

Cysteine desulphurase		Scaffold		ATP-hydrolysing component		Trafficking		
	<i>E. coli</i>	<i>C. jejuni</i> NCTC 11168	<i>E. coli</i> / <i>H.pylori</i>	<i>C. jejuni</i> NCTC 11168	<i>E. coli</i>	<i>C. jejuni</i> NCTC 11168	<i>E. coli</i>	<i>C. jejuni</i> NCTC 11168
ISC/NIF	IscS	Cj0240 (39%)	IscU (<i>Ec</i>) NifU (<i>Hp</i>)	Cj0239 (40%) Cj0239 (69%)	HscA HscB	DnaK (41%) -	IscA	-
SUF	SufS SufE	Cj0240 (27%) -	SufB SufD	- -	SufC	LivG (26%) Cj0669 (28%) Cj1663 (27%)	SufA	-
Other	CsdA	Cj0240 (27%) Cj0791 (22%)					Mrp NfuA ErpA	Cj1606 (37%) Cj1639 (32%) -

Supplementary Table S1. Comparison between the components of the *E. coli* and *C. jejuni* NCTC 11168 Fe-S cluster biosynthesis pathways. Protein function is listed along the top row, and the specific pathways that the listed proteins belong to are in the right-hand column (other referring to those proteins not experimentally linked to any specific pathway) (Py & Barras, 2010). *C. jejuni* homologues to *E. coli* proteins are shown in bold with percentage sequence identity in brackets. Note that Cj0239 is homologous with the characterised NifU protein in *H. pylori* (*Hp*) rather than IscU, so this comparison is also shown. Proteins without homologues in *C. jejuni* are indicated by a – sign.

HerA	/	/	/	/	/	/										
11168	-MTYNEKII 81116	SMNNNDLLDHQHKE -MTYNEKII 81-176	FEIS---- SMNNNDLLDHQHKE FEIS---- SMNNNDLLDHQHKE	KKLSLMNQRVGT KKLSLMNQRVGT KKLSLMNQRVGT	KELKIVLRELLIMINR KELKIVLRELLIMINR KELKIVLRELLIMINR	HFSDEEAFMREIE HFSDEEAFMREIE HFSDEEAFMREIG	YPYINH YPYINH YPYINH	HTRIHKII HTRIHKII HTRIHKII	LEIEEEIII LEIEEEIII LEIEEEIII	SEAKFVNIMTE SEAKFVNIMTE SEAKFVNIMTE	KLNLVVQDFIFK KLNLVVQDFIFK KLNLVVQDFIFK					

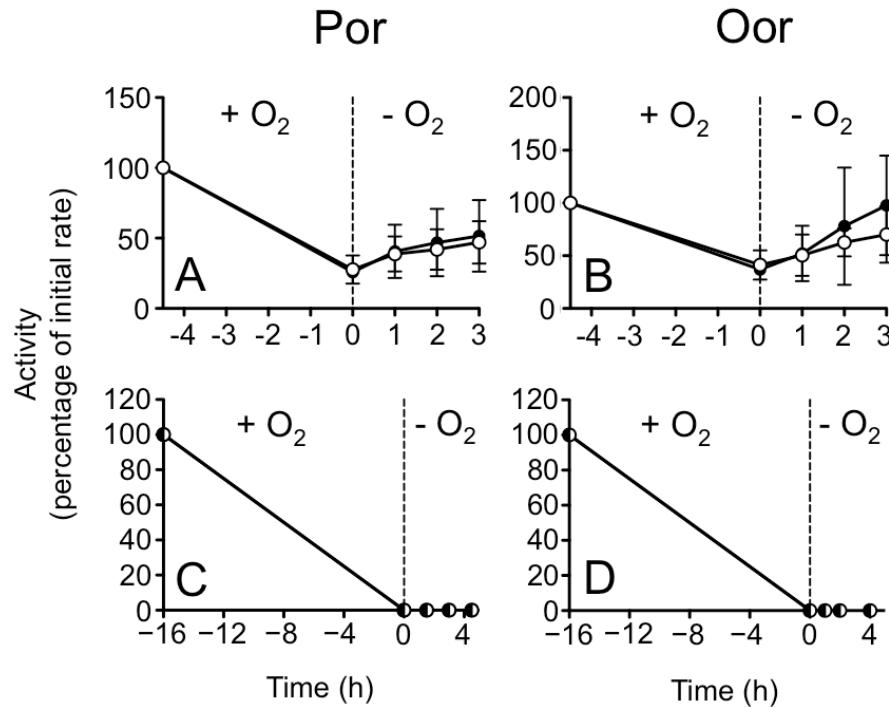
HerB	/	/	/	/	/	/										
11168	MLPKWDNSYSVHN 81116	NAKIDEQHKK MLPKWDNSYSVHN 81-176	FKLA---- NAKIDEQHKK FKLA---- NAKIDEQHKK	AKVEVVS AKVEVVS AKVEVVS	SDRSVSK SDRSVSK SDRSVSK	NEMKDH NEMKDH NEMKDH	HFNDEEK HFNDEEK HFNDEEK	YKMQLIG YKMQLIG YKMQLIG	YPNL YPNL YPNL	HRKIHK HRKIHK HRKIHK	KEIQTMIN KEIQTMIN KEIQTMIN	LIKDIKSTNDL LIKDIKSTNDL LIKDIKSTNDL	KEKLYVAKKW KEKLYVAKKW KEKLYVAKKW			

FedA	/	/	/	/	/	/										
11168	MKVWSRDFS 81116	IKNMQLDKQH MKVWSRDFS 81-176	ELIFEITN ELIFEITN ELIFEITN	LANDLALN LANDLALK LANDLALN	IQDNNTQH IQENNTQY IQDNNTQH	KNDLKQ KDDLQ KNDLKQ	QILV QILAKL QILV	LFQYIK LFQYIK LFQYIK	IHF HF IHF	KDEEKFM DEEKFM KDEEKFM	ESIDFP MESIDFP MESIDFP	LIIE LIEE LIIE	VEKTKE VEKTKE VEKTKE	LLEHSNDIV LLEHSNDIV LLEHSNDIV	KMMSQELS KMMSQELS KMMSQELS	ILTKDWILD ILTKDWILD ILTKDWILD
*:*****																
HerA	/															
11168	...HTAKEDSKIV 81116	KYYEEKFKK KYYEEKFKK	133													
81-17	...HTAKEDSKIV ...HTAKEDSKIV	KYYEEKFKK KYYEEKFKK	133													

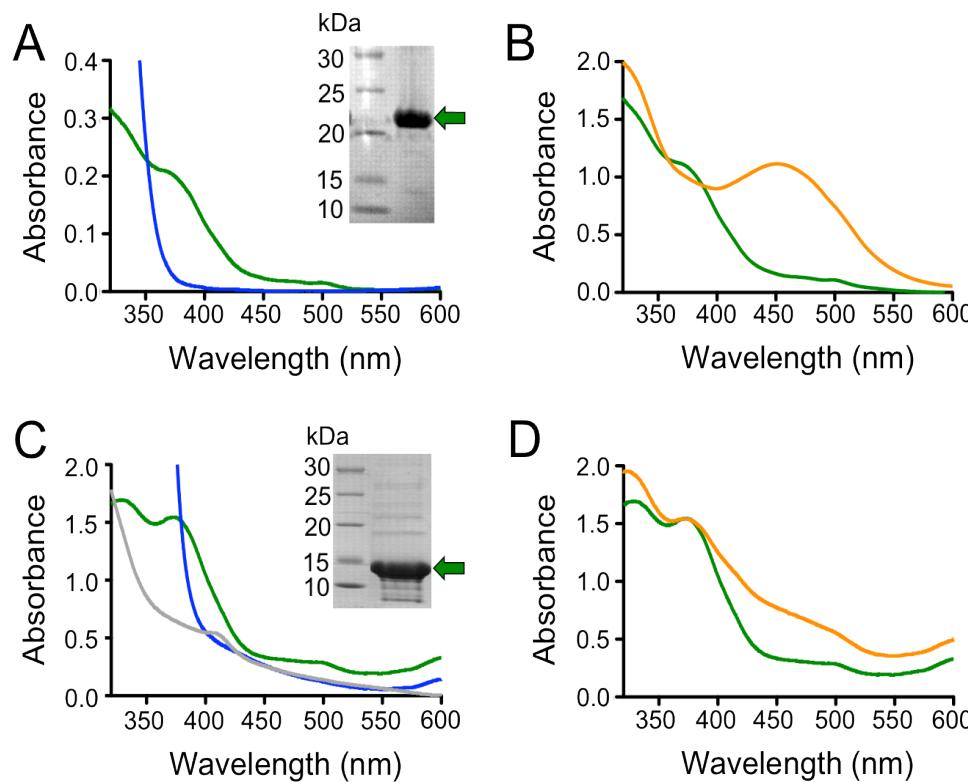
HerB	/	/	/	/	/	/										
11168	...HILYEDMK 81116	VEKRSSLSTDGG DVSFEAAEDE	199													
81-176	...HILYEDMK ...HILYEDMK	VEKRSSLSTDGG DVSFEAAEDE	199													

FedA	/	/	/	/	/	/										
11168	...HFANEDLW 81116	IANFTKKTLH QEIHYTLEQY IYLKSIKQDLRA EAKTYDYICNC SLRIHAVPQT IHQELVSKENT LKCEKGCG QILVHLDY FDLNQN FEKFNA IFEDALQ NHFTTQ ENDMRGGG	240													
81-176	...HFANEDLW ...HFANEDLW	IANFTKKTLH QEIHYTLEQY IYLKSIKQDLRA EAKTHDYICNC SLRIHAVPQT IHQELVSKENT LKCEKGCG QILVHLDY FDLNQN FEKFNA IFEDALQ NHFTTQ KNDMGGG	239													
<i>FedA*</i>	<i>-KS1KQDLRAEKTHDYICNCYLRTHAVPQTIEHEVL SKENTLKCEKGCG QILVHLDY FDLNQN FEKFNA IFEDALQ NHFTTQ KNDMGGG</i>											88				

Supplementary Figure 1: Protein sequence alignments of hemerythrins in *C. jejuni* NCTC 11168 (top line), *C. jejuni* 81116 (middle line) and *C. jejuni* 81-176 (bottom line). HerA = Cj0241, HerB = Cj1224 and FedA = Cj0045 in strain NCTC 11168. Conserved motifs characteristic of hemerythrin proteins are highlighted in yellow, hydrophobic residues potentially lining the oxygen binding pocket are in blue and underlined (French *et al.*, 2008). Potential metal binding CXC and CXXC motifs are highlighted grey. Amino-acid similarity is indicated underneath the alignment: * = fully conserved residue, : = residues sharing strongly similar properties, . = residues sharing weakly similar properties. Sequence length is indicated above each protein; / = 20 residues. *FedA** indicates the missing C-terminal sequence of the 81-176 gene is encoded in a different reading frame.



Supplementary Figure 2. *In vivo* recovery of Por and Oor activity after partial (A, B) or complete (C, D) aerobic inactivation. Assays of Por (A, C) and Oor (B, D) activity in CFE prepared from wild-type (closed circles) and *herA* mutant (open circles) cells incubated aerobically (500 ml cultures in 2.5 L baffled flasks with 250 rpm shaking in air) for 4.5 hours (A, B) or 16 hours (C, D), followed by anaerobic incubation in the presence of chloramphenicol. Dashed vertical lines indicate the point of transfer from aerobic conditions to the anaerobic recovery conditions. All activities are expressed as a percentage of the initial pre-stress activity (mean intial values in $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$ A: wt = 2.4, *herA* = 2.3; B: wt = 0.61, *herA* = 0.61; C: wt = 1.0, *herA* = 0.9; D: wt = 0.18, *herA* = 0.17). The data shown in A and B are from four independent experiments, with error bars showing standard deviation. The data shown in C and D is from a single experiment which was repeated twice with similar results.



Supplementary Figure 3. **(A)** *Inset:* 12% SDS-PAGE gel showing purified NCTC 11168 HerB (Cj1224) protein band of ~23 kDa (green arrow). Sizes of marker bands are also shown. *Main figure:* Absorption spectra of the purified as-isolated HerB (green spectrum) and HerB reduced with sodium dithionite (blue spectrum). **(B)** Absorption spectra of purified as-isolated HerB (green spectrum) and HerB plus 50 mM sodium azide (orange spectrum). **(C)** *Inset:* 12% SDS-PAGE gel showing purified 81-176 FedA protein band (green arrow). *Main figure:* Absorption spectra of as-isolated FedA purified from cells grown in media supplemented with ferrous ammonium sulphate (green spectrum), FedA reduced with sodium dithionite (blue spectrum) and FedA purified from cells grown with no additional ferrous ions in the medium (grey line). **(D)** UV absorption spectra of purified iron-loaded FedA (green spectrum) and FedA with 50 mM sodium azide (orange spectrum).