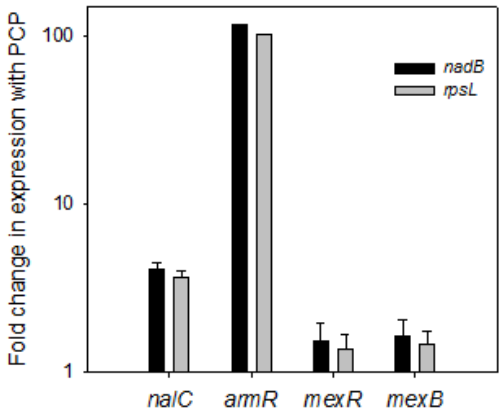
Supplementary Information

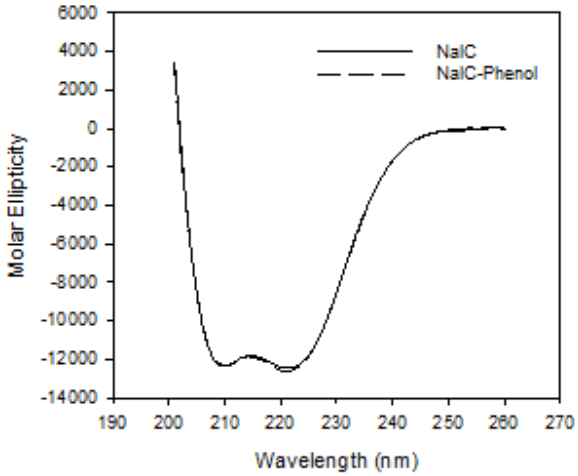
Expression of nalC, armR, mexR and mexB in presence of 120 µM PCP



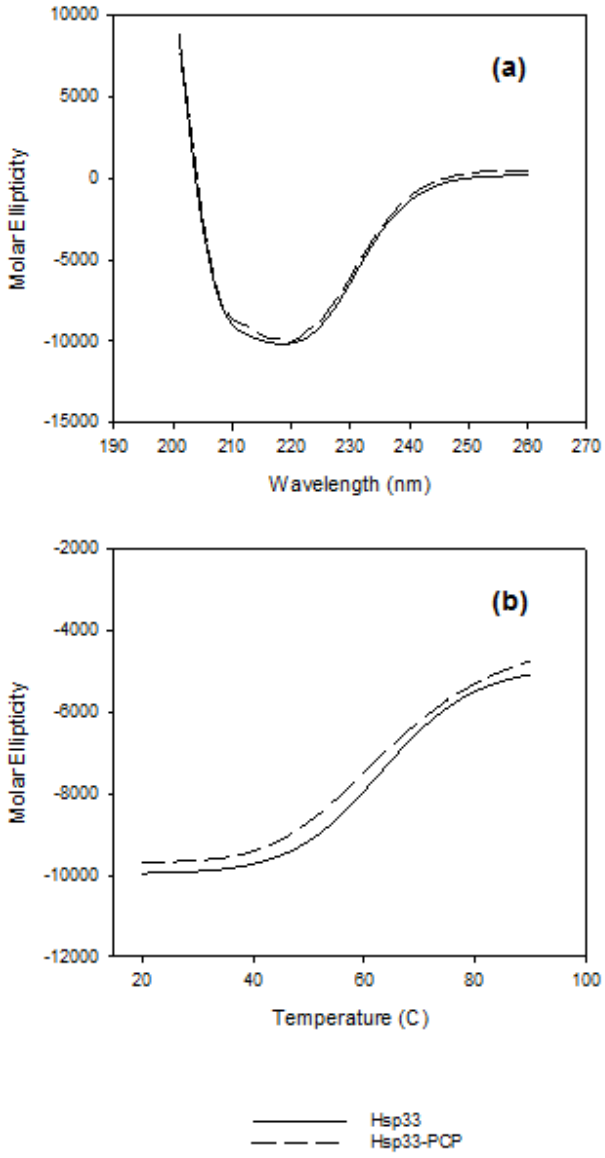
SI Figure 1: Fold change in expression of *nalC*, *armR*, *mexR* and *mexB* normalized to housekeeping genes *nadB* and *rpsL* in mid-log phase batch cultures of *P. aeruginosa* PAO1 with 120 µM of PCP. Bars represent means from 3 separate batch cultures and error bars represent standard deviations about the mean. All increases were statistically significant (p values < 0.05) based on analysis of variance using MacAnova version 5.03.

Circular Dichroism (CD) spectroscopy with NalC with phenol and Hsp33 with PCP

NalC was diluted to 8.7 µM (0.2 mg/mL) in 20 mM KH_2PO_4 buffer (pH 7.8). For monitoring the molar ellipticity of NalC in presence of phenol, 8.7 µM of NalC was pre-incubated with 1.25 mM phenol for 1 hour at room temperature. Far UV CD scans (199-260 nm) for NalC and NalC-phenol were performed using a Jasco J-810 CD spectrophotometer (Jasco Analytical Instruments, Easton, MD). The spectra of buffer alone or buffer with phenol were subtracted from the protein spectra. Six scans were accumulated. Thermal transitions of Hsp33 and Hsp33 with 400 µM PCP were analyzed between 20°C and 90°C (temperature was controlled by a Jasco PTC-423S) and readings were taken at 222 nm. The Hsp33 concentration for both these experiments was 0.2 mg/mL (7 µM). The rate of temperature increase was 1°C/min. Thermal transition data were fitted with sigmoidal curves in SigmaPlot 10.



SI Figure 2: Thermodynamic stability of NalC in presence of phenol. CD spectra of NalC (straight line) and NalC incubated with 1.25 mM phenol (dashed line).



SI Figure 3: Effect of PCP on the thermodynamic stability of Hsp33

(a) CD spectra of Hsp33 (straight line) and Hsp33 in presence of 400 µM PCP (dashed line), **(b)** thermal transitions curves for Hsp33 (straight line) and Hsp33 in presence of 400 µM PCP (dashed line). The samples were heated with a rate of 1°C/min and the CD signal at 222 nm was monitored.

Batch growth experiments

The *mexB* mutant, lacking a functional *mexB* (SI Table 1) was obtained from the University of Washington Genome Center. The *P. aeruginosa* mutant K2276 and *E. coli* S17-1 with pLC1 were obtained from Keith Poole (SI Table 1). Plasmid pLC1 was mobilized into K2276 restoring the *nalC* gene following previously described protocol (Poole *et al.*, 1993), thus creating a *P. aeruginosa* PAO1 mutant lacking only the *armR* gene. *P. aeruginosa* PAO1, and mutants lacking functional *mexB* and *armR* were grown in LB to early logarithmic phase (OD_{600} ~ 0.1). Then 150 µM PCP was added to triplicate cultures and growth was measured by taking periodic OD_{600} readings.

SI Table 1: Bacterial strains and plasmids

Strains/Plasmids	Derivative	Relevant Characteristics	Reference
***P. aeruginosa* strains**			
PW0426	PAO1	mexB-bp02q4D03::ISphoA/hah In frame IsphoA/hah insertion in *mexB*Tc^r	(Jacobs *et al*., 2003)
K2276	K767 (PAO1 prototroph)	K1454 (spontaneous *nalC* mutant of K767) Δ*armR*	(Cao *et al.* 2004)
***E. coli* strains**			
S17-1		*thi pro hsdR recA* Tra^+	(Cao *et al.* 2004)
Plasmids			
pLC1		pDSK519::PA3721	(Cao *et al.* 2004)

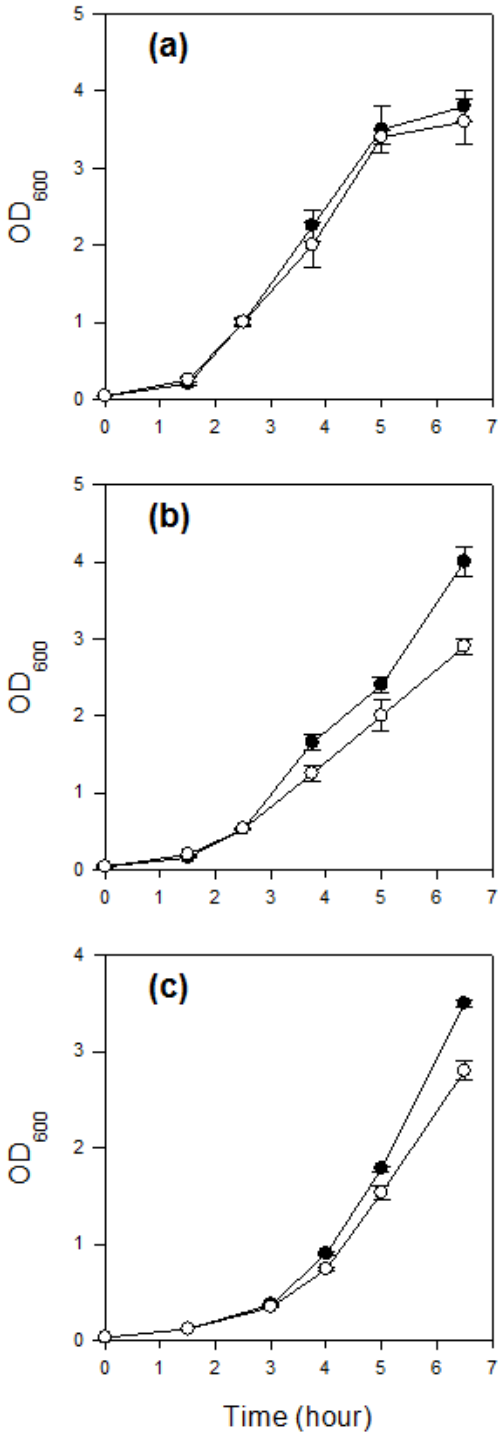
Tc^r Resistant to tetracycline, Ap^r Resistant to Ampicillin

References

Cao, L., Srikumar, R., and Poole, K. (2004) MexAB-OprM hyperexpression in NalC-type multidrug-resistant *Pseudomonas aeruginosa*: identification and characterization of the *nalC* gene encoding a repressor of PA3720-PA3719. *Mol Microbiol* 53: 1423-1436.

Jacobs, M. A., Alwood, A., Thaipisuttikul, I., Spencer, D., Haugen, E., Ernst, S., *et al*. (2003) Comprehensive transposon mutant library of Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A* 100: 14339-14344.

Poole, K., Heinrichs, D. E., and Neshat, S. (1993) Cloning and sequence-analysis of an EnvCD homolog in Pseudomonas aeruginosa – regulation by iron and possible involvement in the secretion of the siderophore pyoverdine *Mol Microbiol* 10: 529-544.



SI Figure 4: Batch growth of (a) *P. aeruginosa* PAO1, (b) a *mexB* defective mutant of PAO1 (PWO426) and (c) an *armR* deletion mutant of PAO1 without (closed circles) and with (open circles) 150 µM PCP added at the 1.5 hours time point.