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Sensitive and Specific Cadmium Biosensor Developed by Reconfiguring Metal Transport and Leveraging Natural Gene Repositories

Mei-Ying He, Yu-Jen Lin, Yi-Ling Kao, Pu Kuo, Cédric Grauffel, Carmay Lim, Yi-Sheng Cheng, and Hsin-Hung David Chou*

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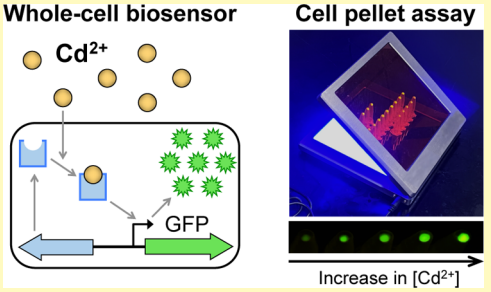
ABSTRACT: Whole-cell biosensors are useful for monitoring heavy metal toxicity in public health and ecosystems, but their development has been hindered by intrinsic trade-offs between sensitivity and specificity. Here, we demonstrated an effective engineering solution by building a sensitive, specific, and high-response biosensor for carcinogenic cadmium ions. We genetically programmed the metal transport system of *Escherichia coli* to enrich intracellular cadmium ions and deprive interfering metal species. We then selected 16 cadmium-sensing transcription factors from the GenBank database and tested their reactivity to 14 metal ions in the engineered *E. coli* using the expression of the green fluorescent protein as the readout. The resulting cadmium biosensor was highly specific and showed a detection limit of 3 nM, a linear increase in fluorescent intensities from 0 to 200 nM, and a maximal 777-fold signal change. Using this whole-cell biosensor, a smartphone, and low-tech equipment, we developed a simple assay capable of measuring cadmium ions at the same concentration range in irrigation water and human urine. This method is user-friendly and cost-effective, making it affordable to screen large amounts of samples for cadmium toxicity in agriculture and medicine. Moreover, our work highlights natural gene repositories as a treasure chest for bioengineering.



KEYWORDS: *cadmium, whole-cell biosensor, trade-off, metal homeostasis, synthetic biology, smartphone detection*

Whole-cell biosensors bear sensing and transducing modules, allowing them to detect and report nutrients or harmful chemicals as optical or electrochemical signals.[1] Microorganisms, in particular, are ideal chassis for whole-cell biosensor development because they are self-renewable, easy to manipulate, and tolerant to harsh environments.[2] Compared with conventional chromatographic analysis, microbial sensors have low instrument requirements, can provide a real-time response, and are simple to operate. These advantages make them a portable and affordable solution for rapid medical diagnosis and on-site monitoring of environmental pollution.

Microbial sensors have been developed to target a wide range of organic and inorganic compounds in recent years,[3] and a major category of analytes is toxic heavy metal ions (abbreviated as metals hereafter), namely, arsenic, cadmium, mercury, and lead.[4] Anthropogenic activities, such as smelting and electronics industries, fossil fuel combustion, mining, and waste disposal, continuously release these pollutants to the environment, posing an ever-increasing threat to public health and ecosystems.[5] The intake of these heavy metals causes acute or chronic damage of the nervous system and organs such as kidneys, muscles, bones, and lungs. Long-term exposure of cadmium and arsenic even results in cancer.[6,7] Microbial sensors have been applied to surveying metal pollution in the environment.[4] Unlike chromatographic analysis, microbial sensors report specifically the bioavailable metals in the specimen, hence providing information more relevant to biotoxicity;[8] yet, metal-detecting microbial sensors typically suffer the drawbacks of low sensitivity, low specificity, or low signal intensities, which hinder their application.[4] Taking the development of microbial cadmium sensors as an example, only 2 out of 25 prior studies reported the generation of cadmium-specific biosensors (Table 1).[9,10] Moreover, nearly half of them showed the signal fold-change (i.e., maximal induction coefficients) < 10.

The shortcomings of microbial metal sensors likely stem from (1) a conflict between biosensor function and cell survival and (2) the physiochemical limitation for specific protein-metal interaction. The genetic module in microbial sensors mainly consists of a transcription factor (TF), which activates the expression of a reporter gene (fluorescent proteins or enzymes) upon binding with intracellular analytes (Figure

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Table 1. Sensitivity, Specificity, and Signal Intensities of Microbial Sensors for Cadmium Ions

microbial chassis	assay method[a]	signal[b]	detection limit (nM)	specificity[c]	maximal induction coefficient	signal transducer	reference[d]
Escherichia coli	I	F	3	○	777	16 kinds	this study
Pseudomonas putida	I	F	10	×	1.3	CadR.Pp	11
E. coli	I	F	18	×	35	ZntR	12
E coli	I	F	45	×	1.8	ZntR	13
Pseudomonas aeruginosa	I	F	89	×	10	CadR.Pp	14, M
E. coli	I	F	89	×	2	CadC	15
E. coli	I	F	100	×	20	CadC	16
E. coli	I	F	100	×	1.2	CadR.Pp[e]	17
E. coli	I	F	500	×	13	CadR.Pp	18
E. coli	I	F	500	×	2.4	split-GFP[f]	19
E. coli	I	F	890	×	22	ZntR	20
E. coli	I	F	1000	×	3.8	ZntR	21
E. coli	I	F	1000	○	6.2	ZntR	10
E. coli	I	F	1000	×	5.6	ZntR	22
Hansenula polymorpha	I	F	1000	×	250	NA[g]	23
E. coli	I	F	5000	×	3	6 kinds	24
Bacillus subtilis	I	L	3.3	×	5	CadC	25, M
P. putida	I	L	5	×	50	CadR.Pp	26
B. subtilis	I	L	10	×	4	CadC	27, M
E. coli	I	L	10	×	70	ZntR	28
E. coli	I	L	100	×	NA	ZntR	29
Staphylococcus aureus	I	L	1000	×	45	CadC	30, M
E. coli	I	E	25	×	NA	ZntR	31
Chlorella vulgaris	I	E	8.9	△	NA	AP[h]	32
Deinococcus radiodurans	I	A	50	○	NA	NA	9
E coli	CL	L	0.1	×	NA	CadC	16
D. radiodurans	CL	A	10	○	NA	NA	9
Saccharomyces cerevisiae	CL	F	200	×	5	PS[h]	33

[a]I, intact cell; CL, cell lysate. [b]A, absorbance; E, electric current; F, fluorescent protein; L, luminescence. [c]○, reactive to just cadmium ions; ×, reactive to additional metal species at the same concentration range; △, not tested. [d]M, multiple microbial chassis are tested. Only the device showing the highest sensitivity is reported. [e]Fluorescent resonance energy transfer transducer built with the CadR.Pp metal-binding loop. [f]Split-GFP with an engineered metal-binding loop. [g]NA, information is not available and cannot be computed based on the presented data. [h]AP, alkaline phosphatase; PS, phytochelatin synthase; both enzymes functioned as signal transducing/reporting elements.

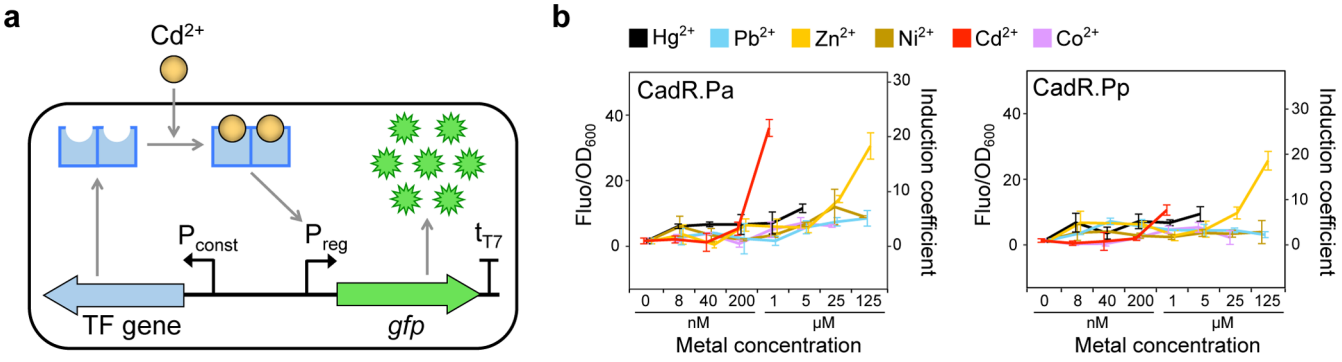

Figure 1. Detection of cadmium by a whole-cell biosensor. (a) Cadmium ions bind to TFs (as homodimers) and activate GFP expression. P_{const}, constitutive promoter; P_{reg}, regulatory promoter; t_{T7}, terminator. (b) Responses of CadR.Pa and CadR.Pp genetic modules to metal induction in *E. coli* MG1655. Error bars indicate standard deviation from the mean (n = 3).

1a). While the sensitivity of microbial sensors depends on the accumulation of analytes in the cytoplasm, the cellular homeostasis system autonomously expels metals to keep their concentrations below the toxic level. On the other hand, the specificity of microbial sensors requires TFs binding only to the target analyte. Nevertheless, specific protein-metal interactions are inherently difficult because, relative to organic compounds, metal ions are smaller in size and short of distinguishable physiochemical features.[34,35] To resolve the aforementioned issues, a rational strategy to construct microbial metal sensors would require (1) selection of analyte-specific TFs with a high signal-transducing capacity and (2) reconfiguring metal transport to enrich target analytes and deprive other interfering metal species in cells. With respect to this engineering proposal, most studies of microbial cadmium sensors recruited three TFs known to react broadly with cadmium, zinc, mercury, and lead (i.e., CadR of *P. putida*, CadC of *S. aureus*, and ZntR of *E. coli*), instead of exploring alternative options (Table 1).[4] On the other hand, few studies attempted to improve the performance of microbial sensors by

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testing the reactivity of a given TF in multiple microbial chassis.[14,25,27,30] The results, however, were often unpredictable and showed a mixed success.

As microbial cadmium sensors suitable for practical application remain to be developed, we sought to build one with high sensitivity, specificity, and signal intensities based on the aforementioned engineering concept. We selected *E. coli* as our chassis because it was amenable to genetic manipulation and had the best understood metal homeostasis system. We used the green fluorescent protein (GFP) gene as the reporter because its fluorescence could be readily detected by basic optical detectors like mobile phones,[36] and the assay did not require cell lysis or adding additional substrates. Through genetically reprogramming the metal transport of *E. coli* and testing the metal reactivity of 16 metal-sensing TFs selected from natural gene repositories, we built a cadmium-specific biosensor capable of measuring cadmium concentrations in irrigation water and human urine using smartphones as the optical detector.

EXPERIMENTAL METHODS

Plasmid and Strain Construction. Primers and plasmids are listed in Tables S1 and S2, respectively. The procedures of plasmid construction are described in the Supporting Information. To shift the balance of metal accumulation, five metal homeostasis genes in *E. coli* MG1655 (*cueR, copA, cusCFBA, zntR, zntA*) were deleted to generate strain EK317, with the assistance of allelic exchange plasmids pWL01, pWL02, pYK16, pJL17, and pWL03 following an established procedure.[37] Gene deletions in *E. coli* EK317 were confirmed by PCR amplification using primer pairs WL01p5/WL01p6, WL02p5/WL02p6, YK16p5/YK16p6, JL17p5/JL17p6, and WL03p5/WL03p6, respectively. To preserve strains, cells were suspended in phosphate buffered saline (PBS, 1×) supplemented with 25% glycerol and stored at −80 °C.

Growth Medium and Metal Supplements. Luria-Bertini (LB) medium consisted of 10 g of sodium chloride (Fisher Scientific), 10 g of tryptone (Becton Dickinson), 5 g of yeast extract (Beckton Dickinson), and 10 g of glucose (Sigma-Aldrich) in 1 L of deionized water (pH 6.9) made by the ELGA Micra Type II system, unless indicated otherwise. Glucose was added because it enhanced GFP expression.[38] LB medium was supplemented with kanamycin sulfate (50 mg/L, Thermo Fisher Scientific) to prevent plasmid loss. To examine the influence of pH on biosensor performance, the pH of water or growth medium was adjusted by adding 0.1 M HCl (Fisher Scientific) or 0.1 M NaOH (Sigma-Aldrich) and determined by a pH meter (Denver Instrument). Metal salts, $AuHCl_4{\cdot}3H_2O$ (Acros Organic), $CaCl_2$ (Hayashi Pure Chemical Industries), $3CdSO_4{\cdot}8H_2O$ (Hayashi Pure Chemical Industries), $CoCl_2$ (Sigma-Aldrich), CsCl (J. T. Baker), $CuSO_4{\cdot}5H_2O$ (J. T. Baker), $FeCl_3$ (AppliChem Gmbh), $HgCl_2$ (Sigma-Aldrich), LiCl (Fisher Chemical), $NiSO_4$ (Sigma-Aldrich), $MgSO_4{\cdot}7H_2O$ (J. T. Baker), $MnSO_4{\cdot}H_2O$ (J. T. Baker), $Pb(NO_3)_2$ (Fisher Chemical), and $ZnSO_4{\cdot}7H_2O$ (Sigma-Aldrich) were dissolved in deionized water, sterilized by filtration, and stored in the dark at 4 °C. Metal solutions were added to LB medium right before growth assays.

Measurement of Cell Growth and GFP Expression. The growth and GFP expression of *E. coli* were monitored by a TECAN Spark 10M plate reader. Each experiment began with the inoculation of 1 μL of frozen stocks into 200 μL of LB medium incubated overnight at 37 °C and 225 rpm. At this pre-culture stage, ethylenediaminetetraacetic acid (EDTA; 50 μM, Riedel-de Haën) was added to LB medium because it chelated residual metal cations, resulting in a lower basal GFP expression.[36] Subsequently, 1 μL of the pre-culture was transferred to 200 μL of LB medium supplemented with metal solutions. The optical density at 600 nm (OD_{600}) at 1 cm path length and GFP fluorescence (excitation/emission: 488 ± 10 nm/528 ± 10 nm) were measured every 20 min. The cellular GFP expression ($Fluo/OD_{600}$) was computed as the average of fluorescence intensities divided by OD_{600} and 100 (i.e., a scaling factor) at OD_{600} = 0.55−0.65 (incubation time ∼2.5 h). Under inhibitory growth conditions (e.g., toxic metal concentrations or suboptimal temperatures and pH), the signal output of biosensors were reported as $Fluo/OD_{600}$ at the early exponential phase of the cell culture instead. For each biosensor construct, its induction coefficient was computed as cellular GFP expression in metal-supplemented LB medium divided by that in the control LB medium.

Cell Pellet Assay Based on Smartphone Detection. Each experiment began with inoculation of 1 μL of frozen stocks into 200 μL of LB medium containing 50 μM EDTA. The pre-culture was incubated overnight at 37 °C and 225 rpm. Subsequently, 6 μL of the pre-culture was transferred to 600 μL of LB medium supplemented with metal solutions. Three types of LB medium, prepared with distilled deionized water (pH 6.9), filter-sterilized irrigation water (pH 7.5, sampled at the campus farm of National Taiwan University), and a 1:1 mixture of distilled deionized water and filter-sterilized human urine (pH 7.6), were tested. After incubation at 37 °C and 225 rpm for 8 h, stationary-phase cells in 200 μL of culture medium were spun down by a cubee Mini-Centrifuge (GeneReach) at 2000*g* for 5 min at room temperature. Cells were washed with 500 μL of 1× PBS and were spun down again through centrifugation. Cell pellets held in 1.5 mL tubes were illuminated by a BluView Transilluminator MBE-200 (Major Science; λ = 470 nm, filter cut-off = 580 nm). Images were taken by iPhone 11 configured by the RCam application (focal length = 4.25 mm, aperture = f/1.8, exposure time = 1/57 s, ISO 500, exposure compensation = −3.27 EV). Each image included the test samples and a constitutive GFP-expressing strain (*E. coli* EK317 bearing pYC08). The fluorescence intensity of each cell pellet in the image was quantified by ImageJ,[39] and the fluorescence of the constitutive GFP-expressing strain served as the reference for signal calibration across images. Residual cadmium ions in growth medium, determined by the inductively coupled plasma-mass spectrometry analysis of Sun Dream Environmental Technology Co. (Taichung, Taiwan), were below the detection limit (0.44 nM). The creatinine concentration in the urine sample was determined as 1.81 g/L by Union Clinical Laboratory (Taipei, Taiwan).

RESULTS

Developing a Whole-Cell Biosensor for Cadmium Detection. We used *E. coli* strain MG1655 (wild-type) to build the prototype of whole-cell biosensors containing a sensing/transducing module located on a low-copy plasmid (Figure 1a; Tables S1, S2). The module consisted of a TF gene expressed by the constitutive promoter P_{cueR} and a GFP gene controlled by the TF-regulated promoter (Figure S1). When cadmium ions entered the cytoplasm and were bound to TF, TF activated the transcription of the regulatory promoter, resulting in an increase in GFP fluorescence (Figure S2). We first constructed two modules with two known cadmium-sensing TFs, CadR of *P. aeruginosa* and *P. putida* (termed CadR.Pa and CadR.Pp, respectively) and their regulatory promoters.[40,41] We examined the response of the two modules in *E. coli* MG1655 at various concentrations of cadmium (Cd), cobalt (Co), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) ions. To minimize the confounding influence of metal toxicity on the readout, we quantified the response of the two modules below growth-inhibiting concentrations (Figure S3a). Both CadR.Pa and CadR.Pp modules exhibited low signal-to-noise ratios, had a detection limit (defined as a statistically significant change relative to the negative control; $P < 0.05$) of just 1 μM for cadmium ions, and responded to cadmium, mercury, lead, and zinc ions (Figure 1b). We reasoned that the sensitivity and signal intensities of the prototype sensors could be improved if more metals were allowed to accumulate in the

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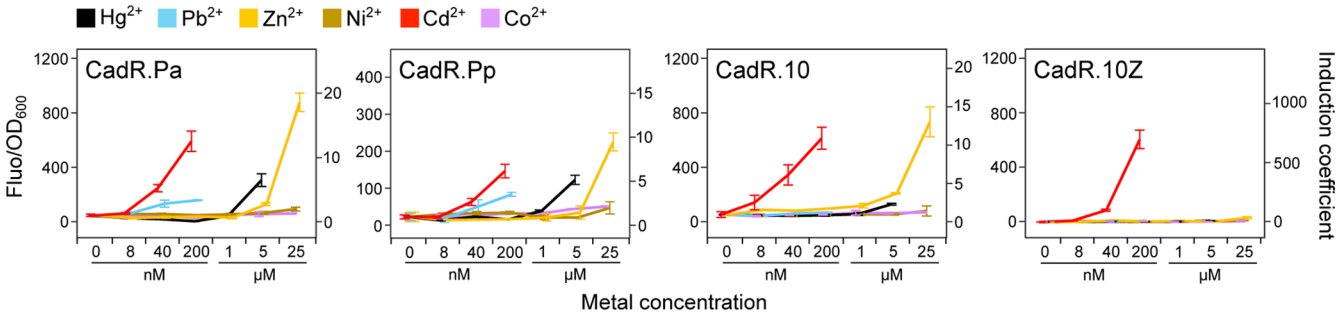

Figure 2. Responses of CadR.Pa, CadR.Pp, CadR.10, and CadR.10Z modules to metal induction in *E. coli* EK317. The vertical axis in each plot is scaled differently to highlight the trend. Error bars indicate standard deviation from the mean (n = 3).

cytoplasm. To achieve this, we removed the coding genes (*zntA*, *copA*, and *cusCFBA*) of the three exporters of transition metals in *E. coli*.[34] Moreover, we deleted *zntR* and *cueR* genes because they encoded homologous TFs that might cross-regulate and cause the nonspecific response of the CadR modules.[40,42]

Disrupting Metal Exporters Enhances the Sensitivity but Compromises the Specificity of Microbial Sensors. The full-length deletion of aforementioned metal homeostasis genes in *E. coli* MG1655 resulted in the engineered strain EK317 (Figure S4). Relative to *E. coli* MG1655, strain EK317 exhibited reduced physiological tolerance to cadmium, lead, and nickel ions (Figure S3b), consistent with the elevation of intracellular metal concentrations. Metal induction assays, conducted at concentrations below the growth-inhibiting level, further confirmed the altered metal homeostasis of *E. coli* EK317. In this strain, both CadR.Pp and CadR.Pa modules showed an overall increase in signal intensities and had a deletion limit of 40 nM for cadmium ions (Figure 2). On the other hand, despite the removal of potential cross-regulators ZntR and CueR, CadR.Pa and CadR.Pp modules still reacted with lead, mercury, and zinc besides cadmium ions. These results suggested the nonspecific response of microbial sensors largely attributed to CadR.Pa and CadR.Pp proteins. Furthermore, the response of CadR.Pa and CadR.Pp modules to lead and mercury ions increased significantly in strain EK317 relative to *E. coli* MG1655, suggesting an intrinsic trade-off between the sensitivity and specificity of biosensors.

Engineered *E. coli* Equipped with 16 TFs Shows Variable Sensitivity, Specificity, and Signal Intensities to Metals. As CadR.Pa and CadR.Pp proteins were likely responsible for the nonspecific response,[40,41] we decided to look for alternative TFs with higher sensitivity and specificity to cadmium ions. We performed a sequence similarity search and selected a total of 711 CadR homologues in the GenBank database for phylogenetic analysis. Based on the result (Figure S5), we chose additional 14 TFs as candidate sensory modules. Among these, only PbrR, PbrR691, and ZccR have been studied before.[43−45] The remaining 11 uncharacterized CadR homologues were named CadR.1 to CadR.11. The 14 TF genes and their regulatory promoters were synthesized and used to construct new sensing/transducing modules with an identical genetic organization (Figure 1a; Tables S3 and S4). These modules showed variable sensitivity, specificity, and signal intensities to metals in *E. coli* EK317 (Table 2, Figure S6). Among them, the high cadmium sensitivity and signal intensities of the CadR.10 module were most promising (Figure 2). The only caveat was that it was significantly induced by zinc ions at high concentrations. Interestingly, most

Table 2. Genetic Modules for Cadmium Detection in *E. coli* EK317 (Summary of Data in Figures 2 and S6)

module	signal intensity at 200 nM[a]	induction coefficient at 200 nM	detection limit (nM)	specificity[b]
CadR.10Z	high	694.7	3	○
CadR.4	high	18.3	8	×
CadR.2	high	15.3	8	×
CadR.10	high	10.9	8	×
CadR.3	high	7.6	8	×
CadR.5	high	6.5	8	×
CadR.Pa	high	12.5	40	×
CadR.1	high	4.8	40	×
CadR.7	medium	31.7	8	×
CadR.6	medium	23.5	8	×
PbrR	medium	15.8	8	×
CadR.9	medium	9.1	8	×
PbrR691	medium	6.7	40	×
CadR.Pp	medium	6.2	40	×
CadR.11	low	22.4	8	×
CadR.8	low	7.7	40	×
ZccR	low	2.1	40	×

[a]The signal intensity (Fluo/OD_{600}) for cadmium detection is defined as low (<40), medium (40−400), and high (>400). [b]○, reactive to just cadmium ions; ×, reactive to cobalt, lead, mercury, nickel, or zinc ions below 200 nM.

of the tested TFs responded to both cadmium and zinc ions, suggesting a fundamental limitation for CadR homologous TFs to distinguish metal ions with similar physicochemical properties.[34,46]

Exogenous Expression of a Zinc Ion Exporter Leads to a Cadmium-Specific Microbial Sensor. To eliminate the response of the CadR.10 module to high zinc concentrations, we attempted to reduce zinc ions in the cytoplasm of *E. coli* EK317 through overexpressing a zinc exporter. We selected the ZitB metal exporter of *E. coli* because a prior work found it preferentially ejecting zinc over cadmium ions.[47] The *zitB* gene controlled by its native promoter was incorporated into the plasmid bearing the CadR.10 module. Remarkably, the "CadR.10 + ZitB" module (abbreviated as CadR.10Z) was highly specific to cadmium ions and showed no reactivity to the other 13 metal species below the growth inhibitory concentration: caesium (Cs), calcium (Ca), cobalt, copper (Cu), gold (Au), iron (Fe), lead, lithium (Li), magnesium (Mg), manganese (Mn), mercury, nickel, and zinc (Figures 2 and 3a). Moreover, it had the advantages of a low basal GFP expression, a low cadmium detection limit (3 nM), high signal intensities (maximal induction coefficient = 777 at 350 nM),

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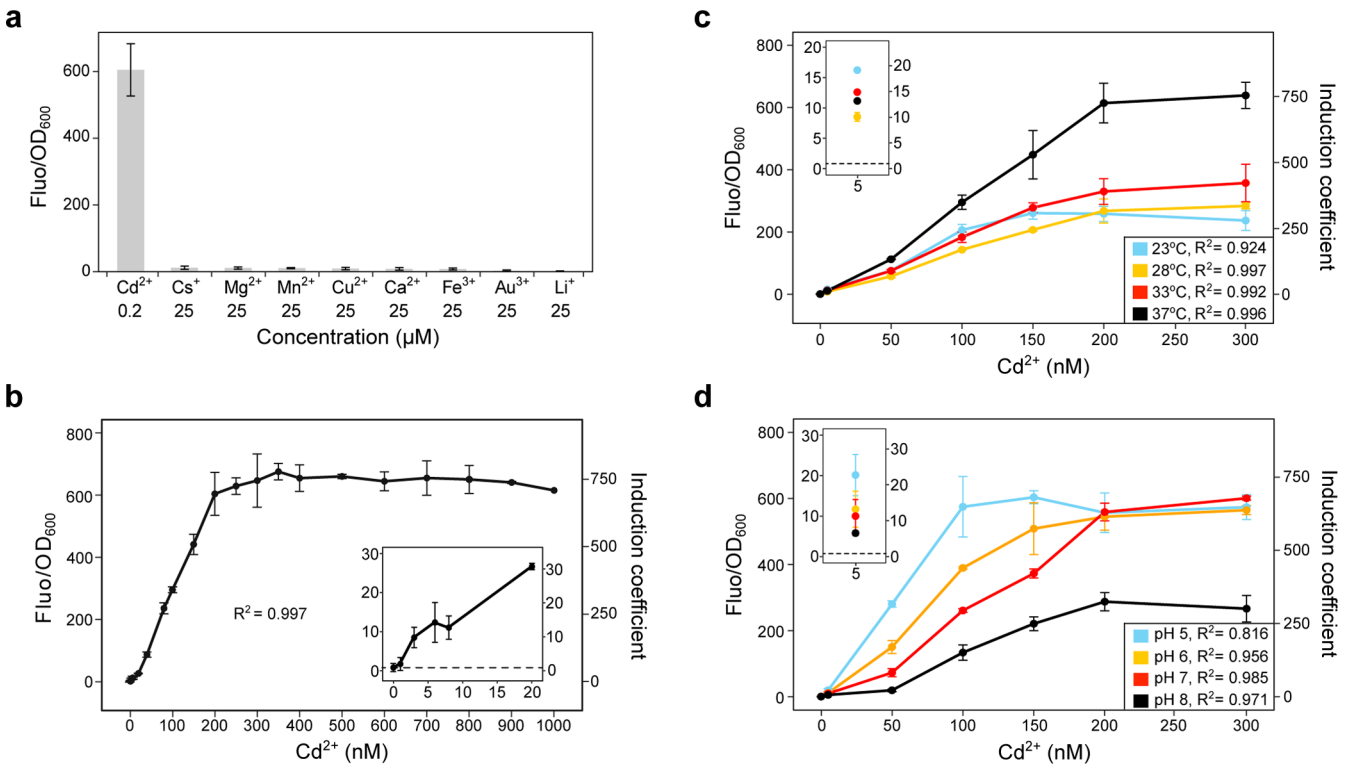

Figure 3. Sensitivity and specificity of the CadR.10Z module in *E. coli* EK317. (a) Response to cadmium and other heavy metals. (b) Response to cadmium concentrations. (c) Influence of incubation temperatures. (d) Influence of the pH of growth medium. Panels (b–d) show the *R*-squared values of linear regression at 0–200 nM and insets highlighting the biosensor response at low cadmium concentrations. The dashed lines in insets indicate Fluo/OD_{600} at 0 nM Cd^{2+}, whose values are similar across tested temperatures and pH ($P < 0.05$). Error bars indicate standard deviation from the mean ($n = 3$).

and a linear response range and robust growth at 0 to 200 nM of cadmium ions ($R^2 = 0.997$; Figures 3b and S3c). In terms of these properties, the resulting microbial cadmium sensor (i.e., *E. coli* EK317 harboring the CadR.10Z module) outperformed those published to date (Table 1). The only microbial cadmium sensor showing higher sensitivity to cadmium ions (detection limit = 0.1 nM) was nonspecific and relied on an enzyme-based cell lysis assay.[16]

To examine the robustness of our microbial cadmium sensor against environmental perturbations, we monitored its cadmium-induced responses under different temperatures and pH of growth medium (Figure 3c,d). Though its signal outputs were quantitatively influenced by these two factors, the cadmium detection limit of our biosensor stayed ≤5 nM under all tested conditions. Moreover, its linear detection range (defined as $R^2 \geq 0.95$) remained 0–200 nM at 28–37 °C and pH 6–8. Because the chemical components of (LB) growth medium exhibited substantial pH buffering capacity to the water source (Figure S7), the above test likely covered a realistic pH variation encountered in practical application.

Development of a Smartphone-Assisted Assay for Practical Application. The performance of most microbial sensors, including ours, was monitored using exponentially growing cells and laboratory-grade instruments, a scenario clearly deviating from the real-world situation.[4] For practical application, we developed a simple cadmium quantification assay using our microbial sensor, a smartphone, and low-tech equipment. We grew the microbial sensor in small volume compatible with the 96-well deep well plate and harvested stationary-phase cells at a fixed time point (i.e., 8 h). Cells in culture medium were spun down by a mini-centrifuge. The GFP fluorescence of cell pellets was excited by a blue LED plate and recorded by the smartphone (Figure 4a,b). We applied this assay to the microbial sensor grown in culture medium prepared with deionized water, irrigation water, and the 1:1 urine-water mixture (Figure 4c). Under the three conditions, our microbial sensor exhibited a linear response to cadmium ions at 0–200 nM. The signal intensities were generally lower in the urine–water mixture, likely caused by the chelation of cadmium ions by urinary proteins or other organic compounds. The detection limits were 8 nM for deionized water and irrigation water and 20 nM for the urine–water mixture. Taking the dilution and the urinary protein content into account, the detection limit of cadmium ions in urine was 40 nM, or 2.48 μg Cd/g creatinine. The detection limits of this smartphone-assisted diagnosis were below the safety thresholds set by the World Health Organization for drinking water (<26.7 nM) and human urine (<5.0 μg Cd/g creatinine).[48]

DISCUSSION

Conventionally whole-cell biosensors are engineered by recruiting well-known metal-sensing TFs, followed by testing their performance in one or multiple microbial chassis.[14,25,27,30] Nevertheless, the suitability of each microbial chassis is unforeseeable, and the results often show limitations in either sensitivity, specificity, or signal intensities, raising concerns about the reliability of whole-cell biosensors.[49] To overcome this, we sought an alternative strategy by reprogramming the metal transport system of *E. coli* and exploring the utility of uncharacterized TFs. Though the engineering process involves several steps of genetic manipulation, the effort is worthy and

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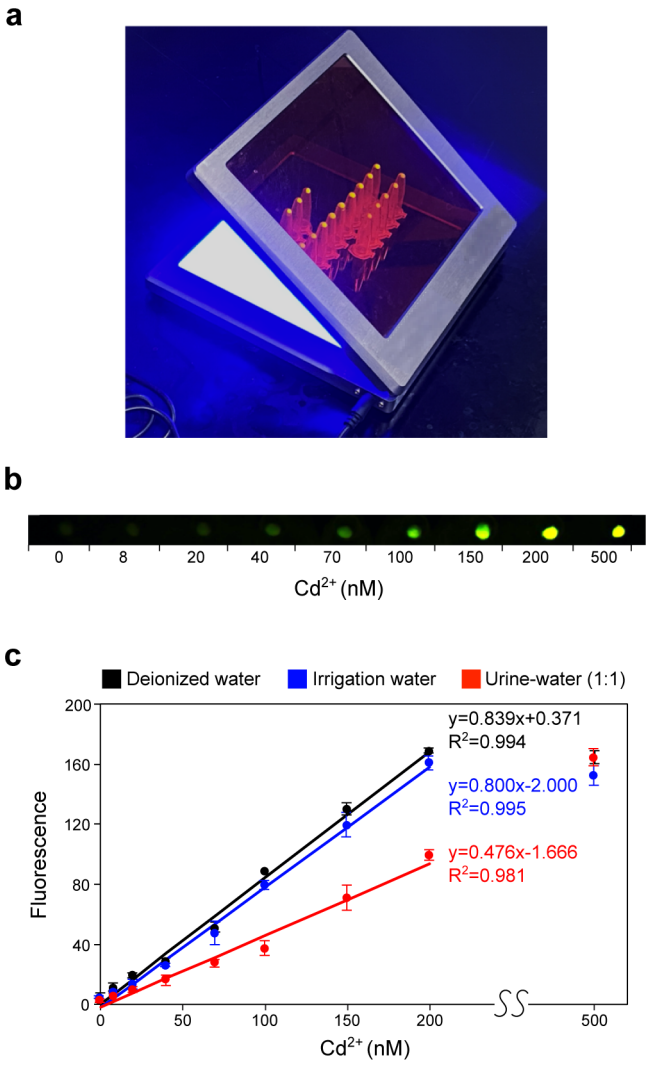

Figure 4. Smartphone-assisted cell pellet assay for cadmium quantification. (a) Cell pellets of *E. coli* EK317 bearing the CadR.10Z module emit fluorescence on a blue LED plate. (b) Smartphone image shows the fluorescence of cell pellets increasing with the cadmium concentration. (c) Fluorescent intensities of cell pellets. *E. coli* EK317 is grown in LB medium prepared with deionized water, irrigation water, or a urine-deionized water mixture (1:1), and spiked with $CdSO_4$. Cells reaching the stationary phase (i.e., 8 h) are spun down by a mini-centrifuge and photographed by iPhone 11. The regression lines at 0−200 nM Cd^{2+} are shown. Error bars indicate standard deviation from the mean (n = 3).

necessary as it yields a sensitive, specific, and high-response microbial cadmium sensor outperforming those published since 1993 (Table 1). The idea to optimize biosensor function via disrupting metal exporters has been tested previously but showed limited success.[10,26,50] Similar to an earlier work, our study finds this modification insufficient as it sacrifices the specificity of microbial sensors for increased sensitivity; yet, we show that this intrinsic trade-off could be surmounted by employing TFs with higher analyte specificity and by increasingly expelling interfering metal species. Particularly, the best performing TF in our biosensor comes from a GenBank search and has not been characterized before. This finding highlights the synergy between bioinformatics and synthetic biology to leverage natural gene repositories for bioengineering.[51−53]

To fit our cadmium biosensor for practical application, we developed a sensitive fluorescence assay using cell pellets, low-tech equipment, and smartphone detection. Though collecting cell pellets may seem primitive, the practice amplifies weak fluorescent signals, allowing the smartphone to achieve comparable detection limits as laboratory-grade instruments. Notably, this method could detect cadmium ions in irrigation water and human urine below the safety standard defined by the World Health Organization, demonstrating its applicability to monitoring cadmium pollution in agriculture and medical diagnosis of harmful cadmium exposure. This simple biosensor assay is cost-effective, scalable to screen large amounts of samples, and suitable for monitoring cadmium toxicity at remote locations. Apart from whole-cell biosensors, nanoparticles,[54−58] DNA aptamers,[59] and monoclonal antibodies[60,61] have also been employed to develop real-time and on-site cadmium quantification methods. With respect to our microbial sensor, these noncell-based approaches enjoy a shorter response time (30 s to 15 min), but most of them exhibit lower sensitivity (detection limits: 33.3 nM to 20 μM),[54−58,60] lower specificity,[54−58,60,61] and/or lower induction coefficients[54−61] (1.7−16-fold signal change). Despite a lower signal fold change, the DNA aptamer-based assay is cadmium-specific and shows a detection limit (5 pM) significantly lower than ours.[59] Collectively, our work underscores the value of whole-cell biosensors for the detection of heavy metals and provides an innovative and effective engineering strategy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.0c02204.

Supplementary experimental methods; transcriptional activity of PcueR; correlation between gfp transcripts and GFP fluorescence; growth of *E. coli* in response to heavy metals; confirmation of gene deletions in *E. coli* EK317; summary of the phylogenetic relationship of 711 CadR homologues; responses of multiple genetic modules to metal induction in *E. coli* EK317; pH buffering capacity of growth medium; primers; plasmids; native operon structure and Preg sequence of the 16 CadR homologues; sequence of synthetic DNA fragments; and references (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hsin-Hung David Chou − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan; Genome and Systems Biology Degree Program, Academia Sinica and National Taiwan University, Taipei 106, Taiwan;* orcid.org/0000-0001-6003-5609; Email: chouhh@ntu.edu.tw

Authors

Mei-Ying He − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan*

Yu-Jen Lin − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan*

Yi-Ling Kao − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan*

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Pu Kuo − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan*

Cédric Grauffel − *Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan;* orcid.org/0000-0002-9478-4699

Carmay Lim − *Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan;* orcid.org/0000-0001-9077-7769

Yi-Sheng Cheng − *Department of Life Science, National Taiwan University, Taipei 106, Taiwan; Genome and Systems Biology Degree Program, Academia Sinica and National Taiwan University, Taipei 106, Taiwan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssensors.0c02204

Author Contributions

M.-Y.H. and Y.-J.L. contributed equally to this work. H.-H.D.C. conceived the idea. M.-Y.H. and H.-H.D.C. designed the experiments. M.-Y.H., Y.-J.L., Y.-L.K., and P.K. performed the experiments. M.-Y.H., Y.-J.L., C.G., C.L., Y.-S.C, and H.-H.D.C. analyzed the data. H.-H.D.C. and M.-Y.H wrote the manuscript. All the authors have given approval to the final version of the manuscript.

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Notes

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