Supporting Information

Concerted Motions Networking Pores and Distant Ferroxidase Centers Enable Bacterioferritin Function and Iron Traffic

Huili Yao,[†] Huan Rui,[§] Ritesh Kumar,[§] Kate Eshelman,[†] Scott Lovell,[‡] Kevin P. Battaile,[¥] Wonpil Im,[§] and Mario Rivera[†]*

[†]Department of Chemistry, University of Kansas, Multidisciplinary Research Building, 2030 Becker Dr., Lawrence, KS 66047.

[‡]Del Shankel Structural Biology Center, University of Kansas, 2034 Becker Dr., Lawrence, KS 66047.

[§]Department of Molecular Biosciences and Center for Bioinformatics, University of Kansas, 2030 Becker Dr., Lawrence, KS 66047.

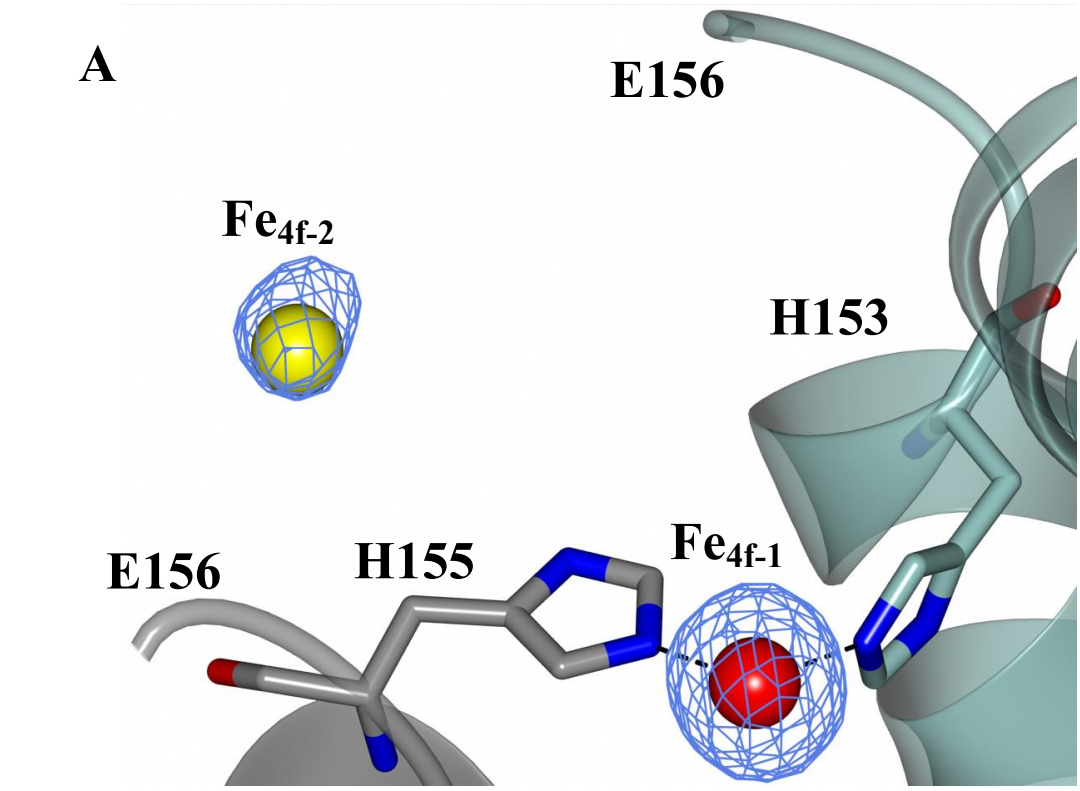
[¥]IMCA-CAT, Hauptman Woodward Medical Research Institute, 9700 S. Cass Avenue, Bldg. 435A, Argonne, IL 60439.

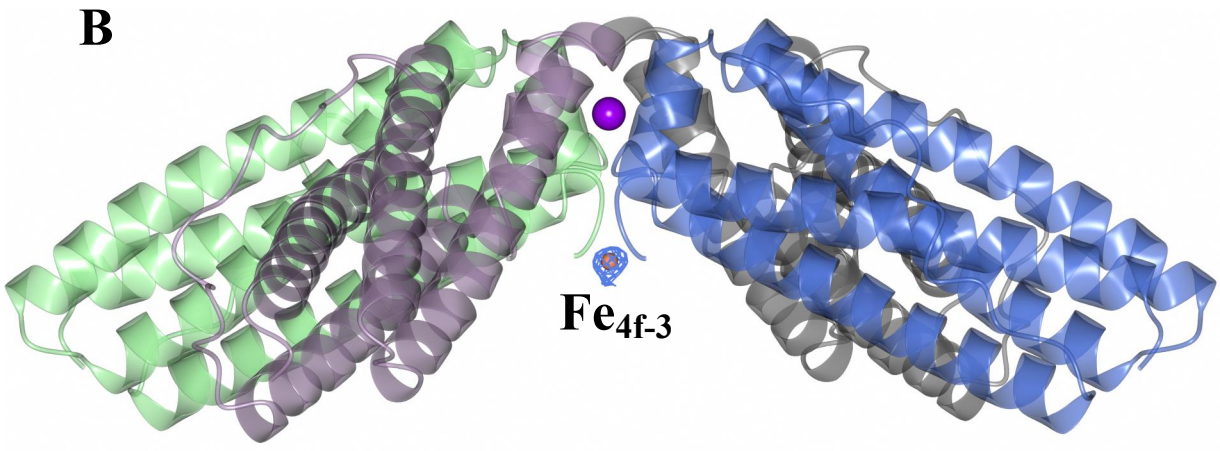
*To whom correspondence should be addressed: Email: mrivera@ku.edu

Sequence of primer pairs used to introduce the site directed mutations in the *bfrB* gene:

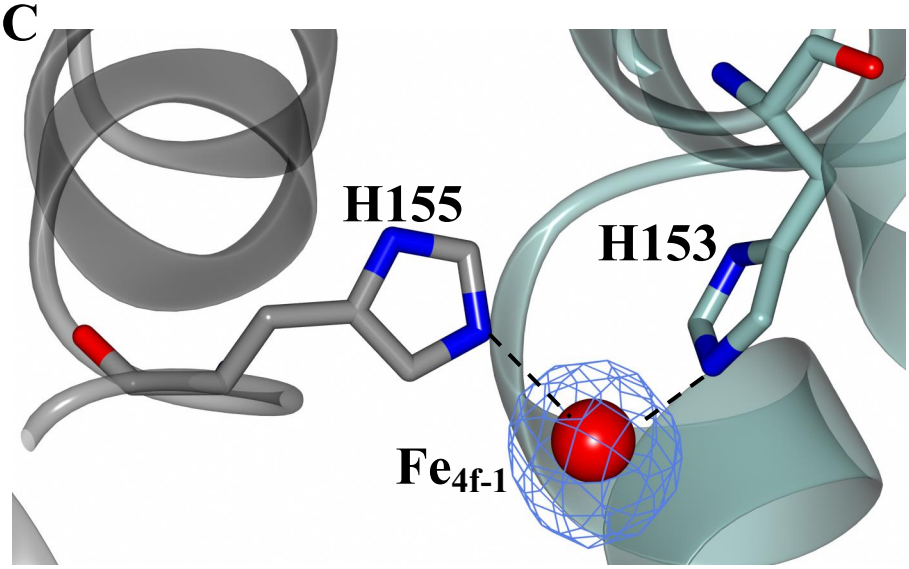
D34F:	Forward: 5'GCATAGCCGCATGTGGAATTTTTGGGGCCTGAAACGTCTGG3'
	Reverse: 5'CCAGACGTTTCAGGCCCCAAAAATTCCACATGCGGCTATGC3'
N148L:	Forward: 5'CAGAAAGTGGGCCTGGAACTGTATCTGCAGAGCCATATGC3'
	Reverse: 5' GCATATGGCTCTGCAGATACAGTTCCAGGCCCACTTTCTG 3'
Q151L:	Forward: 5' GCCTGGAAAACTATCTGCTGAGCCATATGCATGAAG 3'
	Reverse: 5' CTTCATGCATATGGCTCAGCAGATAGTTTTCCAGGC 3'
C89S:	Forward: 5' CACCCAGGAAATGCTGCAGAGCGATCTGAATCTGGAAC 3'
	Reverse: 5' GTTCCAGATTCAGATCGCTCTGCAGCATTTCCTGGGTG 3'
K96C:	Forward: 5' GCGATCTGAATCTGGAACTGTGCGCGACCAAAGATCTGCGTG 3'
	Reverse: 5' CACGCAGATCTTTGGTCGCGCACAGTTCCAGATTCAGATCGC 3'

Figure S1. Phased anomalous difference maps (blue mesh) contoured at 3σ for various iron atom sites. (**A**) Fe-soaked BfrB C89S/K96C showing Fe_{4f-1} and the nearby Fe_{4f-2} atom. (**B**) The 4-fold pore for Fe-soaked BfrB N148L Fe_{4f-3} located at the entrance of the 4-fold pore from the interior cavity. The potassium ion in the pore is drawn as a purple sphere. (**C**) Fe_{4f-1} in Fe-soaked Q151L BfrB and (**D**) Fe-soaked D34F BfrB.





C



D

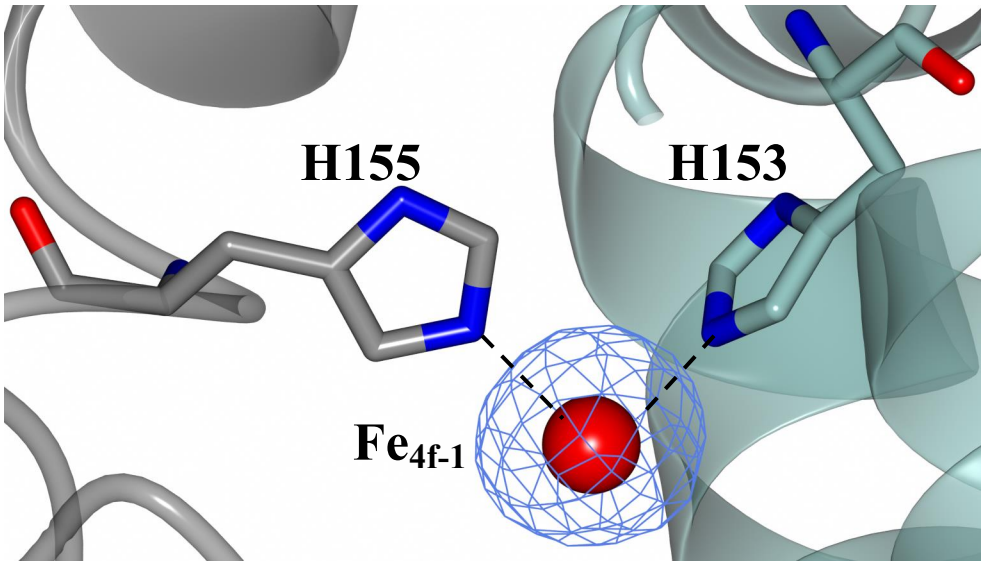


Figure S2. Phased anomalous difference maps contoured at 3σ for the potassium ion in the 4-fold pore of Fe-soaked BfrB C89S/K96C. Maps were computed using data collected at wavelengths of 1.7398 Å (blue mesh) and 1.7463 Å (orange). The peak height increases from 5.5 σ to 9.4 σ using data collected at 1.7398 Å and 1.7463 Å, respectively, supporting the assignment of a potassium ion. The anomalous difference density disappears at the iron sites for the longer wavelength data.

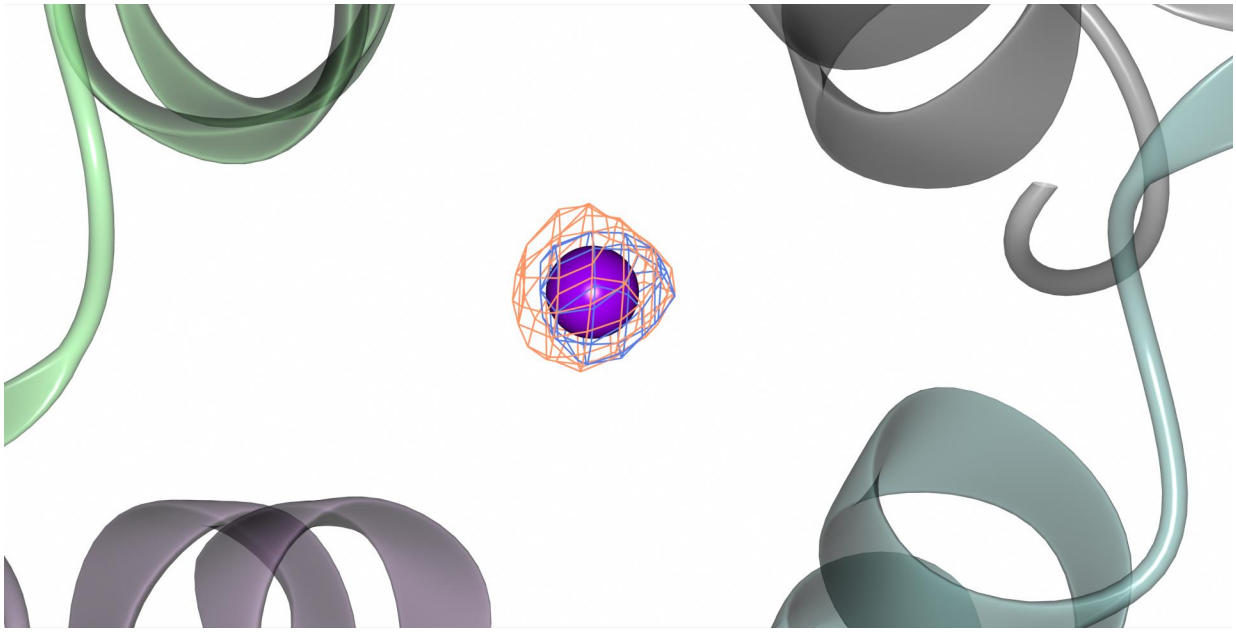


Figure S3. Phased anomalous difference maps contoured at 3σ (blue mesh) for the iron atoms (Fe_{3f}) and 2Fo-Fc maps (green mesh) contoured at 1σ for the sulfate ions in the 3-fold pores of (**A**) Fe-soaked C89S/K96C BfrB, (**B**) Fe-soaked N148L BfrB, (**C**) Fe-soaked Q151L BfrB and (**D**) Fe-soaked D34F BfrB.

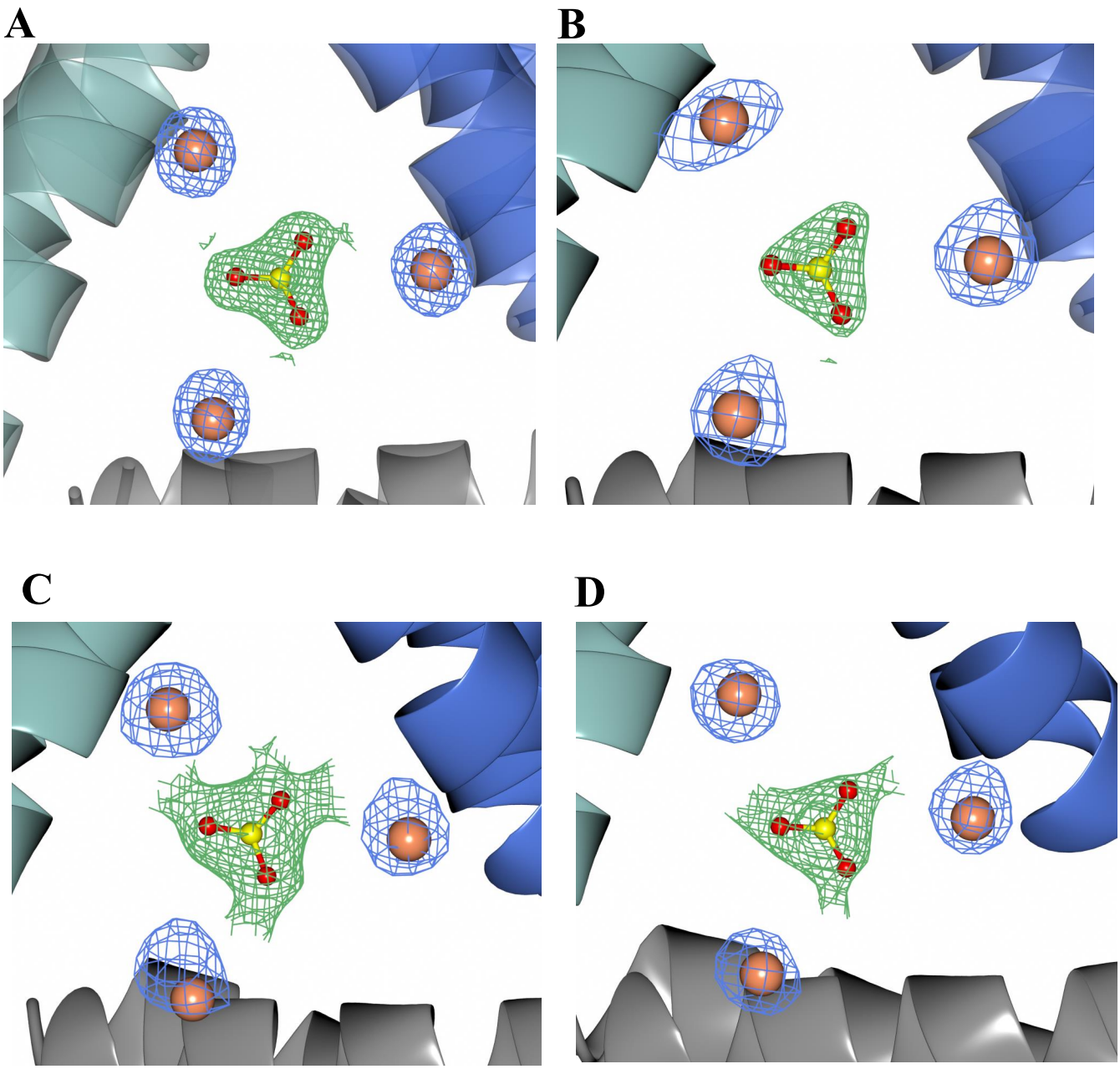


Figure S4. 2Fo-Fc maps (blue mesh) contoured at 1σ for the sulfate ions in the 3-fold pores of as-isolated-2 BfrB C89S/K96C. (**A**) Viewed along the 3-fold pore. (**B**) Viewed approximately perpendicular to the 3-fold pore.

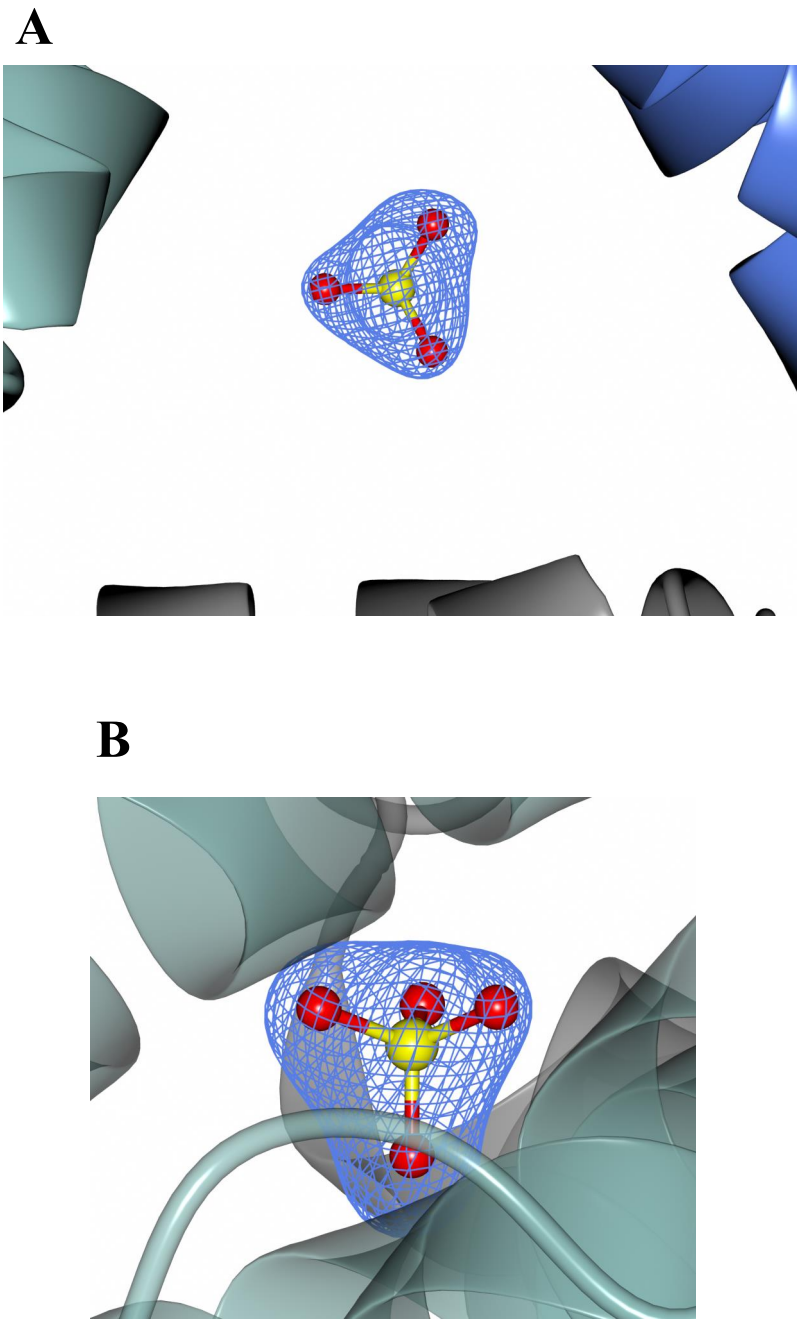


Figure S5. Phased anomalous difference maps contoured at 3σ (blue mesh) for the iron atoms in the B-pores of (**A**) Fe-soaked D34F BfrB and (**B**) Fe-soaked C89S/K96C BfrB.

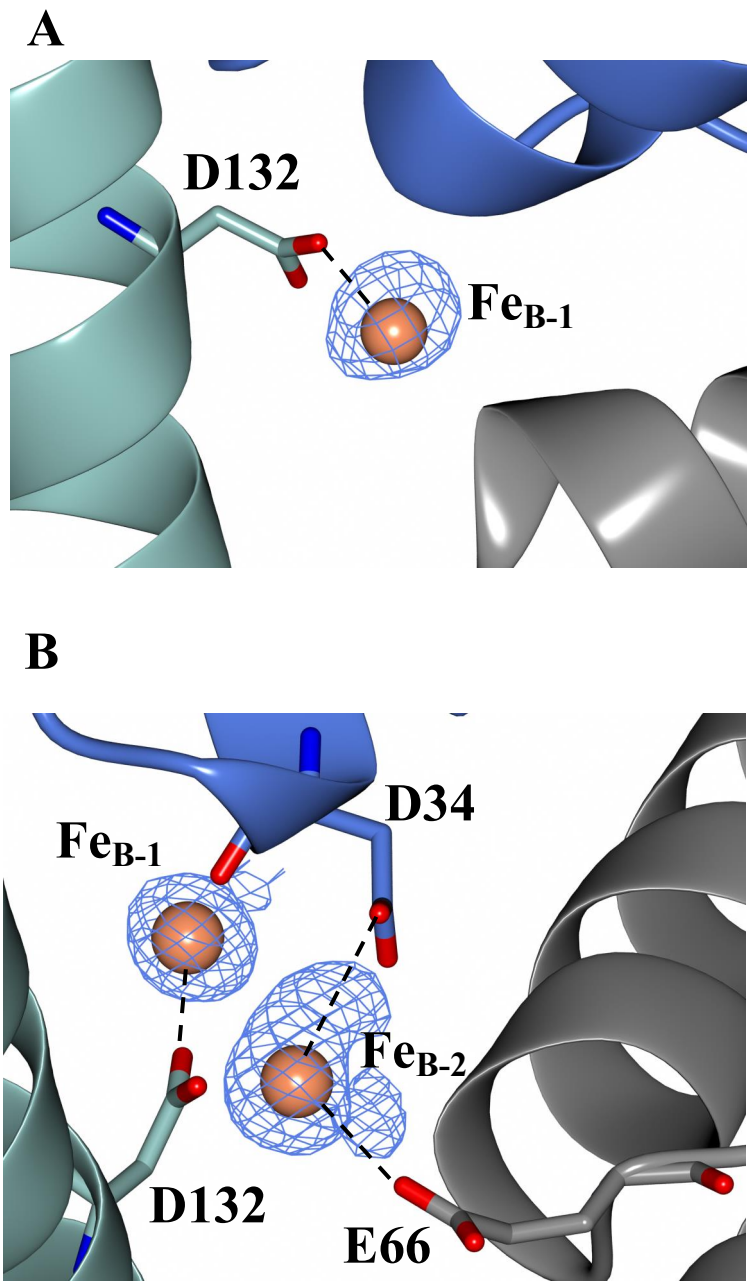


Figure S6. Phased anomalous difference maps contoured at 3σ (blue mesh) for the iron atoms in the ferroxidase center of (**A**) Fe-soaked C89S/K96C BfrB, (**B**) Fe-soaked N148L BfrB, (**C**) Fe-soaked Q151L BfrB and (**D**) Fe-soaked D34F BfrB.

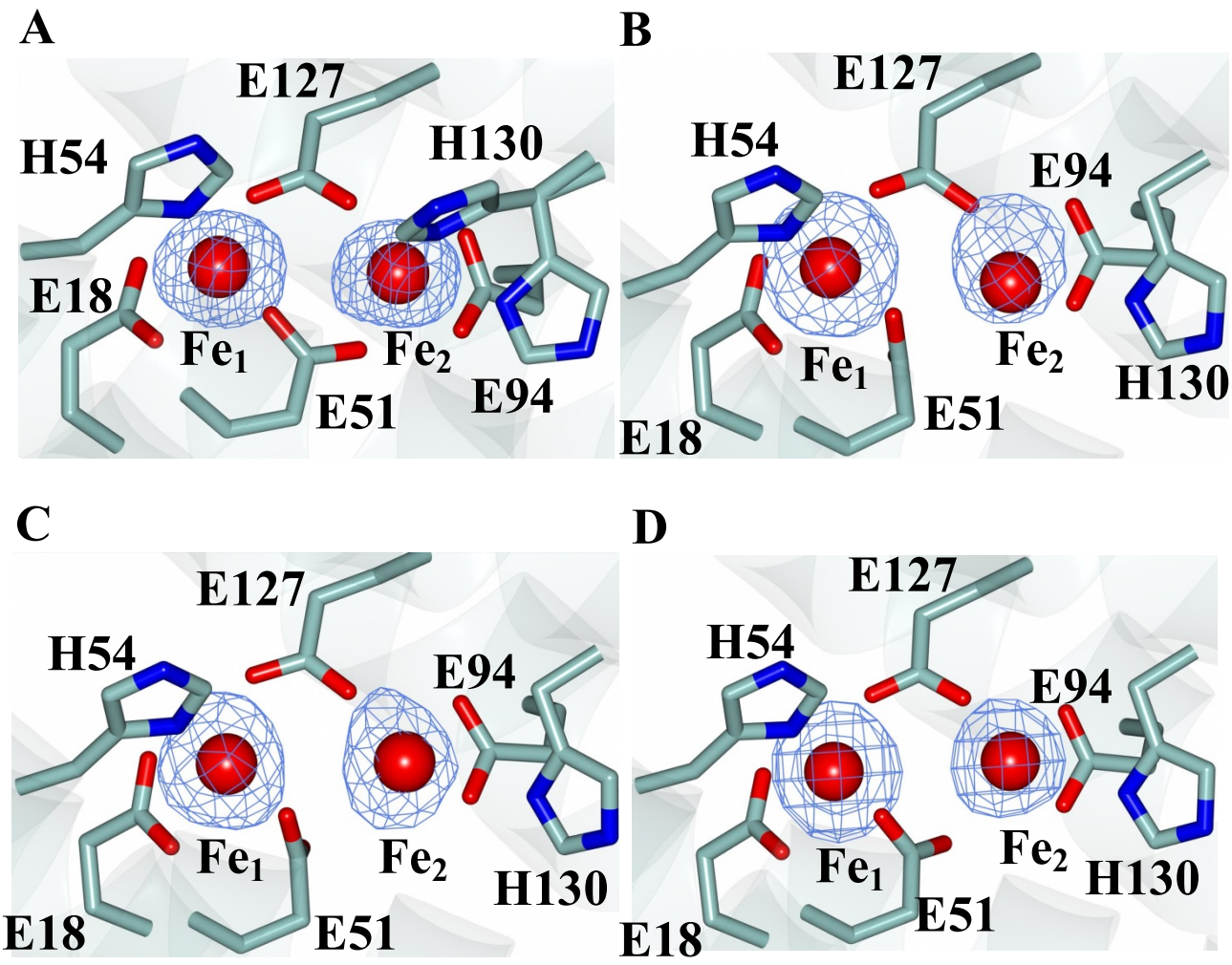


Figure S7. Phased anomalous difference maps contoured at 3σ (blue mesh) for the iron atom located below the ferroxidase center ($Fe_{b\text{-}fc}$) in Fe-soaked BfrB C89S/K96C. Subunit A of this structure is depicted for which alternate conformations were observed for H46 and H130. Residue E50 is near (~4.5 Å) $Fe_{b\text{-}fc}$ but is not coordinated.

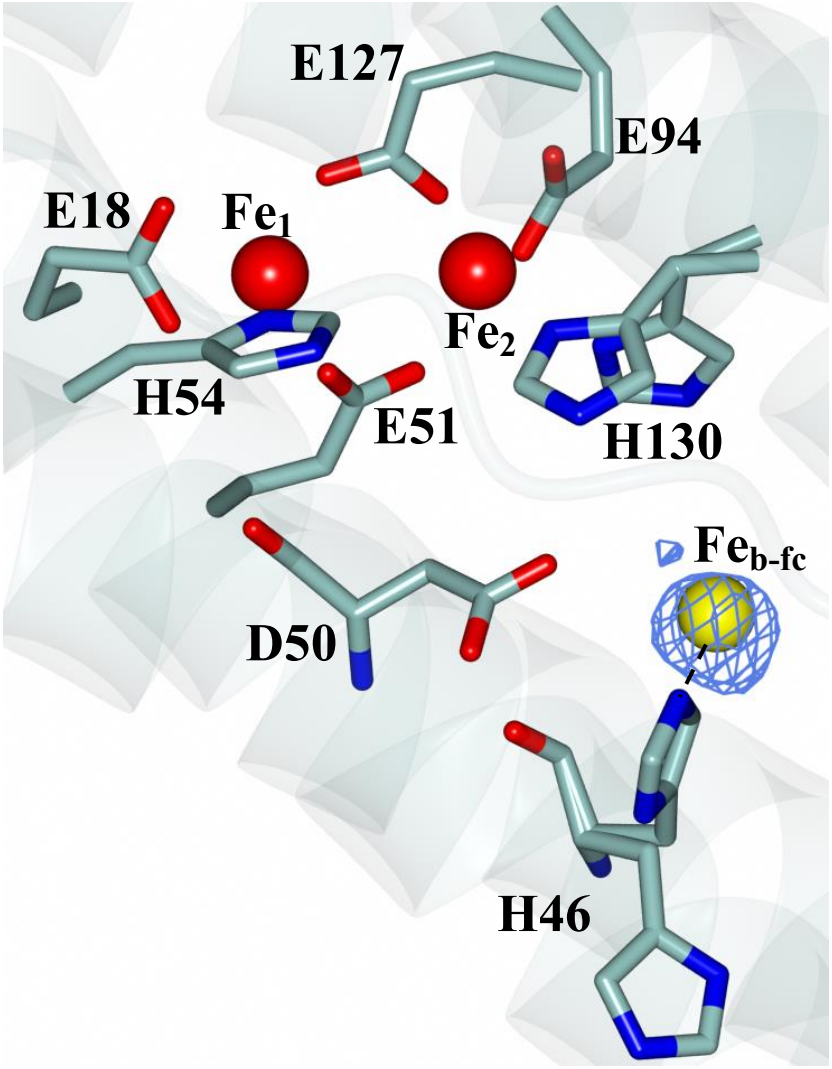


Figure S8. Zoomed-in view of the region near C96 (subunit A) in the as-isolated C89S/K96C BfrB structures. Residue Cys 96 in the asymmetric unit is colored magenta and its counterpart related by the symmetry operator (Y, X, -Z) is colored gray. **A)** In as-isolated BfrB C89S/K96C, Cys 96 adopts two conformations with a distance of 4.7 Å between sulfur atoms. **B)** In the as-isolated-2 BfrB C89S/K96C structure, the Cys 96 residues are brought closer together and form an intermolecular disulfide bond.

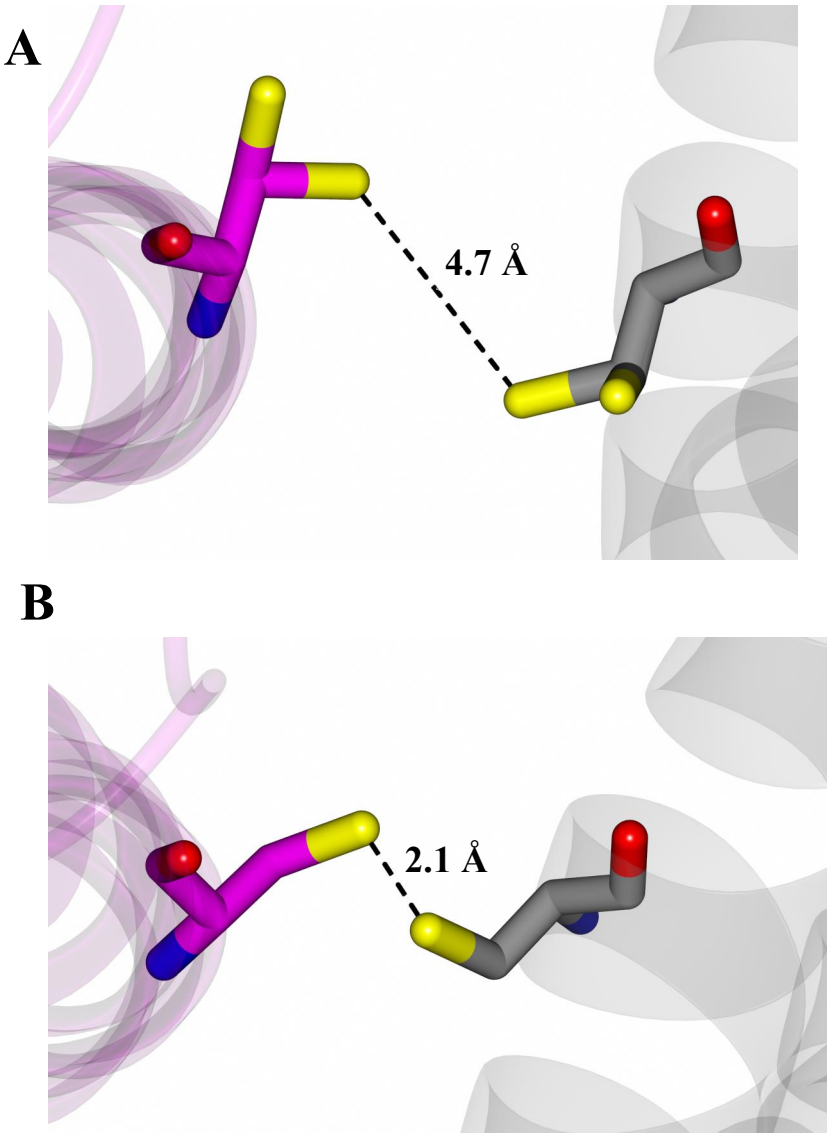


Table S1. Anomalous peak heights and *B*-factors for the Fe atoms in the iron soaked C89S/K96C BfrB structure.

Fe #	Location	Anomalous Peak Height (σ)	*B*-factor (Å^2)
13	$Fe_{4f\text{-}1}$	51.9	18.8
6	Fe_1	43.3	22.3
10	Fe_2	50.1	22.3
12	Fe_2	35.9	22.7
15	$Fe_{4f\text{-}1}$	46.7	22.9
14	$Fe_{4f\text{-}1}$	47.8	23.0
5	Fe_1	43.1	23.0
7	Fe_1	39.9	23.2
16	$Fe_{4f\text{-}1}$	46.2	24.0
8	Fe_1	40.0	24.3
11	Fe_2	42.7	24.5
2	Fe_{out}	39.8	28.1
1	Fe_{out}	37.1	29.5
9	Fe_2	32.9	29.9
4	Fe_{out}	38.4	30.7
3	Fe_{out}	35.0	30.8
18	Fe_{3f}	28.2	37.0
19	Fe_{3f}	22.8	39.4
20	Fe_{3f}	26.7	39.5
17	Fe_{3f}	25.7	40.3
21	$Fe_{B\text{-}2}$	24.6	45.3
24	$Fe_{B\text{-}2}$	22.0	50.3
28	$Fe_{B\text{-}1}$	18.0	51.8
22	$Fe_{B\text{-}2}$	21.4	52.1
23	$Fe_{B\text{-}2}$	20.8	52.4
27	$Fe_{B\text{-}1}$	17.4	53.7
30	$Fe_{b\text{-}fc}$	16.3	56.4
31	$Fe_{b\text{-}fc}$	13.6	62.2
26	$Fe_{B\text{-}1}$	13.3	62.2
25	$Fe_{B\text{-}1}$	16.4	63.0
32	$Fe_{b\text{-}fc}$	12.4	65.4
29	$Fe_{b\text{-}fc}$	9.5	80.4
36	$Fe_{4f\text{-}2}$	8.1	81.4
34	$Fe_{4f\text{-}2}$	7.3	96.4
33	$Fe_{4f\text{-}2}$	8.9	96.8
37	$Fe_{4f\text{-}2}$	8.1	104.5

- Anomalous peak heights for the heme Fe atoms were 33.1 σ, 28.3 σ and 23.9 σ.
- Fe_{out} are on the exterior surface, and are equivalent to Fe_{out} reported previously (ref. 21); PDB ID: 3IS8.

Table S2. Anomalous peak height and *B*-factors ranges for the Fe atoms in the iron soaked BfrB N148L, Q151L and D34F mutant structures. The anomalous peak heights are listed from low to high and *B*-factor ranges from high to low.

	N148L	**Q151L**	**D34F**
Anomalous Peak Height Range (σ)	8.1 – 42.8	7.5 – 32.8	7.4 – 29.0
B-factor Range (Å^2)	91.0 – 21.8	84.8 – 32.6	76.1 – 21.8
# of Fe atoms	126	120	144
Anomalous Peak Height Range (σ) for Heme	18.2 – 21.3	23.6 – 27.6	32.0 – 42.0