Supplementary information

Structure of sirohydrochlorine ferrochelatase SirB: The last of the structures of the class II chelatase family

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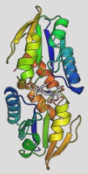
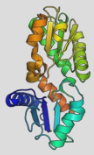
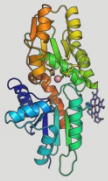
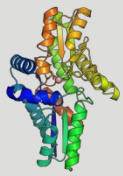
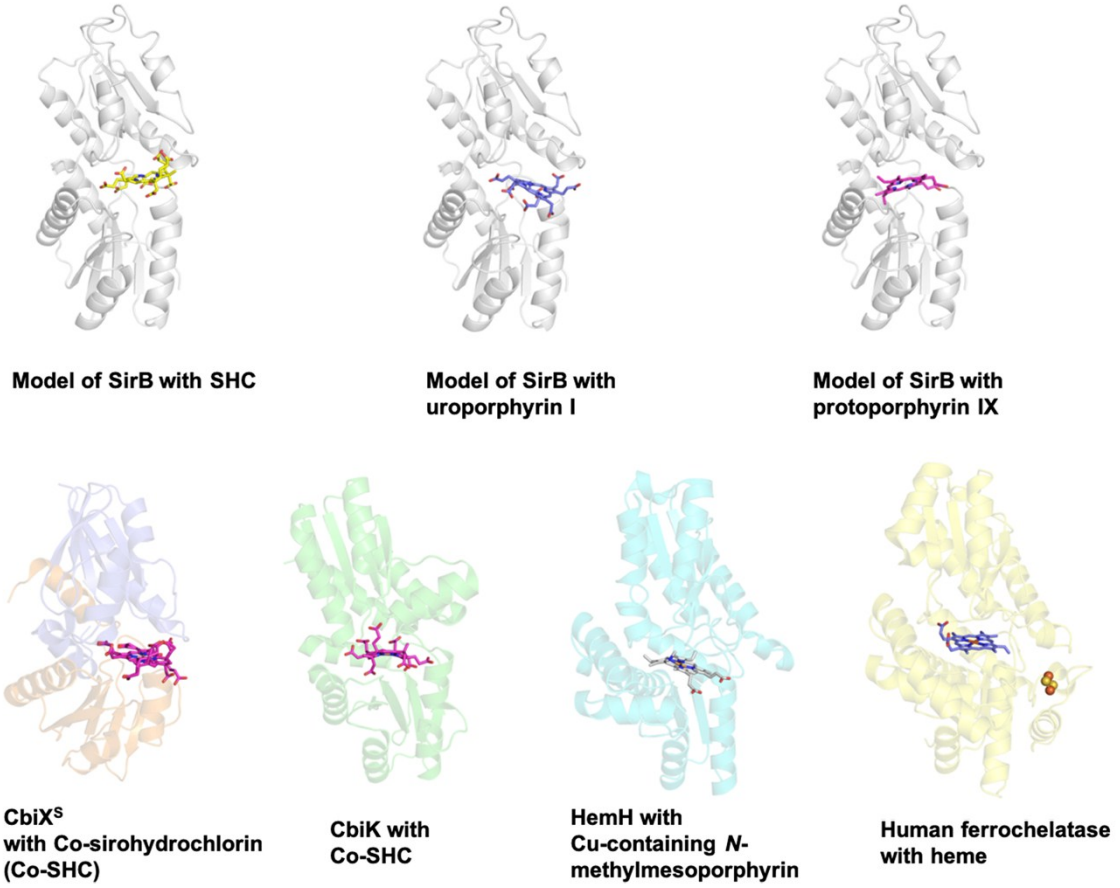
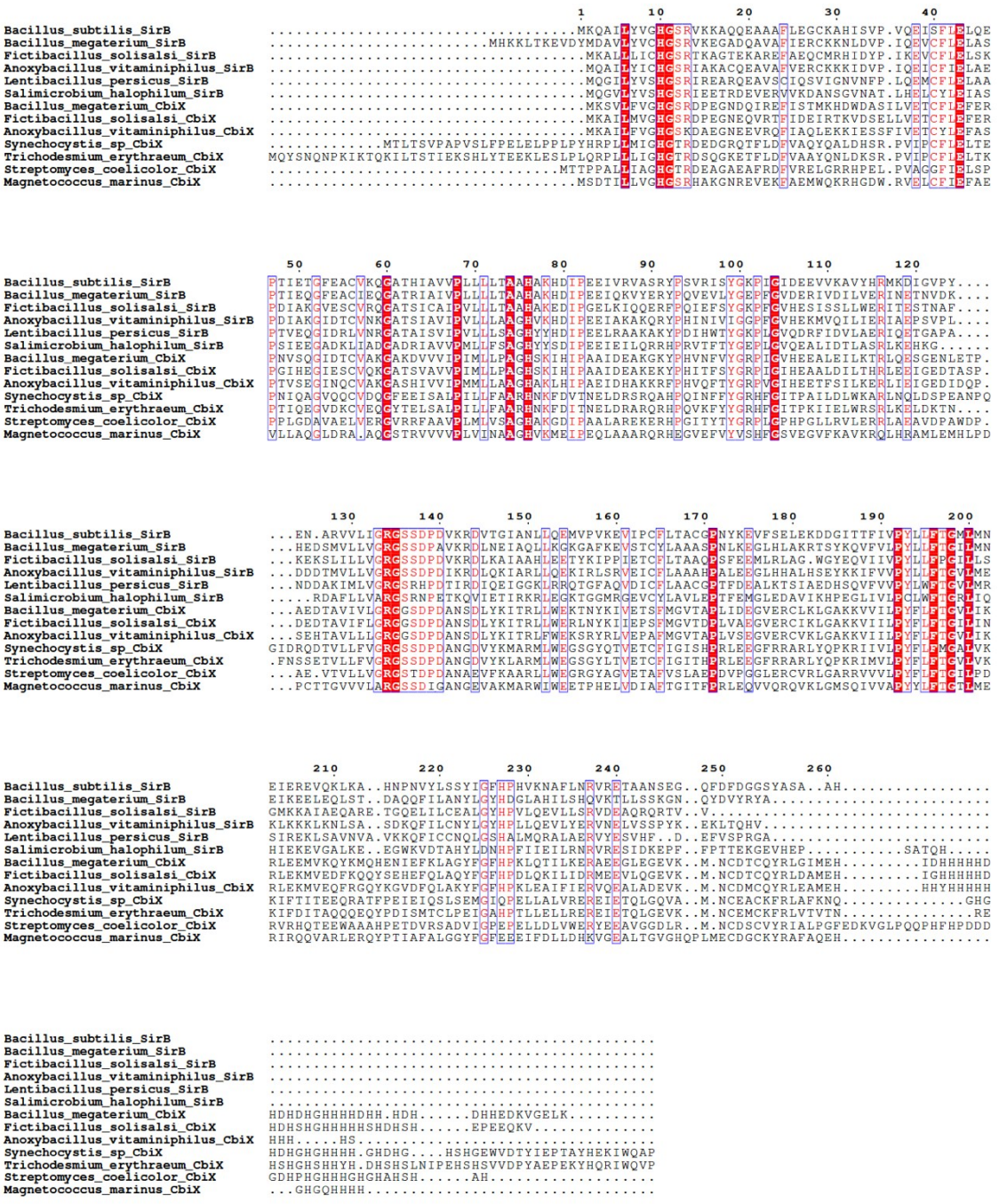
name	"Ancestral" CbiX^{S} (or CfbA)	CbiX	SirB	CbiK	HemH
Structure					
Organization	Homodimer	monomer	monomer	monomer	monomer
Active site	Central dimer interface	N-terminal domain	N-terminal domain	C-terminal domain	C-terminal domain
Physiological function	Co-chelatase (Ni-chelatase)	Co-chelatase	Co-chelatase	Co-chelatase	Fe-chelatase

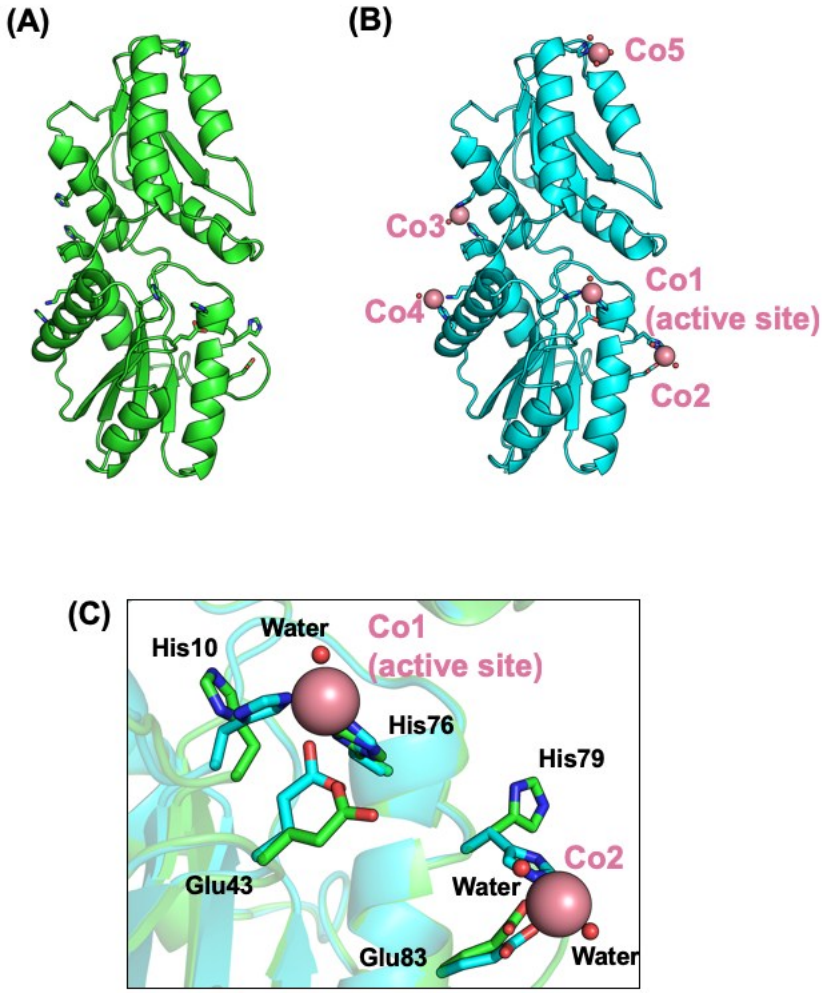
Supplementary Figure 1. Summary of evolutionarily related class II chelatase family proteins, and the SirB structure determined in this work. The cobalt-sirohydrochlorin chelatase CbiX^{S},[1, 2] which has recently been characterised as nickel chelatase CfbA[3, 4] involved in the biosynthesis of coenzyme F430 in methanogenic archaea, is regarded as an "ancestral" class II chelatase. SirB[5, 6] and CbiX[7] are also cobalt-chelatases catalysing the formation of cobalt-sirohydrochlorin, and are classified as class II chelatases having metal-binding active sites at their N-terminal domains. In contrast, HemH[8-11] and CbiK[2, 12, 13] have their metal-binding active sites at the C-terminal domains. These two chelatases, HemH and CbiK, however, play different roles: HemH, for the biosynthesis of heme, using iron and protoporphyrin IX; CbiK, for the biosynthesis of cobalt-sirohydrochlorin. The protein structures were depicted using PyMOL (Schrödinger, LLC) and rainbow-colored from the N-terminus (blue) to the C-terminus (red).



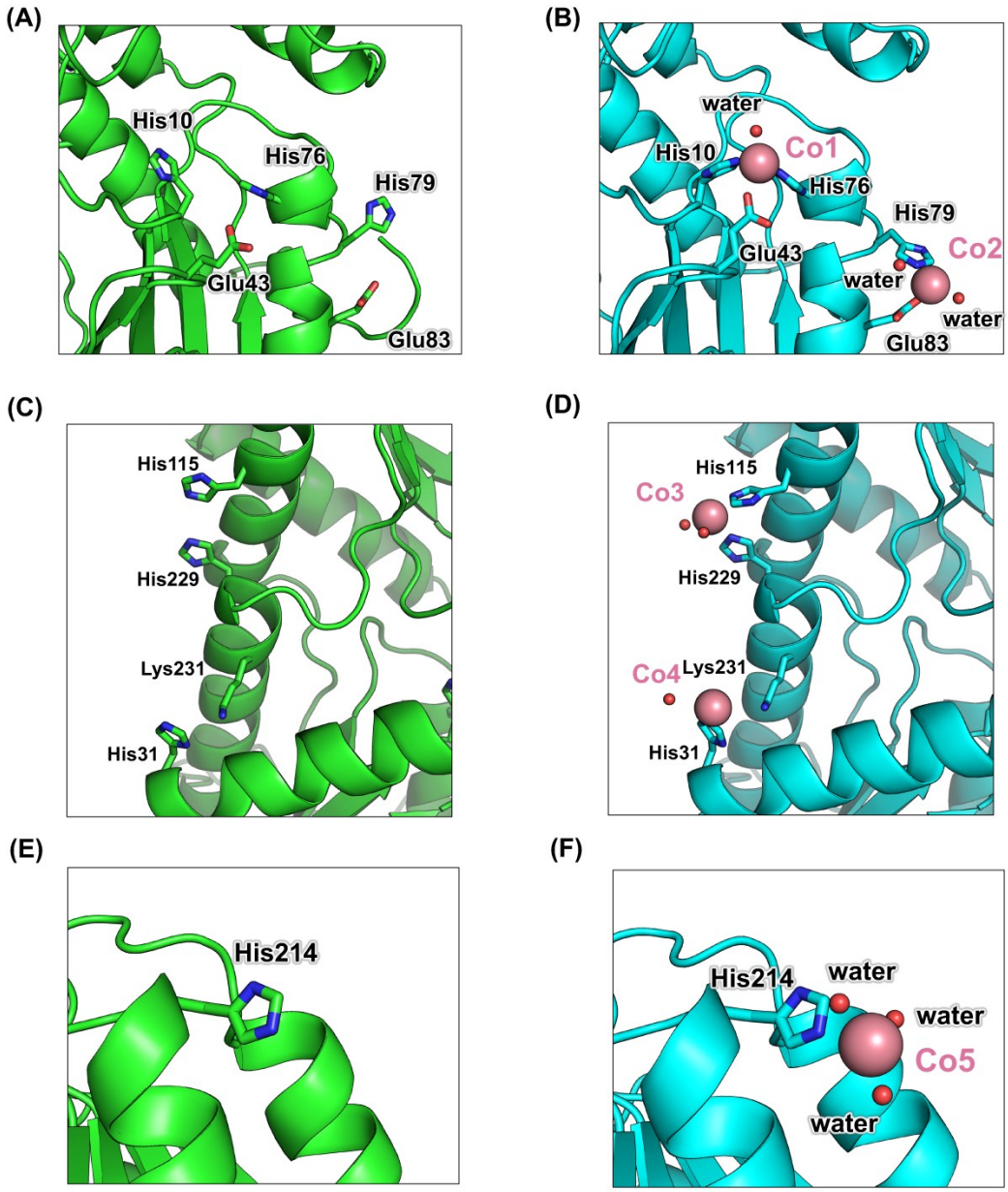
Supplementary Figure 2. Model structures of Co^{2+}-bound SirB with sirohydrochlorin (SHC), protoporphyrin IX, and uroporphyrin I. These docking models were compared with the crystal structures of $CbiX^S$ with cobalt-SHC (PDB ID: 2XWQ),[2] CbiK with cobalt-SHC (PDB ID: 2XWP),[2] HemH with copper-containing *N*-methyl mesoporphyrin (PDB ID: 1C9E),[14] and human ferrochelatase with heme (PDB ID: 3HCN).[14] Tetrapyrroles were shown as stick models. In human ferrochelatase, iron and sulphur of the [2Fe-2S] cluster are shown as yellow and brown spheres, respectively.



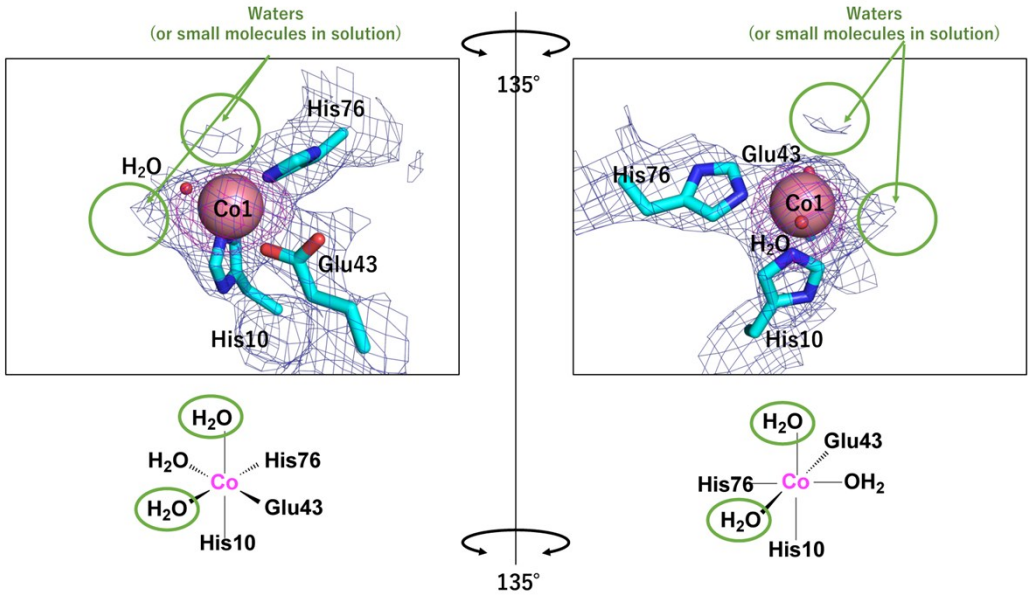
Supplementary Figure 3. Amino acid sequence alignments of SirB and CbiX, using Clustal omega.[15] The number of amino acid residues of *Bacillus subtilis* SirB are shown on the alignments. His10, Glu43, and His76 of *B. subtilis* SirB, which are ligands for cobalt/iron substrate binding, are strictly conserved in both SirB and CbiX. The figure was made using ESPript 3.[16] White letters on a red background indicate strictly conserved amino acid residues. Red letters in blue boxes indicate well-conserved amino acids.



Supplementary Figure 4. Overall structures of (A) metal-free SirB and (B) Co^{2+}-bound SirB. (C) Superimposition of active sites of metal-free and Co^{2+}-bound SirB. Co^{2+} and water molecules are shown in pink and small red spheres, respectively. The amino acid side chains ligating to the Co^{2+} ions are shown as stick models.



Supplementary Figure 5. Comparison of metal-binding sites between metal-free SirB and Co^{2+}-bound SirB. (A) Metal-free SirB and (B) Co^{2+}-bound SirB at the Co1 and Co2 sites; (C) Metal-free SirB and (D) Co^{2+}-bound SirB at the Co3 and Co4 sites; (E) Metal-free SirB and (F) Co^{2+}-bound SirB at the Co5 site.



Supplementary Figure 6. The geometry of the Co1 site. Green circles with arrows indicate plausible $2F_o$-F_c electron density corresponding to waters or solvents, although the density was too small to model anything. The geometry appears to be octahedral with slight distortion, not tetrahedral. The $2F_o$-F_c electron density and Co-anomalous difference maps were contoured at 1σand at 4σ, respectively.

Supplementary Table 1. The *B*-factor of each metal site of Co^{2+}-bound SirB. The occupancy of each metal was 1.0.

Co^{2+}-bound SirB	
Site	*B*-factor (Å^2)
Co1	68.5
Co2	128.3
Co3	82.8
Co4	102.8
Co5	119.8

Supplementary Table 2. List of primers. The *Nco*I and *Xho*I restriction sites are underlined.

Primer	**Sequence**
NcoI-BsSirA-F	5'-ATACCATGGGGAAAGTATATATTGTAGGAG-3'
XhoI-BsSirC-R	5'-GTGCTCGAGGATCCTTATTTCTTTCTTCTCATCTGATAAAGCC-3'
His6-BsSirB-F	5'-ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCATGAAACAAGCAATTTTATATGTC-3'
BsSirA-R	5'-TTTTACAACGCCTCGCTTAAATC-3'

Supplementary Table 3. Data collection and refinement statistics.

	SirB (metal-free)
Data collection	
Temperature (K)	100
Wavelength (Å)	1.000
Resolution range[(a)]	39.6– 2.0 (2.1–2.0)
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	69.7 53.8 80.4
α, β, γ (°)	90 105.4 90
Total reflections[(a)]	258734 (24477)
Unique reflections[(a)]	38718 (3817)
Redundancy[(a)]	6.7 (6.4)
Completeness (%)[(a)]	99.7 (99.1)
I/σ_I[(a)]	20.2 (2.55)
R_{sym} (%)[(a), (b)]	7.5 (77.2)
$CC_{1/2}$[(a)]	1.00 (0.84)
Refinement	
Resolution range (Å) [(a)]	31.4– 2.0 (2.1–2.0)
R_{work} (%)[(a), (c)]	18.5 (26.4)
R_{free} (%)[(a), (d)]	21.6 (30.1)
Rmsd bond length (Å)[(e)]	0.005
Rmsd bond angle (°)[(e)]	1.06
Clashcore, all atoms	4.9 (98th percentile)
Average *B*-factor (Å^2)	38.0
PDB ID	5ZT8

[a]The values in parentheses are for the highest resolution shell. [b]$R_{sym} = \sum\sum|I - \langle I \rangle|/\sum I$, where I is the intensity of each reflection. [c]$R_{work} = \sum||F_o| - k|F_c||/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. [d]R_{free} was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. [e]rmsd, root mean square deviation.

	Co^{2+}-bound SirB	Co^{2+}-bound SirB (Co peak)
Data collection		
Temperature (K)	100	100
Wavelength (Å)	1.000	1.605
Resolution range[(a)]	41.8– 2.9 (3.0–2.9)	41.8– 3.1 (3.2–3.1)
Space group	$P2_1$	$P2_1$
Cell dimensions		
a, *b*, *c* (Å)	65.0 52.1 75.5	65.0 52.2 75.6
α, β, γ (°)	90 112.1 90	90 112.1 90
Total reflections[(a)]	66609 (6910)	42871 (390)
Unique reflections[(a)]	10156 (1024)	7555 (250)
Redundancy[(a)]	6.6 (6.7)	5.7 (1.5)
Completeness (%)[(a)]	99.8 (100.0)	86.9 (29.7)
I/σ_I[(a)]	12.68 (1.8)	13.7 (1.1)
R_{sym} (%)[(a), (b)]	11.0 (102.0)	9.0 (57.9)
$CC_{1/2}$[(a)]	1.00 (0.76)	1.00 (0.66)
Refinement		
Resolution range (Å) [(a)]	32.4– 2.9 (3.0–2.9)	
R_{work} (%)[(a), (c)]	24.5 (35.9)	
R_{free} (%)[(a), (d)]	27.6 (41.3)	
Rmsd bond length (Å)[(e)]	0.007	
Rmsd bond angle (°)[(e)]	1.53	
Clashcore, all atoms	12.0 (96th percentile)	
Average *B*-factor (Å^2)	88.7	
PDB ID	5ZT7	

[a]The values in parentheses are for the highest resolution shell. [b]$R_{sym} = \sum\sum|I - \langle I \rangle|/\sum I$, where I is the intensity of each reflection. [c]$R_{work} = \sum||F_o| - k|F_c||/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. [d]R_{free} was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. [e]rmsd, root mean square deviation.

	Co^{2+}-bound SirB (Co inflection)	Co^{2+}-bound SirB (Co remote)
Data collection		
Temperature (K)	100	100
Wavelength (Å)	1.606	1.566
Resolution range[(a)]	41.8– 3.1 (3.2–3.1)	41.8– 3.1 (3.2–3.1)
Space group	$P2_1$	$P2_1$
Cell dimensions		
a, *b*, *c* (Å)	65.0 52.2 75.6	65.0 52.2 75.6
α, β, γ (°)	90 112.1 90	90 112.1 90
Total reflections[(a)]	42871 (390)	42987 (433)
Unique reflections[(a)]	7555 (257)	7613 (283)
Redundancy[(a)]	5.7 (1.5)	5.6 (1.5)
Completeness (%)[(a)]	87.0 (30.5)	87.4 (33.4)
I/σ_I[(a)]	13.7 (1.1)	13.8 (1.1)
R_{sym} (%)[(a), (b)]	9.0 (57.9)	9.2 (62.9)
$CC_{1/2}$[(a)]	1.00 (0.66)	1.00 (0.58)
Refinement		
Resolution range (Å) [(a)]		
R_{work} (%)[(a), (c)]		
R_{free} (%)[(a), (d)]		
Rmsd bond length (Å)[(e)]		
Rmsd bond angle (°)[(e)]		
Clashcore, all atoms		
Average *B*-factor (Å^2)		
PDB ID		

[a]The values in parentheses are for the highest resolution shell. [b]$R_{sym} = \sum\sum|I - \langle I \rangle|/\sum I$, where I is the intensity of each reflection. [c]$R_{work} = \sum||F_o| - k|F_c||/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. [d]R_{free} was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. [e]rmsd, root mean square deviation.

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