

Supporting Information

The structure of the BfrB-Bfd complex reveals protein-protein interactions enabling iron release from bacterioferritin

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	1	10	20	30	40	50
		↓ ↓			↓ ↓	
<i>Pseudomonas aeruginosa</i> PAO1	-----MYVCLCQGVTDNQIRDAIYEGCCS-YREVREATGVGTQCGKCACLAQVVR	ETL				
<i>Escherichia coli</i> O157	-----MYVCLCNGVSDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Escherichia coli</i> K12	-----MYVCLCNGISDKKIRQAVRQFSPHSFQQLKKFI	PVGNQCGKCVRAREVMQDEL				
<i>Salmonella typhimurium</i>	-----MYVCLCNGISDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Salmonella enterica</i> serovar Typhi	-----MYVCLCNGISDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Salmonella enterica</i> choleraesuis	-----MYVCLCNGISDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Klebsiella pneumoniae</i>	-----MYVCLCNGVSDKKIRQVVRQFHPQSFQQLRKFI	PVGNQCGKCVRAREVMQDEL				
<i>Yersinia pestis</i>	-----MYVCLCNAVSDKVIKAVRQHPHTVKQLRLVPI	IGSDCGKCIRQAREILIEEL				
<i>Yersinia pseudotuberculosis</i>	-----MYVCLCNAVSDKVIKAVRQHPHTVKQLRLVPI	IGSDCGKCIRQAREILIEEL				
<i>Yersinia enterocolitica</i>	-----MYVCLCNAVSDKVIKAVRQHPHTVKQLRLVPI	IGSDCGKCIRQAREILIEEL				
<i>Aeromonas salmonicida</i>	-----MYVCLCRGITDQIRKAVQAGKSE-FRQLKQSL	EVGAQCGKCVRMTMEIIAAELD				
<i>Vibrio cholerae</i> El	-----MYVCLCHGVSDKKIRRLVAEQGITDIKGIK	RCTALGSQCGKCVRAKEIIEESL				
<i>Vibrio cholerae</i> 0395	-----MYVCLCHGVSDKKIRRLVAEQGITDIKGIK	RCTALGSQCGKCVRAKEIIEESL				
<i>Vibrio vulnificus</i>	-MSCNM	MYVCLCHGVSDKKIRKLVIEQGVADIRGIKCTALGSQCGKCIRMAKEIINETA				
<i>Shigella flexneri</i> 2a 2457T	-----MYVCLCNGVSDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Shigella flexneri</i> 2a str301	-----MYVCLCNGVSDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Shigella dysenteriae</i>	-----MYVCLCNGVSDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCIRAAREVMQDEL				
<i>Erwinia carotovora</i>	-----MYVCLCNAISDKAIRNAVRRQHPQSMQQLRK	LVPVIGTDCGKCIRQARVIFEEEQ				
<i>Enterobacter cloacae</i>	-----MYVCLCNGVSDTKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCVRAREIMQDEL				
<i>Serratia</i> sp.	-----MYVCLCNAVTDKAIKAVRQHNPHTMKQLREL	VPIGTDCGKCIRQARQIMVEES				
<i>Citrobacter koseri</i>	-----MYVCLCNGVSDKKIRQAVRQFHPQSFQQLRKFI	PVGNQCGKCVRAREVMQDEL				
<i>Acinetobacter</i> sp.	MRMTLMYVCLCRGITDQDKDAVASGAES-YREVRERLDL	GTCGRCAPAKSINIIEEL				
	^^^ ^^ ^ ^^	^^ ^^ ^^				
	60	70				
<i>Pseudomonas aeruginosa</i> PAO1	-----NDLQSAQPVPAFGTTAFVAA					
<i>Escherichia coli</i> O157	-----MQMPEFKEIA	-----				
<i>Escherichia coli</i> K12	-----MQLPPEFKESA	-----				
<i>Salmonella typhimurium</i>	-----TQMPEFKEIA	-----				
<i>Salmonella enterica</i> serovar Typhi	-----TQMPEFKEIA	-----				
<i>Salmonella enterica</i> choleraesuis	-----TQMPEFKEIA	-----				
<i>Klebsiella pneumoniae</i>	-----TTMPEFKEIA	-----				
<i>Yersinia pestis</i>	-----ANIPENMDVA	-----				
<i>Yersinia pseudotuberculosis</i>	-----ANIPENMDVA	-----				
<i>Yersinia enterocolitica</i>	-----ANIPENMDVA	-----				
<i>Aeromonas salmonicida</i>	KAELETAPLYYEVA	-----				
<i>Vibrio cholerae</i> El	-----IAQFKLAS	-----				
<i>Vibrio cholerae</i> 0395	-----IAQFKLAS	-----				
<i>Vibrio vulnificus</i>	-----PALFKQAS	-----				
<i>Shigella flexneri</i> 2a 2457T	-----MQMPEFKEIA	-----				
<i>Shigella flexneri</i> 2a str301	-----MQMPEFKEIA	-----				
<i>Shigella dysenteriae</i>	-----MQMPEFKEIA	-----				
<i>Erwinia carotovora</i>	-----AKIPDMYKVA	-----				
<i>Enterobacter cloacae</i>	-----MQIPEYKEIA	-----				
<i>Serratia</i> sp.	-----GTIIPMHEVA	-----				
<i>Citrobacter koseri</i>	-----MQMPEFKEIA	-----				
<i>Acinetobacter</i> sp.	-----AEIAARISVA	-----				

Figure S1a. Amino acid sequence of *Pa*-Bfd aligned with Bfd-like sequences from different bacterial species. Conserved cysteine residues binding iron in the [2Fe-2S] cluster are highlighted by an arrow (↓) and residues buried at the *Pa*-BfrB-Bfd interface are denoted by (^). Conserved residues across the alignment are in red, conservative substitutions in green and semi-conservative substitutions in blue, sequence numbering according to *Pa*-Bfd

	10	20	30	40	50	60
<i>Pseudomonas aeruginosa</i> PAO1	MKGDKKVIQHLNKLILGNELIAINQYFLHSRMWN	DWGLKRLGAHEYHESIDEMKHADKLIIE				
<i>Escherichia coli</i> O157	MKGDVKKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLMRINDVEYHESIDEMKHADKYIE				
<i>Escherichia coli</i> K12	MKGDTVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLKRLNDVEYHESIDEMKHADRYIE				
<i>Salmonella typhimurium</i>	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLTRLNDVEYHESIDEMKHADKYIE				
<i>Salmonella enterica</i> serovar Typhi	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLTRLNDVEYHESIDEMKHADKYIE				
<i>Salmonella enterica</i> choleraesuis	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLTRLNDVEYHESIDEMKHADKYIE				
<i>Klebsiella pneumoniae</i>	MKGDVKIISYLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDIEYHESIDEMKHADKYIE				
<i>Yersinia pestis</i>	MKGDKKIIAHLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDKEYHESIDEMKHADKYIE				
<i>Yersinia pseudotuberculosis</i>	MKGDKKIIAHLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDKEYHESIDEMKHADKYIE				
<i>Yersinia enterocolitica</i>	MKGDKKIIAHLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDKEYHESIDEMKHADKYIE				
<i>Aeromonas salmonicida</i>	MKGDPKIIAHLNKVLANELIAINQYFLHARIYDD	WGDLKALGHKEYHESIDEMKHADLVLE				
<i>Vibrio cholerae</i> E1	MKGDVPVIIQHNLKVLANELIAINQYFLHARMYKD	WGDLKHLADKEYHESIDEMKHADHIVE				
<i>Vibrio cholerae</i> 0395	MKGDVPVIIQHNLKVLANELIAINQYFLHARMYKD	WGDLKHLADKEYHESIDEMKHADHIVE				
<i>Vibrio vulnificus</i>	MKGDPIIIQHNLKVLGNELIAINQYFLHARMYKD	WGDLKHLADKEYHESIDEMKHADHLVE				
<i>Shigella flexneri</i> 2a 2457T	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDVEYHESIDEMKHADKYIE				
<i>Shigella flexneri</i> 2a str301	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDVEYHESIDEMKHADKYIE				
<i>Shigella dysenteriae</i>	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDVEYHESIDEMKHADKYIE				
<i>Erwinia carotovora</i>	MKGDKKVIITHNLKLLGNELVAINQYFLHARMFKN	NWGLTRLNDHEYHESIDEMKHADRYIE				
<i>Enterobacter cloacae</i>	MKGDGVKIIISYLNKLLGNELVAINQYFLHARMFKN	NWGLTRLNDVEYHESIDEMKHADKYIE				
<i>Serratia sp.</i>	MKGDKKIIAHLNKLLGNELVAINQYFLHARMFKN	NWGLMRLNDKEYHESIDEMKHADRYIE				
<i>Citrobacter koseri</i>	MKGDVKIINYLNKLLGNELVAINQYFLHARMFKN	NWGLTRLNDVEYHESIDEMKHADKYIE				
<i>Acinetobacter sp.</i>	MKGNRDVINQLNVLYLHHLTAINQYFLHSRMFN	DWGIEKLSGEYHESIRQMKHADKIIIE				
	70	80	90	100	110	120
<i>Pseudomonas aeruginosa</i> PAO1	RILFLEGLPNLQDLGKLLIGENTQEMLQCDDL	NLELKATKDLEAIIVHCQVHDYVSRDILL				
<i>Escherichia coli</i> O157	RILFLEGIPNLQDLGKLIGEDVDEEMLQSDLR	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Escherichia coli</i> K12	RILFLEGIPNLQDLGKLNINGEDVDEEMLRSDL	LALELDGAKNLRATIGYADSVHDYVSRDMM				
<i>Salmonella typhimurium</i>	RILFLEGIPNLQDLGKLIGEDVDEEMLRSDL	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Salmonella enterica</i> serovar Typhi	RILFLEGIPNLQDLGKLIGEDVDEEMLRSDL	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Salmonella enterica</i> choleraesuis	RILFLEGIPNLQDLGKLIGEDVDEEMLRSDL	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Klebsiella pneumoniae</i>	RILFLEGIPNLQDLGKLIGEDVDEEMLRSDL	LRELEGAQNLRATIGYADSVHDYVSRDMM				
<i>Yersinia pestis</i>	RILFLEGIPNLQELGKLNIGEDVDEEMLKSDLA	LESAGATDLREGIAYADSIHDYVSRDILL				
<i>Yersinia pseudotuberculosis</i>	RILFLEGIPNLQELGKLNIGEDVDEEMLKSDLA	LESAGATDLREGIAYADSIHDYVSRDILL				
<i>Yersinia enterocolitica</i>	RILFLEGIPNLQDLGKLNIGEDVDEEMLKSDLA	LESAGATDLREGIAYADSIHDYVSRDILL				
<i>Aeromonas salmonicida</i>	RVLFLEGLPNLQDLGKLRIGETVDEEMLRCDSL	LEMDAIPDLKVAIAYAESVQDYISRDLF				
<i>Vibrio cholerae</i> E1	RILFLEGIPNLQDLGKLIMIGEDVDEEMLCDDL	KLLELIAIPDLKEAIAYAEKVDRDYVSRDLF				
<i>Vibrio cholerae</i> 0395	RILFLEGIPNLQDLGKLIMIGEDVDEEMLCDDL	KLLELIAIPDLKEAIAYAEKVDRDYVSRDLF				
<i>Vibrio vulnificus</i>	RILFLEGIPNLQDLGKLIMIGEDVDEEMLCDDL	KLLEMAAIPDLKAAIAYAEVDH DYVSRDLF				
<i>Shigella flexneri</i> 2a 2457T	RILFLEGIPNLQDLGKLIGEDVDEEMLKSDLR	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Shigella flexneri</i> 2a str301	RILFLEGIPNLQDLGKLIGEDVDEEMLKSDLR	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Shigella dysenteriae</i>	RILFLEGIPNLQDLGKLIGEDVDEEMLKSDLR	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Erwinia carotovora</i>	RILFLEGIPNLQDLGKLNINGEDVDEEMLKSDLR	LQLELDGAKNLRATIGYADSVHDYVSRDMM				
<i>Enterobacter cloacae</i>	RILFLEGIPNLQDLGKLIGEDVDEEMLRSDL	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Serratia sp.</i>	RILFLEGIPNLQDLGKLNINGEDIETIELRSDL	LALELDGAKNLRATIGYADSIHDYVSRDMM				
<i>Citrobacter koseri</i>	RILFLEGIPNLQDLGKLIGEDVDEEMLKSDLR	LRELEGAKDLREAIAYADSVHDYVSRDMM				
<i>Acinetobacter sp.</i>	RILFLEGIPNLQHLGKLYIQHTQEVVINC DV	RVRVKENIETLKKAVEISEIQQDYVSRDILV				
	130	140	150			
<i>Pseudomonas aeruginosa</i> PAO1	KDILESEEEHIDYLETQLGLIQKVGLENYLQSHMH	HEDDI				
<i>Escherichia coli</i> O157	IEILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-ND				
<i>Escherichia coli</i> K12	IEILRDEEGHIDWLETELDLDIGIKIGLQNYLQA	QIRE-EG				
<i>Salmonella typhimurium</i>	IEILADEEGHIDWLETELDLDIAKLGMQNYLQS	QIKV-TD				
<i>Salmonella enterica</i> serovar Typhi	IEILADEEGHIDWLETELDLDIAKLGMQNYLQS	QIKV-TD				
<i>Salmonella enterica</i> choleraesuis	IEILADEEGHIDWLETELDLDIAKLGMQNYLQS	QIKV-TD				
<i>Klebsiella pneumoniae</i>	IEILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-SD				
<i>Yersinia pestis</i>	KEILVDEEEHIDWLETELSLIDRLGIQSYSAQA	LA--KD				
<i>Yersinia pseudotuberculosis</i>	KKILVDEEEHIDWLETELSLIDRLGIQSYSAQA	LA--KD				
<i>Yersinia enterocolitica</i>	KEILADEEGHIDWLETELSLIERGLQNYSAQA	LA--KD				
<i>Aeromonas salmonicida</i>	QDILEDEEEHV DWLETEQLDLIDRIGLENYQSQ	LHHGSS				
<i>Vibrio cholerae</i> E1	QEILEDEEEHV DWLETEQLGLIDMTGTQNYLQAQ	FVDDDE				
<i>Vibrio cholerae</i> 0395	QEILEDEEEHV DWLETEQLGLIDMTGTQNYLQAQ	FVDDDE				
<i>Vibrio vulnificus</i>	QDILEDEEEHV DWLETEQLGLIQLTGIENYLAQA	FVDDDE				
<i>Shigella flexneri</i> 2a 2457T	IEILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-KD				
<i>Shigella flexneri</i> 2a str301	IEILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-KD				
<i>Shigella dysenteriae</i>	IEILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-KD				
<i>Erwinia carotovora</i>	IEILADEEGHIDWLETELDLDIARLGIQNYLQAQ	LK--AE				
<i>Enterobacter cloacae</i>	IQILADEEGHIDWLETELDLDIGIKIGLQNYLQS	QIKV-ED				
<i>Serratia sp.</i>	IEILADEEGHIDWLETELDLDIARLGIQNYAQAQ	ILERKD				
<i>Citrobacter koseri</i>	IEILTDEEGHIDWLETELDLDIAKIGLQNYLQS	QIKV-DD				
<i>Acinetobacter sp.</i>	QEILEKEEEYDWTTTQDLIDIESIGIENYIQSQV	----				

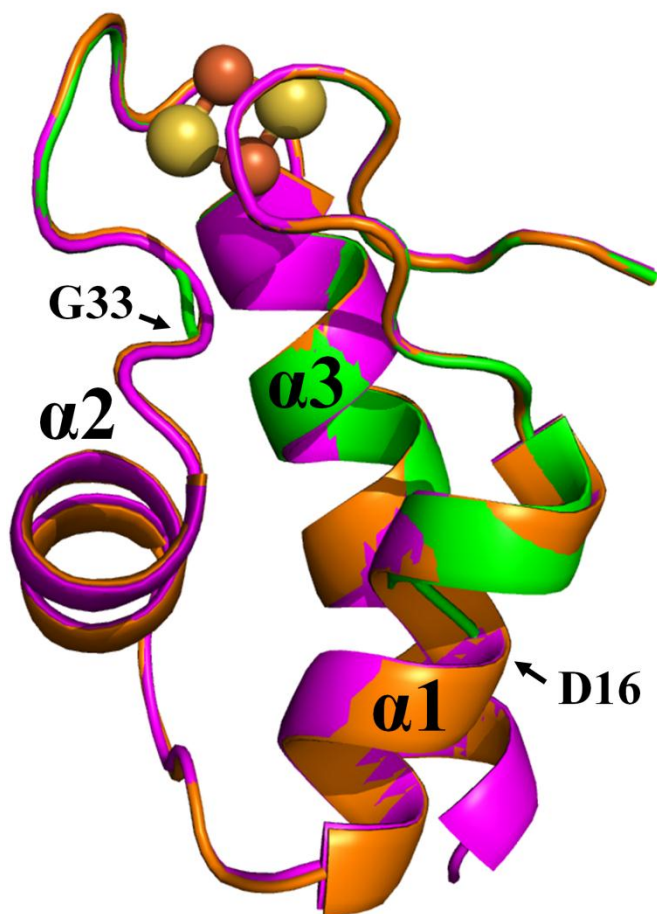


Figure S2. Superposition of Bfd chains G (magenta), H (green) and I (orange) shows that the three Bfd chains are structurally identical. The arrows delimit the stretch of sequence where electron density is missing in chain H (residues D16-G33).

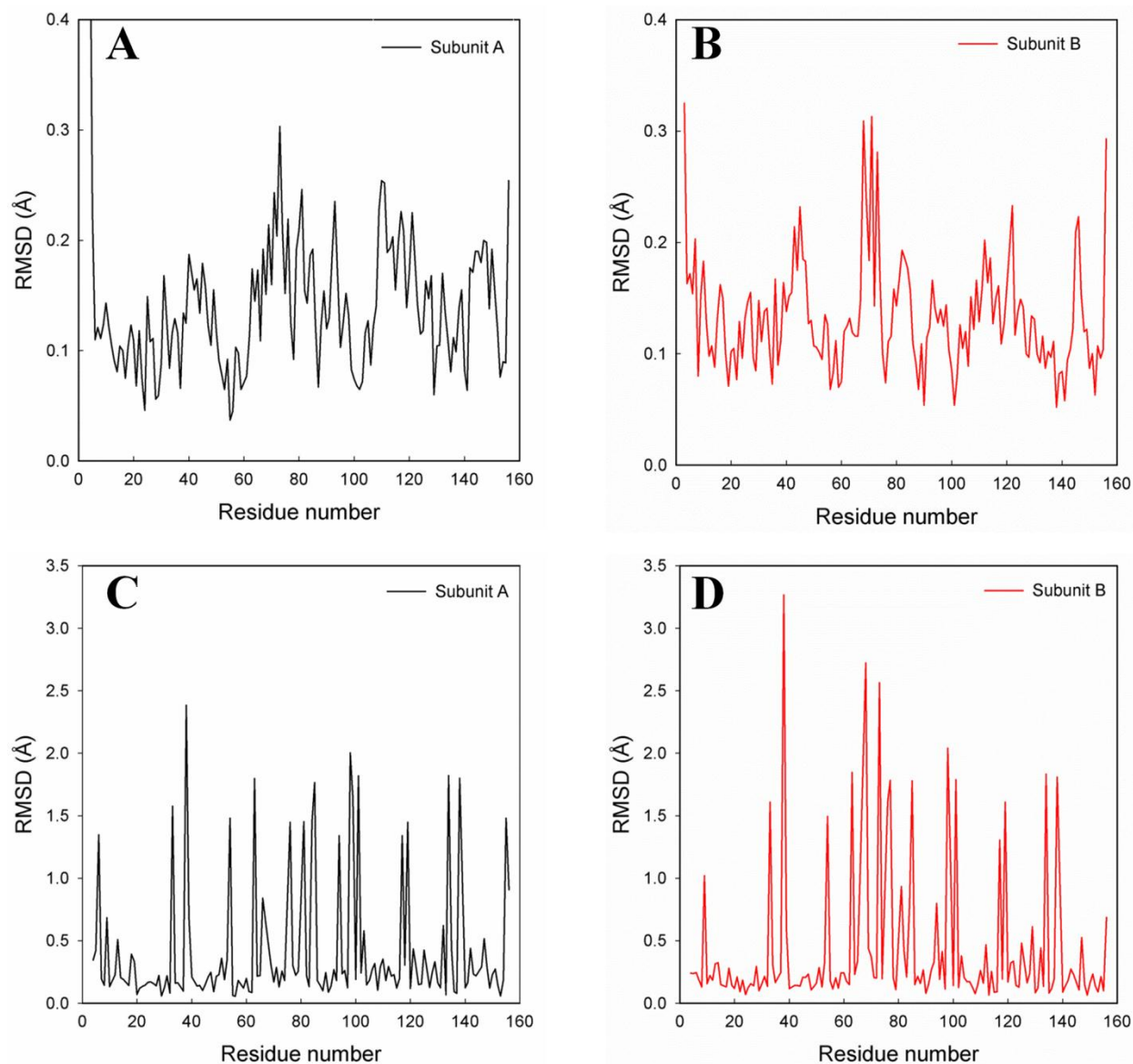


Figure S3. A and B: Per-residue plots of RMSD obtained from comparing main chain atoms in subunits A (**A**) and B (**B**) of *Pa*-BfrB in complex with Bfd with *Pa*-BfrB alone (PDB: 3IS7). **C** and **D:** Per-residue plots of average RMSD obtained from comparing side chain atoms in subunits A (**C**) and B (**D**) of *Pa*-BfrB in complex with Bfd with *Pa*-BfrB alone (PDB: 3IS7). Average RMSD are 0.14 Å between C_{α} atoms and 0.67 Å between side chains.

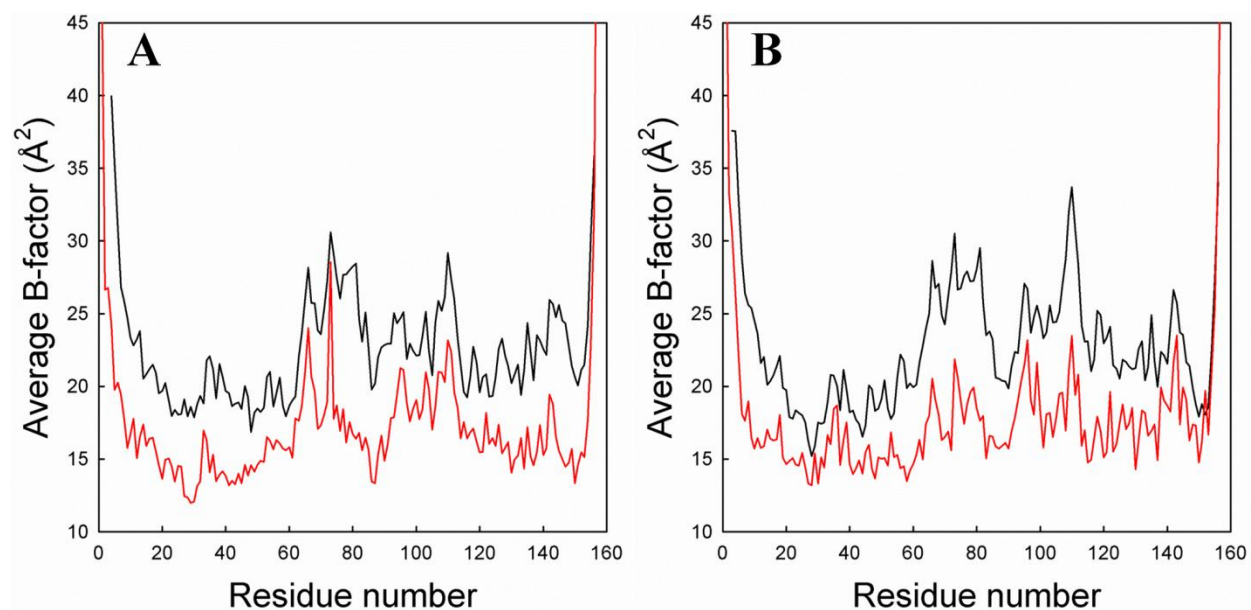


Figure S4. Main chain average B-factors comparing **(A)** subunit A in *Pa*-BfrB alone (black) (PDB: 3IS7) with subunit A in the *Pa*-BfrB-Bfd complex (red), and **(B)** subunit B in *Pa*-BfrB alone (black) (PDB: 3IS7) with subunit B in the *Pa* BfrB-Bfd complex (red).

Table S1. Bfr and Bfd genes are clustered in a number of pathogenic bacteria.

Microorganism	Locus ID	
	Bfr	Bfd
<i>Pseudomonas aeruginosa</i> PAO1	PA3531	PA3530
<i>Escherichia coli</i> O157	Z4695	Z4696
<i>Escherichia coli</i> K12	b3336	b3337
<i>Salmonella typhimurium</i>	STM3443	STM3444
<i>Salmonella enterica</i> serovar Typhi	t4062	t4061
<i>Salmonella enterica</i> choleraesuis	SCH_3378	SCH_3377
<i>Klebsiella pneumonia</i>	KPN_03723	KPN_03722
<i>Yersinia pestis</i>	YPO0206	YPO0205
<i>Yersinia pseudotuberculosis</i>	YPTB3700	YPTB3701
<i>Yersinia enterocolitica</i>	YE3925	YE3926
<i>Aeromonas salmonicida</i>	ASA_0467	ASA_0466
<i>Vibrio cholerae</i> El	VC_0365	VC_0364
<i>Vibrio cholerae</i>	VC0395_A2776	VC0395_A2775
<i>Vibrio vulnificus</i>	VV1_1341	VV1_1340
<i>Shigella flexneri</i> 2a 2457T	S4407	S4406
<i>Shigella flexneri</i> 2a str301	AAN44818.1	AAN44819.1
<i>Shigella dysenteriae</i>	SDY_3498	SDY_3499
<i>Erwinia carotovora</i>	ECA4034	ECA4033
<i>Enterobacter cloacae</i>	ECL_04700	ECL_04701
<i>Serratia</i> sp.	SerAS12_4645	SerAS12_4646
<i>Citrobacter koseri</i>	CKO_04743	CKO_04744
<i>Acinetobacter</i> sp.	ACIAD3330	ACIAD3328