Supplemental Material :

WrpA is an atypical flavodoxin-family protein under regulatory control of the *Brucella abortus* general stress response system

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Table S1: Primers used for this study.

Primer	Sequence	Restriction sites	Plasmid
wrpA1-UP	TACTTCCAATCCAATGCCATGGTAAAGATGCTGGTGCTGTATTATTC	Ligation Independent Cloning (LIC)	pMCSG68 (AmpR)
wrpA1-LO	TTATCCACTTCCAATGTTAGCCATGCAGTTTCGCGGTAATTTC		
wrpA2-UP	TATA**CATATG**GTAAAGATGCTGGTGCTG	NdeI	pET28c (KanR)
wrpA2-LO	TATA**CTCGAG**TCAGCCATGCAGTTTCGCG	XhoI	
*wrpA*_Y80F-UP	GCAACGCGCTTTGGCATGATG	-	
*wrpA*_Y80F-LO	CATCATGCCAAAGCGCGTTGC	-	
$wrbA_{Ec}$-UP	TATA**CATATG**GCTAAAGTTCTGGTGCTTT	NdeI	
$wrbA_{Ec}$-LO	TATA**CTCGAG**TTAGCCGTTAAGTTTAACTGC	XhoI	
wrpA-UP	TATA**GGATCC**GGCGCGCATAATTGTCGCC	BamHI	pNPTS138 (KanR)
Δ*wrpA*_Mid-LO	GATTAACGGAGATGTCAGCCAAACTGGGCAGTTGCG	-	
Δ*wrpA*_Mid-UP	CGCAACTGCCCAGTTTGGCTGACATCTCCGTTAATC	-	
wrpA-LO	TATA**GCATGC**TTTCAACGGCTTTGGAAATAAA	SphI	

Table S2: Strains used for this study.

Strain #	Organism	Plasmid	Description	Reference
HTRL1	*E. coli* Top10	pMCSG68-*wrpA*	Strain carrying the expression vector for *B. abortus* WrpA.	This study.
HTRL2	*E. coli* BL21(DE3)-Gold strain	pMCSG68-*wrpA*	Strain used to overexpress *B. abortus* WrpA (crystal form $P4_32_12$)	This study.
FC2162	*E. coli* Top10	pET28-*wrpA*	Strain carrying the expression vector for *B. abortus* WrpA.	This study.
FC2163	*E. coli* Rosetta (DE3) pLysS	pET28-*wrpA*	Strain used to overexpress *B. abortus* WrpA (crystal form $P4_222$)	This study.
FC2454	*E. coli* Top10	pET28-*wrbA*	Strain carrying the expression vector for *E. coli* WrbA.	This study.
FC2455	*E. coli* Rosetta (DE3) pLysS	pET28-*wrbA*	Strain used to overexpress *E. coli* WrbA.	This study.
FC2452	*E. coli* Top10	pET28-*wrpA(Y80F)*	Strain carrying the expression vector for *B. abortus* WrpA(Y80F) mutant protein.	This study.
FC2453	*E. coli* Rosetta (DE3) pLysS	pET28-*wrpA(Y80F)*	Strain used to overexpress *B. abortus* WrpA(Y80F) mutant protein.	This study.
FC2164	*E. coli* Top10	pNPTS138-Δ*bab1_1070*	Strain used to delete *bab1_1070* (coding WrpA) in *B. abortus* chromosome	This study.
FC2456	*E. coli* Top10	pNPTS138-*bab1_1070*	Strain used to complement *B. abortus* Δ*bab1_1070* strain with *bab1_1070.*	This study.
6834	*B. abortus* 2308	-	Wild-type strain.	This study.
6544	*B. abortus* 2308	-	*B. abortus* Δ*rpoE1.* Strain deleted for σ^{E1}.	Kim H.S. *et al.* (2014) Mol. Microbiol.
6899	*B. abortus* 2308	-	*B. abortus* Δ*bab1_1070*. Strain deleted for *wrpA.*	This study.
7248	*B. abortus* 2308	-	*B. abortus* Δ*bab1_1070* complemented with *bab1_1070.*	This study.

Table S3: Data collection and refinement statistics.

	WrbA (PDB ID: 5F51)	WrbA + FMN (PDB ID: 5F4B)
Wavelength (Å)	12.66 keV (0.979 Å)	12.66 keV (0.979 Å)
Resolution range (Å)	44.32 - 2.53	44.7 - 2.49
Space group	P $4_2$22	P $4_3 2_1 2$
Unit cell	a= 61.28 b= 61.28 c= 128.34 (α=β=γ=90˚)	a= 63.58 b= 63.58 c= 188.53 (α=β=γ=90˚)
# molecules in ASU	1	2
Total reflections	110348	25226
Unique reflections	8691	13940
Multiplicity	12.7	1.8
Completeness (%)	99.7	98.5
Mean I/sigma(I)	23.5	11.1
R-merge	0.063	0.122
Reflections used for R-free	412	758
R-work	0.239	0.230
R-free	0.268	0.269
RMS(bonds)	0.003	0.003
RMS(angles)	0.71	0.62
Ramachandran favored (%)	94.34	98.78
Ramachandran allowed (%)	1.89	1.22
Ramachandran outliers (%)	3.77	0

Table S4: Biolog conditions with a *B. abortus* Δ*rpoE1* growth defect. Conditions include those outside the 99% confidence interval presented in Figure 2, and 10 additional conditions (*) identified in which the rate of growth was significantly slower at earlier culture time points, but which showed no significant Abs 630 nm difference at 72 hours (i.e. compounds outside the 99% interval in Figure 2). A *wrpA* null strain did not exhibit a growth defect in any of these conditions.

Condition	Plate	Well	Compound
6	1	A6	D-Galactose
8	1	A8	L-Proline
33	1	C9	a-D-Glucose
63	1	F3	*m-Inositol
170	2	G2	L-Alaninamide
176	2	G8	Hydroxy-L-Proline
248	3	E8	D-Glucosamine
697	8	C1	*Lys-Gly
828	9	E12	7% Urea
835	9	F7	7% Sodium Lactate
836	9	F8	8% Sodium Lactate
837	9	F9	9% Sodium Lactate
838	9	F10	10% Sodium Lactate
839	9	F11	11% Sodium Lactate
840	9	F12	12% Sodium Lactate
859	9	H7	10mM Sodium Nitrite
860	9	H8	*20mM Sodium Nitrite
976	11	B4	Amoxicillin
1151	12	H11	Dodecyltrimethyl ammonium bromide
1224	13	F12	Thallium (I) acetate
1260	14	A12	Sanguinarine chloride
1308	14	E12	Sodium metaborate
1330	14	G10	*Sodium Nitrite Conc. 2
1332	14	G12	Sodium Nitrite Conc. 4
1380	15	C12	*1,10-Phenanthroline Monohydrate
1387	15	D7	*Domiphen bromide
1392	15	D12	*Nordihydroguaiaretic acid
1425	15	G9	Menadione, sodium bisulfite
1451	16	A11	*5-Chloro-7-iodo-8-hydroxy-quinoline Conc. 3
1452	16	A12	*5-Chloro-7-iodo-8-hydroxy-quinoline Conc. 4
1467	16	C3	Dichlofluanid
1478	16	D2	Chlorodinitrobenzene
1548	17	A12	Thiosalicylate
1551	17	B3	*Salicylate, sodium
1678	18	D10	Lidocaine
1722	18	H6	2- Phenylphenol
1827	20	A3	Amitriptyline
1845	20	B9	**Tetrazolium violet
1908	20	G12	8-Hydroxyquinoline

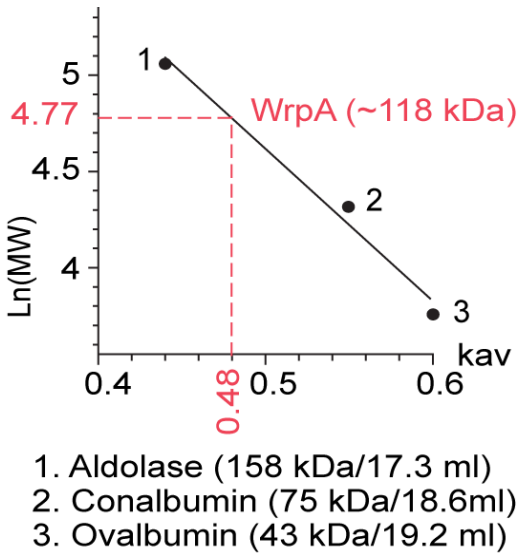

Figure S1: Relative elution volumes of protein standards as a function of the natural log of protein molecular weights. Blue dextran [2,000 kDa; void, ~11.9 ml], aldolase [158 kDa; elution, ~17.2 ml], conalbumin [75 kDa; elution, ~18.7 ml], and ovalbumin [43 kDa; elution, ~19.1 ml]) on a Superdex 200 10/300 GL column. Relative elution volume of *B. abortus* WrpA is reported on the graph.

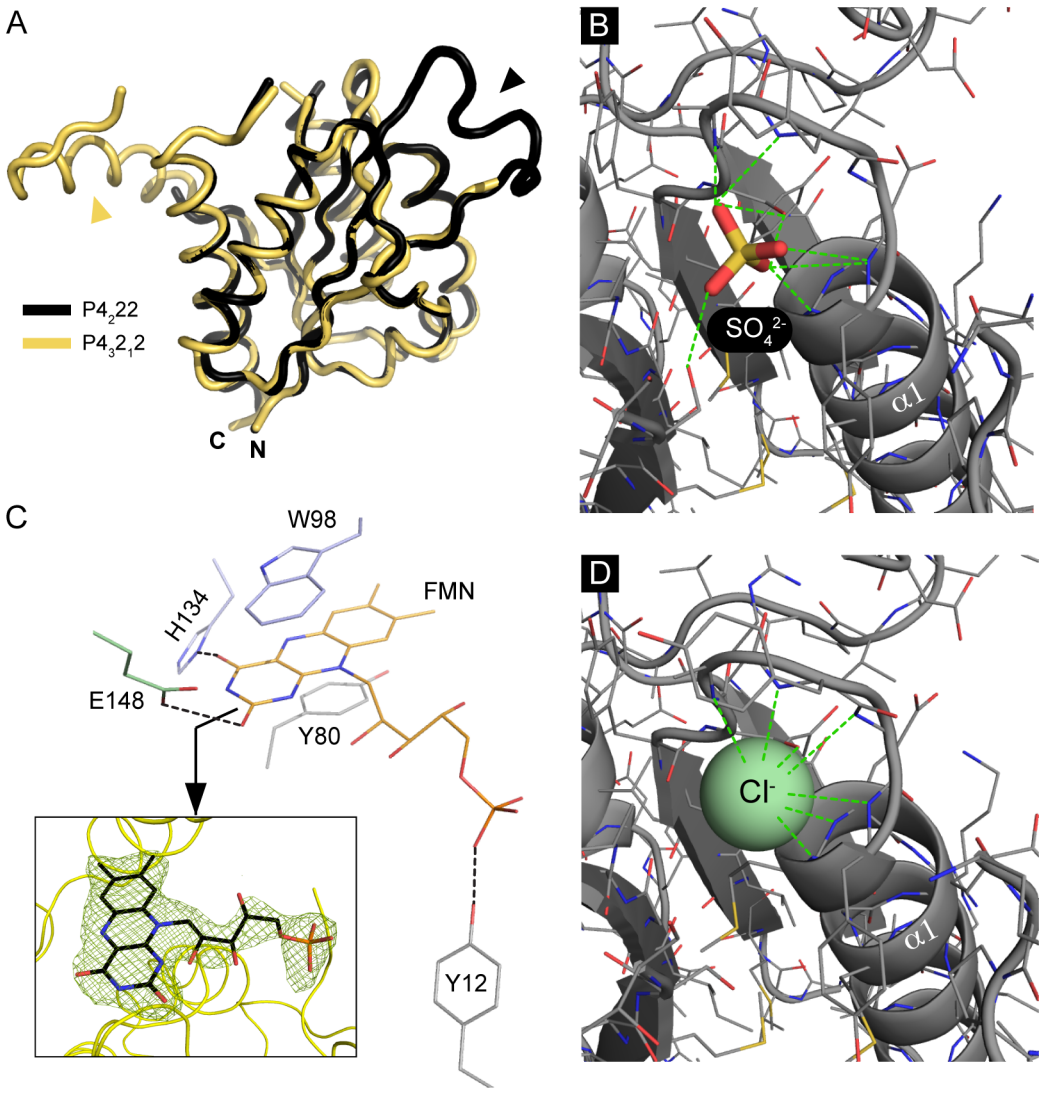

Figure S2: A) Structural alignment of WrpA monomers from the two crystal forms of *B. abortus* WrpA. $P4_32_12$ structure is in yellow, with α-helix α' (Y144-G157) highlighted with a yellow triangle; $P4_222$ structure is in black with disordered loop L41-I54 highlighted with a black triangle (RMSD of alpha carbons=0.35). B) Interaction map (dotted green line) of the sulfate ion (yellow/red sticks) in WrpA $P4_222$ FMN-binding site. C) Cofactor-protein interactions of FMN (yellow/orange lines) with WrpA in the $P4_32_12$ crystal form. Residues of monomer 1 (Y12, Y80) are in grey; monomer 2 residues that interact with the same FMN cofactor are in violet (W98, H134), and residue E148 from the third monomer is in green. A simulated annealing composite omit map (contoured at 1σ) of the FMN is inset. D) Zoom view of the chloride ion (green sphere) interaction site in the $P4_32_12$ WrpA crystal structure.

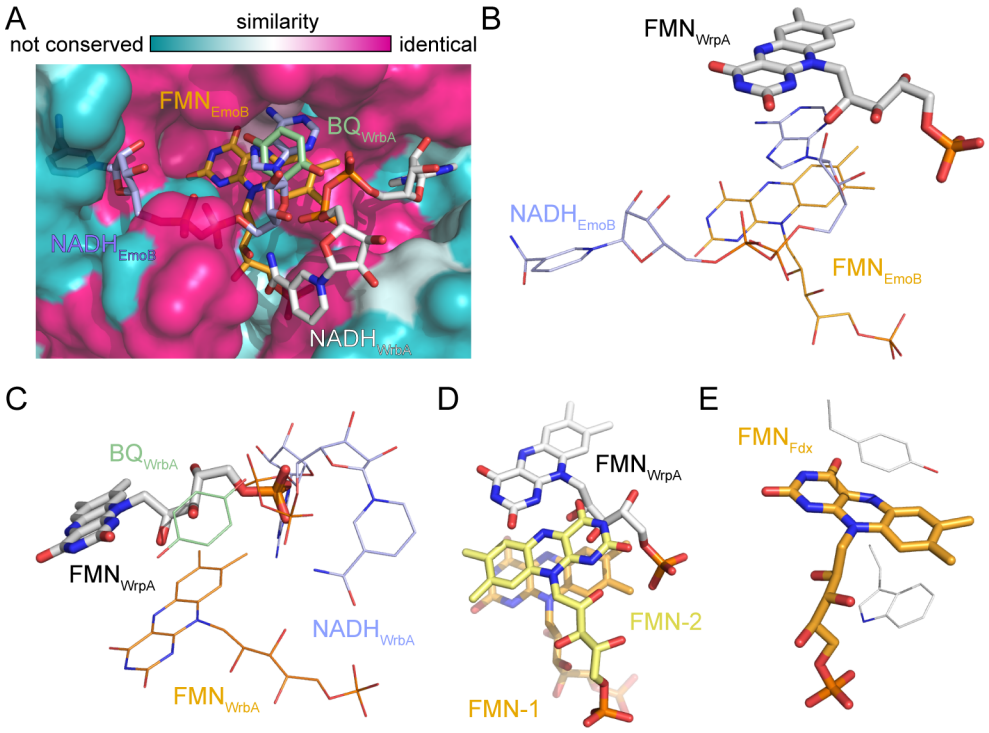

Figure S3: A) WrpA cavity modeled with FMN (orange) and NADH (blue) from the EmoB structure (PDB ID: 4LTN) (77) and NADH (white) and benzoquinone (BQ, green) from *E. coli* WrbA structures (PDB ID: 3B6J and 3B6K) (33) showing that the WrpA cavity can accommodate all these molecules. Conservation of WrpA and $WrbA_{Ec}$ active sites has also been compared: color scale ranges from identical (magenta) to unconserved (blue). B) Superposition of WrpA and EmoB cavities. WrpA FMN (white) adopts a position incompatible with NADH (blue) binding. However, the position of EmoB FMN (orange) is compatible with the presence of NADH in the cavity. FMN and NADH were modeled from the coordinates of EmoB structure (PDB ID: 4LTN) (77). C) WrpA and $WrbA_{Ec}$ active site cavities were structurally superimposed. WrpA FMN (white) adopts a position incompatible with NADH (blue) or benzoquinone (BQ, green) binding. However, the position of FMN (orange) in $WrbA_{Ec}$ structure is compatible with NADH or benzoquinone binding. FMN, NADH and benzoquinone were modeled from $WrbA_{Ec}$ PDB structures 3B6J, 3B6K and 3B6M (33). D) Comparison of EmoB FMN positions (FMN-1 and FMN-2, orange and yellow) to WrpA FMN (white) position. E) Classic interaction pattern of FMN with flavodoxin proteins. As we observed in FMN soaked crystals of *B. abortus* WrpA, the FMN (orange) cofactor is stacked between the side chains (white) of a tryptophan residue and a tyrosine residue (see PDB 1FLV of *Anabaena* 7120 (92)).