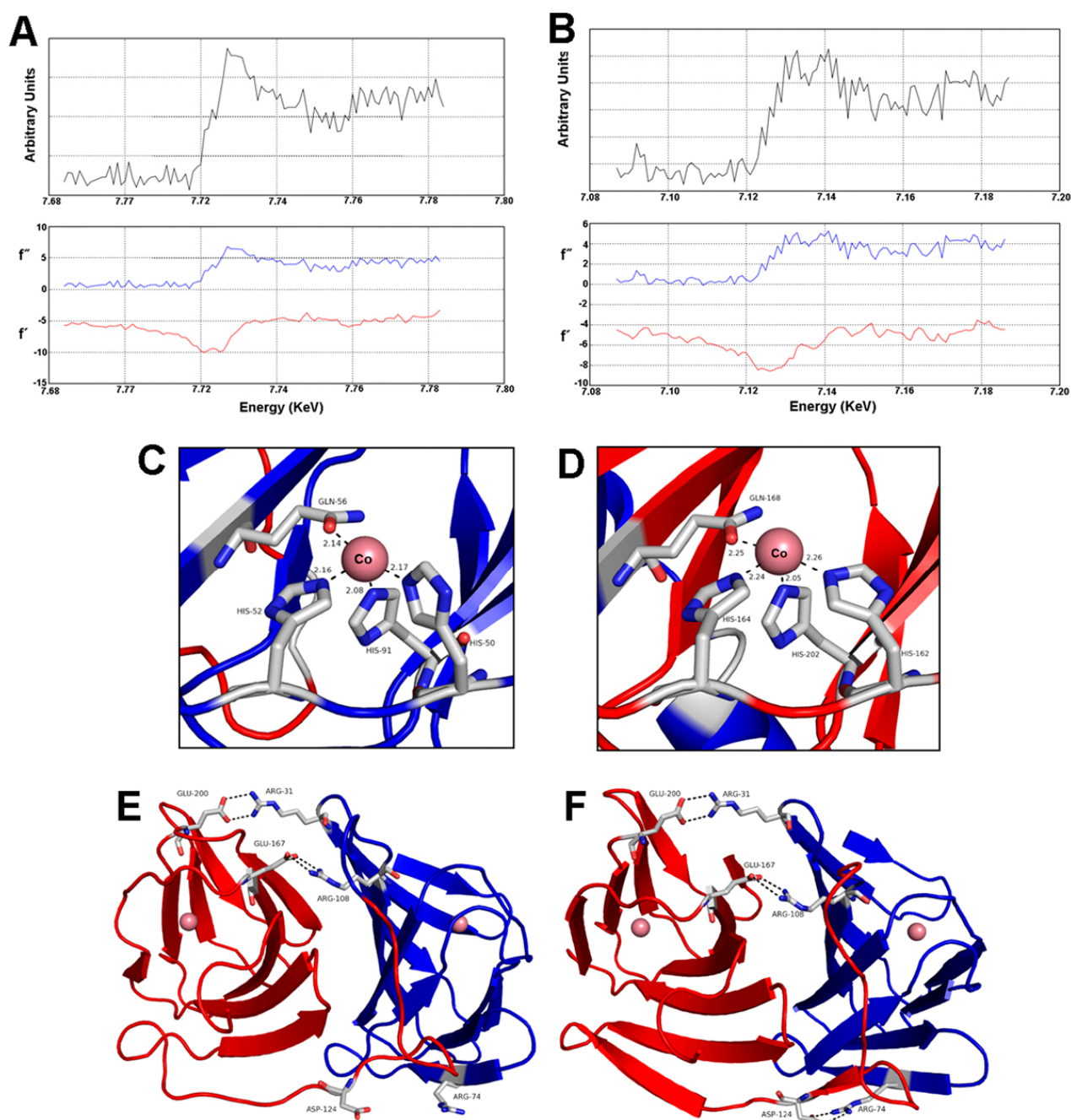
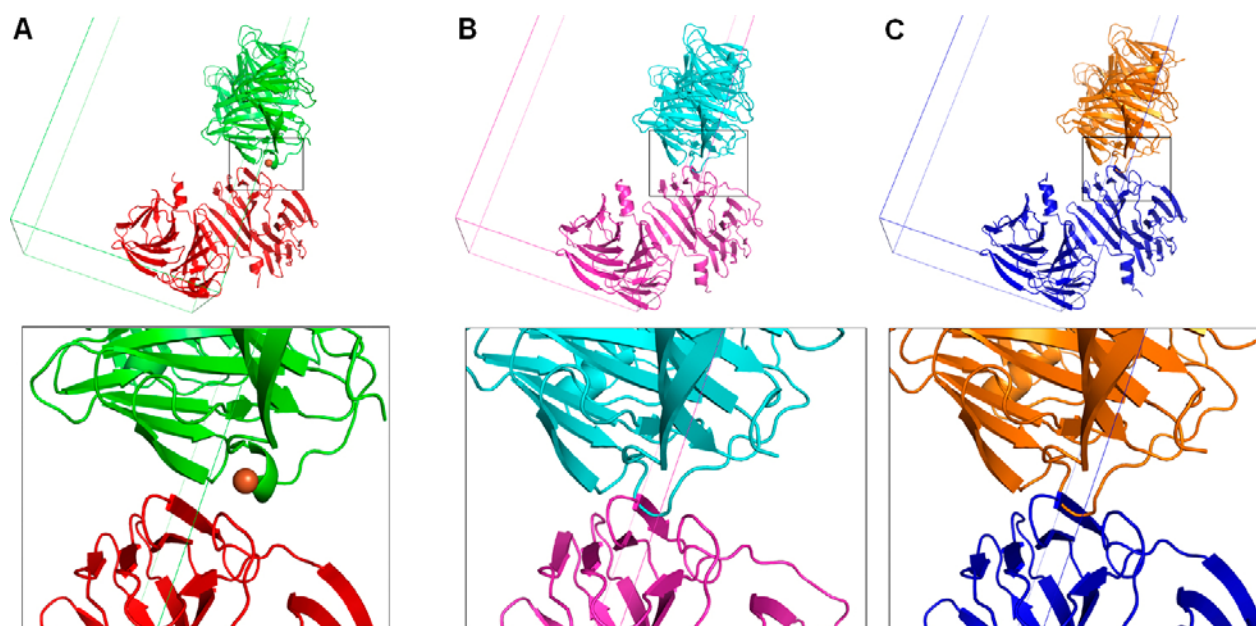


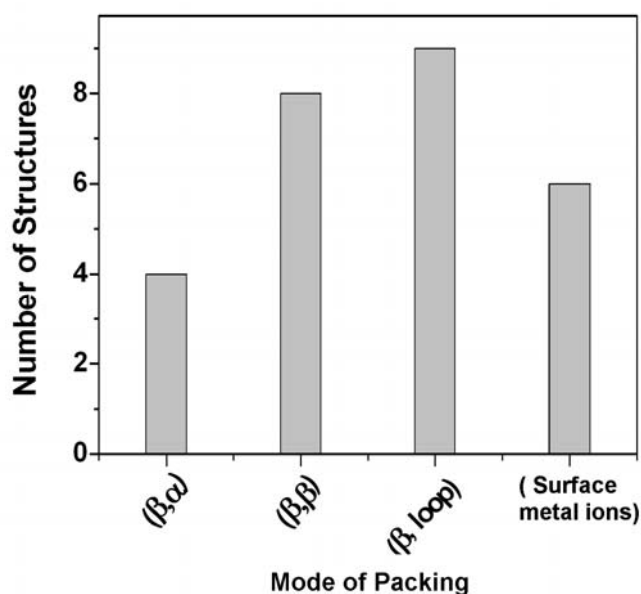
## Supplementary Material



**Supplementary Figure 1: X-ray absorption spectra recorded using crystals of the monoclinic form of BacB. A.** An energy scan for Cobalt showed an absorption edge around 7.72KeV. **B.** The energy scan for Iron showed an absorption edge around 7.13KeV. The  $f'$  and  $f''$  values are shown in blue and red respectively. **C and D. Metal ions at the active site of the N and C-terminal cupin domains of BacB.** The residues coordinating the metal ion at the active site of the N-terminal domain are His50, His52, His91 and Gln56. The metal ion at the C-terminal domain is coordinated by His162, His164, His202 and Gln168. **E and F. Salt bridges in the triclinic, monoclinic and tetragonal crystal forms.** The triclinic form and monoclinic forms of BacB contains two salt bridges between Arg31- Glu200 and Arg108-Glu167. The tetragonal form of BacB contains an additional salt bridge apart from the first two between Arg74 – Asp124. The third salt bridge reduces the flexibility of a loop (residues 114-126) and leads to a more compact packing arrangement.



**Supplementary Figure 2: Packing of BacB in the tetragonal cell.** **A.** Tetragonal form BacB in the tetragonal cell. The ordered loop in this form allows a snug fit of BacB monomers in this cell (A magnified view of the interface is shown in the boxes below). **B.** Monoclinic form BacB in a tetragonal cell. The loop segment hinders this packing arrangement. **C.** Triclinic form BacB in a tetragonal cell.



**Supplementary Figure 3: Comparison of crystal packing in cupin proteins.** A dataset of 39 cupin structures (compiled in Supplementary Table 4) were used for this analysis. The criterion used to limit this dataset (from over 250 structures) was the sequence identity (these 39 structures share less than 30 % sequence identity) and resolution (better than 2.5 Å). The bar-graph shows the preferred mode of crystal contacts in these structures. While interactions between a β-strand and a loop dominate lattice interactions in cupin proteins, metal ions are also seen to play a significant role in this process.

**Supplementary Table 1: ICP-AES(Inductively Coupled Plasma Atomic Emission Spectroscopy) analysis**

	Analyte and Wavelength(nm)	Mean Corrected Intensity	Sample conc. Units	Std. Dev.	RSD(%)
BacB 250μg	Fe 238.204	8858.5	0.082mg/L	0.0009	1.11
	Fe 239.562	7528.8	0.080mg/L	0.0001	0.15
BacB 500μg	Fe 238.204	14757.3	0.135mg/L	0.0009	0.68
	Fe 239.562	12792.1	0.135mg/L	0.0006	0.46
BacB 250μg	Co 228.616	318.1	0.006mg/L	0.0001	1.74
	Co 238.892	626.5	0.005mg/L	0.0003	5.97
BacB 500μg	Co 228.616	225.3	0.004mg/L	0.0004	6.42
	Co 238.892	468.0	0.004mg/L	0.0001	2.42

Note: This spectroscopic data is consistent with the presence of Fe<sup>2+</sup> and Co<sup>2+</sup> in BacB samples.

Std. Dev.: Standard Deviation; RSD (%): Relative Standard Deviation

**Supplementary Table 2: Analysis of the coordination distances of Fe<sup>2+</sup> and Co<sup>2+</sup> in BacB**

Crystal forms of BacB	Coordination distances of metal ions with protein ligands, ligands and water molecules (Å)						
<b>P4(1)2(1)2 lattice</b>							
Co (N-terminal domain)	His50- (2.17)	His52- (2.25)	His91- (2.23)	Gln56- (2.20)	HOH- (2.2)	HOH- (2.07)	
Co (C-terminal domain)	His162- (2.27)	His164- (2.21)	His202- (2.15)	Gln168- (2.18)	PPY- (2.1)	PPY- (2.09)	
Surface Iron	Glu119- (2.3)	Glu119- (2.35)	Phe122- (2.37)	*Asp120- (2.32)	HOH- (2.25)	HOH- (2.6)	
<b>P2(1) lattice</b>							
Co (N-terminal domain)	His50- (2.17)	His52- (2.16)	His91- (2.08)	Gln56- (2.14)	HOH- (2.21)	HOH- (2.09)	
Co (C-terminal domain)	His162- (2.26)	His164- (2.24)	His202- (2.05)	Gln168- (2.25)	PPY- (2.14)	PPY- (1.85)	
Surface Iron 1	Asn155- (2.2)	Glu209- (2.42)	Glu209- (2.50)	*Glu113- (2.53)	HOH- (2.43)	HOH- (2.37)	HOH- (2.34)
Surface Iron 2	Asp33- (2.41)	Asp33- (2.55)	Glu167- (2.51)	Glu197- (2.60)	HOH- (2.42)	HOH- (2.33)	HOH- (2.34)
<b>P1 lattice</b>							
Co (N-terminal domain)	His50- (2.31)	His52- (2.25)	His91- (2.40)	Gln56- (2.37)	HOH- (2.32)	HOH- (2.86)	
Co (C-terminal domain)	His162- (2.21)	His164- (2.25)	His202- (2.24)	Gln168- (2.19)	PPY- (2.2)	PPY- (2.0)	
Surface Iron	Asn155- (2.37)	Glu209- (2.43)	Glu209- (2.51)	*Glu113- (2.41)	HOH- (2.56)	HOH- (2.58)	HOH- (2.48)

\* Residues from other asymmetric unit partners

Interaction	Fe		Co	
	Reported <sup>a</sup>	Observed	Reported <sup>a</sup>	Observed
O (carboxyl, mono)	2.11±0.14	-N. A. -	2.22±0.19	2.04±0.10
N (Imidazole)	2.08±0.11	-N. A. -	2.07±0.12	2.21±0.08
O (hydroxyl, water)	2.19±0.19	2.42±0.11	2.22±0.31	2.29±0.29
O (Asn/Gln)	2.45±0.41	2.42±0.10	2.26	2.22±0.08
O (backbone carbonyl)	2.74±0.28	2.35±0.05	-N. A.-	-N. A.-

<sup>a</sup> (Values from Dokmanic *et al.*, 2007)

**Supplementary Table 3: Dynamic Light Scattering experiment**

	<b>R<sub>h</sub>(nm)</b>	<b>Std. Dev.</b>	<b>RSD(%)</b>
Native BacB	2.6	0.18	7.2
BacB incubated with CoCl <sub>2</sub> (5μM)	3.3	0.22	6.8
BacB incubated with CoCl <sub>2</sub> (20μM)	12.2	0.85	7.0

Note: R<sub>h</sub>: hydrodynamic radius; RSD(%): Relative Standard Deviation (polydispersity). Increase in metal ion concentration of BacB protein samples leads to an increase in the polydispersity index.

**Supplementary Table 4: Comparison of crystal packing in cupin proteins.**

<b>PDB</b>	<b>(<math>\beta</math>, <math>\alpha</math>)</b>	<b>(<math>\beta</math>, <math>\beta</math>)</b>	<b>(<math>\beta</math>, loop)</b>	<b>Surface</b>	<b>metal ion</b>
1J1L			1J1L		
1JUH					
1O5U	1O5U				
1SEF		1SEF			
1VJ2		1VJ2			
1X82			1X82		
1XE7					
1XRU					
1Y3T					
1YFU			1YFU		
2B5H					
2BNM			2BNM		
2COZ		2COZ	2COZ		
2EA7					
2ET1					
2F4P					
2FCT					
2NVN			2NVN		
2O8Q					
2OPK					
2OPQ			2OPQ		
2OZJ		2OZJ			
2PFW		2PFW			
2Q1Z					
2QNK					
2V09					
3BCW					
3CEW	3CEW		3CEW		
3D82	3D82				
3ES1		3ES1			
3ES4					
3FJS					
3G7D	3G7D			3G7D	
3H1W		3H1W			
3H8U			3H8U		
3H50				3H50	
3I7D				3I7D	
3IBM				3IBM	
3H7J				3H7J	
3H7Y		3H7Y		3H7Y	
3H9A				3H9A	

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