

Supplemental Information

Sulfane sulfur is an intrinsic signal for the organic peroxide sensor OhrR of *Pseudomonas aeruginosa*

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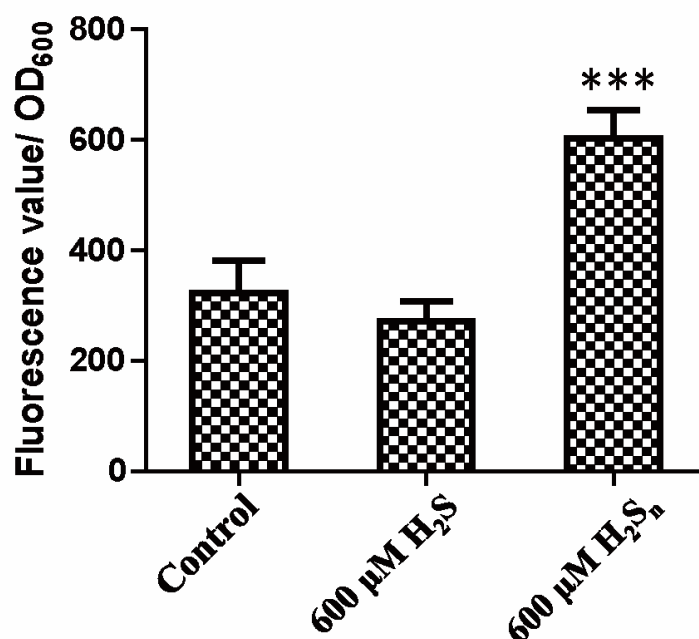


Figure S1. OhrR senses sulfane sulfur rather than H₂S. *E. coli* BL21 containing pBBR5-OhrR-P_{ohr}-mKate were induced by 600 μM H₂S or H₂S_n. The control was uninduced *E. coli*. Symbol *** indicate the sample is significantly different from the control ($p < 0.001$).

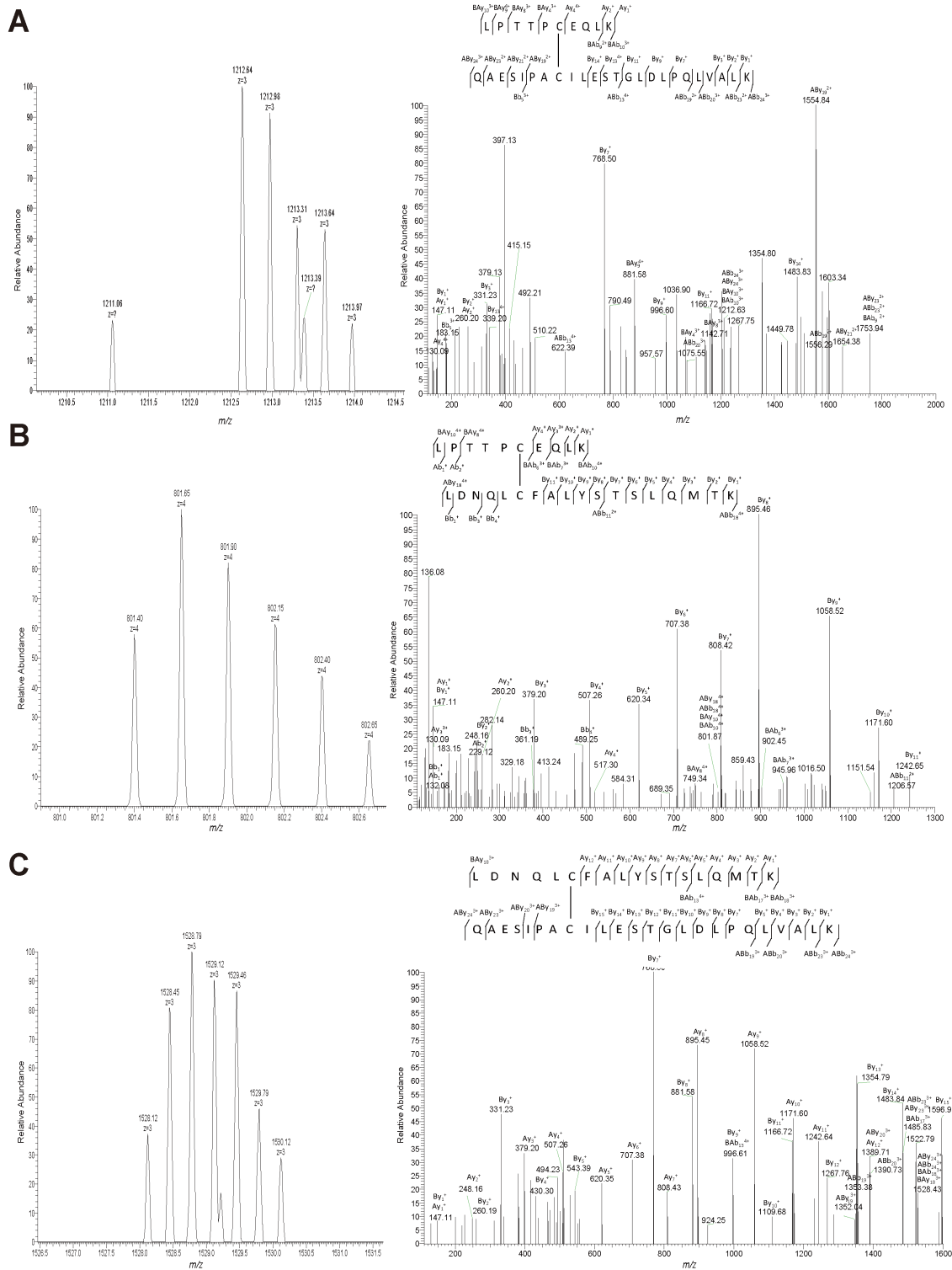


Figure S2. LC-MS/MS analysis of H₂S_n-treated OhrR. Mass spectrum of individual tryptic peptides of H₂S_n-treated OhrR. A) Left: The 3+ charged peak (m/z:1212.64) corresponding to the Cys⁹-Cys¹²¹ disulfide-containing peptide of interest (theoretical molecular mass: 3635.93Da). Right: MS/MS fragmentation of the 3+ charged peptide (m/z: 1212.64). B) Left: The 4+ charged peak (m/z: 801.40)

corresponding to the Cys⁹-Cys¹⁹ disulfide-containing peptide of interest (theoretical molecular mass: 3202.59 Da). Right: MS/MS fragmentation of the 4+ charged peptide (m/z: 801.40). C) Left: The 3+ charged peak (m/z: 1528.12) corresponding to the Cys¹⁹-Cys¹²¹ disulfide-containing peptide of interest (theoretical molecular mass: 4582.35 Da). Right: MS/MS fragmentation of the 3+ charged peptide (m/z: 1528.12).

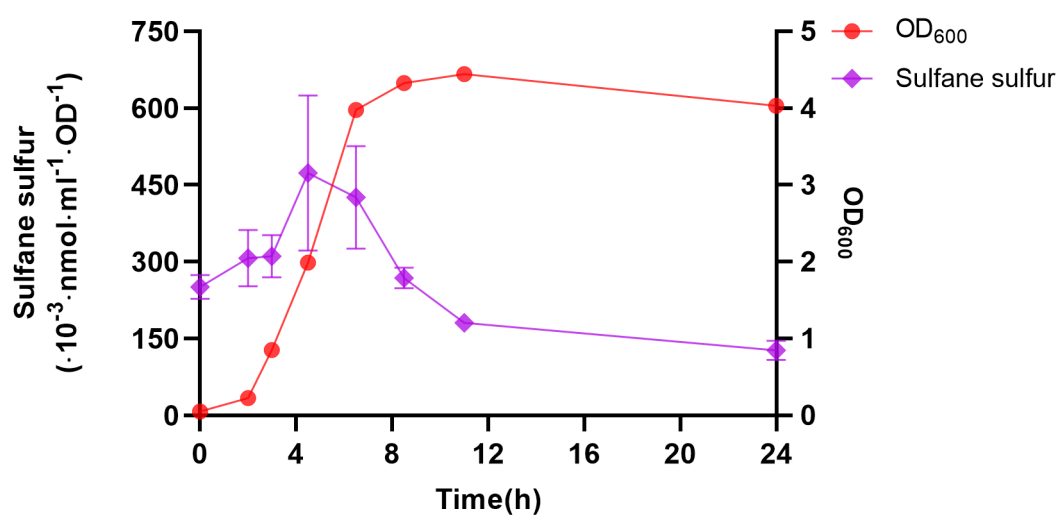


Figure S3. Intracellular sulfane sulfur of the reporter bacterium in LB medium changed with growth phases. Intracellular sulfane sulfur contents (■) and OD_{600nm} (●) were measured associated with growth phases. *E. coli* BL21 (pBBR5-OhrR-Pohr-mKate) was cultured in LB. The data are average of at least three samples with standard deviation (error bar).

Table S1. Strains and plasmids used in this study.

Strain/plasmid	Characteristic	Source
<i>Escherichia coli</i> BL21(DE3)	Cloning strain	Invitrogen
<i>Pseudomonas aeruginosa</i> P <i>AO1</i>	Wild type	ATCC 15692
Plasmids		
pBBR1mcs5	Gm, broad host range	Kovach ^a
pBBR5-OhrR –P _{ohr} -mKate	pBBR1mcs5 vector with <i>ohr</i> promotor, <i>ohrR</i> , and <i>mkate</i> genes	This study
pBBR5-OhrR –P _{ohr} -mKate- P _{t_{re}} -pdo	pBBR1mcs5 vector with <i>ohr</i> promotor, <i>ohrR</i> , <i>mkate</i> and <i>pdo</i> genes	
pET28a	Kmr, expression vector	Invitrogen
pET28a-OhrR	pET28a containing OhrR with N terminal his-tag	This study
pET28a-OhrR/C19S	pET28-OhrR with Cys19Ser	This study
pET28a-OhrR/C121S	pET28-OhrR with Cys121Ser	This study
pET28a-OhrR/C19SC121S	pET28-OhrR with Cys19Ser, Cys121Ser	This study
pET28a-OhrR/C9S	pET28-OhrR with Cys9Ser, Cys19Ser	This study
pET28a-OhrR/C9SC19S	pET28-OhrR with Cys9Ser, Cys19Ser	This study
pET28a-OhrR/C9SC121S	pET28-OhrR with Cys9Ser, Cys121Ser	This study

Table S2. Primers used in this study.

Primers	Sequence (5'-3')	Usage
ohrR-1	AGCAAATGGGTCGCGGATCCATGTCCCGAC TGCCCACCA	OhrR recombinant expression
ohrR-2	GGTGGTGGTGGTGGTCTCGAGTTCAATCCGGT GCTTGCAGGTTACC	
ohrR-C9S-1	CACCCCTTCCGAGCAGCTCAAGCTGGACAA CCAG	OhrR/C9S recombinant expression
ohrR-C9S-2	GCTGCTCGGAAGGGGTGGTGGGCAGTCG	
ohrR-C19S-1	CCAGCTGTCCTTCGCCCTGTATTCCACCTCG CTG	OhrR/C19S recombinant expression
ohrR-C19S-2	GGGCGAAGGACAGCTGGTTGTCCAGCTTGA GCT	
ohrR-C121S-1	TCCCGCCTCCATCCTGGAGAGTACCGGCCT CG	OhrR/C121S recombinant expression
ohrR-C121S-2	CCAGGATGGAGGCGGGAATGCTCTCGGC	
ohrR+Pohr F	TCTAGAGAAAGAGGAGAAATACTAGATGTC CCGACTGCCCACCA	
ohrR+Pohr R	CTAGTATTTCTCCTCTTTCTCTAGAGAGAGT CACCTGTCTGATTTGTACGT	
mKate F	TCTAGAGAAAGAGGAGAAATACTAGATGTC AGAATTAATTAAGAAAATATGCACATG	Primers for construction of pBBR5-OhrR-P _{ohr} -m Kate
mKate R	CTTACAATTTCCATTCGCCATTTCAACGATG TCCTAATTTTCGACG	
pBBR1MCS-5-F	CTAGTATTTCTCCTCTTTCTCTAGACAACAT ACGAGCCGGAAGCATAAAG	
pBBR1MCS-5-R	AATGGCGAATGGAAATTGTAAGCG	

^a A ribosome binding site (sbs) sequence (gaggag) was inserted before *mkate*.

Table S3. Mass data of OhrR from LTQ-Orbitrap tandem mass spectrometry.

OhrR	Modification	Observed mass	Calculated mass	
		(MH⁺)	(MH⁺)	
Peptide 1	R ₉ S-SR' ₁₂₁	3635.9218	3635.9326	107
				108
				109
Peptide 2	R ₉ S-SR' ₁₉	3202.6056	3202.5884	110
Peptide 3	R ₁₉ S-SR' ₁₂₁	4582.3669	4582.3544	111
				112

Peptide mass was calculated on the website:

<http://db.systemsbio.org:8080/proteomicsToolkit/FragIonServlet.html>