagnostics. *Sensors* **2017**, *17*, 1−17.
(22) Koebnik, R.; Locher, K. P.; Van Gelder, P. Structure and Function of Bacterial Outer Membrane Proteins: Barrels in a Nutshell. *Mol. Microbiol.* **2000**, *37*, 239−253.
(23) Bereza-Malcolm, L. T.; Mann, G.; Franks, A. E. Environmental Sensing of Heavy Metals Through Whole Cell Microbial Biosensors: A Synthetic Biology Approach. *ACS Synth. Biol.* **2015**, *4*, 535−546.
(24) Gupta, S.; Sarkar, S.; Katranidis, A.; Bhattacharya, J. Development of a Cell-Free Optical Biosensor for Detection of a Broad Range of Mercury Contaminants in Water: A Plasmid DNA-Based Approach. *ACS Omega* **2019**, *4*, 9480−9487.
(25) Cerminati, S.; Soncini, F. C.; Checa, S. K. A Sensitive Whole-Cell Biosensor for the Simultaneous Detection of a Broad-Spectrum of Toxic Heavy Metal Ions. *Chem. Commun.* **2015**, *51*, 5917−5920.
(26) Shingler, V. Integrated Regulation in Response to Aromatic Compounds: From Signal Sensing to Attractive Behaviour. *Environ. Microbiol.* **2003**, *5*, 1226−1241.
(27) Diaz, E.; Prieto, M. A. Bacterial Promoters Triggering Biodegradation of Aromatic Pollutants. *Curr. Opin. Biotechnol.* **2000**, *11*, 467−475.
(28) Fuchs, G.; Boll, M.; Heider, J. Microbial Degradation of Aromatic Compounds — from One Strategy to Four. *Nat. Rev. Microbiol.* **2011**, *9*, 803−816.
(29) Jin, H. M.; Kim, J. M.; Lee, H. J.; Madsen, E. L.; Jeon, C. O. Alteromonas as a Key Agent of Polycyclic Aromatic Hydrocarbon Biodegradation in Crude Oil-Contaminated Coastal Sediment. *Environ. Sci. Technol.* **2012**, *46*, 7731−7740.
(30) Gerischer, U. Specific and Global Regulation of Genes Associated with the Degradation of Aromatic Compounds in Bacteria. *J. Mol. Microbiol. Biotechnol.* **2002**, *4*, 111−121.
(31) Tropel, D.; van der Meer, J. R. Bacterial Transcriptional Regulators for Degradation Pathways of Aromatic Compounds. *Microbiol. Mol. Biol. Rev.* **2004**, *68*, 474−500.
(32) Kurbatov, L.; Albrecht, D.; Herrmann, H.; Petruschka, L. Analysis of the Proteome of Pseudomonas Putida KT2440 Grown on Different Sources of Carbon and Energy. *Environ. Microbiol.* **2006**, *8*, 466−478.
(33) Arenghi, F. L. G.; Berlanda, D.; Galli, E.; Sello, G.; Barbieri, P. Organization and Regulation of Meta Cleavage Pathway Genes for Toluene and O-Xylene Derivative Degradation in Pseudomonas Stutzeri OX1. *Appl. Environ. Microbiol.* **2001**, *67*, 3304−3308.
(34) Xue, H.; Shi, H.; Yu, Z.; He, S.; Liu, S.; Hou, Y.; Pan, X.; Wang, H.; Zheng, P.; Cui, C.; Viets, H.; Liang, J.; Zhang, Y.; Chen, S.; Zhang, H. M.; Ouyang, Q. Design, Construction, and Characterization of a Set of Biosensors for Aromatic Compounds. *ACS Synth. Biol.* **2014**, *3*, 1011−1014.
(35) Serganova, I.; Blasberg, R. G. Molecular Imaging with Reporter Genes: Has Its Promise Been Delivered? *J. Nucl. Med.* **2019**, *60*, 1665−1681.
(36) Ehrt, S.; Ornston, L. N.; Hillen, W. RpoN (Σ54) Is Required for Conversion of Phenol to Catechol in Acinetobacter Calcoaceticus. *J. Bacteriol.* **1994**, *176*, 3493−3499.
(37) Schirmer, F.; Ehrt, S.; Hillen, W. Expression, Inducer Spectrum, Domain Structure, and Function of MopR, the Regulator of Phenol Degradation in Acinetobacter Calcoaceticus NCIB8250. *J. Bacteriol.* **1997**, *179*, 1329−1336.
(38) Griva, E.; Pessione, E.; Divari, S.; Valetti, F.; Cavaletto, M.; Rossi, G. L.; Giunta, C. Phenol Hydroxylase from Acinetobacter Radioresistens S13: Isolation and Characterization of the Regulatory Component. *Eur. J. Biochem.* **2003**, *270*, 1434−1440.
(39) O'Neill, E.; Ng, L. C.; Sze, C. C.; Shingler, V. Aromatic Ligand Binding and Intramolecular Signalling of the Phenol-Responsive Σ54-Dependent Regulator DmpR. *Mol. Microbiol.* **1998**, *28*, 131−141.
(40) Gupta, S.; Saxena, M.; Saini, N.; Kumar, R.; Kumar, A. An Effective Strategy for a Whole-Cell Biosensor Based on Putative Effector Interaction Site of the Regulatory DmpR Protein. *PLoS One* **2012**, *7*, No. e43527.
(41) Abdullah, J.; Ahmad, M.; Karuppiah, N.; Heng, L. Y.; Sidek, H. Immobilization of Tyrosinase in Chitosan Film for an Optical Detection of Phenol. *Sens. Actuators B* **2006**, *114*, 604−609.

https://doi.org/10.1021/acssensors.1c00329
ACS Sens. XXXX, XXX, XXX−XXX

(42) Leedjärv, A.; Ivask, A.; Virta, M.; Kahru, A. Analysis of Bioavailable Phenols from Natural Samples by Recombinant Luminescent Bacterial Sensors. *Chemosphere* **2006**, *64*, 1910−1919.
(43) Hernández-Sánchez, V.; Molina, L.; Ramos, J. L.; Segura, A. New Family of Biosensors for Monitoring BTX in Aquatic and Edaphic Environments. *J. Microbial. Biotechnol.* **2016**, *9*, 858−867.
(44) Dai, Y.; Liu, C. C. Recent Advances on Electrochemical Biosensing Strategies toward Universal Point-of-Care Systems. *Angew. Chem. Int. Ed.* **2019**, *58*, 12355−12368.
(45) Shingler, V.; Bartilson, M.; Moore, T. Cloning and Nucleotide Sequence of the Gene Encoding the Positive Regulator (DmpR) of the Phenol Catabolic Pathway Encoded by PVI150 and Identification of DmpR as a Member of the NtrC Family of Transcriptional Activators. *J. Bacteriol.* **1993**, *175*, 1596−1604.
(46) Abril, M. A.; Michan, C.; Timmis, K. N.; Ramos, J. L. Regulator and Enzyme Specificities of the TOL Plasmid-Encoded Upper Pathway for Degradation of Aromatic Hydrocarbons and Expansion of the Substrate Range of the Pathway. *J. Bacteriol.* **1989**, *171*, 6782−6790.

https://doi.org/10.1021/acssensors.1c00329
ACS Sens. XXXX, XXX, XXX−XXX