Species-specific residues calibrate SoxR sensitivity to redox-active molecules

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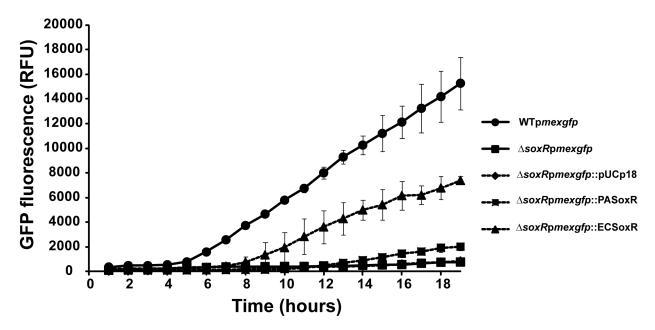
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Supplementary Figure S1.



A. Colony morphology assay

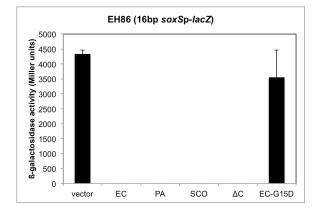
B. Phenazine-dependent activation of *P. aeruginosa* SoxR regulon



Supplementary Figure S2.

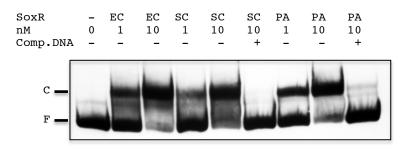
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A. His-SoxR expression in *E. coli*

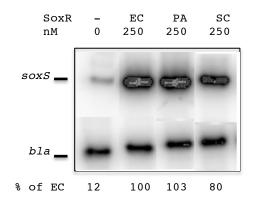


B. soxS promoter binding in vivo

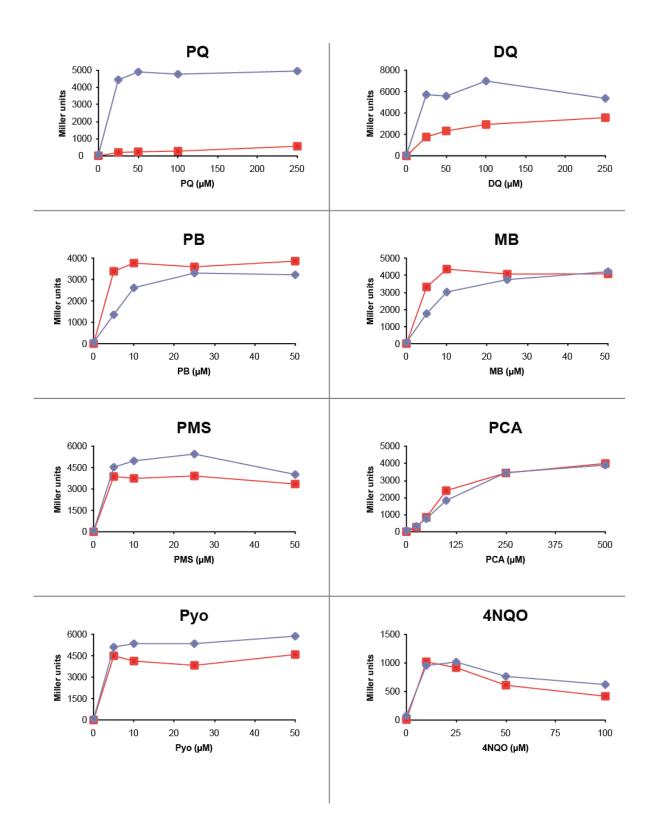
C. soxS promoter binding in vitro



D. In vitro transcription of soxS gene



Supplementary Figure S3



Supplementary Figure S4

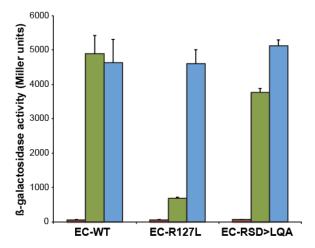
A. Allignment of SoxR [2Fe-2S] cluster domain



Pseudomonas fluorescens Pf0-1 Pseudomonas aeruginosa PAO1 Salinisphaera shabanensis E1L3A Pseudomonas mendocina NK-01 Pseudoxanthomonas suvonensis 11-1 Reinekea blandensis MED297 Pseudoalteromonas haloplanktis ANT/5 Shewanella denitrificans OS217 Mesorhizobium loti MAFF303099 Rhizobium etli CIAT 894 CCC Phizobium etli CIAT 894 CCC Mesorhizobium etli CIAT 894 CCC Rhizobium etli CIAT 894 CCC CCC Pseudoxanthomonas tubia tri, Lupac 08 Amycolicioccus subflavus DQS3-9A1 Intrasporangium roseum DSM 43021 Streptomyces coelicolor A3(2) Kribbella flavida DSM 17836 Thermomonospora curvata DSM 43183 Tsukamurella paurometabola DSM 201 Mycobacterium gilvum PYR-GCK Beutenbergia cavernae DSM 1233 Sphaerobacter thermophilus DSM 20745 Nocardia farcincia IFM 10152 Rhodococcus erythropolis PR4 Sinorhizobium meliloti 1021 CCC
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Thiomonas intermedia K12 CI C Azospirillum brasilense Sp245 CI C
Phenylobacterium zucineum HLK1 CLC
Bradýrhizobiaceae bacterium SG-6C Candidatus Koribacter versatilis Ellin345 C
Xanthomonas campestris pv. campestri Stenotrophomonas maltophilia D457
Comamonas testosteroni ATCC 11996
Bordetella pertussis Tohama I
Cupriavidus taiwanensis LMG 19424 Cupriavidus necator N-1
Raistonia eutropha H16 CIC Hahella chejuensis KCTC 2396 CIC
Opitutus terrae PB90-1 Methylosinus trichosporium OB3b
Xanthobacter autotrophicus Py2
Pseudomonas putida BIRD-1 CLC Achromobacter xylosoxidans AXX-A CLC
Providencia alcalifaciens DSM 30120 Acinetobacter calcoaceticus PHEA-2
Rhodopseudomonas palustris BisB5
Agrobacterium tumefaciens 5A CLC
Brevundimonas diminuta ATCC 11568 CLC Ochrobactrum anthropi ATCC 49188
Acetobacteraceae bacterium AT-5844 CLC Rheinheimera sp. A13L
Erwinia amylovora CFBP1430 CLG Pantoea ananatis LMG 20103 CLG
Pantoea vagans C9-1 CLC
Plautia stali symbiont CLC Escherichia blattae DSM 4481 CLC
Escherichia hermannii NBRC 105704 Yokenella regensburgei ATCC 43003
Cronobacter sakazakii ATCC BAA-894
Enterobacter aerogenes KCTC 2190
Enterobacter hormaechei ATCC 49162
Enterobacter cancerogenus ATCC 35316 Enterobacter cloacae EcWSU1
Shigella sonnei 53G Salmonella bongori NCTC 12419
Salmonella enterica subsp. arizonae se
Escherichia fergusonii ATCC 35469
Escherichia albertii TW07627 Pseudomonas nitroreducens
Shigella flexneri CDC 796-83 Shigella dysenteriae 155-74
Shigella boydii 5216-82
Escherichia coli Sphingopyxis alaskensis RB2256

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B. Activity of *E. coli* SoxR mutants



Untreated
200 µM PQ
20 µM PMS

Supplementary information figure legends

Figure S1. Complementation of a *P. aeruginosa* $\Delta soxR$ mutant by *E. coli* or *P. aeruginosa* SoxR.

A. Colony morphology assay. Cultures were grown for 16 h in LB medium supplemented with 300 μ g mL⁻¹ carbenicillin. Ten microliters of each culture was spotted on agar plates (1% tryptone, 1% agar, supplemented with 20 μ g mL⁻¹ Coomassie Blue and 40 μ g mL⁻¹ Congo Red) for two days before imaging (Keyence VHX1000 microscope). As reported previously (Dietrich *et al.*, 2008), wild type PA14 started to wrinkle after two days of growth while the $\Delta soxR$ mutant remained smooth. Complementation of the $\Delta soxR$ mutant with *P. aeruginosa* SoxR ($\Delta soxR:PAsoxR$) or *E. coli* SoxR ($\Delta soxR:ECsoxR$) restored the wrinkled colony morphotype of wild type.

B. Expression of *E. coli* SoxR in the *P. aeruginosa* PA14 $\Delta soxR$ mutant mediates phenazinedependent activation of the SoxR regulon. The *mexGHI-ompD* operon encodes an efflux pump that is part of *P. aeruginosa's* SoxR regulon. It is expressed in a SoxR- and phenazinedependent manner in the stationary phase of planktonic cultures. To test if *E. coli* SoxR could complement a PA14 $\Delta soxR$ mutant, we made transcriptional *gfp* reporter constructs under the control of the *mexG* promoter. Strains were diluted to an optical density of 0.05 at 500 nm and cell density and GFP fluorescence were monitored for 19 hours. Data represent the mean and standard deviation of three experiments. Complementation with *P. aeruginosa* and *E. coli* SoxR both restored *mexG* expression in the $\Delta soxR$ mutant.

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Figure S2. SoxR protein expression, *soxS* promoter binding, and *in vitro* transcription of the *soxS* gene.

A. SoxR protein levels in *E. coli*. EH46 cells expressing empty vector (pSE380) or histidinetagged SoxR proteins from *E. coli* (His-EC SoxR), *P. aeruginosa* (His-PA SoxR), or *S. coelicolor* (His-SC SoxR) were grown at 37°C for 2.5 h. Total cell extract (50 µg per lane) was resolved on a 15% SDS-polyacrylamide gel and subjected to immunoblot analysis using anti-histidine antibody (GE Healthcare). Purified histidine-tagged *E. coli* SoxR (10 ng) was loaded as a control. His-SC SoxR migrates as a higher molecular weight species than His-EC and His-PA SoxR.

B. SoxR binding to *soxS* promoter *in vivo*. *E. coli* strain EH86 ($\Delta soxRS$ lysogenized with λ [16bp *soxS* promoter-*lacZ* reporter]) was transformed with vector control, histidine-tagged *E. coli*, *P. aeruginosa* or *S. coelicolor soxR* genes, C-terminal truncated *S. coelicolor soxR*, or the *E. coli* DNA binding variant G15D. The wild type *soxS* promoter has a 19-bp spacer separating the -10 and -35 elements. The shortened (16-bp) *soxS* promoter renders *soxS* transcription constitutive and promoter occupation by SoxR prevents access to RNA polymerase (Hidalgo and Demple, 1997). Low ß-galactosidase activity in this background thus indicates specific promoter binding by SoxR, while high ß-galactosidase activity indicates defective promoter binding as demonstrated by cells expressing the *E. coli* SoxR DNA-binding mutant G15D (Fig. 2D, Chander *et al.* 2003). The values shown represent the means and standard errors of three independent experiments.

C. SoxR protein binding to *soxS* promoter *in vitro*. A DIG-end-labeled fragment (180 bp)
containing the *soxS* promoter was incubated with 1 nM or 10 nM purified histidine-tagged SoxR
proteins from *E. coli* (EC), *S. coelicolor* (SC), or *P. aeruginosa* (PA). Protein-bound complexes
(C) were separated from free DNA (F) on a 5% native polyacrylamide gel. SoxR binding

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specificity was demonstrated by the addition of a 500-fold molar excess of unlabeled probe (Comp. DNA).

D. Transcription of the *soxS* gene *in vitro*. Purified histidine-tagged SoxR proteins (250 nM) from *E. coli* (EC), *P. aeruginosa* (PA), or *S. coelicolor* (SC) were incubated with a plasmid containing the *soxS* and *bla* genes, *E. coli* σ^{70} -RNA polymerase, and four ribonucleotide triphosphates for 15 min at 37°C. The *soxS* and *bla* transcripts were quantified by primer extension analysis as described (Chander and Demple, 2004). Reactions were electrophoresced on 8% polyacrylamide, 6 M urea gels and quantified on a Storm phosphorimager. The *bla* gene is a SoxR-independent transcript and serves as a loading control. The amount of *soxS* mRNA is reported as a percent of the amount obtained with *E. coli* SoxR.

Figure S3. Comparison of the transcriptional response of *E. coli* and *P. aeruginosa* SoxR to varying doses of redox-active molecules.

E. coli strain EH46 ($\Delta soxRS$ lysogenized with $\lambda[soxS$ promoter-*lacZ* promoter] expressing histidine-tagged *E. coli* SoxR (blue lines) or *P. aeruginosa* SoxR (red lines) were treated with the indicated concentrations of various redox-active drugs for 1 h before the assay for ß-galactosidase activity (Miller units).

Figure S4. The [2Fe-2S] cluster domain of SoxR has a hypervariable stretch of three amino acids.

A. Shown is a sequence allignment of the [2Fe-2S] cluster domain of the SoxR proteins represented in the phylogenetic tree in Fig. 3B. The blue tree on the left of the allignment is a different representation of the SoxR tree from Fig. 3B, and was generated accordingly. [2Fe-2S] clusters containing the RSD motif are highlighted in red.

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B. β-galactosidase activity was measured in EH46 cells (*soxS* promoter-*lacZ* reporter)
expressing wild type or mutant *E. coli soxR* alleles. The mutations are within the [2Fe-2S]
cluster domain. Activity was measured in untreated cells (red columns) or cells treated for 1 h
with 200 μM PQ (green columns) or 20 μM PMS (blue columns).

Cloning	Sequence $(5' \rightarrow 3')$				
pET-F	CGC GTC GAC TCA CTA TAG GGG	AAT TGT G			
pET-R	GCT TTG TTA GCA GCC G				
380F-Bam	CCG CCG GAT CCG ACA TCA TAA	CGG TTC TG	GC		
380R-Bam	GCA GAT CTG TCA TGA TG				
pmexG-F	TAC CAA GCT TCT CGT GGC CAA CCA GAA TAG				
pmexG-R	TTG CGA ATT CGT CGT TCC TTG TGC TGG TC				
PA and EC					
Mutagenic*	Sequence $(5' \rightarrow 3')$				
PA-V64I	AAG GTC GCC CAG CGG <u>A</u> TC GGC ATT CCC CTC G				
PA-R82H	CCC TGC CGG CCG GGC <u>A</u> CA GCC CTA GCG CGG C				
PA-P84L	CGG CCG GGC GCA GCC <u>T</u> TA GCG CGG CGG ACT G				
PA-A94S	TGG GCG CGC CTG TCG <u>T</u> CG CAG TGG AAG GAG G				
PA-L125R	GCG GCT GCC TGT CGC GCC AGG CCT GCC CGT TG				
PA-Q113E	CTG CTG TTG CGC GAC <u>G</u> AA CTG GAC GGC TGC A				
PA-Q126S	GGC TGC CTG TCG CTC <u>TC</u> G GCC TGC CCG TTG CG				
PA-A127D	GCC TGT CGC TCC AGG ACT GCC CGT TGC GCA AC				
PA-RSD	GCG GCT GCC TGT CGC <u>G</u> C <u>T C</u> GG <u>A</u> CT GCC CGT TGC GCA AC				
PA-AAA	TGC GGC TGC CTG TCG GCC GCG GCC TGC CCG TTG CGC				
PA-L125A	TGC GGC TGC CTG TCG GCC CAG GCC TGC CCG TTG				
PA-ASD	TGC GGC TGC CTG TCG <u>GC</u> C TCG GAC TGC CCG TTG				
PA-C118A	GAC CAA CTG GAC GGC <u>GC</u> C ATC GGT TGC GGC TG				
EC-LQA	TGT GGC TGC CTT TCG CTC CAA GCT TGC CCG TTG CGT AAC				
SCO Mutagenic*	Sequence (5' → 3')				
SCO-S154stop	GGA GCG CCG CGG CT <u>G A</u> AC CG	C CAG GGG C	;		
		Amuliaan			
qRT-PCR	Sequence $(5' \rightarrow 3')$	Amplicon	Size (bp)		
hrdB-F	CAT GCG CTT CGG ACT CA	hrdB	95		
hrdB-R	ACT CGA TCT GGC GGA TG	0001170			
1178-F	TCA AGG TCC GGC AGG TCT A	SCO1178	82		
1178-R		0000470	104		
2478-F	GAG ATC ACC CCG AAA CTG G	SCO2478	104		
2478-R	AAG TGC CAG TCG ATG ACG TT				
4266-F	GAT GGG CAT CCT CCA GTT C	SCO4266	104		
4266-R	CGT TCT TCG CGT ACT GCA C				

Table S1. Primers used in this study

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* Sequence of forward primers used to mutagenize *P. aeruginosa, E. coli* and *S. coelicolor soxR* genes cloned in plasmid pSE380 using either Invitrogen's GENEART site-directed mutagenesis kit (for *P. aeruginosa* and *E. coli*) or Stratagene QuikChange site-directed mutagenesis kit (for *S. coelicolor*). Underlined sequence indicates change from original nucleotide. Reverse primers are complementary to forward primers.