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Design, Construction, and Characterization of a Set of Biosensors for Aromatic Compounds

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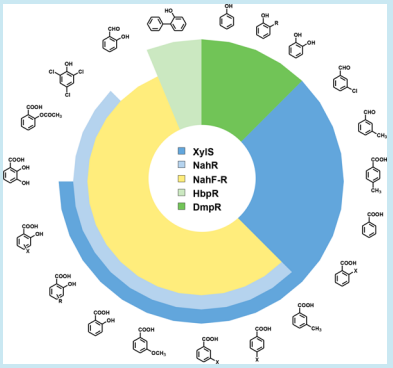
Supporting Information

ABSTRACT: Aromatic pollutants in the environments pose significant threat to human health due to their persistence and toxicity. Here, we report the design and comprehensive characterization of a set of aromatic biosensors constructed using green fluorescence protein as the reporter and aromatics-responsive transcriptional regulators, namely, NahR, XylS, HbpR, and DmpR, as the detectors. The genetic connections between the detectors and the reporter were carefully adjusted to achieve fold inductions far exceeding those reported in previous studies. For each biosensor, the functional characteristics including the dose−responses, dynamic range, and the detection spectrum of aromatic species were thoroughly measured. In particular, the interferences that nontypical inducers exert on each biosensor's response to its strongest inducer were evaluated. These well-characterized biosensors might serve as potent tools for environmental monitoring as well as quantitative gene regulation.



Many environmental microorganisms (e.g., bacterial strains from *Pseudomonas* genus) naturally possess transcription factors (TFs) that activate degradation pathways of specific aromatic compounds in response to their exposure to these compounds; these TFs would serve as valuable tools for the quantitative measurement of aromatic compounds. In this study, to build our aromatic biosensors, we employed four aromatics-responsive transcriptional activators from three *Pseudomonas* strains: *nahR* from naphthalene catabolic pathway,[1] *xylS* from benzoate catabolic pathway,[2] *hbpR* from 2-hydroxybiphenyl catabolic pathway,[3] and *dmpR* from phenol catabolic pathway[4] (see more discussion in Supporting Information, Part I).

RESULTS AND DISCUSSION

Design and Construction of Biosensors. The TFs were constitutively expressed and cotransformed with the superfold GFP gene (*sfgfp*) controlled by TFs' cognate promoters (Figure 1a). The primary constructs for NahR and DmpR biosensors performed well (see below). However, for XylS and HbpR biosensors, the fold inductions were quite low due to high basal expression of sfGFP (the promoter of TFs is J23106; the ribosome binding site of sfGFP is B0034; see Supporting Information Figure 2a and Figure 3). To improve their performance, the expression levels of TFs and sfGFP were fine-tuned, respectively, using libraries of constitutive promoters and ribosome binding sites (RBSs) with strengths varying across 3 orders of magnitude (Figure 1a; see detailed methods in Supporting Information, Part II). The construct variants of XylS and HbpR biosensors with the highest fold inductions were selected, together with NahR and DmpR biosensors, for the further characterizations as shown below.

Detection Spectrum and Dynamic Properties. The aromatic species that can induce a given biosensor's response (detection spectrum) were first determined for each biosensor. We selected 44 archetypical aromatic compounds covering the spectrum of environmental aromatics and examined their ability to activate each biosensor using saturated induction; the potential cytotoxity was also assessed (Supporting Information Figure S4). Results show that the NahR biosensor responds mainly to salicylate and its derivatives; XylS to benzoate, salicylate and their derivatives; HbpR specifically to 2-hydroxybiphenyl and 2-aminobiphenyl; and DmpR to phenol and its derivatives (Figure 1b; see more summary in Supporting Information, Part III.A). Notably, 2,4,6-TClPhl, a kind of polychlorinated phenol that is particularly hazardous, is a strong inducer for NahR biosensor; such a sensing capability has not been reported in previous studies. Three novel inducers (2-MePhl, 2-ClPhl and Cat) for DmpR are also identified, rather than previously reported Phl only.

To allow these biosensors to perform quantitative measurements, the accurate dose−responses of biosensors to their archetypical inducers should be obtained. To faithfully capture any possible heterogeneity in GFP expression level across a

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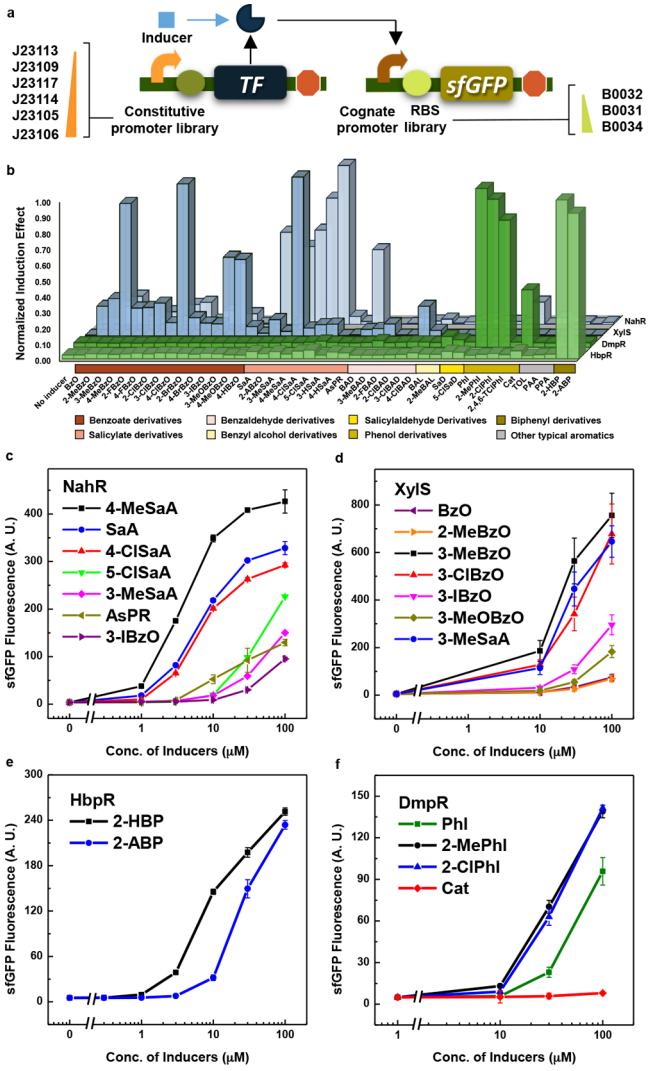

Figure 1. Genetic constructs and dynamic properties of NahR, XylS HbpR, and DmpR biosensors. (a) Genetic constructs of aromatic biosensors and fine-tuning strategy. Constitutive promoter and RBS libraries used to tune the expression level of TFs and sfGFP are also listed. (b) The induction capability of 44 archetypical aromatic compounds to four biosensors. The compounds are grouped and color-coded according to their chemical properties. 5-ClSaD, 2,4,6-TClPhl, 2-HBP, and 2-ABP were added at 100 μM concentration due to severe toxicity at 1000 μM concentration. All the other compounds were added at 1000 μM concentration. See Supporting Information Figure S4 for the cytotoxity data; Supporting Information Figure S5 for the detailed data of detection spectra of individual biosensors. The induction effect was calculated by normalizing the GFP fluorescence values to the highest (5-ClSaA) among them. (c–f) Dose–response curves of NahR (c), XylS (d), HbpR (e), and DmpR (f) biosensors to their typical (strong) inducers. Full names and structures of the 44 aromatic compounds are available in Supporting Information Table S5. The GFP fluorescence was calculated using cytometry data based on geometric means. Error bars represent s.d.; n = 3.

bacterial population, we utilized flow cytometry to determine the dose–responses of each biosensor at the single-cell level (Figure 1c–f). Reassuringly, from the GFP fluorescence histograms obtained in each dose–response experiment, it can be observed that the GFP-expressing populations were homogeneous for all four biosensors (Supporting Information Figure S6). This forms a sharp contrast to the conventional bimodal expression profile of an arabinose-induced population.[9] Having verified that no heterogeneity will be masked, we chose geometric mean value as the quantitative representation of GFP expression at the population level in all the experiments. Results showed that all biosensors exhibited fold inductions far exceeding those reported in previous studies.[5−8] 4-Methyl salicylic acid (4-MeSaA), 3-methyl benzoate (3-MeBzO), 2-hydroxybiphenyl (2-HBP), and 2-methyl phenol (2MePhl) were the strongest inducers for NahR, XylS, HbpR, and DmpR biosensors, respectively; they each induced 120-, 110-, 45- and 20-fold GFP expression in corresponding biosensors at 100 μM concentration.

Three Types of Interference Caused by Noninducer Compounds. For a given biosensor, when aromatic chemicals that are able or unable to induce significant response (typical inducers and nontypical inducers, respectively) coexist, nontypical inducers might interfere with the response of the biosensor to other inducers, although they could not induce any response by themselves alone (Figure 2a). Moreover, if the typical inducer-biosensor pairs exhibit crosstalk to some extent, there would be constraints for the combination of these biosensors in practical measurement of aromatic compounds. To evaluate such potential interference, we chose 4-MeSaA, 3-MeBzO, 2-HBP, and Phl as typical inducers for NahR, XylS, HbpR, and DmpR biosensors, respectively, because these chemicals were among the strongest inducers of their corresponding biosensors. For each given biosensor, typical inducers chosen for the other three biosensors were regarded as interfering inducers, because in all four cases typical inducers of other biosensors showed little induction effect on the given biosensor (Supporting Information Figure S5). Interfering inducers of a given biosensor were combined individually with the biosensor's typical inducer to conduct dose–response measurements. GFP fluorescence measured in the presence of interfering inducers was plotted against GFP fluorescence measured without them. In case of zero interference, the regression line of the data points will have a slope of 1; similarly, for positive interference the slope would be greater than 1 and for negative interference, smaller than 1 (Figure 2b). For XylS biosensor, 4-MeSaA exerted significant negative interference; for DmpR biosensor, 4-MeSaA exerted a mild positive interference while 2-HBP a mild negative interference; surprisingly, for HbpR biosensor, all interfering inducers significantly reduced GFP fluorescence; no interference was observed in other tests (Figure 2c–n). These results highlight the importance of considering the complex crosstalk between different aromatic compounds and biosensors for practical applications (see more discussion in Supporting Information, Part III.B).

Expanding the Detection Spectrum. The detection spectrum of the biosensors can be further expanded. For example, salicylaldehyde (SaD) cannot be sensed by any of the biosensors but can be degraded into SaA by the enzyme NahF from naphthalene catabolic pathway.[10] Thus, enzyme NahF, when combined with NahR biosensor, will expand the sensor's detection spectrum by serving as an adaptor that converts undetectable compounds into detectable compounds (Figure 3a; Supporting Information Figure S7). To prove the concept, constitutively expressed NahF protein was combined with NahR biosensor to construct a NahF-R biosensor, and its dose–response to SaD and SaA was measured, respectively. Results show that NahF-R biosensor can indeed respond to SaD while NahR biosensor alone cannot (Figure 3b). In fact,

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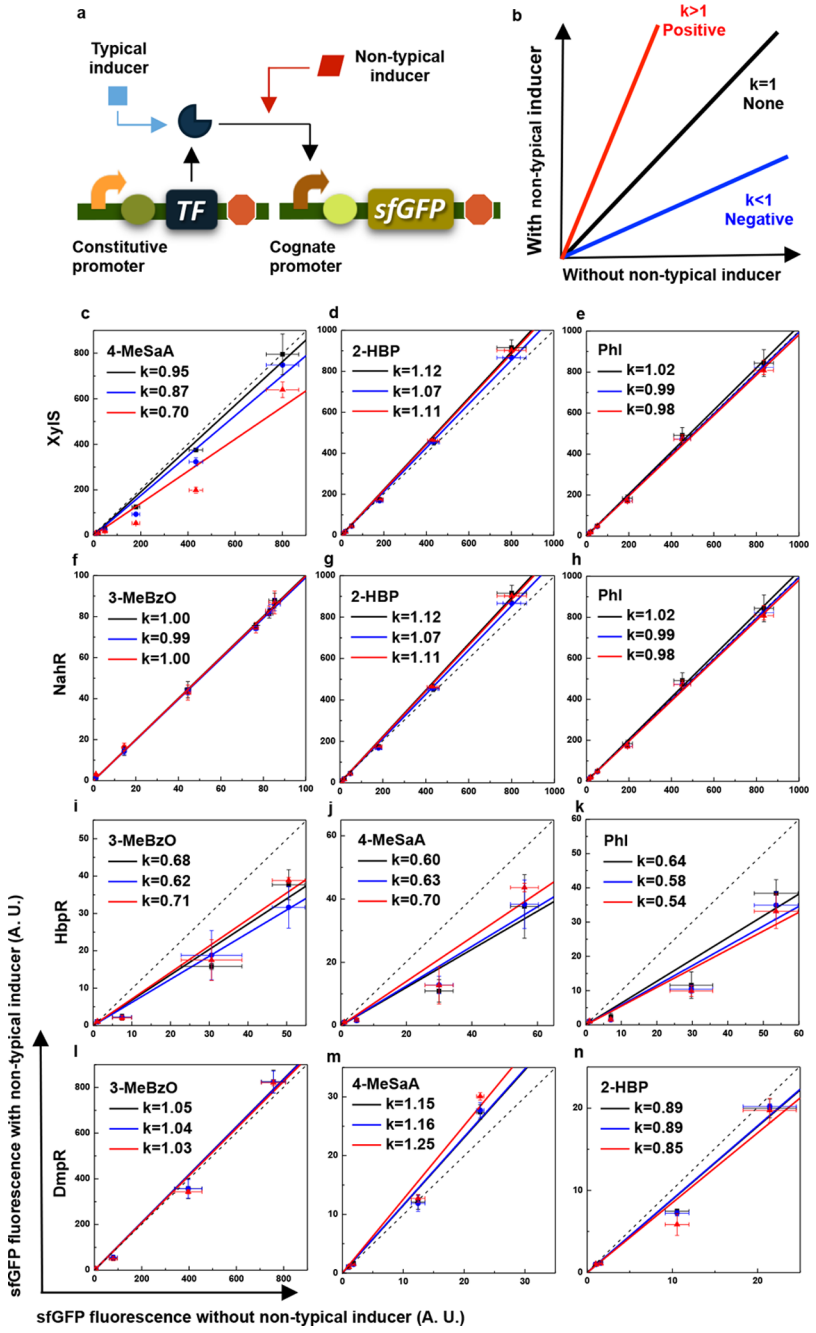

Figure 2. Interference on the sensing capability of biosensors caused by noninducer compounds. (a) The response of a specific biosensor to a given typical (strong) inducer might be interfered by a nontypical (inefficient) inducer. (b) When the GFP fluorescence measured with an interfering inducer (i.e., a nontypical inducer) was plotted against the fluorescence measured without interfering inducer, the slope of regression line reflects the type of interference (positive, none, or negative). (c–n) Plots of interference examination for XylS (c, d, e), NahR (f, g, h), HbpR (i, j, k), and DmpR (l, m, n) biosensors. The aromatic compounds used as the interfering inducers in each test were indicated in the upper left of each plot. The interfering inducers were supplied at three different concentrations: 10 μM (black), 30 μM (blue), and 100 μM (red). The R^2 values are no smaller than 0.94 for all plots. The cells were assayed using flow cytometry. Error bars represent s.d.; n = 4.

NahF-R biosensor is equally sensitive to SaD and SaA (Figure 3c), indicating that NahF efficiently converts SaD into SaA (for more discussion on the converting efficiency, see Supporting Information, Part V). Furthermore, NahF-R and NahR biosensors show similar response to SaA (Figure 3d), indicating that incorporation of NahF protein does not interfere with NahR protein's original function. Such a strategy can be generalized (e.g., to XylS biosensor to further expand its detection range).

Conclusion. We have designed and optimized four aromatic biosensors, characterized their dynamic performance, and evaluated the interference between the aromatic compounds and the biosensors. These biosensors with clear detection spectrum, low detection threshold, and wide dynamic range might be valuable to quantitative measurements of aromatics compounds. They might also serve as novel inducible systems for gene expression in molecular biology.

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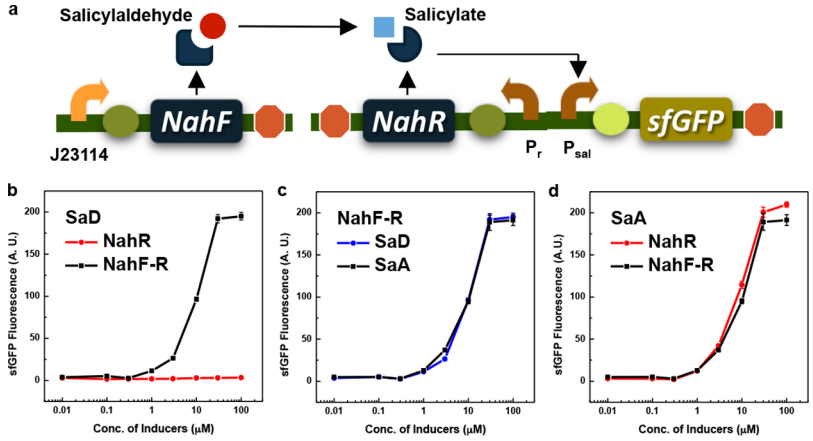

Figure 3. Genetic construct and dynamic properties of the NahF-R biosensor. (a) Genetic construct of the NahF-R biosensor. NahF is constitutively expressed under the promoter J23114 to convert SaD into SaA to be sensed by the NahR biosensor. (b) NahR and NahF-R biosensors' dose–response curves to SaD. (c) NahF-R biosensor's dose–response curves to SaD and SaA. (d) NahR and NahF-R biosensors' dose–response curves to SaA. See Supporting Information Figure S7 for more detailed characterization data. The GFP fluorescence was recorded using flow cytometry. Error bars represent s.d.; $n = 3$.

METHODS

The detailed methods including plasmid construction, chemicals usage, and test protocols can found in Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Detailed descriptions on the materials and methods used in this study; supplementary figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖H.X., H.S., Z.Y., and S.H. contribute equally to this work. H.M.Z. and S.C. conceived the project. H.X., H.S., S.H., Z.Y., S.L., Y.H., X.P., H.W., P.Z., C.C., H.V., J.L., and Y.Z. performed the experiment. H.X., H.S., and Z.Y. analyzed the data. Z.Y., H.S., and H.M.Z wrote the manuscript.

Notes

The authors declare no competing financial interest.

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