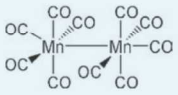
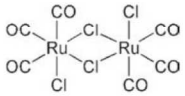
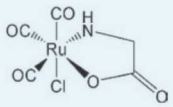
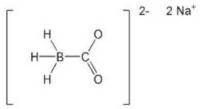
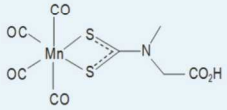
Table 1.2. Chemical properties of CO-RMs.

Name	Chemical formula	Structures	Chemical properties	References
CORM-1 (dimanganese decacarbonyl)	$[Mn_2(CO)_{10}]$		Soluble in DMSO. CO released by photodissociation. CO release to Mb is gradual over time.	Motterlini *et al*. (2002)
CORM-2 (tricarbonyldichloro-ruthenium (II) dimer)	$[(RuCl_2(CO)_3)_2]$		Soluble in DMSO. CO released by ligand exchange with DMSO. 0.7 mol CO released per mol compound to Mb *in vitro* with a half-life of 1 min.	Motterlini *et al*. (2002)
CORM-3 (tricarbonylchloro (glycinato) ruthenium(II))	$[Ru(CO)_3Cl(glycinate)]$		Soluble in water. Mechanism of CO release is uncertain, but likely occurs via ligand exchange. 1 mol CO released per mol compound to Mb *in vitro* with a half-life of less than 2 min.	Clark *et al*. (2003)
CORM-A1 (sodium boranocarbonate)	$Na_2[H_3BCO_2]$		Soluble in water. CO release is pH- and temperature-dependent. 1 mol CO released per mol compound to Mb *in vitro* with a half-life of c. 21 min.	Motterlini *et al*. (2005b)
CORM-401	$[Mn(CO)_4\{S_2CNMe(CH_2CO_2H)\}]$		Soluble in water. CO release is likely to occur via ligand exchange. 3.2 mol CO released per mol compound to Mb *in vitro* with a half-life of 0.8 min for the first CO.	Crook *et al*. (2011)

Abbreviations: Dimethyl sulfoxide (DMSO), myoglobin (Mb).

Table 1.3. Summary of the current literature on the effects of CO/CO-RMs on bacteria.

Paper	Bacteria tested	CO-RMs utilised	Application of CO gas	Key findings
Nobre *et al.* (2007)	*Escherichia coli* *Staphylococcus aureus*	CORM-2, CORM-3, ALF 021, ALF 062	Yes, but at higher concentrations than for CO-RMs, and the effect was less pronounced.	CO-RMs significantly reduced bacterial viability. CO-RMs were more effective under near-anaerobic conditions. ALF 062 enters cells, as determined by intracellular Mo levels.
Kumar *et al.* (2008) & Shiloh *et al.* (2008)	*Mycobacterium tuberculosis*	N/A	Yes	CO activates the dormancy regulon of MTB. The DosS/DosT/DosR two-component system is required for the response to CO. CO was not directly toxic to MTB.
Nobre *et al.* (2009)	*Escherichia coli*	CORM-2	No	Transcriptomic analysis revealed a multifaceted response to stress; genes in almost all functional categories were affected*. Gene expression changes were greater in anaerobically grown cells, but a large number of genes were commonly affected under aerobiosis and anaerobiosis following CO-RM addition.
Davidge *et al.* (2009)	*Escherichia coli*	CORM-3	Yes, but equimolar concentrations to CORM-3 were ineffective.	CORM-3 was more bactericidal under aerobic conditions. Carbonmonoxy adducts of terminal oxidases were identified. Transcriptomic profiling and complimentary mathematical modelling supported CO-targeting of respiration, and revealed a number of additional responses following CO-RM exposure*. CORM-3 enters bacterial cells, as determined by ICP-AES and myoglobin 'competition assays'.
Desmard *et al.* (2009)	*Pseudomonas aeruginosa*	CORM-3	Yes, but at higher concentrations than for CORM-3, and the effect was less pronounced.	CORM-3 was bactericidal against PAO1 and three clinical isolates. The compound was as effective against PAO1 as typically used antibiotics. Carbonmonoxy adducts of terminal oxidases were identified and respiratory inhibition was observed following CORM-3 addition. Thiols alleviated the effects elicited by CORM-3. CORM-3 rescued mice from *P. aeruginosa*-induced bacteraemia at concentrations that were non-toxic to the animals.

Smith *et al*. (2011)	*Campylobacter jejuni*	CORM-3	No	Bacterial growth was unaffected by CORM-3. CO-RM entry into cells was confirmed using myoglobin 'competition assays'. Carbonmonoxy adducts of terminal oxidases were identified and respiration of bacterial suspensions was inhibited, which was accompanied by the generation of ROS.
Tavares *et al*. (2011)	*Escherichia coli*	CORM-2, ALF 062	No	Both CO-RMs stimulated ROS production in bacterial cells and CORM-2 caused ROS generation in the absence of cells. CORM-2 resulted in DNA damage, destruction of Fe-S clusters and accumulation of intracellular iron. Thiols alleviated the effects elicited by the CO-RM.
Desmard *et al*. (2012)	*Pseudomonas aeruginosa*	CORM-2, CORM-3, CORM-A1, CORM-371	No	CO-RM effects, and the inhibitory action of thiols on CO-RM activity, were shown to be compound-specific. CO-RM-induced respiratory inhibition was shown to be a separate event from bactericidal activity.
Murray *et al*. (2012)	*Pseudomonas aeruginosa*	CORM-2	No	CORM-2 killed planktonic PAO1, decreased biofilm formation and bacterial colonisation of human bronchial epithelial cells. The compound prevented biofilm maturation to a similar extent as a commonly used antibiotic. Thiols alleviated the effects elicited by CORM-2. ROS generation was observed following CORM-2 treatment, but the toxicity of the compound was shown to be an independent event. CORM-2 resulted in differential inhibition of biofilm formation and planktonic growth in a number of clinical isolates. Activity of the compound was abolished in rich media.

* More detailed descriptions of transcriptomic studies are given in Chapter 5 where they have direct relevance to the work presented in this thesis.

Abbreviations: Mo (molybdenum), haemoglobin (Hb), *Mycobacterium tuberculosis* (MTB), inductively coupled plasma atomic emission spectroscopy (ICP-AES), laboratory strain of *P. aeruginosa* (PAO1), reactive oxygen species (ROS).

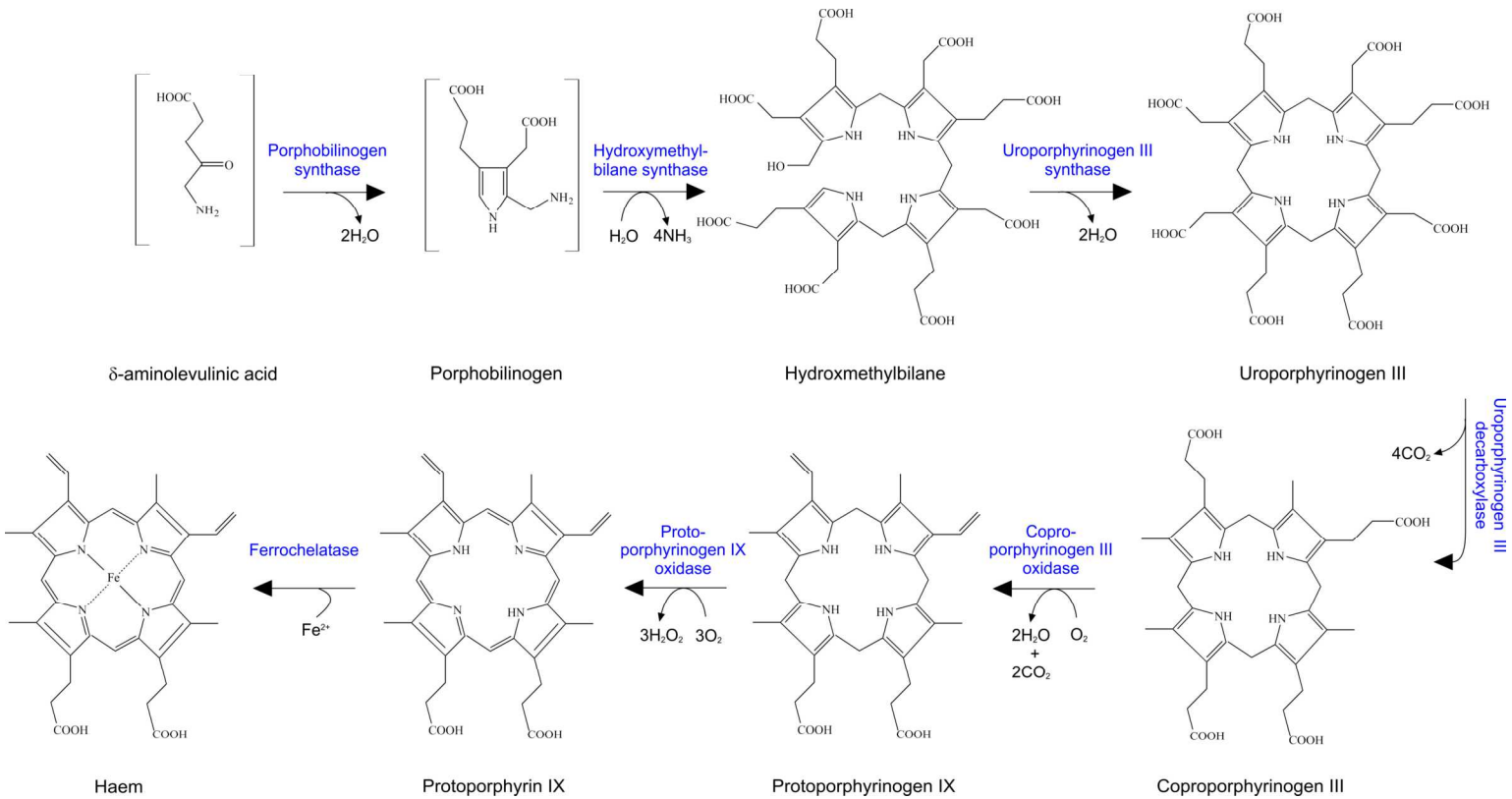
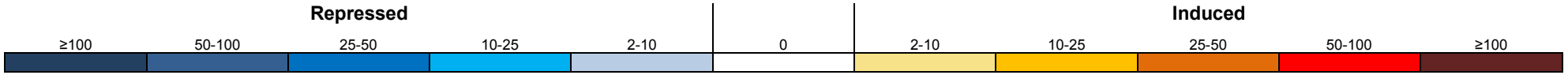


Figure 4.2. Tetrapyrrole biosynthesis. The names of enzymes catalysing each step are shown in blue. The initial three steps involve formation of the first cyclic intermediate, urorporphyrinogen. Urorporphyrinogen III decarboxylase catalyses decarboxylation of four acetate residues resulting in corresponding methyl groups. Protoporphyrinogen IX is yielded by coproporphryinogen III oxidase which oxidatively decarboxylates the propionate side-chains. The final tetrapyrole shape is generated by oxidation of the ring by protoporphyrinogen IX oxidase. Insertion of iron into protoporphyrin IX is the last stage of haem biosynthesis. The information enabling the generation of this figure was sourced from Heinemann *et al*. (2008).



Gene	Product/Function	Fold change with CORM-3										Fold change with iCORM-3					Relevant regulatory proteins
		hemA time-course (min)					Wild type time-course (min)					*hemA* time-course (min)					
		10	20	40	60	120	10	20	40	60	120	10	20	40	60	120	
bfd	Bacterioferritin-associated ferredoxin																-
entA	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase						*							*			CRP+, Fur-
entB	2,3-dihydro-2,3-dihydroxybenzoate synthetase, isochroismatase						*	*									
entC	Isochorismate synthase 1						*										
entE	2,3-dihydroxybenzoate-AMP ligase component of enterobactin synthase					*	*										
entF	Enterobactin synthase multienzyme complex component						*										FNR+, Fur-, H-NS+
exbB	Membrane-spanning proteins in TonB-ExbB-ExbD complex																Fur-
exbD																	
fecI	KpLE2 phage-like element; RNA polymerase, sigma 19 factor										*						
fecR	KpLE2 phage-like element; transmembrane signal transducer for ferric citrate transport								*	*							
feoA	Ferrous iron transport protein				*												FNR+, Fur-
fepA	Outer membrane receptor for ferric enterobactin						*										CRP+, Fur-
fepB	Iron-enterobactin transporter subunit																Fur-
fepC	ATP-binding component of ferric enterobactin transport																Fur-
fepD	Iron-enterobactin transporter subunit																
fepG	Ferric enterobactin transport protein										*						
fes	Enterobactin/ferric enterobactin esterase						*										FNR+, Fur, H-NS+
fhuA	Ferrichrome outer membrane transporter																Fur-
fhuB	Hydroxamate-dependent iron uptake																
fhuC	ATP-binding component of hydroxymate-dependent iron transport																
fhuD	Hydroxamate-dependent iron uptake																
fhuE	Outer membrane receptor for ferric iron uptake							*									
fhuF	Ferric iron reductase involved in ferric hydroximate transport															*	Fur-, OxyR-
ftnA	Ferritin iron storage protein																Fur+, H-NS-
ryhB	sRNA; reduces iron consumption under low-iron conditions																Fur-
tonB	Membrane-spanning protein in TonB-ExbB-ExbD complex																Fur+/-

Figure 5.6. Differential expression of genes involved in iron transport and acquisition. The colour-scale bar shows mean fold changes in individual genes of the haem-deficient mutant of *E. coli* (*hemA*) and the corresponding wild type grown anaerobically in defined medium (modified for the growth of haem-deficient bacteria) after addition of 100 μM CORM-3 or, for the mutant only, 100 μM iCORM-3. Unless otherwise stated, *p* values were ≤ 0.05; * indicates a *p* value that exceeds 0.05.

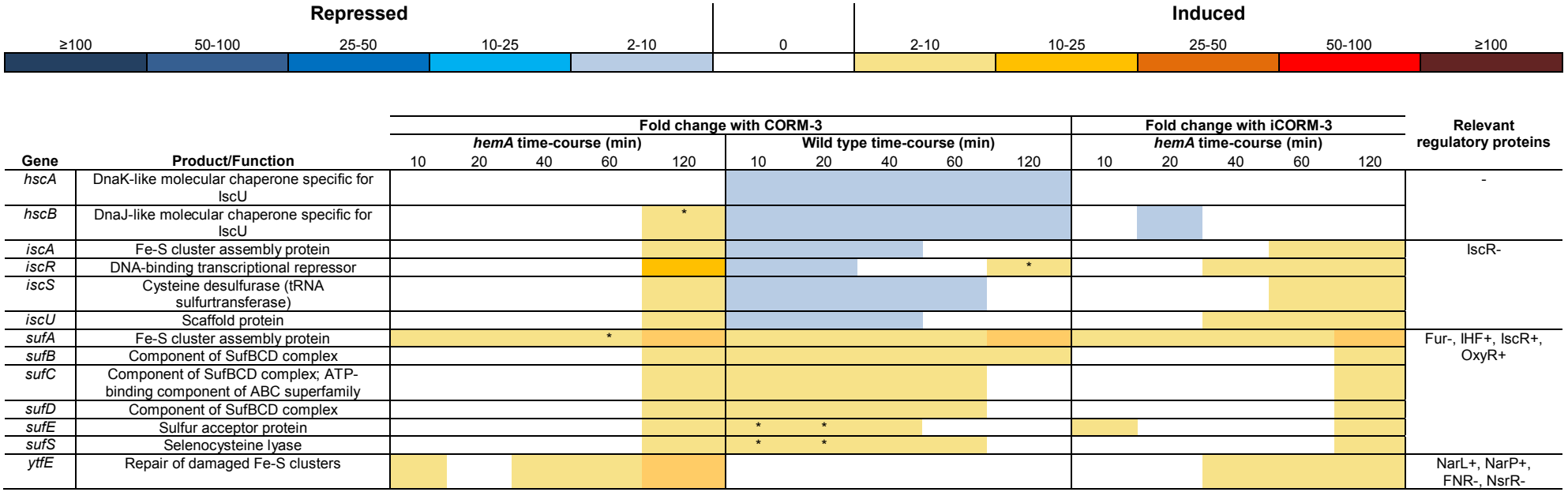
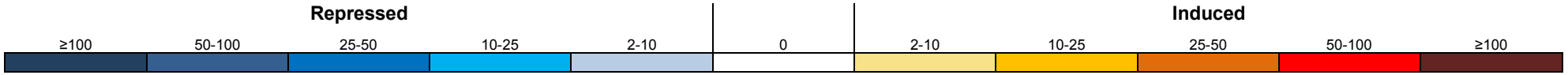
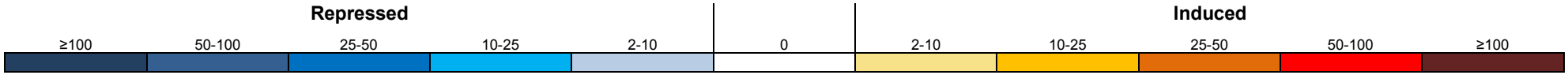


Figure 5.7. Differential expression of genes involved in Fe-S cluster assembly and repair. The colour-scale bar shows mean fold changes in individual genes of the haem-deficient mutant of *E. coli* (*hemA*) and the corresponding wild type grown anaerobically in defined medium (modified for the growth of haem-deficient bacteria) after addition of 100 μM CORM-3 or, for the mutant only, 100 μM iCORM-3. Unless otherwise stated, *p* values were ≤ 0.05; * indicates a *p* value that exceeds 0.05.



Gene	Product/Function	Fold change with CORM-3										Fold change with iCORM-3					Relevant regulatory proteins
		hemA time-course (min)					Wild type time-course (min)					*hemA* time-course (min)					
		10	20	40	60	120	10	20	40	60	120	10	20	40	60	120	
baeS	Sensory histidine kinase																BaeR+, CpxR+
baeR	DNA-binding response regulator																
bhsA	Multiple stress resistance and biofilm formation	*			*									*			CRP-
bssR	Regulator of biofilm formation																-
bssS	Regulator of biofilm formation																-
clpB	Protein disaggregation chaperone; heat shock protein	*		*	*												-
cpxP	Inhibitor of the *cpx* response																CpxR+
hmp	Fused nitric oxide dioxygenase				*												FNR-, Fur-, NsrR-
hns	Global DNA-binding transcriptional dual regulator	*			*												Fis+, GadX+, H-NS-
htpX	Heat shock protein																CpxR+
ibpA	Heat shock chaperone				*				*								IHF+
ibpB	Heat shock chaperone																
mdtA	Multidrug efflux system; subunit A																BaeR+, CpxR+
mdtB	Multidrug efflux system; subunit B										*						
mdtC	Multidrug efflux system; subunit C																
oxyS	sRNA; oxidative stress response	*														*	OxyR+
spy	Envelope stress protein																BaeR+, CpxR+
yodA	Divalent metal binding protein; Cd, Zn, Ni; general stress response																Fur+, SoxS+
znuA	Zinc transporter; periplasmic-binding component								*								Zur-
znuB	Zinc transporter; membrane component																
znuC	Zinc transporter; ATP-binding component																

Figure 5.8. Differential expression of genes involved in general stress response, signal transduction and zinc homeostasis. The colour-scale bar shows mean fold changes in individual genes of the haem-deficient mutant of *E. coli* (*hemA*) and the corresponding wild type grown anaerobically in defined medium (modified for the growth of haem-deficient bacteria) after addition of 100 μM CORM-3 or, for the mutant only, 100 μM iCORM-3. Unless otherwise stated, p values were ≤ 0.05; * indicates a p value that exceeds 0.05.



Gene	Product/Function	Fold change with CORM-3										Fold change with iCORM-3					Relevant regulatory proteins
		hemA time-course (min)					Wild type time-course (min)					*hemA* time-course (min)					
		10	20	40	60	120	10	20	40	60	120	10	20	40	60	120	
cysA	Sulfate/thiosulfate transporter subunit									*							CysB+, H-NS-
cysB	DNA-binding transcriptional dual regulator																CysB-
cysC	Adenosine 5'-phosphosulfate kinase								*								CysB+
cysD	Sulfate adenylyltransferase; subunit 2		*						*								
cysE	Serine acetyltransferase																-
cysH	3'-phosphoadenosine 5'-phosphosulfate (PAPS) reductase					*			*								CysB+, IHF+
cysI	Sulfite reductase; beta subunit					*			*								
cysJ	Sulfite reductase; alpha subunit								*								
cysK	Cysteine synthase A (O-acetylserine sulfhydrolase A)																CysB+
cysM	Cysteine synthase B (O-acetylserine sulfhydrolase B)			*	*				*								CysB+, H-NS-
cysN	Sulfate adenylyltransferase; subunit 1								*								CysB+
cysP	Thiosulfate transporter subunit									*							CysB+, H-NS-
cysU(T)	Sulfate/thiosulfate transporter subunit										*						CysB+, H-NS-
cysW	Sulfate/thiosulfate transporter subunit									*							CysB+, H-NS-
cysZ	Sulfate transport																CysB+
sbp	Sulfate transporter subunit; periplasmic sulfate-binding protein					*											-
cbl	DNA-binding transcriptional activator of cysteine biosynthesis		*	*	*												CysB+

Figure 5.9. Differential expression of genes involved in cysteine biosynthesis and sulfate assimilation. The colour-scale bar shows mean fold changes in individual genes of the haem-deficient mutant of *E. coli* (*hemA*) and the corresponding wild type grown anaerobically in defined medium (modified for the growth of haem-deficient bacteria) after addition of 100 μM CORM-3 or, for the mutant only, 100 μM iCORM-3. Unless otherwise stated, p values were ≤ 0.05; * indicates a p value that exceeds 0.05.

Table 5.2. Transcription factors with activity in only one condition.

hemA CORM-3 but not iCORM-3		*hemA* iCORM-3 but not CORM-3		*hemA* but not wild type (CORM-3)		Wild type but not *hemA* (CORM-3)	
ArgP	Arginine transport and DNA replication	AgaR	Negatively controls the *aga* gene cluster (aminosugar utilisation)	AllR	Anaerobic utilisation of allantoin as a nitrogen source	AgaR	Negatively controls the *aga* gene cluster (aminosugar utilisation)
CueR	Primary copper homeostasis system	AraC	Arabinose catabolism and transport	ArgP	Arginine transport and DNA replication	CaiF	Carnitine metabolism
HyfR	Proton-translocating formate hydrogenase system and formate transport	Cbl	Aliphatic sulfonate utilisation and homeostatic response to sulfate starvation	CdaR	Uptake and metabolism of galactarate and glucarate	CytR	Transport and utilisation of ribonucleosides and deoxyribonucleosides
IdnR	L-idonate metabolism	CytR	Transport and utilisation of ribonucleosides and deoxyribonucleosides	CueR	Primary copper homeostasis system	DgsA	Global regulator of carbohydrate metabolism
NarP	Nitrate/nitrite response regulator	DcuR	C4-dicarboxylate metabolism	GadE	Maintenance of pH homeostasis	DnaA	Initiates chromosomal replication
OmpR	Outer membrane protein regulator	DgsA	Global regulator of carbohydrate metabolism	GalR	Represses transport and catabolism of D-galactose	DpiA	Anaerobic citrate catabolism
PspF	Induced under extracytoplasmic stress	DpiA	Anaerobic citrate catabolism	GlpR	Repressor of the glycerol-3-phosphate regulon	EvgA	Acid resistance and multidrug resistance
TrpR	Negatively regulates the *trp* regulon	FucR	Fucose transport and degradation	HyfR	Proton-translocating formate hydrogenase system and formate transport	MalT	Maltose catabolism and transport
		GadW	Controls the principal acid resistance system	IdnR	L-idonate metabolism		
		MalT	Maltose catabolism and transport	ModE	Transport of molybdenum, synthesis of molybdoenzymes and molybdate-related functions		
		PaaX	Catabolism of phenylacetic acid	NarL	Nitrate/nitrite response regulator		
				NarP	Nitrate/nitrite response regulator		
				NhaR	Adaptation to Na^+ and alkaline pH		
				OxyS	Oxidative stress regulator (sRNA)		
				PspF	Induced under extracytoplasmic stress		
				TorR	Utilisation of trimethylamine N-oxide (TMAO) as an alternate electron acceptor		
				TrpR	Negatively regulates the *trp* regulon		

Supplementary document for:

The Anti-Microbial Effects of Carbon Monoxide and Carbon Monoxide-Releasing Molecule-3 (CORM-3)

by

Jayne Louise Wilson

September 2012