

## **Supplemental Information**

### **Development of a bioavailable Hg(II) sensing system based on MerR-regulated visual pigment biosynthesis**

Yan Guo<sup>1</sup>, Chang-ye Hui<sup>2\*</sup>, Lisa Liu<sup>3</sup>, Min-peng Chen<sup>1</sup> & Hong-ying Huang<sup>1</sup>

<sup>1</sup> National Key Clinical Specialty of Occupational Diseases, Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, China

<sup>2</sup> Department of Pathology & Toxicology, Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, China

<sup>3</sup> Lewis Katz School of Medicine, Temple University, Pennsylvania, USA

Correspondence and requests for materials should be addressed to C.H. (email: hcy\_sypu@hotmail.com)

**AGATCT**CTAAGGCATAGCTGACCTTGCCAGGCCTGCTTCGCCCTGTAGT  
GACGCGATCAACGGGCAGGAAACATTCCCCTTTCGTGCATGGCAGGCG  
CACACGAGTTCAGACAGCACGGTTTCCATGCGCGCCAAGTCGGCCATCT  
TCTCGCGCACGTCTTGAGCTTGTGTTTCGGCCAGGCTGCTGGCCTCCTC

*merR*

GCAGTGGGTGCCATCGTCGAGCCGCAACAGCTCGGCAATCTCGTCCAG  
ACTGAACCCCAGCCGCTGTGCCGATTTACGAATTTACCCGAACCACG  
TCCGCCTCCCCATAGCGGCGGATGCTGCCGTAAGGCTTGTCCGGTTCC  
CGCAACAGGCCCTTGCGCTGATAGAAGCGGATTGTCTCCACGTTGACCC  
CGGCCGCCTTGCCAAAACGCCAATGGTCAGTTTTTCAAATTATTTTCC

mer bidirectional promoter region

ATATCGCTTGACTCCGTACATGAGTACGGAAGTAAGGTTACGCTATCCAA  
TCCAAATTCAAAGGGCCAACGT**TCTAGAAATAATTTGTTAACTTTAAG**

rbs

*NdeI*

Violacein expression cassette

*SacI*

**AAGGAG**ATATACAT**ATG** **vioA-vioB-vioC-vioD-vioE** **TAA****GAGCTCCGT**  
CGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCTGAGA  
TCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCAC

T7 terminator

CGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACGGGTCTTG  
AGGGGTTTTTTG

**pPmer-vio**

**AGATCT**CTAAGGCATAGCTGACCTTGCCAGGCCTGCTTCGCCCTGTAGT  
GACGCGATCAACGGGCAGGAAACATTCCCCTTTCGTGCATGGCAGGCG  
CACACGAGTTCAGACAGCACGGTTTCCATGCGCGCCAAGTCGGCCATCT  
TCTCGCGCACGTCTTGAGCTTGTGTTTCGGCCAGGCTGCTGGCCTCCTC

*merR*

GCAGTGGGTGCCATCGTCGAGCCGCAACAGCTCGGCAATCTCGTCCAG  
ACTGAACCCCAGCCGCTGTGCCGATTTACGAATTTACCCCGAACCACG  
TCCGCCTCCCCATAGCGGCGGATGCTGCCGTAAGGCTTGTCCGGTTCC  
CGCAACAGGCCCTTGCCTGATAGAAGCGGATTGTCTCCACGTTGACCC  
CGGCCGCTTGGCAAAAACGCCAATGGTCAGGTTTTCAAATTATTTCC

mer bidirectional promoter region

ATATCGCTTGACTCCGTACATGAGTACGGAAGTAAGGTTACGCTATCCAA  
TCCAAATTCAAAGGGCCAACGT**TCTAGA**AATAATTTGTTAACTTTAAG

rb

*NdeI*

**AAGGAG**ATATACAT**ATG**GTTTCTAAAGGCGAAGAACTGTTACCGGCGTT  
GTTCCGATCCTGGTTGAACTGGATGGTGTGTTAACGGCCACAAATTCAG  
CGTCAGCGGCGAAGGCGAAGGCGATGCGACCTACGGCAAACCTGACCCCT  
GAAATTCATCTGCACCACCGGTAAACTGCCGGTTCCGTGGCCGACCCTG  
GTTACCACCCTGACCTACGGCGTTCAGTGCTTCAGCCGTTACCCGGATCA  
CATGAAACAGCACGATTTCTTCAAAGCGCGATGCCGGAAGGCTACGTTT  
AGGAACGTACCATCTTCTTCAAGGATGATGGCAACTACAAAACCCGTGCG

*eGFP*

GAAGTTAAATTCGAAGGCGATACGCTGGTTAACCGTATCGAACTGAAAGG  
CATCGATTTCAAAGAAGATGGTAACATCCTGGGGCACAAACTGGAATACA  
ACTACAACAGCCACAACGTTTATATCATGGCCGACAAACAGAAAAACGGA  
ATCAAAGTTAATTTCAAGATTCGCCACAATATCGAAGACGGTTCTGTGCAA  
CTTGCAGATCATTACCAGCAAAACACCCCAATTGGCGATGGACCCGTCCT  
GCTGCCGACAAACCATTACCTGTGACACAGTCAGCGCTGTCCAAGGAT  
CCGAACGAAAAACGTGATCACATGGTTCTGCTGGAATTCGTTACCGCGGC

*HindIII*

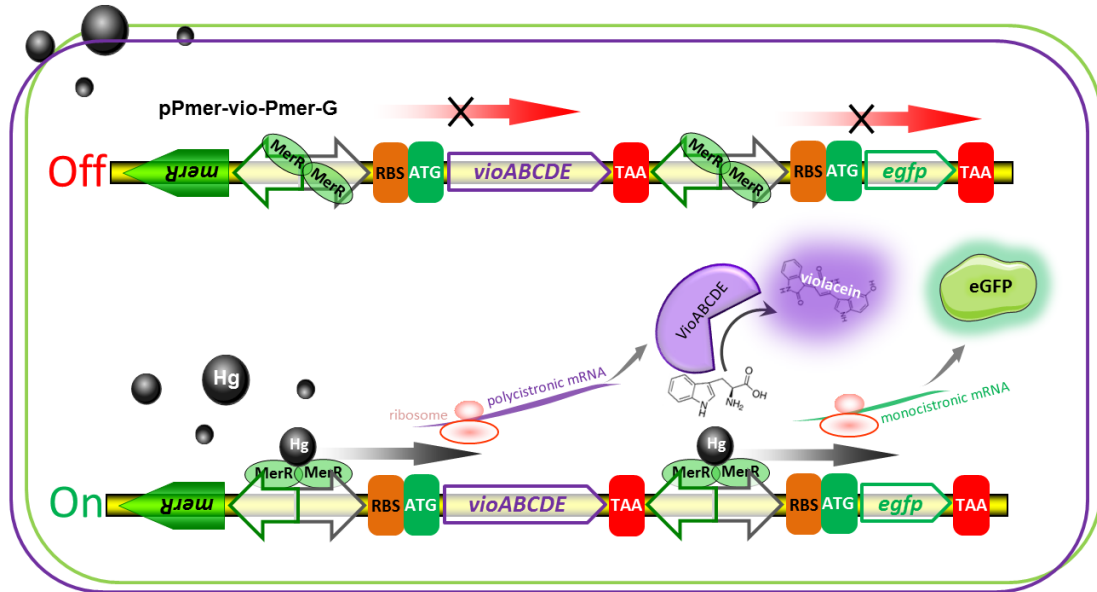
GGGCATCACCTGGGTATGGATGAACTGTACAAA**TAA****AAGCTT**GCGGCC  
GCACTCGAGCACCACCACCACCACCTGAGATCCGGCTGCTAACAAAG  
CCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGC  
ATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

pPmer-G

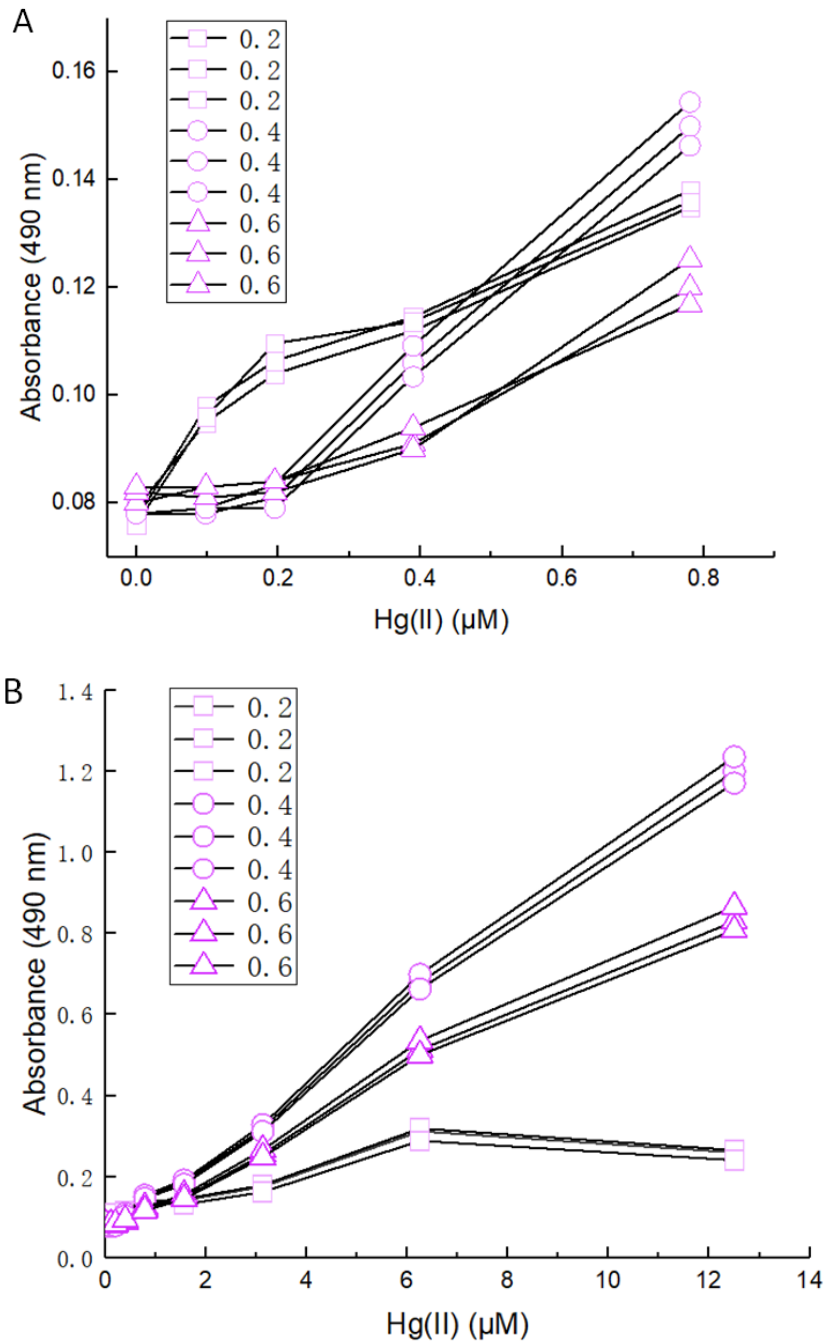
**AGATCT**CTAAGGCATAGCTGACCTTGCCAGGCCTGCTTCGCCCTGTAGT  
 GACGCGATCAACGGGCAGGAAACATTCCCCTTTCGTGCATGGCAGGCG  
 CACACGAGTTCAGACAGCACGGTTTCCATGCGCGCCAAGTCGGCCATCT  
 TCTCGCGCACGTCCTTGAGCTTGTGTTTCGGCCAGGCTGCTGGCCTCCTC  
*merR*  
 GCAGTGGGTGCCATCGTCGAGCCGCAACAGCTCGGCAATCTCGTCCAG  
 ACTGAACCCCAGCCGCTGTGCCGATTTACGAATTTACCCGAACCACG  
 TCCGCCTCCCCATAGCGGCGGATGCTGCCGTAAGGCTTGTCCGGTTCC  
 CGCAACAGGCCCTTGCGCTGATAGAAGCGGATTGTCTCCACGTTGACCC  
 CGGCCGCTTGGCCAAAACGCCAATGGTCAGGTTTTCAAATTATTTTC  
mer bidirectional promoter region  
 ATATCGCTTGACTCCGTACATGAGTACGGAAGTAAGGTTACGCTATCCAA  
 TCCAAATTCAAAGGGCCAACGT**TCTAGAA**AATAATTTTGTTTAACTTTAAG  
rbs *NdeI* Violacein expression cassette *SacI*  
 AAGGAGATATACAT**ATG**—**vioA-vioB-vioC-vioD-vioE**—**TAA****GAGCTC**ATC  
 GCTTGACTCCGTACATGAGTACGGAAGTAAGGTTACGCTATCCAATCCAA  
rbs *NotI*  
 ATTCAAAAGGGCCAACGTGAAGGAGATATACC**ATG**—*eGFP*—**TAA****GCGG**  
**CCGC**ACTCGAGCACCACCACCACCACCTGAGATCCGGCTGCTAACAA  
 AGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTA  
 GCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

**pPmer-vio-Pmer-G**

**Fig S1.** The cloning/expression region of vectors used in this study. DNA sequence and annotation data are all marked.



**Fig S2.** Genetic construct for sensing bioavailable Hg(II) with two reporters. The violacein biosynthesis module and eGFP reporter module were placed under the control of its own *mer* promoter separately in one genetic construct. Upon binding of bioavailable Hg(II) to the dimeric MerR, the protein-metal-ion complex switches from a repressor to an activator to allow the transcription of two reporters.



**Fig S3.** The response of whole-cell biosensor TOP10/pPmer-vio induced with increased concentrations of Hg(II). Early exponential-phase ( $OD_{600}=0.2$ ), exponential-phase ( $OD_{600}=0.4$ ), and late exponential-phase ( $OD_{600}=0.6$ ) cultures (with three repeats) were induced with 0, 0.098, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, and 12.5  $\mu\text{M}$  Hg(II) at 37 °C for 12 h. Whole-cell biosensor dose-response curves with Hg(II) concentrations range from 0-0.78  $\mu\text{M}$  (A), and dose-response curves with Hg(II) concentrations range from 0-12.5  $\mu\text{M}$