

Electronic supplementary information

A prodigiosin-based whole-cell biosensor for sensitive colorimetric detection of Hg(II)

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Materials and methods

Bacteria, vectors, and reagents

The bacterial strains and plasmids used in this study are listed in Table S1. *Escherichia coli* (*E. coli*) harboring recombinant plasmids was cultivated in LB medium (5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl) supplemented with 50 µg/mL ampicillin at 37 °C and 250 rpm. Stock solutions of HgCl₂, Pb(NO₃)₂, CdCl₂, ZnSO₄, MgSO₄, CuSO₄, MnSO₄, and CaCl₂ were freshly prepared using analytical-grade chemicals. 4-Methoxy-2,2'-bipyrrole-5-carbaldehyde (MBC) and 2-methyl-3-amylypyrrole (MAP) were purchased from Sigma-Aldrich. Oligonucleotide primers and synthetic DNA fragments were synthesized by Sangon Biotech (Shanghai, China).

Table. S1 Bacterial strains and plasmids used in this study

Strains and plasmids	Genotypes or description	Reference
<i>E. coli</i> strains		
TOP10	F ⁻ Φ80 <i>lacZ</i> ΔM15 Δ <i>lacX</i> 74 <i>recA</i> 1	Invitrogen
BL21(DE3)	F ⁻ <i>ompT hsdS_B (r_B⁻ m_B⁻) gal dcm</i> (DE3)	Merck
Plasmids		
pT7lac-PigC	pET-21a derivative containing the <i>pigC</i> gene from <i>Serratia marcescens</i> cloned into <i>Nde</i> I and <i>Sac</i> I sites	This study
pT7lac-HapC	pET-21a derivative containing the <i>hapC</i> gene from <i>Hahella chejuensis</i> , cloned into the <i>Nde</i> I and <i>Sac</i> I sites	This study
pT7lac-TreaP	pET-21a derivative containing the <i>treaP</i> gene from <i>Pseudoalteromonas citrea</i> , cloned into <i>Nde</i> I and <i>Sac</i> I sites	This study
pT7lac-TamQ	pET-21a derivative containing the <i>tamQ</i> gene from <i>Pseudoalteromonas tunicata</i> , cloned into <i>Nde</i> I and <i>Sac</i> I sites	This study
pHg-PigC	pT7lac-PigC derivative containing a redesigned Hg(II)-sensory element cloned into <i>Bgl</i> III and <i>Xba</i> I sites	This study
pHg-HapC	pT7lac-HapC derivative containing a redesigned Hg(II)-sensory element cloned into <i>Bgl</i> III and <i>Xba</i> I sites	This study
pHg-TreaP	pT7lac-TreaP derivative containing a redesigned Hg(II)-sensory element cloned into <i>Bgl</i> III and <i>Xba</i> I sites	This study
pHg-TamQ	pT7lac-TamQ derivative containing a redesigned Hg(II)-sensory element cloned into <i>Bgl</i> III and <i>Xba</i> I sites	This study

Plasmid construction and sensor assembly

Four prodiginine condensation enzyme genes (*pigC*, *hapC*, *treaP*, and *tamQ*) were codon-optimized for *E. coli* based on their origins listed in Table S2 and chemically

synthesized by Sangon Biotech (Shanghai, China). The optimized DNA sequences and encoded amino acids are detailed in Table S3. Each coding sequence was cloned into the pET-21a backbone via *NdeI/SacI* sites downstream of a T7lac promoter, generating constitutive expression vectors pT7lac-PigC, pT7lac-HapC, pT7lac-TreaP, and pT7lac-TamQ. All recombinant plasmids were transformed into *E. coli* BL21(DE3) for protein expression validation.

Table. S2 Prodiginine condensation enzymes used in this study.

Condensation enzymes	Origins	GenBank accession
PigC	<i>Serratia marcescens</i>	WP_049190811.1
HapC	<i>Hahella chejuensis</i>	WP_216738636.1
TreaP	<i>Pseudoalteromonas citrea</i>	WP_138598219.1
TamQ	<i>Pseudoalteromonas tunicata</i>	WP_009837228.1

To construct Hg(II)-responsive sensors, a redesigned mercury-sensing regulatory element MerR-Pmer¹ was inserted as a *BglIII-XbaI* fragment into the above expression vectors, replacing the original T7lac promoter (Table S3). This regulatory module comprises the coding sequence of the Hg(II)-binding transcription factor MerR and its cognate operator, the Pmer promoter, forming an "OFF-ON" inducible expression system. The resulting sensor plasmids pHg-PigC, pHg-HapC, pHg-TreaP, and pHg-TamQ were transformed into *E. coli* TOP10 to generate whole-cell sensor strains.

Table. S3 DNA sequences and deduced amino acid sequences of biological modules used in this study.

Vectors	sequence	Description
pT7lac-PigC	CATATGAACCCGACCCTGGTTGTTGAACCTGAGCGGTGACAAAACCCTGG AACCGCACCGTCTGGGCGGCAAAGCTCACTCCCTGAACCACCTGATCCAC GCGGGTCTGCCGGTTCGCCGGCGTTTTGCATTACGGCGCAGGCGTATCG TCAGTTTATTGAATTTGCCGTACCGGGCGCGCTGCTGGATACCGGGCGCGC CGGGTAACGTGCGTGACATGATCCTGAGCGCAGCAATCCCGGCTCCGCTG GATCTGGCTATCCGTCACGCGTGTAAACAGCTGGGCGACGGCGCCAGCCT GGCAGTTCGCAGCTCCGCGCTGGAAGAGGACGGTCTGACCCACTCCTTCG CGGGTCAGTACGATACCTACCTGCACGTCCGCGGTGACGATGAAGTAGTC CGCAAAGTGCAGAGCTGCTGGGCGTCCCTGTGGGCGGAACGCGCGGCGC AGTACTCTCGTACCAGCGCAGCTCAGTCCGATATCGCTGTTGTGCTGCAG ATCATGGTTGACGCAGATGCTGCGGGCGTGATGTTCACTCAGGATCCACT GACCGGCGACGCAAACCACATTGTTATTGATTCTTGGTGGGGTCTGGGCG AAGGCGTGGTTAGCGGTCAGGTAACACTGATTCTTTTATCCTGGATAAA GCGAGCGGCGAAATCCGTGAACGCCAGATCCGTCACAAACCGCACTACT GTCAGCGTGACCCGACGGCCCGTGTGACTCTGCTGCAGACCCCAAGAGC CCGCCGTGATGCCCTAGCCTGACCCCGGAACAGCTGCAGCAGCTGGCA CGTCTGGCCCCCAGACCCGCATGATCTATGGTGCAGGAACTGGATATTGA ATGGGCTGTCAAAGATGATCGTGTGGCTGCTGCAGGCACGTCCGATCA CCACCCAGGCGAAACCAGTTCAGATGTTGTACGCTAACCCGTGGGAATCT GACCCGACTATTAAAGAGCGTGCGTTCTTCTCTCGTATGGATACCGGCGA AATCGTGACCGGCTGATGACCCCGCTGGGTCTCAGCTTCTGCCAGTTCT ATCAGAAACACATCCATGGTCCGGCAATCAAACCATGGGGCTGGCCGA TATCGGTGATTGGCAAATCTACATGGGCTATCTGCAGGGCTACGTCTACC	<i>pigC</i> gene (<i>NdeI-SacI</i>)

	<p>TGAACATCTCTGGTAGCGCCTACATGCTGCGCCAGTGCCCGCCGACCCGT GATGAAATGAAATTCACCACCCGTTATGCTACCGCGGATATCGATTTTC TGGCTACAAAAATCCGTACGGCCCTGGTGTTCAGGGCTGGGCGTACCTGA AAAGCGCATGGCACTGGCTGAAACAGCAGCGTCATAACCTGCGCTCTGC GGTGGCAGCCGTTGACGCAATGATCGCGCTGCGCCAGCGGAAACCCGT CGTTCCCTGGCACTGGACCTGACTACCATGACTCACCAGGAACCTGGAACG TGAAGTGTCTCGCATCGACGGCTATTTCTGGACTCTTGTGCGGCTTACAT GCCGTTCTTCTGCAGTCTTTTGGCGTGTACGACGCGCTGGCGTGACGT GCGAGCGCTATCTGAAAGGCCGTGGCAACGCTCTGCAGAACCCTATCAA AGCTTCCATGAACAACCTGCGTACTATCGAAGTTACCCTGGGTATCCTTT CTTTAGTGGAAACGGTTAACCGCCAACCGGCGTTGAAAGCGCTGTTGAA CGTCACTCCGCACAGGAACCTGTTACCGTTCTGCCGACCGACCCAGAATC TCGCGCCTTCTGGCAATCCGATTTTCTGCGTTTCTGTTTGAATTCGGCGC ACGCGGTGCGCAAGAATTTGAACTGAGCCTGCCGCGTTGAAACGATGAT CCGAGTTACCTGCTGCAGGTTATGAAAATGTACCTGCAGCACCCAGTAGA TTTGATACCAAACCTGCGTGAAACCGAACGCCTGCGCCATGAAGATTCTG CGGCACTGCTGAAAGCTATGCCGTGGTTTGGTTCGATGAAACTGAAATTC ATTACCAAACCTGATGGCGTGATGGCAGAACGTCGCGAAGCGACCCGCC CAACCTTCGTTACCGAAACCTGGTTCTATCGTCGTATCATGCTGGAAGTT CTGCGCCGCTTGGAAAGCCAGGGCCTGGTTAAATCTGCCGATCTGCCGTA CGTTGACTTTGAACGCTTTCGTGCATTTCATGGCCGGTGAACAGTCCGCGC AGGAAGCATTTCGCGGCAGACCTGATTGAACGCAACCGTCATCAGCACCT GTTGAACCTGCACGCGGAAGAACCGCCGATGGCGATCGTGGGTGGCTAC CAGCCGCGTATGAAAGCACCGACCGCTGAAAACGCTGCTGGTATGCTGA GCGGCTGGCAGCCTCTCCGGGTAAGTTGTTGCTAAAGCACGTGTGATT ACCGATCTGCTGGCCAGGCGGGTGAAGTGCAGCCGAATGAAATTCTGG TTGCCCGTTTACCGATGCGTCTTGGACCCCGCTGTTTCGCGCTGGCAGCG GGTATCGTTACCGACATTGGCTCCGCCCTGAGCCATTCTTGTATTGTGGC ACGTGAATTCGGTATCCCGGCAGCAGTTAATCTGAAAAACGCGACCCAG CTGATTAACCTCCGGTGACACCCTGATCCTGGATGGTGATAGCGGCACTGT GATTATCCAGCGTGGTGAACGTGCTGACGGTTAAAGAGCTC</p> <p>Protein: PigC Length = 888 amino acids Molecular Weight = 99186.00 Daltons</p> <p>MNPTLVVELSGDKTLEPHRLGGKAHSLNHLIHAGLPVPPAFCITAQAYRQFIE FAVPGALLDTGAPGNVRDMILSAAIPAPLDLAIHACKQLGDGASLAVRSSA LEEDGLTHSFAGQYDITYLHVVRGDDDEVVRKVQSCWASLWAERAAQYSRTSA AQSDIAVVLQIMVDADAAGVMFTQDPLTGDANHIVIDSCWGLGEGVVSQ VTTDSFILDKASGEIRERQIRHKPHYCQRDPQGRVTLTQTPEARRDAPSLTPE QLQQLARLARQTRMIYGAELDIEWAVKDDRVLWLLQARPITQAKPVQMLY ANPWESDPTIKERAFPSRMDTGEIVTGLMTPGLSFCQFYQKHIHGAIKTMG LADIGDWQIYMGYLQGYVYLNISGSAYMLRQCPTRDEMKFTRYATADID FSGYKNPYGPGVQGWAYLKSAAHWLWKQQRHNLRSAGATVDAMIALRQRE TRRFLALDLTMTMTHQELERELSRIDGYFLDSCAAAYMPFFLQSFALYDALALT CERYLKGRGNGLQNRKASMNLRITIEVTLGILSLVETVNRQPALKALFERH SAQELVTVLPDPESRAFVQSDFSAFLEFGARGRQEFELSLPRWNDDPSYL LQVMKMYLQHPVDLHTKLRETERLRHEDSAALLKAMPWFGRMKLKFITKL YGVMAERREATRPTFVTETWFYRRIMLEVLRRLEAQGLVKSADLPYVDFER FRAFMAEQSAQEAFAADLIERNRHQHLNLHAEPPMAIVGGYQPRMKAP TAENAAGMLSLAASPGKVVAKARVITDLLAQAGELQPNEILVARFTDASW TPLFALAAGIVTDIGSALSHSCIVAREFGIPAAVNLKNATQLINSGDTLLLDGD SGTVIIQRGERADG</p>	
<p>pT7lac- HapC</p>	<p>CATATGACCTATGATAGCCACATCGTTGAAGTTAGCGGTCAGCAGAACA GTAACGCGCAGTCCCTGGGCGGTAAAGCGTCTAGTCTGAATACCCTGGCA GCTGCGGGCTTCCCGGTTCCGCGTGCCTACTGCCTGACCGTGGACGCTTA CGCTAGCTTCTGCGTGAAAGCGGCCTGGACCAGTGGATTAGCGGCCTGG ATCAGCAGGATGCGAGCGCGTTCACCCAGATCCGTCAGCGCATTGAAGA AACCCAGCTGCCGTCCTCTCTGCGTCAGGCGATCATTGGCGGTACAAAAG ACATTGGCGCAGAACGTTGGCGGTACGTTCTTCCGCAATTTCTGAAGAT TCTGAAGAACAACCTCGTTCGCGGGTCAATATGACACCTACCTGCACGTGGA GAACGAAAGAACCCCTGGTTGACTGCGTGAAACGCTGCTGGGGTAGCTTC TGGACCGAACGTGCGCATGCTTATGAAGGCCGTCAGGATCAGCGCCGCC GCTCTTCCGATGCGGACGCTCCGATGCAGGGTATTGCAGTTGTGATCCAG GCCATGATTGACGCAGATGCCGCGGGTGTCTTTTTACCGCGGACCCGCT</p>	<p><i>hapC</i> gene (<i>NdeI-SacI</i>)</p>

GAACGGCGATCCGCAGCGCACCGTTATTGAAAGCTGCTGGGGCCTGGGT
 GAAGGTGTTGTTAGCGGTCAGGTGACCACTGATACTTTTGTGATCGACAA
 CCAGAAAATGGAGCTGCTGGAACAGACCCCTCCGTGAGAAACCGCTCATG
 AGCACCCGTGCGGAAAATGGTGGTGTTCCTGCGTAAAACCGCCGAAA
 ACAAATCCGTGCGCCGACTCTGAACGAAAACGAAGCGCTGGCGCTGGC
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 CACCGTTACTCCGACTAACAGCGACAACCGCCTGTTTTCGGACGCAGACG
 AAAGCAACAGCTACATTCGTGACAACGCTCTGTTTAGCCGTATGGATACC
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 GGATATTGGTGATAGCCAGCACTATATGGGTTACCTGCAGGGCCATGTTT
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 CGTGATGCGCTGAAATTCACCAAACGTTACTCTACCGAAGAAGTGGACCT
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 CTGGAACCCCGCTGGAACAGCTGGCTCAGGCGCTGAAAACGATGATG
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 GTTACAGCTGGAATCCCGCTGGAAGAAATCGATCGTAAACGTGATGACG
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 CCCGTCCGACCTACATCGCGCAGACCTGGTTCTACCGCAAGATCATTGTT
 GAAGTAATGCGTCTGCTGTCTGCTGAAAAAGTGTGAAAAATGGAGGACC
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 AGCCACAGCAGGCGTTTAGCGCCGAACTGATTGAACGCAACCCGCGTT
 CTCATCTGCTGAACCAGCGCCTGCAGGAGCCCGGATGGCGATCGTTGGC
 GGCCATGAACCGCGCCGCGAAACCGCGGAAACCACCCCGACCGACGATA
 ACCAGGTTTTCCAGGGCATCGGCGCAAGTCCGGGCGTTGCGATGGGCCGT
 GCTCGCGTGATACCGATCTGCCGCGCCAGGCGGAAGAATTCCAGCAAG
 GCGAAATCTTAGTTGCGCGTTTACCGATGCGAGCTGGACCCCGCTGTTT
 GTTCTGGCGGCAGGTGTCGTTGCTGACGTTGGTAGCATGCTGTCTCACAG
 CTCCATCGTTTTCCCGCAATTCCGCATCCCGGCCATTGTGAACACGAAAA
 CCACCACCGTTCGATACCGGTGATATGCTGTACCTGGACGCGGAT
 GCGGGCGTGGTTCGTATTGAAGAACGTAAAGAAGATGCGTCCCAGTAA
 GCTC

Protein: HapC
 Length = 908 amino acids
 Molecular Weight = 102243.95 Daltons

MTYDSHIVEVSGQQNSNAQSLGGKASSLNTLAAAGFPVPRAYCLTVDAYAS
 FLRESGLDQWISGLDQQDASAFTQIRQRIEETQLPSSLRQAIIGAYKDIGAERV
 AVRSSAISEDSEEHSFAGQYDYLHVENEETLVDCVKRCWGSFWTERAHAY
 EGRQDQRRRSSDADAPMQGIAVVIQAMIDADAAGVLTADPLNGDPQRTVI
 ESCWGLGEGVVSQVTTDTFVIDNQKMELLEQTLREKPLMSTRAENGGVCL
 RKTAENKIRAPTLNENEALALAGYANAIRSHYGREMDIEWALKDGIWILQ
 ARPITVPTNSDNRLFADADESNSYIRDNALFSRMDTGEIVTGLMTPGLSFC
 RFYQHNVHGPAAIKTMGLLDIGDSQHMYMGYLQGHVYLNISASAMLLTQCPT
 RDALKFTKRYSTEEVDLNFYTNPYGKAPQGLQYLKSAGYWTLYQVRNLVT
 AERTVRDMISLRKRETERFLKLDLSAMSLGELNAELQRIDRYFLESCAAYMP
 FFLQSFALYDALAELCEKWLGDGKGLQNRKASLSNLRITIEVTRGVCDLAA
 SVQRSSHLRQLVLETPLEQLAQLQNDDEGARFWNDFMAFLRDYGARGR
 QEFELSIKRWADDPSYLLQVIRMYLTSQVLESRLLEEIDRKRDDDDTRALLSGL
 PLRIRLLFKFVISTYAKMAERREATRPTYIAQTWYFVKIIVEVMRRLSRAKVL
 KMEDLPYIDFNELRDYVAGRKSAQQAFSAELIERNRRSHLLNQRLQEPMAI
 VGGHEPRRETAETPTDDNQVFQIGASPGVAMGRARVITDLPRQAEFFQQ
 GEILVARFTDASWTPLFVLAAGVVADVGSMLSHSSIVSREFGIPAIVNTKTTT
 RRIRTDMLYLDGDAGVVRIERKEDASQ

<p>pT7lac- TreaP</p>	<p>CATATGACCGTTAATAGCCTGGCAATTGAACAGCCGCTGAACGATGGCAT CGTTCACCTTCGATTCTCAGCAGAGCGCTTCTGTTGAACTGGTGGGTGGTA AAGGCGCGTCCCCTGTGCGCTATGCACAACGCCGGCCTGCCGGTACCGATC GGTACTGCATCACCGTTAACGTTTTTGAAGAATTCCTGCAGCAGACCGA ATTGCTGAGCCGTTTTGACCCGCTAAAGGCGTGACTGAATGGCGTGAC TGGCGACCGGATCACTAGCGTTGCTATGCCGGAACAGATTGCAACCCA AATCTCTAACGTTCCGCGCTGCTGATTGGCCCGGTGGCTGTGCGTTCCA GCGCAACTGATGAAGATTCCGATTCCCATAGCTTTGCAGGCCAGCACGTA ACTAAATTAGCGTTGAAGGCATTGACCATATCCTGGAAGCGGTGCAGG CATGCTGGGCGTCTGCTTTCTCCGAAAGCGGCTTCCAGTATCGCCAGACC ACCGGCCAGACTACTGATATCCCGAAAATCGCGTTGTAGTTCAGCAGAT GCAGTTCGGTGACGCTTCCGGTGTGGCGTTCGGCCAGCACCCGACCCT TCGCGAAAAACGCATTTGTGATTGAAGGCTGTTACGGCCTGTGTGAAGGT CTGGTGAGCGGTCAAGTTAGCTCTGATTCCCTATGAAATTGATAAACTGAG CGGTGAACTGATTGCGCACACAGTTCAGCCGAAACAGCATCAGATCGTTC ACATCGATGGTAGCATTAAAAAACTGGCTGTCCCGCCGGCGCTGCAGAA CGAACAGGTTCTGAATCACCTGGCGCAGCAGTCTCTCTATAGCGTGTAC GTCAGGTTGAATCTTACTACGGCTGCCCCGAGGACGTGCAATTGACCCCTG AGTGGTAAATTTGGCTGTTACAGTCTCGTCCGATCACCATAAAAA CACCAAAGCTGATCCGTTCTACCTGGGCAACAAATCTGAACATGTTTCA AAGATATCCTGTGGTCGCGCATGGACATCGGCGAAAATCTTTACTGGTCTG ATGACCCCACTGGGTCTGAGCTTCGCGCACTATTACCAGACTCGCGTTCA TATCGACTGCGGCAAAAACTCGGTCTGCTGGATCTGGGTGAAGCAAA GAAACCATTGCTTATCACAAGGCCACGTTTATCTGAACGTAAGCTATAC CGTTGGCAGCTGGCGCAGACCCCGGTAGGTGAAGATCAGACCCCGTTC CTGGCGGTTTTCTTTCGGAAGCGATTGATCTGACCGGTTATGAAAACCC GTACGGTGCTAAACACAGCCATCCGAACATGACGCCTAAACAGTGCCTG CGTTACTGGATCAAACAGAACGTTAAAGAACTGTTCCAGGCTAAACGCC GCGCGAAATCCATGGTGAACAGCCGTTACCAGGAATTTGACCGCGTTCA GTCTCAGGACATCACGAACCTGTCTCACGATGAACTGCGTTACGAACGG ATCACTGTCTGGGTTACTTCAAAGCCATGCACGAGGGTTATCTGCCATAT TACATTAACGCCTTTGCATGTACGGCTGCTGGAAGAAGTGTCCGCAAA ATGGTGGGTGATAAAAAGCAAACACCTGCAGAAACAAAATTAAGGTGAC ATGAGCAACCTGCGTACCCTGCGCTCTGCTCAGGAAGTGTGGCAGCTGTG TAAAGTGCTGAACGCCTGGCCGACGTTAAAGATGTTTTTACCAAACAAA GACATTGCAGATATCGAAGATTTCTGACCTCTACTCCGCTGGGCATCCG TTTACCAACGGCCCGCTGGCGGAATTCATGCGCGACAACGGGGTGCCTG GGCCGTGAAGAGATGGAATTGACCCACCCGCGCTGGCTCGACGACCGTA CCTACGTTCTGCAGATGATTAATTTGACCTGAAACAGGGGTACGAAGTT GACGACGCACTGGTTGAACAGACCCAGAAACAGCAAAAAATCTCTGATG AACTGCTGAACGAAGTCTGCTGGACCCAGCGTTTTACCATCAACCGCGTT ATCAGCCTGTATAGCGGCTGTGCAAACTGCGCGAAGAGACCCGATGTG CCATGACCACCTCTATCTGGCTGGTGCCTGCTGTTGATACGAAGTGGCG CGCCGCTGATCGACAAATCCCTGCTGTCTAGCTTCGACGACGTTGCGTA CCTGGACTTCGAGAACGTTATTCAATATCTGAACAACACCATTACTGCGG AACAGCTGACCGATGCCCTGCTGATCTCAAAAAACCGTGCAGTACCA GCAGTGCAGGCACAGCCAGACCCGCTCTGACGTTTATCGGTAAACCA TCTTCCAGCCTGATCTGATCTTCGACCCGTCCTGACTTCTCTGGAAGGT CTGGCTACCTCCGAAGGCTTCTGCCGCGCGCTGCGTGCCTTACTGTA TCTGGCTAGCCAGGCAGGCGAATTCCAGAAAGGCGATATTCTGATCGCTC CGTTCACCGATGCGAGCTGGACCCCGCTGTTCCGCTTGGCTTCCGCGGTG GTGACCGATATCGGTTCTATGCTGCTCACTCCAGTATCGTAGCTCGTGA ATTCCGCATTCCGTGCGTGGTGAACACCCATCACGCTAGCGCGCTGATTA ACACCGCGATATCATCACCGTTGATGCACATAAAGGTCTGATCATCGTT AACAAAGAAGATTAAGAGCTC</p> <p>Protein: TreaP Length = 898 amino acids Molecular Weight = 100337.47 Daltons</p> <p>MTVNSLAIEQPLNDGIVHFDSSQSSASVELVGGKGLASLCAMHNAGLPVPIGYC ITVNVFEEFLQQTELLSRFDPSKGVTEWRALATAITSVAMPEQIATQISNASE LLIGPVAVRRSATDEDSHSHFAGQHVTKLAVEGIDHILEAVQACWASAFSE AAFQYRQTTGQTTDIPKIAVVVQMQFGDASGVAFGQHPTTFAKNAFVIEG CYGLCEGLVSGQVSSDSYEIDKLSGELIAHTVQPKQHIVHIDGSIKKLAVPP ALQNEQVLNHLAQQSLYSVLRQVESYYGCPQDVEWTLSDGKIWLLQSRPIT</p>	<p><i>treaP</i> gene (<i>NdeI-SacI</i>)</p>
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	<p>TKNTKADPFYLGKSEHVQKDILWSRMDIGEIFTGRMTPLGLSFAHYQTRV HIDCGKGLGLDLGEANETIAYHKGHVYLVNSYTAWQLAQTPVGEDQTPFL ARFSSEIDLTYENPYGAKHSHPNMTPKQCLRYWIKQNVKELFQAKRRRAK SMVNSRYQEFDRVQSDITNLSHDELRYELDHCLGYFKAMHEGYLPYYINA FACYGLLEELSAKWLGDKSKHLQNKIKGDMNSLRTVASAQELWQLCKVLN AWPQVKDVFTNKDIADIEDFLTSTPLGIRFTNGPLAEFMRDNGVRGREEMEL THPRWLDDRTYVLQMIKLYLKQGYEVDALVEQTQKQKISDELLNELSW TQRFTINRVISLYSGCAKLREETRMSMTTSIWLVRVRYEVARRLIDKSLSS FDDVAYLDFENVIQYLNNTITAEQLTDALLISKNRAQYQQWQAQPEPLTFI GKPSQPDLIFDPSLTSLEGLATSEGFCRARACVITDLASQAGEFQKGDILAP FTDASWTPLFALASAVVTDIGSMLSHSSIVAREFGIPCVVNTHHASALINTGDI ITVDAHKGLIIVNKED</p>	
<p>pT7lac- TamQ</p>	<p>CATATGATCAACGCGGCATGAAACTGAGCACCGAATCTGACAACATCT TCCGTTTACCGATATGCCGCTGCAGAGCGTTAGCCACATTGGCGGTA GGTGCTAGCCTGTGCGCGATGAGCGCCGCGGCCTGCCGTTCCGGCGG GTGTTTGTCTGAGCGTTGCGCTGTTTGTATGATTCGCGGCGCACATTGAA CTGCTGACCCGTTTTGATCTGACCGAAGGTTATGAACAGTGGCAGGCGGC TGCAAAAGCGATTAAAGAATCCCCGCTGCCGAACACCCTGCAGGATAAC ATTTCTGCGGCGATCGCTCACCTGAAAGGCCCGTGGCGGTGCGCTTAG CGCGCTGGATGAAGATGGTGAAGCTCTAGCTTTGCGGGCCAGCAGCTG ACGAAACTGGCACTGTGCGGTCTGGATAACATTCTGGATGCAATCAAAG AATGCTGGGCTTCTGCGTTCTCCGAAGCTGCCTACCGTTACCGCCAGCAT ACCGATAAACACCTGGACATGCCGCGTATGGCAGTGGTTGTTGAGCAGAT GCAGTTCGGTGATGTTAGCGGTGTTGCGTTCCGCCAGCACCCGACCACCT TTGCACGCGACGCATTCTGGTTGAAAAGTCTTCCGGCCTGTGCGAAGGC CTGGTGAGCGGCGAGGTGGTTTCTGATAGCTGGCAGATTGATAAAGCTC CGGTGAAGTTCGCGAAGTGACCATCGCTGACAAAATCTGAACAGATCATCT ATCTGGACGCGCAAATTCAGAAAGTTGCAAGTCCGACGAACAGCGTCA GCAGCCGCCCCTGGGCCGATGGCACAGCAGGCACTGTACACCCTGCTG TGCAAAAGTTGAAGCTTATTTGGTGTTCGCGAGGACGTTGAATGGACCTG GTCTGAAGGCAAATCTGGCTGCTGCAGAGCCGTCCTATCACACGCATG CGCCGAGCTTGACGACTTTTACCTGGGTAGCCGTGATGTTGATCTGCAG AAAACCATGCTGTGGTCTCGCATGGACATTGGCGAAATTTTACCGGTG CATGACCCCGCTGGGCGTGTCTTTCGCCCCTACTACCAGCAGCAGGTTT ACACCGGTTGCGGTAAGGTCTGGGTCTGCTGGACCTGGGCGAAGCCGA CGAAACCATCGCGTACTACCGTGGCCACGTTTACCTCAACGTGAGCTATA CCGCCTGGCAGCTGGCCAGACCCCGATCGGCGAAGATCAGCTGCCGTTT CTGCAGCGCTTTTCTAGCGAAGCCATCGATCTGGCTGGCTATCGTAACCC GTACGGTGAAAAACACTCCCACCAGGATTATAGCGCGAAACAGTGCACC CGCTATTGGCTCAAAAAGAACATCAAAGAAGTCTTCTGCGCAAAACGTC CGCGCGTAGCATGATCGCCTCCCGTTATAAGCAGTTCGACCGTGCACAA CAGAAACCGCTGCAGCAGCTGAGCCTGGAGCAGCTGGCAGCTGAAATGC GCCACGCTCTTGCCTACTTCAAAGCGATGCACGAAGGCTACCTGCCGTAC TACATCAACCGCTTCGCGTGTATGGTATCCTGGAAGAAGTGCAGCGCGCT GTGGCTGGGTGACAAAGGCAAAACAGCTGCACAACCAGATCAAAGGTGAT ATGTCTAACCTGCGTACGGTGGCATCGGCACAGGAGCTGTGGCTGCTGTG TCAGCAGCTGGCGGATTGGCCGAGGTTAAACAGGCCCTTCTGGATCTGG AACTGGACGAACTGGAAGTGTCTGTAACGAGCACCAGGCTGGTATCAA ATACAGCCGTTGGCCGCTGGCGCAGTTTATGCGCGAAAACGGCGTGCCT GCGCGTGAAGAGATGGAAGTACCCACCCGCGTTGGCTGGACGACCGTA CCTATCTGCTGCAGATGATTAAGTTTACCTGCACCAGGGCTATGCTGTT GATGACGCTATCGCGACTCAACAGCGCAAAACAGCGTAAAAACAGCAACG AAATTCTGAAAACGATCCCCTGGTACCAGCGTCTGTGATGAAACTGGTC ATCAGCCTGTACAGCGGCTGCGCTAAACTGCGTGAAGAAACCCGATGTC TATGGTGACCTCTATTTGGCTGGTGGTTCGATCGTCTATGAACTGGGTC AGCGCCTGGTTGCGACTCAGCAGCTGACCTCCCTGGATGATATTGCGTAC CTGGACTTCGCGGAAATCCTGAGCTATCTGGAAAAACGCATTGACGTTCA CCAGCTGACCGAACCAGGCGAAACTGGCTCAGCGTCAGAGCGCGTACCAC CTGTGGCAGAGCATGCCGGAACCCGCGTACCTTCATTGGTTCTCCGAA ATCCAGCCGGATCTGATCCTGGACCCGACCCTGACCCAGCTGTCTGGCC TGGCGACACAGCGCGGTTTCTGCCGTGGCAAAGCTTGGTGTATCACCGAT CTGGCTAAACAGGCGGGCAGTTCAAAAAGGTGACATCCTGATCCTGCG CGTTACCGACGCATCCTGGACCCCGCTGTTGCTCTGGCCGGCGCTGTG GTGACCGACATCGGCTCCATGCTGAGCCATTCTAGCATCGTGGCGCGTGA ATTCGGCATCCCCTGTGTGGTGAACACCAACCATGCGTCTGCGCTGTTA AATCCGGCGACATGCTGATCGTTGATGCACAGAATGGCATCGTTGTTCTG</p>	<p><i>tamQ</i> gene (<i>NdeI-SacI</i>)</p>

	<p>GATAAAGAATCT TAAGAGCTC</p> <p>Protein: TamQ Length = 897 amino acids Molecular Weight = 100589.65 Daltons</p> <p>MINAAMKLSTESDNIFRFTDMPLQSVSHIGGKGASLCAMSAAGLPVPAGVCL SVALFDDFAAHIELLTRFDLTEGYEQWQAAAKAIKESPLPNTLQDTISAAIAH LKGPLAVRSSALDEDESSFAGQHLTKLALCGLDTILDALKECWASAFSEA AYRYRQHTDKHLDMPRMAVVVVQQMQFGDVSQVAFGQHPPTTFARDAFLVE NCFGLCEGLVSGQVVSQWIDKASGEVREVTIADKSEQIHYLDGEIQKVAVS DEQRQPALGPMQAQALYTLCKVEAYFGVQDVEWTWSEGIWLLQSRPI TTHAPQLDDFYLGSRDVLQKTMWSRMDIGEIFTGRMTPLGLSFARYYQQ QVHTGCGKGLGLDLGEADETIAYYRGHVYLVNSYTAWQLAQTPIGEDQLP FLQRFSSAIDLGYRNPYGEKSHQDYSKQCTRYWLKKNIKELFCARR ARSMIASRYKQFDRAQQKPLQQLSLEQLAAEMRHALAYFKAMHEGYLPYYI NAFACYGILEELSALWLGDKGKQLHNQIKGDMNSLRTVASAQELWLLCQQ LADWPQVKQAFDLDELEVEFLNEHQAGIKYSRGLAQFMRENGVRAREE MELTHPRWLDDRTYLLQMIKVYLHQGYAVDDAIATQQRKQRKNSNEILKTI PWYQRRVMKLVISLYSGCAKLRETRMSMVTISIWLVRRIYVELGQRLVATQ QLTSLDDIAYLDFAEILSYLEKRIDVHQLTEPAKLAQRQSAYHLWQSMPEPPL TFIGSPKSQPDILDLPTLTQLSGLATSAGFCRGKACVITDLAKQAGQFKKGD LIAPFTDASWTPLFALAGAVVTDIGSMLSHSSIVAREFGIPCVVNTNHASALF KSGDMLIVDAQNGIVVLDKES</p>	
<p>pHg-PigC</p> <p>pHg-HapC</p> <p>pHg-TreaP</p> <p>pHg-TamQ</p>	<p><u>AGATCT</u> CTAAGGCATAGCTGACCTTGCCAGGCCTGCTTCGCCCTGTAGTG ACGCGATCAACGGGCAGGAAACATTCCCCTTTCGTGCATGGCAGGCGCA CACGAGTTCAGACAGCACGGTTTCCATGCGCGCCAAGTCGGCCATCTTCT CGCGCACGTCCTTGAGCTTGTTGTTCCGCCAGGCTGCTGGCCTCCTCGCAG TGGGTGCCATCGTCGAGCCGCAACAGCTCGGCAATCTCGTCCAGACTGAA CCCCAGCCGCTGTGCCGATTACGAATTTACCCGAACCACGTCCGCT CCCCATAGCGGCGGATGCTGCCGTAAGGCTTGTCGGTTCCCGCAACAGG CCCTTGCGCTGATAGAAGCGGATTGTCTCCACGTTGACCCGGCCGCTT GGCAAAAACGCCAATGGTCAGGTTTCCAAATTATTTTC CATCGTTCGCG CAACGCTCCTGGTACCTTGTGTTAAAGGGTTTCAACAGATATTC AATCGCTTGACTCCGTACATGAGTACGGAAGTAAGGTTACGCTATCCAAT CCAAATTCAAAGGGCCAACGTTCTAGA</p>	<p>Hg(II) sensory element (<i>BglII-XbaI</i>)</p>

The start codon is shown in **green**. The end codon is shown in **red**. The Hg(II)-responsive divergent Promoter P_{mer} is shown on a **grey background**. The constitutive promoter P_{ceuR} is shown in **light blue**. Restriction sites are underlined.

Heterologous expression of PigC, HapC, TreaP, and TamQ homologs in *E. coli*

Recombinant strains BL21(DE3)/pT7lac-PigC, BL21(DE3)/pT7lac-HapC, BL21(DE3)/pT7lac-TreaP, and BL21(DE3)/pT7lac-TamQ were inoculated from single colonies into 3 mL LB liquid medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C, 250 rpm for 12 h as seed culture. The overnight culture was then diluted 1:100 (v/v) into 20 mL fresh medium, grown at 37 °C for 3 h to the early exponential phase, and induced with IPTG to a final concentration of 1 mM. After induction at 37 °C, 250 rpm for 4 h, cells were harvested by centrifugation (8,000 \times g, 10 min, 4 °C). Total protein was separated by 10% SDS-PAGE and stained with Coomassie Brilliant Blue R-250 for expression level analysis using a gel imaging system.

Optimization of prodigiosin biosynthesis induction

Time-course and substrate addition strategy optimization

Seed cultures of pT7lac-PigC and pT7lac-HapC were prepared in LB medium containing $50 \mu\text{g}\cdot\text{mL}^{-1}$ ampicillin at 37°C , 250 rpm for 12 h. Overnight cultures were diluted 1:100 (v/v) into fresh medium and grown at 37°C for 3 h to the early exponential phase. IPTG was added to a final concentration of 1 mM, simultaneously with two synthetic precursors—MBC and MAP—at 100 μM each. Cultures were incubated at 37°C , 250 rpm. Sampling (1 mL) began when the culture started turning red (indicating prodigiosin production) at 1 h intervals for 5, 6, 7, and 8 h post-inoculation.

Each time-point sample was processed in two ways: (i) 100 μL culture was directly used to measure OD_{600} for biomass monitoring²; (ii) the remaining 900 μL was centrifuged ($12,000\times g$, 5 min), and the cell pellet was resuspended in 200 μL acidified ethanol extraction solution [4% (v/v) 1 M HCl-ethanol], vortexed for lysis, and centrifuged again³. The supernatant (100 μL) was transferred to a 96-well plate for scanning absorption spectra (400–750 nm) and recording absorbance at the characteristic prodigiosin peak of 535 nm.

To evaluate prodigiosin stability in acidified ethanol, acidified ethanol extracts from IPTG-induced BL21(DE3)/pT7lac-PigC were stored at room temperature for 0–120 h, with periodic sampling (100 μL) for absorption spectral scanning.

Optimization of IPTG concentration and cultivation temperature

To reduce background expression and improve induction efficiency, the coupled effects of IPTG concentration and cultivation temperature were examined. Seed culture preparation and inoculation were performed as described above. After 3 h growth at 37°C to the early exponential phase, IPTG concentration gradients (0, 0.001, 0.01, 0.1, 1 mM) and temperature gradients (27 , 32 , 37°C) were applied, followed by induction at 250 rpm. Sampling (1 mL) was performed at 0, 1, 2, 3, 4, 5, and 6 h post-IPTG induction, with MBC and MAP substrates added to each sample (final concentration, 100 μM), followed by a 10 min incubation at room temperature.

Each time-point sample was processed as follows: (i) 100 μL culture for OD_{600} measurement; (ii) remaining 900 μL centrifuged ($12,000\times g$, 5 min), cell pellet resuspended in 200 μL acidified ethanol [4% (v/v) 1 M HCl-ethanol], and absorbance at 535 nm measured after centrifugation.

Cell morphology and aggregation assessment

Single colonies of *E. coli* TOP10/pHg-PigC were inoculated into LB medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C, 250 rpm for 12 h as seed culture. The overnight culture was diluted 1:100 (v/v) into fresh medium supplemented with Hg(II) standard solutions (final concentrations: 0, 0.007, and 0.125 μM) and incubated at 37 °C, 250 rpm for 4 h. Following exposure, bacterial suspensions were spread on clean glass slides with a drop of physiological saline, air-dried, and heat-fixed by rapidly passing through the outer flame of an alcohol lamp three times. Samples were stained with crystal violet solution (covering the entire bacterial film) for 1 min, then gently rinsed with slow-running water to remove excess stain. Cell morphology and aggregation state were examined under an optical microscope at 400 \times magnification and photographed.

Dose-response curve construction and preliminary sensor performance screening

Single colonies of recombinant strains TOP10/pHg-PigC, TOP10/pHg-HapC, TOP10/pHg-TreaP, and TOP10/pHg-TamQ were inoculated into 3 mL LB liquid medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C, 250 rpm for 12 h as seed culture. In parallel, a negative control strain TOP10/pET-21a (harboring the empty vector without *pigC* or *hapC* genes) was prepared to confirm that MBC and MAP substrates do not spontaneously produce colored products in the absence of the condensation enzyme. Overnight cultures were diluted 1:100 (v/v) into 1 mL of fresh LB medium (50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin) in 15 mL tubes. Hg(II) standard solutions were added by two-fold serial dilution⁴ to final concentrations: 0, 0.007, 0.015, 0.031, 0.062, 0.122, 0.244, 0.488, 0.977, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, 2000 nM. All treatment groups were induced at 37 °C, 250 rpm for 4 h.

After induction, MBC and MAP substrates were added to each tube (final concentration, 100 μM each), vortexed, and incubated at room temperature for 10 min to develop color. Samples were then analyzed: (i) 100 μL culture for OD₆₀₀ measurement; (ii) remaining 900 μL centrifuged (12,000 \times g, 5 min), cell pellet resuspended in 200 μL acidified ethanol [4% (v/v) 1 M HCl-ethanol], and absorbance at the characteristic prodigiosin peak of 535 nm measured after centrifugation.

Organomercury response assay

Single colonies of *E. coli* TOP10/pHg-PigC were inoculated into 3 mL LB medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C, 250 rpm for 12 h as seed

cultures. The overnight culture was diluted 1:100 (v/v) into 1 mL fresh LB medium (supplemented with 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin) in 15 mL centrifuge tubes. MeHgCl standard solutions were added by two-fold serial dilution to final concentrations of 0, 0.03, 0.05, 0.10, 0.20, 0.39, 0.79, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, and 200 nM. All treatments were incubated at 37 °C, 250 rpm for 4 h. After induction, MBC and MAP substrates were added to each tube (final concentration 100 μM), vortexed, and incubated at room temperature for 10 min. Samples were then analyzed: (i) 100 μL culture for OD₆₀₀ measurement; (ii) remaining 900 μL centrifuged (12,000 \times g, 5 min), the pellet resuspended in 200 μL acidified ethanol [4% (v/v) 1 M HCl-ethanol], and absorbance at 535 nm measured.

Metal selectivity assay of the sensor

Single colonies of recombinant strains TOP10/pHg-PigC and TOP10/pHg-HapC were inoculated into 3 mL LB liquid medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C and 250 rpm for 12 h as a seed culture. Overnight cultures were diluted 1:100 (v/v) into fresh medium and exposed to individual metal ions: target ion Hg(II) at low, medium, and high concentrations (62.5, 125, 250 nM); interference ions Mg(II), Ca(II), Cd(II), Mn(II), Cu(II), Pb(II), or Zn(II) at 6.25, 12.5, 25 μM . All treatment groups were cultured at 37 °C, 250 rpm for 4 h, with a blank control without metal addition cultured in parallel.

After induction, MBC and MAP substrates were added to each tube (final concentration 100 μM), vortexed, and incubated at room temperature for 10 min. Samples were analyzed: (i) 100 μL culture for OD₆₀₀ measurement; (ii) remaining 900 μL centrifuged (12,000 \times g, 5 min), cell pellet resuspended in 200 μL acidified ethanol [4% (v/v) 1 M HCl-ethanol], and absorbance at 535 nm measured. All experiments were performed with three biological replicates.

Anti-interference assay of the sensor

Single colonies of *E. coli* TOP10/pHg-PigC and TOP10/pHg-HapC were inoculated into 3 mL LB liquid medium containing 50 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin and cultured at 37 °C and 250 rpm for 12 h as a seed culture. Overnight cultures were diluted 1:100 (v/v) into fresh medium for binary metal co-exposure experiments: all treatment groups contained fixed Hg(II) at 50 nM or 100 nM, plus individual interfering metal ions (Pb(II), Cd(II), Zn(II), Mg(II), Cu(II), Mn(II), or Ca(II)) at low and high concentrations (5 μM and 10 μM). Mixed-metal exposure systems were cultured at 37 °C and 250 rpm for 4 h, with

positive controls containing only 50 nM or 100 nM Hg(II) and negative controls without metal addition.

After exposure, MBC and MAP substrates (10 μ L each, final concentration 100 μ M) were added, vortexed, and incubated at room temperature for 10 min. Samples were analyzed: (i) 100 μ L culture for OD₆₀₀ measurement; (ii) remaining 900 μ L centrifuged (12,000 \times g, 5 min), cell pellet resuspended in 200 μ L acidified ethanol [4% (v/v) 1 M HCl-ethanol], and absorbance at 535 nm measured.

Cell viability assay by colony-forming unit (CFU) counting

Single colonies of *E. coli* TOP10/pHg-PigC were inoculated into LB medium containing 50 μ g \cdot mL⁻¹ ampicillin and cultured at 37 °C, 250 rpm for 12 h as seed culture. LB agar was prepared, autoclaved, and cooled to approximately 50 °C. Hg(II) standard solutions were added to the molten agar to achieve final concentrations of 250 nM and 1000 nM, respectively, before pouring into sterile Petri dishes. Aliquots (100 μ L) of the seed culture were spread evenly onto the agar surface. The plates were incubated at 37 °C for 12 h. Colony-forming units were subsequently counted and photographed. Experiments were performed in triplicate.

Hg(II) response of the sensor in environmental water matrices

Environmental water samples were collected from laboratory deionized water, municipal tap water, Xianhu Park surface water (Luohu District, Shenzhen, Guangdong, China), and Daya Bay seawater (Yantian District, Shenzhen, Guangdong, China). Samples were clarified by centrifugation (10,000 rpm, 10 min) and sterilized through 0.22 μ m membrane filters (Millipore, Billerica, MA, USA).

Detection culture systems were prepared by mixing environmental water samples with 10 \times LB, 10 \times NaCl-free LB, and sterile water in the proportions shown in Table S4.

Table. S4 Detection culture system for different environmental water samples

Component	deionized water	Tap water	Surface water	Seawater
Water samples	90%	90%	90%	50%
10 \times LB	10%	10%	10%	-
10 \times NaCl-free LB	-	-	-	10%
Sterile water	-	-	-	40%

Overnight cultures of TOP10/pHg-PigC and TOP10/pHg-HapC were inoculated at 1% (v/v) into each culture-detection system containing 50 µg/mL ampicillin. Hg(II) was added by two-fold serial dilution to final concentrations of 0, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 nM, followed by induction at 37 °C, 250 rpm for 4 h. Cell density and pigment signal were measured as described above.

Statistical analysis

All experiments were performed with at least three independent biological replicates. Data are presented as mean ± standard deviation. Student's t-test or one-way ANOVA was used to determine statistical significance, with Tukey's post hoc test in GraphPad Prism 9.0. A P-value < 0.05 was considered statistically significant.

For LOD determination, the limit of blank (LOB) was first calculated as the mean absorbance of blank samples plus 1.645 times the standard deviation of the blank. The LOD was then calculated as LOB plus 1.645 times the standard deviation of a low-concentration sample.

Results

Growth characteristics of recombinant strains

The growth profiles of BL21(DE3) strains expressing pT7lac-PigC, pT7lac-HapC, pT7lac-TreaP, and pT7lac-TamQ were monitored after induction with 1 mM IPTG during early log phase. All strains exhibited typical growth curves (Fig. S1), with no significant growth inhibition observed under standard expression conditions, confirming their suitability for downstream applications.

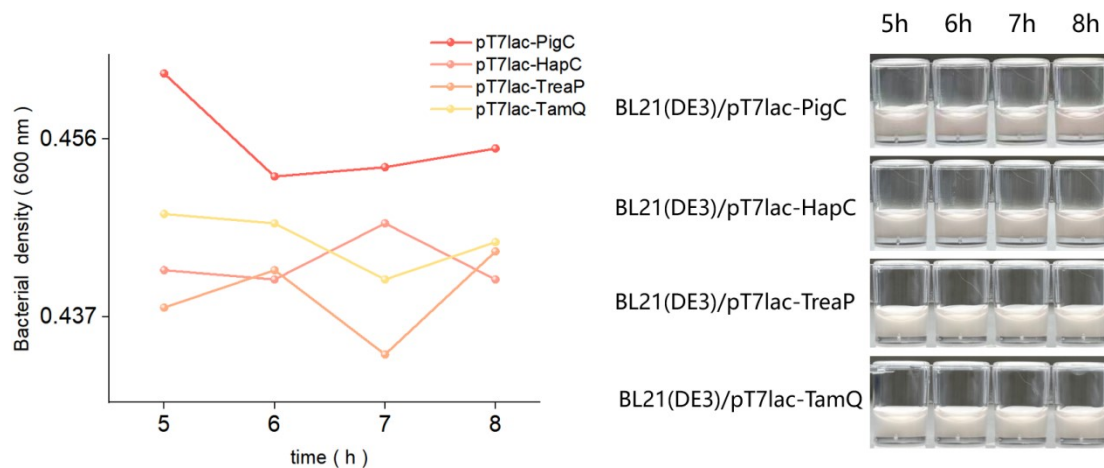


Fig. S1 Growth curves (left) and culture photographs (right) of BL21(DE3)/pT7lac-PigC, BL21(DE3)/pT7lac-HapC, BL21(DE3)/pT7lac-TreaP, and BL21(DE3)/pT7lac-TamQ induced with 1 mM IPTG. Data are representative of three independent experiments.

Stability of prodigiosin in acidified ethanol

To verify that the extraction solvent does not degrade the pigment over time, we monitored the absorption spectra and color of acidified ethanol extracts from IPTG-induced BL21(DE3)/pT7lac-PigC cultures stored at room temperature for 0–120 h. The extracts showed no significant spectral shift or color fading over the entire period (Fig. S2), confirming that acidified ethanol [4% (v/v) 1 M HCl-ethanol] provides a stable environment for the storage and measurement of prodigiosin.

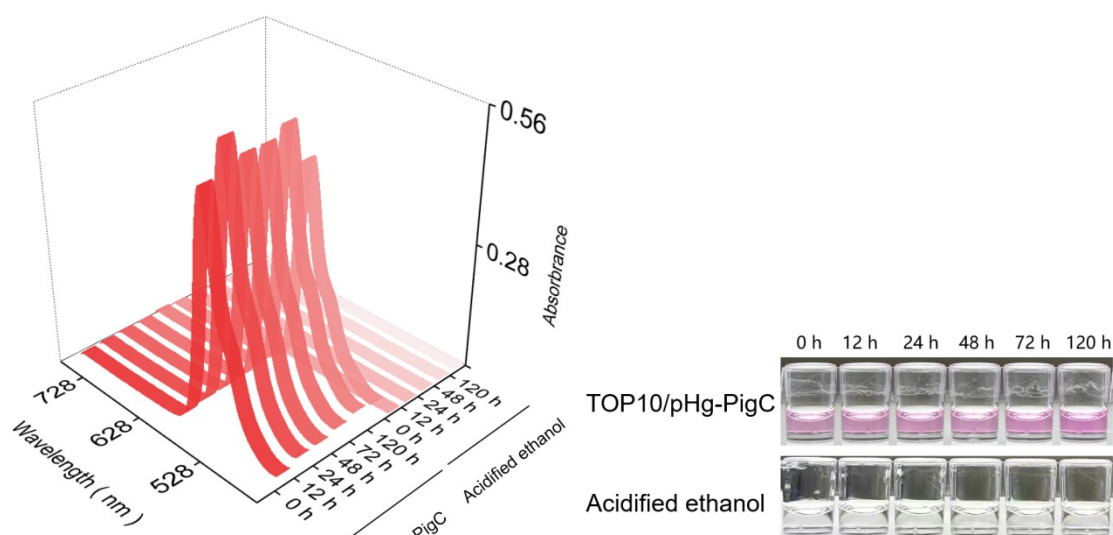


Fig. S2 Stability of prodigiosin in acidified ethanol. Absorption spectra (left) and color changes (right) of acidified ethanol extracts from IPTG-induced BL21(DE3)/pT7lac-PigC stored at room temperature for 0–120 h. Data are representative of three independent experiments.

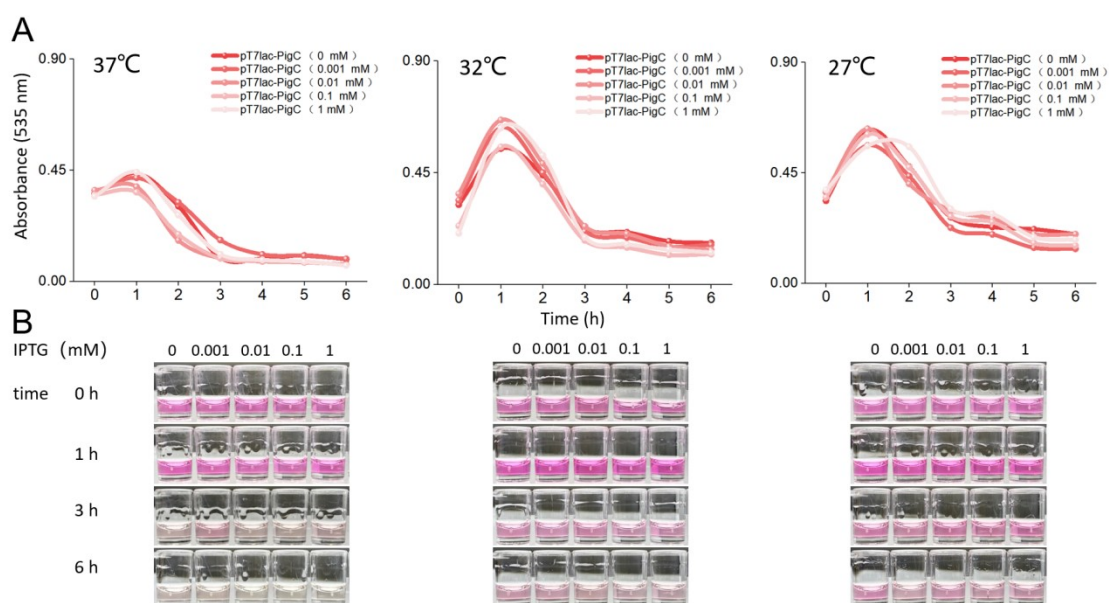
X-ray diffraction (XRD) analysis was not performed as prodigiosin is an intrinsically amorphous pigment; its long-term stability and optical properties are governed by molecular structure and the acidified ethanol environment (as evidenced by the consistent absorbance spectra in Fig. S2), rather than by crystalline lattice parameters.

Optimization of prodigiosin synthesis kinetics and induction conditions

To determine the optimal time window for prodigiosin production and minimize

background signal, we systematically evaluated the effects of induction temperature, IPTG concentration, and reaction time on pigment output.

Time-course analysis revealed that pigment accumulation peaked within 1 h after substrate addition for both PigC and HapC (Fig. S3A-D), followed by a gradual decline in absorbance, suggesting intracellular degradation or conversion of the product^{3,5}. The substrates MAP and MBC were added at a final concentration of 100 μ M simultaneously with IPTG induction. The background signal analysis showed that PigC exhibited higher baseline pigment production than HapC, without IPTG induction (Fig. S3B vs. S3D). TreaP and TamQ showed no significant pigment changes under any condition (Fig. S3E-H). Based on these results, a 10-minute substrate condensation time was selected for all subsequent experiments to ensure maximal signal capture while maintaining stability.



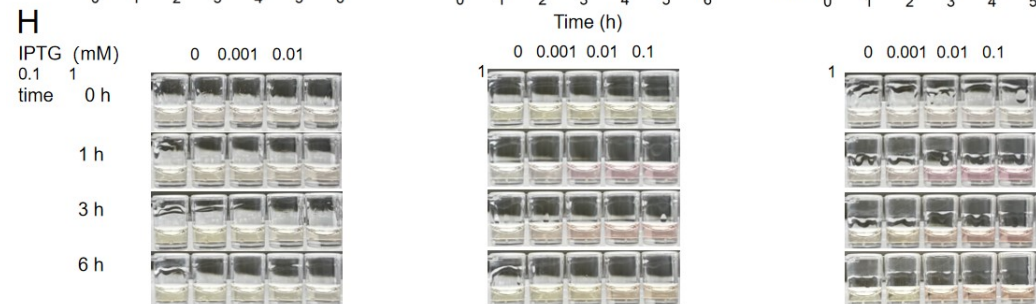
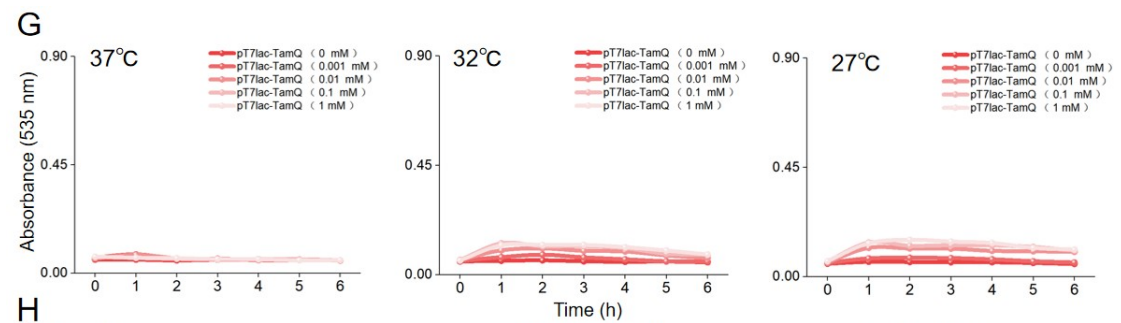
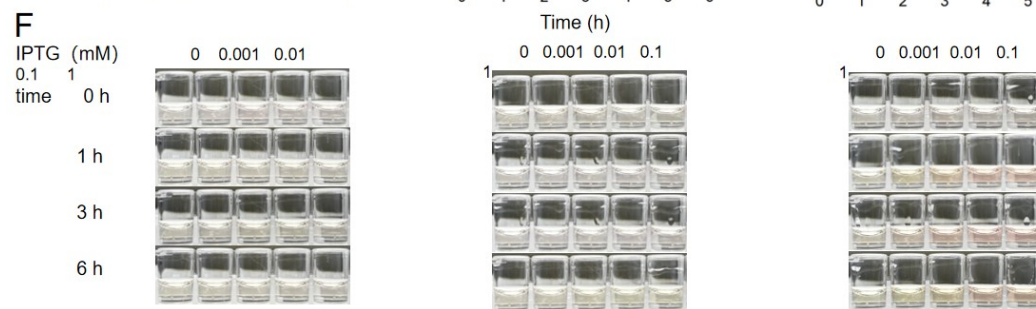
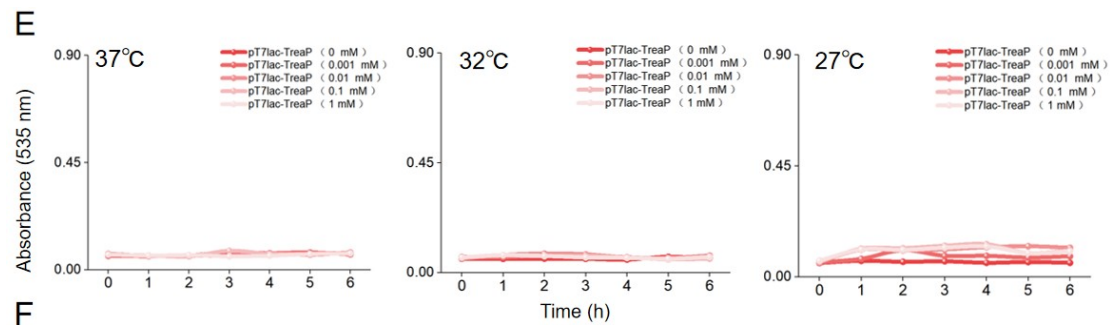
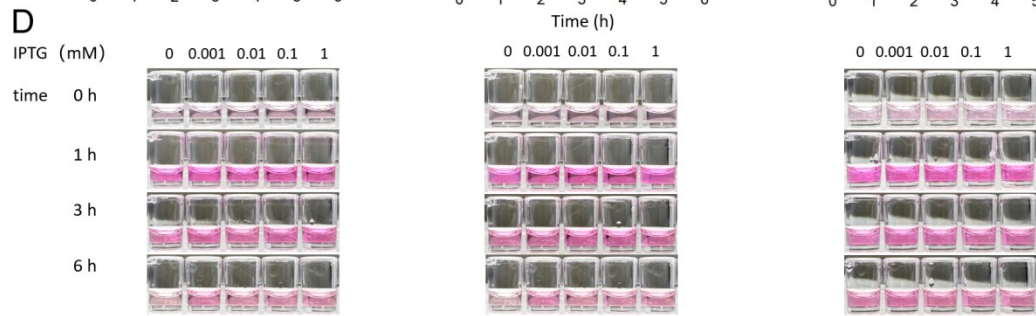
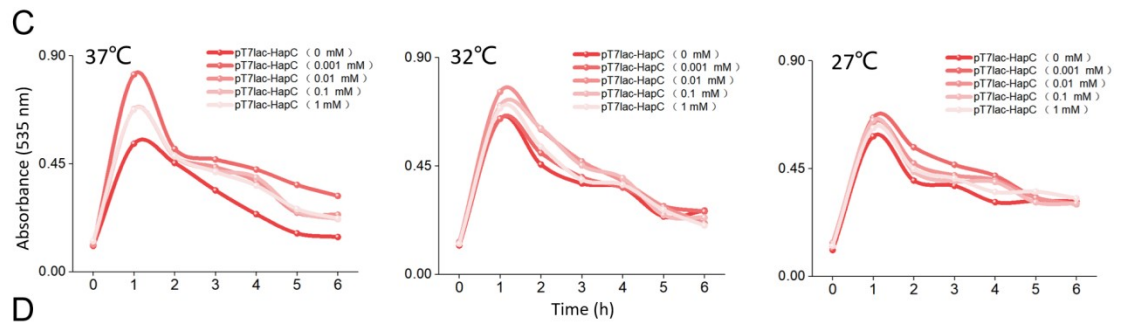


Fig. S3 Optimization of prodigiosin synthesis kinetics and induction conditions. Time-dependent A_{535} changes and color development of acidified ethanol extracts from BL21(DE3)/pT7lac-PigC (A, B), BL21(DE3)/pT7lac-HapC (C, D), BL21(DE3)/pT7lac-TreaP (E, F), and BL21(DE3)/pT7lac-TamQ (G, H) under different temperatures, IPTG concentrations, and induction durations. Data are representative of three independent experiments.

Effect of Hg(II) on sensor strain growth

To evaluate potential cytotoxic effects, we monitored the growth of TOP10/pHg-PigC, TOP10/pHg-HapC, and TOP10/pET-21a at various Hg(II) concentrations. The growth curves (Fig. S4) showed that Hg(II) concentrations up to 100 nM did not significantly inhibit cell growth. At the same time, the characteristic red color developed clearly after incubation with MBC and MAP substrates for 10 min, confirming the sensor's operational range.

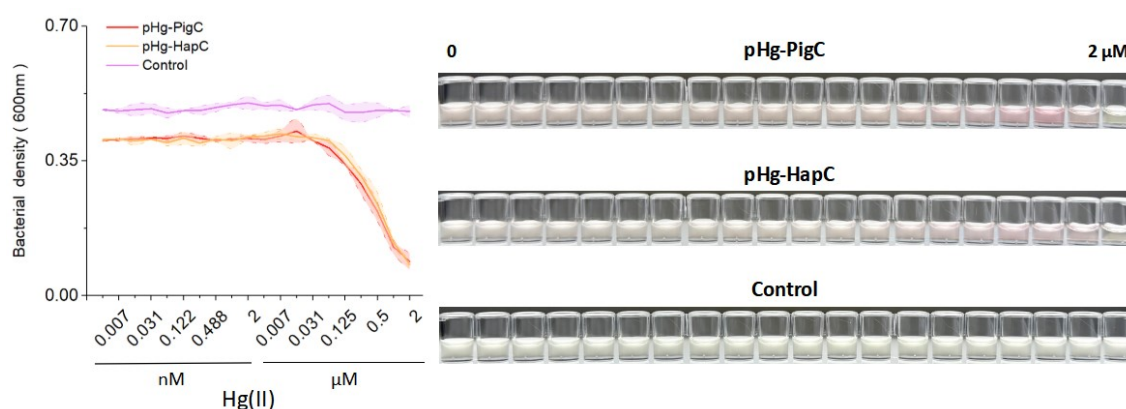


Fig. S4 Growth curves (left) and culture photographs (right) of TOP10/pHg-PigC, TOP10/pHg-HapC, and TOP10/pET-21a (control) at different Hg(II) concentrations. MBC and MAP were added 10 min before measurement.

Crystal violet staining and optical microscopy (400×) revealed a slight concentration-dependent trend toward cellular aggregation in TOP10/pHg-PigC following Hg(II) exposure, with marginally more clumping observed at 0.125 μM compared to the metal-free control (Fig. S5). However, this did not compromise pigment extraction efficiency, as evidenced by the white appearance of cell pellets post-extraction and the negligible residual absorbance ($A_{535} < 5\%$ of primary extraction) in secondary extracts, confirming that prodigiosin recovery remained quantitative across all tested concentrations.

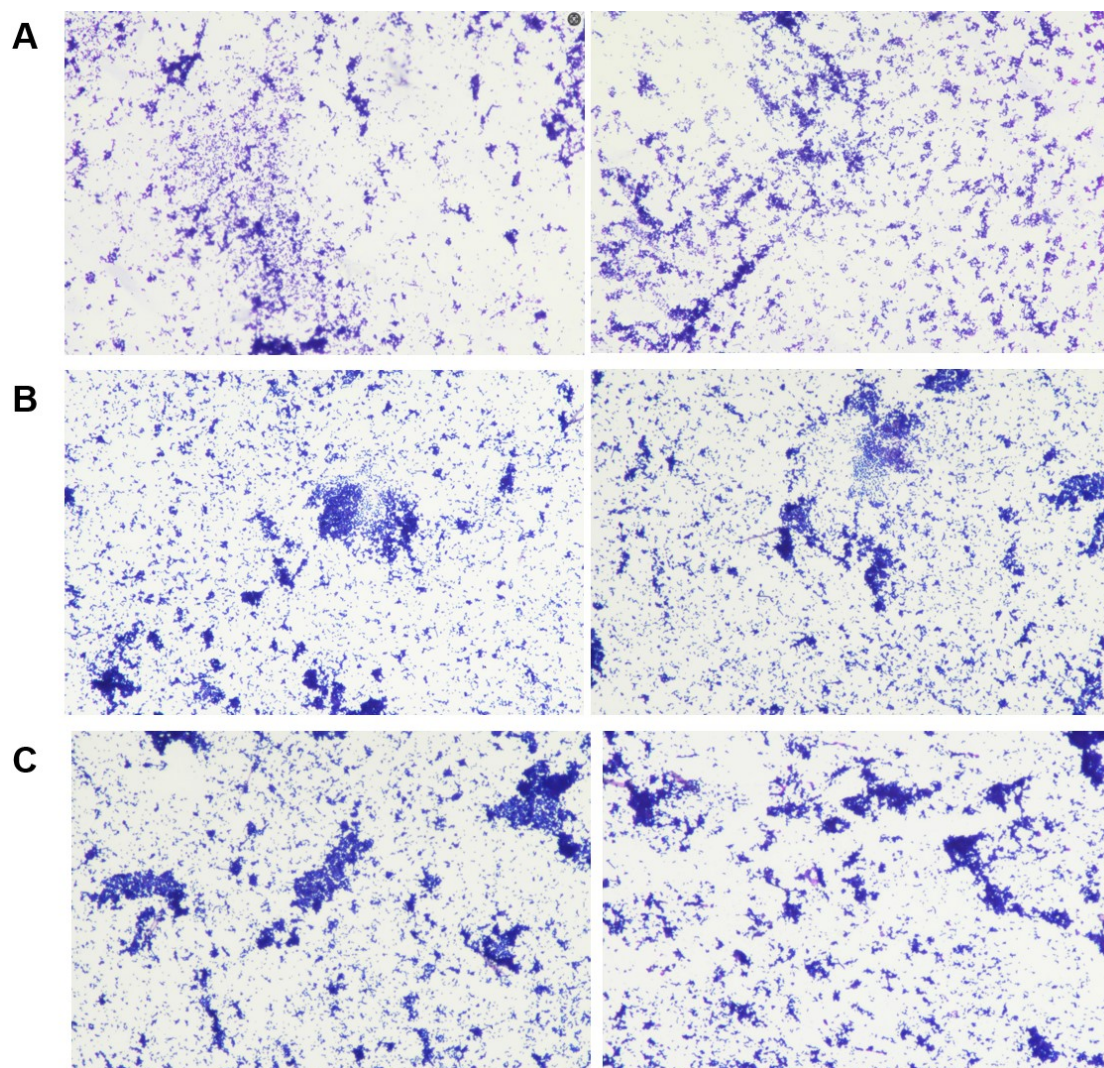


Fig. S5 Morphology and aggregation state of TOP10/pHg-PigC after exposure to Hg(II). Representative optical micrographs (400 \times) of crystal violet-stained cells following 4 h exposure to (A) 0 μ M, (B) 0.007 μ M, and (C) 0.125 μ M Hg(II).

Notably, the sensor TOP10/pHg-PigC showed negligible response to methylmercury (CH_3Hg^+) across the tested concentration range (0–200 nM) (Fig. S6), consistent with the established specificity of MerR for free Hg(II) ions rather than organomercury species⁶.

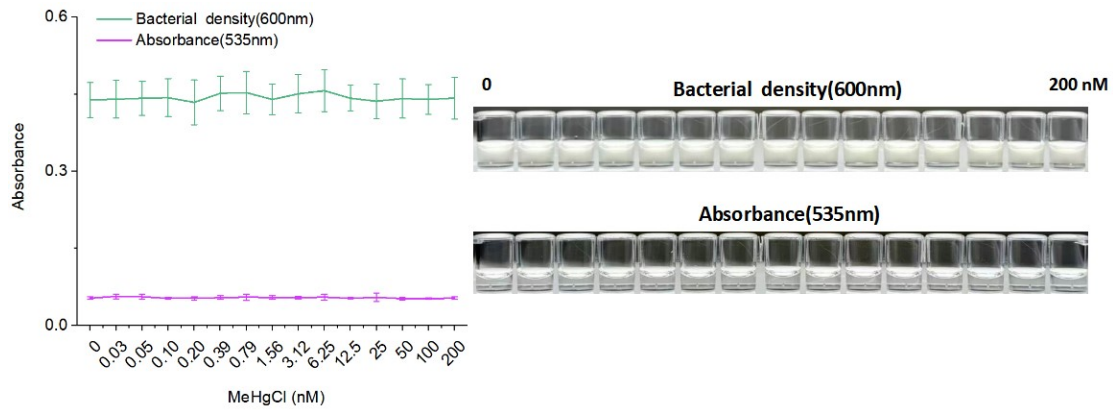


Fig. S6 Growth curves and absorbance at 535 nm of acidified ethanol extracts of TOP10/pHg-PigC exposed to 0–200 nM MeHgCl. Data are mean \pm standard deviation ($n = 3$).

Cytotoxicity assessment under metal co-exposure conditions

To evaluate whether co-occurring metal ions affect sensor viability, TOP10/pHg-PigC and TOP10/pHg-HapC were co-exposed to Hg(II) in the presence of interfering metals. Specifically, cells were treated with 50 nM Hg(II) plus 5 μ M of individual interfering metals (Pb(II), Cd(II), Zn(II), Mg(II), Cu(II), Mn(II), or Ca(II)), or 100 nM Hg(II) plus 10 μ M of the same interfering metals. Cell density was monitored by measuring OD₆₀₀ after 4 h of exposure. No significant growth inhibition was observed under any co-exposure condition compared to Hg(II)-only controls, confirming that the presence of interfering metals does not compromise sensor performance.

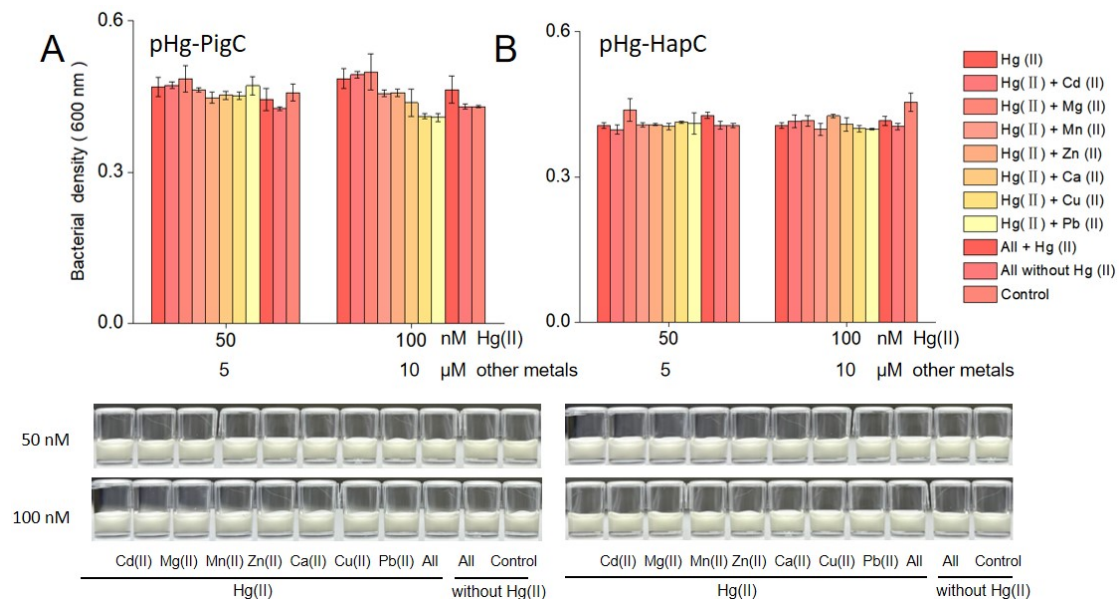


Fig. S7 Growth of TOP10/pHg-PigC (A) and TOP10/pHg-HapC (B) co-exposed to Hg(II) and interfering metal ions. Cells were incubated with 50 nM Hg(II) + 5 μ M

interfering metals or 100 nM Hg(II) + 10 μ M interfering metals for 4 h. Data are mean \pm standard deviation (n = 3).

Effect of high Hg(II) concentrations on cell viability

To directly assess whether the signal attenuation observed at supra-optimal Hg(II) concentrations (>250 nM) correlates with reduced cell viability, colony-forming unit (CFU) assays were performed. As shown in Fig. S8, the number of viable colonies on agar plates containing 250 nM Hg(II) was approximately three-fold higher than those on plates containing 1000 nM Hg(II). This significant reduction in viable cell counts at elevated mercury concentrations supports the interpretation that metabolic burden or toxicity-induced stress contributes to the observed decrease in pigment production at high Hg(II) levels.

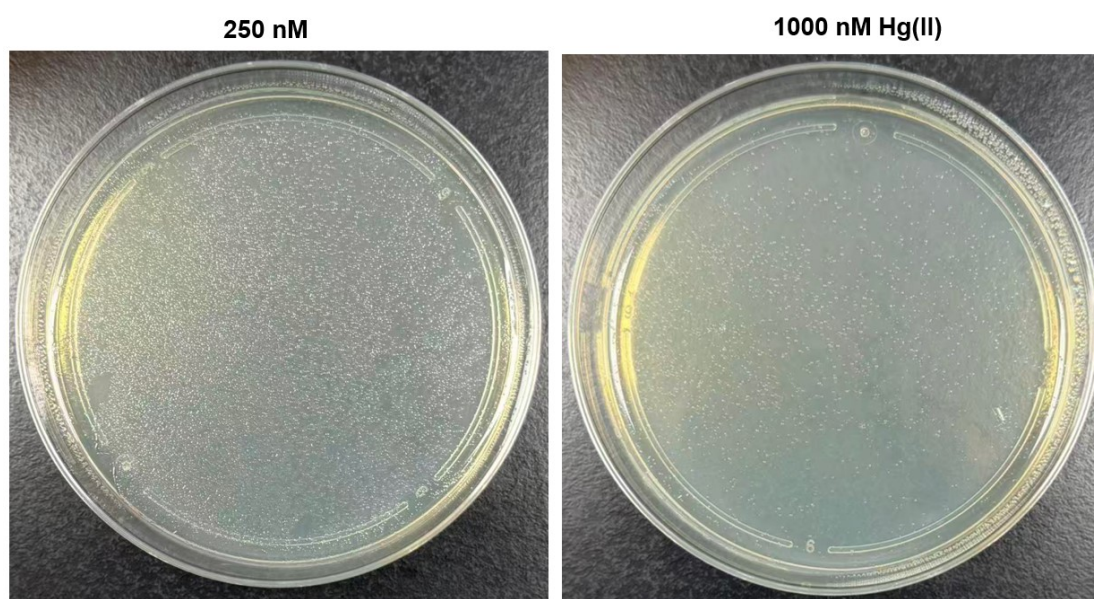


Fig. S8 Colony-forming unit viability assay of TOP10/pHg-PigC exposed to high Hg(II) concentrations. Representative photographs of LB agar plates showing colony formation at 250 nM and 1000 nM Hg(II), respectively, demonstrating markedly reduced viability at the higher concentration.

Performance in environmental water matrices

To validate real-world applicability, TOP10/pHg-PigC and TOP10/pHg-HapC were challenged with Hg(II) in four water matrices. The sensors maintained robust growth in all matrices (Fig. S9A, B) and exhibited consistent dose-dependent responses even

in high-salinity seawater. The 535 nm absorption spectra of extracts from 100 nM Hg(II) treatments showed characteristic prodigiosin peaks across all water types (Fig. S9C), confirming matrix compatibility.

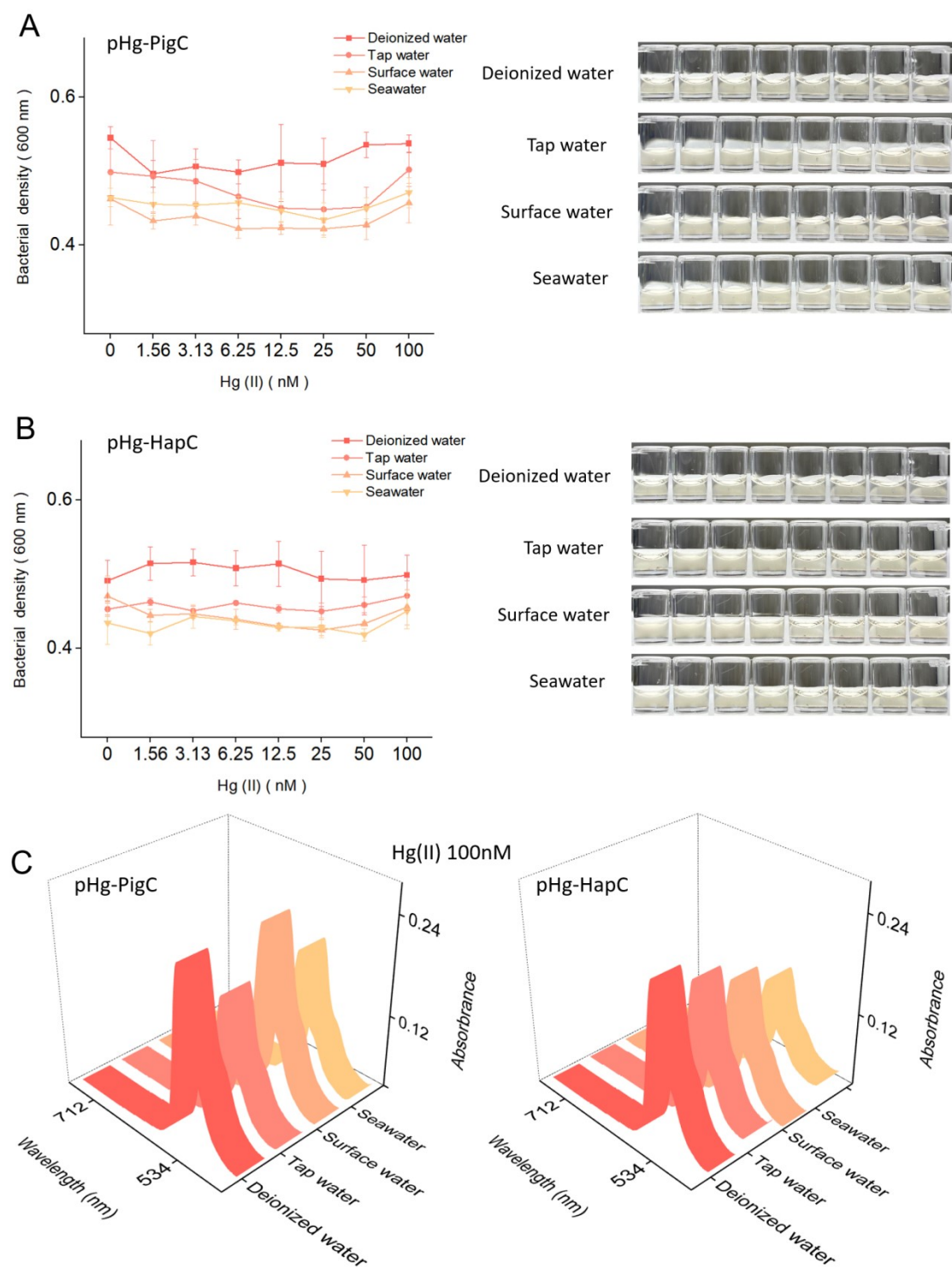


Fig. S9 Hg(II) detection in environmental water matrices. Growth curves (A, B) and representative culture photographs of TOP10/pHg-PigC and TOP10/pHg-HapC in four water matrices exposed to gradient Hg(II) concentrations. (C) Absorption spectra of acidified ethanol extracts responding to 100 nM Hg(II).

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