Immune Interference in Mycobacterium tuberculosis Intracellular Iron Acquisition through Siderocalin Recognition of Carboxymycobactins

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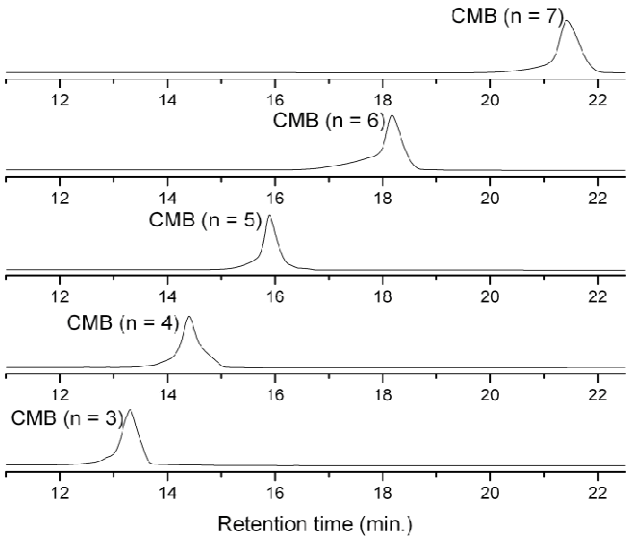
Supplementary Information

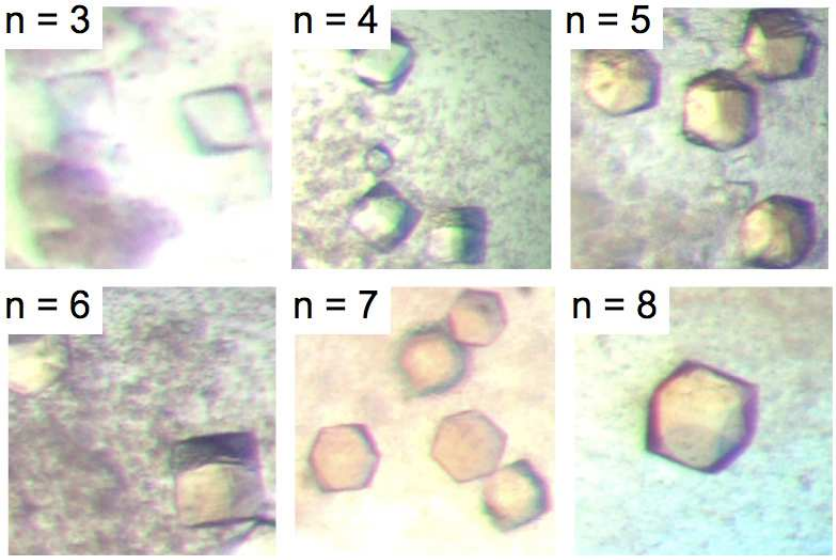
Supplemntary Figure 1. LCMS of separated ferric CMB isoforms with detection at 450 nm. The ferric complex of each CMB isoform has a distinct retention under the experimental LCMS solvent conditions (see supplementary materials and methods). The successful separation of each isoform was confirmed by the presence of a peak with the expected retention time and mass (see ESI data in Supplementary Table 1) and the absence of other peaks in the LCMS trace.



Supplementary Table 1. Electrospray Ionization (ESI) data for each LCMS peak shown in Figure S1. The calculated mass for the ionized ferric complex is listed first, followed in parentheses by the experimentally measured mass.

Fe^{III}CMB (by *n*)	**ESI(-) Calc.(Found)**
3	755.55 (755.21)
4	769.58 (769.23)
5	783.24 (784.24)
6	797.26 (797.26)
7	811.27 (811.27)
8	825.29 (825.29)

Supplementary Figure 2. Cocrystals of Scn with Fe^{III}CMB (n = 3–8). The reddish color of a crystal indicates close association of the protein with the ferric complex, i.e. specific binding within the calyx. The crystals are 0.2 - 0.3 mm in size.



Supplementary Figure 3. An overlay of the modeled ligands in pocket 1 in the protein crystal structures of Scn bound to ferric Ent and CMB. The 2,3-catecholamide binding unit from Ent is shown in yellow and the HPO binding unit from CMB is shown in blue; iron atoms are shown as separate spheres.

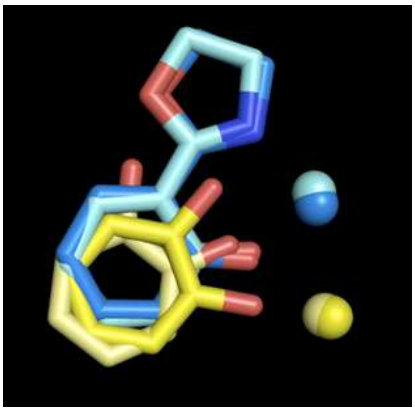
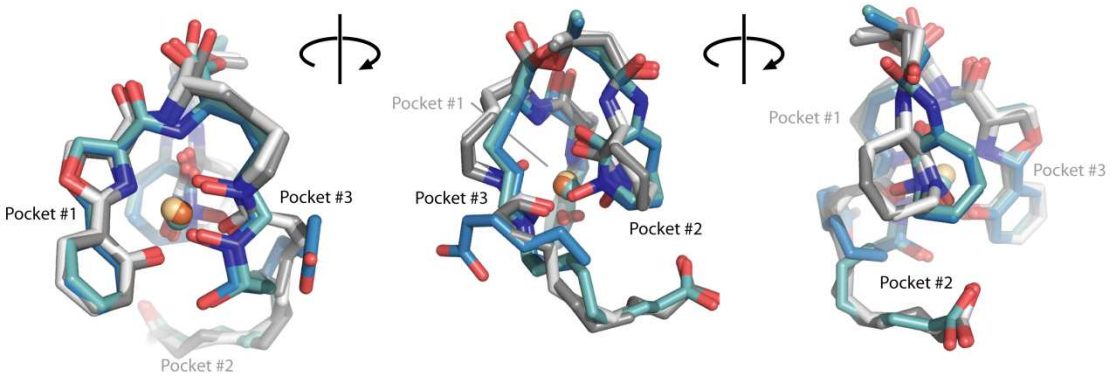


Figure S4. Comparison of the structures of Scn bound to Fe^{III}CMB-5 (light and dark blue), Fe^{III}CMB-6 (light grey), Fe^{III}CMB-7 (dark grey). The original structure of Fe^{III}CMB-6 (PDB accession code 1X89) and Fe^{III}CMB-7 show little difference in the overall binding mode. In the structure of Fe^{III}CMB-5, the hydroxamate in pocket #3 shifts down into the calyx by ~2.0 Å to accommodate the shorter tail length. Additionally, the fatty acid tail adopts both "tail in" and "tail out" alternate conformations.



Supplementary Table 2. Crystallographic Statistics				
Data Collection				
Protein	Wild Type	Wild Type	Wild Type	Wild Type
Ligand	CMB-756	CMB-770	CMB-784	CMB-812
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Unit cell (Å)	a=b=114.8, c=119.0	a=b=115.2, c=118.6	a=b=115.0, c=119.0	a=b=114.1, c=118.5
Resolution (Å)	50-3.0 (3.11-3.0)	50-2.3 (2.38-2.3)	50-2.2 (2.28-2.2)	50-2.3 (2.38-2.3)
Rmerge (%)	0.076 (0.34)	0.049 (0.37)	0.048 (0.35)	0.054 (0.31)
I/σI	25.0 (6.3)	36.8 (5.7)	37.7 (5.6)	24.9 (5.5)
Redundancy	7.7 (7.6)	8.1 (8.1)	7.3 (7.0)	6.4 (6.4)
Completeness (%)	100 (100)	99.4 (100)	100 (100)	99.8 (100)
Unique Reflections	16475 (1598)	35725 (3505)	41091 (4024)	35471 (3502)
Structure Refinement				
Rwork (%)	-	-	24.8	20.8
Rfree [b] (%)	-	-	27.3	24.4
Number of atoms	-	-	4398	4416
Protein	-	-	4056	4087
Siderophore	-	-	54	165
Water	-	-	228	159
Est. Coor. Error (Å) [c]	-	-	0.14	0.14
Geometry				
RMSD Bonds Å	-	-	0.001	0.009
RMSD angles (°)	-	-	0.860	1.286
RMSD chiral ($Å^3$)	-	-	0.111	0.045
Average B ($Å^2$)	-	-	44.5	41.8
Protein monomer B factors (A, B, C; $Å^2$)	-	-	35.7, 75.8, 28.9	48.3, 63.5, 42.4
Siderophore B factors (A, B, C; $Å^2$)	-	-	NA, NA, 55.2	53.8, 60.0, 70.3
Water B factors $Å^2$	-	-	26.2	52.4
Ramachandran [d]		-		
Most favored (%)	-	-	90.4	90.5
Additionally allowed (%)	-	-	7.4	7.9
Generously allowed (%)	-	-	0.7	0.2
Disallowed (%)	-	-	1.6	1.3
PDB Accession Code	-	-		

[a] Numbers in parentheses correspond to the highest resolution shells
[b] Calculated on 10% of the data (Kleywegt and Brugner, 1996) and matched between the original structure PDB code1L6M
[c] Based on maximum likelihood in refmac (Murshudov et al., 1997)
[d] Calculated with PROCHECK (Laskowski et al., 1992)
NA: not applicable

Supplemental References:

Kleywegt GJ, Brünger AT. Checking your imagination: applications of the free R value. Structure. 1996 Aug 15;4(8):897-904.

G.N. Murshudov, A.A.Vagin and E.J.Dodson, "Refinement of Macromolecular Structures by the Maximum-Likelihood Method", (1997) Acta Cryst. D53, 240-255.

Laskowski R A, MacArthur M W, Moss D S & Thornton J M (1993). PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst., 26, 283-291.