

## **A *Burkholderia thailandensis* multidrug efflux pump with unexpected roles in antibiotic resistance**

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**Table S1. Thermal stability of reduced and oxidized OstR**

<b>Protein: Ligand</b>	<b>T<sub>m</sub> (°C)</b>
<b>OstR (reduced)</b>	34.1 ± 0.1
<b>OstR: Bipyridyl</b>	33.4 ± 0.2
<b>OstR: DNA (1: 2)</b>	39.3 ± 0.2
<b>OstR: ZnCl<sub>2</sub> (1: 100)</b>	37.2 ± 0.1
<b>OstR: H<sub>2</sub>O<sub>2</sub> (1: 100)</b>	38.5 ± 0.3
<b>OstR: CHP (1: 100)</b>	39.9 ± 0.3
<b>OstR: tBHP (1: 100)</b>	41.6 ± 0.1

**Table S2. Affinity of OstR variants for *emrB* promoter DNA**

Protein	Kd (nM)	Hill coefficient (n <sub>H</sub> )
OstR	6.7 ± 0.8	1.9 ± 0.3
H <sub>2</sub> O <sub>2</sub> -OstR	9.7 ± 1.1	1.9 ± 0.4
CuCl <sub>2</sub> -OstR	6.5 ± 1.0	1.8 ± 0.4
OstR-C3A	17.5 ± 2.3	1.6 ± 0.3
OstR-C4A	23.4 ± 3.2	1.2 ± 0.3
OstR-C169A	539 ± 58	1.9 ± 0.2

**Table S3. Thermal stability of cysteine variants**

Protein	Reduced	Bipyridyl	ZnCl <sub>2</sub> (1:100)	DNA (1:2)	H <sub>2</sub> O <sub>2</sub> (1:100)	CuCl <sub>2</sub> (1:100)
OstR-C3A	44.7 ± 0.1	41.5 ± 0.1	50.1 ± 0.5	67.5 ± 0.6	nt	nt
OstR-C4A	50.6 ± 0.1	50.4 ± 0.2	51.1 ± 0.3	50.2 ± 0.2	nt	nt
OstR-C169A	nt	nt	56.8 ± 0.3	41.2 ± 0.2	nt	nt

T<sub>m</sub> ± SD (°C). nt = no transition.

**Table S4. Primer sequences**

Cloning, mutagenesis, and amplification of <i>emrB</i> promoter DNA	
OstR_tran_Fw	CGTTTTTCCTCATATGCACTGTTGC
OstR_tran_Rv	CGAGGGAATTCCATGCTCTTTC
I21_C3A_Fw	GCGGCAGCCATATGCACGCGTGC GGTTTCGCGCAATCGACCGGAC
I21_C3A_Rv	GAACCGCACGCGTGCATATGGCTGCCGCGCGGCACCAGGC

I21_C4A_Fw	GCGGCAGCCATATGCACTGTGCCGGTTCGCGCAATCGACCGGAC
I21_C4A_Rv	GAACCGGCACAGTGCATATGGCTGCCGCGCGGCACCAGGC
I21_C169A_Fw	GATCCGCGAGCCGCCGCGTCGATCGCCGAGCCGCCGC
I21_C169A_Rv	GATCGACGCGGCGGCTGCGGAATCCGCGGGCGCCC
BTH_22int_Fw	GGTAAGCGCTGACCGGGA A
BTH_22int_Rv	AGCCATGCGTCTCTCCT
<b>Complementation and mutant verification</b>	
LacZ_148	GGGTAACGCCAGGGTTTTCC
BTH_21_Fw	CACCGGGCAAGGCGGC
Cre_del21_Fw	CACCGGGCAAGGCGGC
Cre_del21_Rv	GACGAAGCCCTTCTTCTCG
Tra_delE_Fw	TGACAAGACACTTACTGTCGGTC
Tra_delE_Rv	GAGATCACCCACGTGCCTTC
OstR_XbaI_Fw	GATCTTCTAGACGCGATGCGGACCGGACA
OstR_KpnI_Rv	AAGCGGGTACCGTCGCAGCCA
Con_pBBR_XbaI	GTAATACGACTCACTATAGGGC
Con_pBBR_KpnI	GCAATTAACCCTCACTAAAGG
<b>qRT-PCR</b>	
OstrR_auto_Fw	ATGCACTGTTGCGGTTTCGC
OstR_auto_Rv	ATAGCCGAGGCTCGATTCGAG
EmrB_F1	GGATCACCGACAACACTACACG
EmrB_R1	TTGAACCAGTCACGGTCCTT
AmrB_Fw	TGTCGATGAGCAAGGTCGTG
AmrB_Rv	TGATCTGCTTCATCGCCTTCA C
BpeF_Fw	GATCACCGTCACGTTCAAGCT
BpeF_Rv	GGCGATCCTTCACGTTGATGAG
Glusyn_qPCR_Fw	GCAAGAAGAGCCACGAAATC
Glusyn_qPCR_Rv	CCATCTCCTCGCGATAGAAC