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Crystal structures of *Mycobacterial* MeaB and MMAA-like GTPases

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Abstract

The methylmalonyl Co-A mutase-associated GTPase MeaB from *Methylobacterium extorquens* is involved in glyoxylate regulation and required for growth. In humans, mutations in the homolog methylmalonic aciduria associated protein (MMAA) cause methylmalonic aciduria, which is often fatal. The central role of MeaB from bacteria to humans suggests that MeaB is also important in other, pathogenic bacteria such as *Mycobacterium tuberculosis*. However, the identity of the mycobacterial MeaB homolog is presently unclear. Here, we identify the *M. tuberculosis* protein Rv1496 and its homologs in *M. smegmatis* and *M. thermoresistibile* as MeaB. The crystal structures of all three homologs are highly similar to MeaB and MMAA structures and reveal a characteristic three-domain homodimer with GDP bound in the G domain active site. A structure of Rv1496 obtained from a crystal grown in the presence of GTP exhibited electron density for GDP, suggesting GTPase activity. These structures identify the mycobacterial MeaB and provide a structural framework for therapeutic targeting of *M. tuberculosis* MeaB.

Keywords

MeaB; metallochaperone; methylmalonic aciduria associated protein; MMAA; Mycobacterium; Ras-like GTPase; structural genomics; tuberculosis

Introduction

In *Methylobacterium extorquens* AM1, the gene encoding the membrane associated GTPase MeaB clusters with genes encoding methylmalonyl-CoA mutase (*mcm*) and other genes involved in coenzyme B₁₂ (cobalamin) transport [1]. A mutation in the *Methylobacterium extorquens* AM1 MeaB prohibited growth due to the inability to convert methylmalonyl-CoA to succinyl-CoA caused by an inactive form of MCM [2]. Later it was shown that MeaB protects MCM from inactivation [1] and that MeaB is a small G-protein involved in loading coenzyme B₁₂ to MCM [3]. A human ortholog of *MeaB* encodes methylmalonyl

associated protein A (MMAA), and mutations in MMAA are associated with the fatal disease methylmalonyl aciduria [4]. Crystal structures have been reported for MeaB [5] and MMAA [6], which reveal N- and C-terminal α -helical domains and a Ras-like GTPase domain.

The importance of MeaB from bacteria to humans points to a general essential role in bacterial metabolism and a conserved function in pathogenic bacteria. The deadly respiratory disease tuberculosis is caused by the pathogenic bacterium *Mycobacterium tuberculosis*. The emergence of multi-drug resistant strains has made current therapies involving a number of antibiotics less effective, necessitating intensive medicinal research to discover new therapies. Because mutations in MMAA are fatal in humans and mutations in MeaB prohibit bacterial growth, we sought to characterize MMAA/MeaB homologs in *Mycobacteria*.

In *M. tuberculosis*, Rv1496 has been proposed to be a MeaB ortholog due to sequence similarity and proximity to the *mcm* ortholog gene [7]. However, Rv1496 was originally annotated as an arginine/ornithine/lysine (LAO) transport system ATPase, presumably based on sequence comparison to the lysine/arginine/ornithine transport system ATPase *ArgK* from *Escherichia coli* [8]. Incidentally, that same protein (*ArgK*) from *Escherichia coli* is also named YgfD and was shown recently to exhibit GTPase activity and interact with sleeping beauty mutase (SBM), a homolog of MCM [9]. Thus, not only the early annotation of *E. coli* LAO ATPase may be incorrect, but the incorrect annotation may have propagated to other organisms. To identify the mycobacterial MeaB, resolve the contradictory annotation, and understand the structure and function of these *Mycobacteria* proteins, we solved the crystal structures of Rv1496 and its orthologs from *M. smegmatis* and *M. thermoresistible*. All three proteins exhibit a global fold that is similar to MeaB and MMAA, including an N-terminal α -helical domain, a Ras-like GTPase domain that binds GDP, and a C-terminal α -helical dimerization domain. These data identify Rv1496 and its orthologs as the mycobacterial MeaB.

Material and Methods

Cloning, expression, and purification

The 330-residue *M. tuberculosis* Rv1496 gene (UniProt accession code P9WPZ1, NP_216012.1), the 325-residue *M. smegmatis* Rv1496 ortholog gene (UniProt accession code A0QX37, MSMEG_3160), and the 326-residue *M. thermoresistible* Rv1496 ortholog gene (UniProt G7CAR0) were amplified from genomic DNA and cloned into the pAVA0421 expression vector encoding an N-terminal histidine affinity tag followed by the human rhinovirus 3C protease cleavage sequence (the entire tag sequence is MAHHHHHHMGTLEAQTQGPGS-ORF) using ligation independent cloning [10]. Sequence alignment of various protein sequences without the expression tag are shown in Figure 1.

The clones were transformed into *E. coli* BL21 (DE3) Rosetta cells, and starter cultures were grown in LB broth with ampicillin (50 μ g/ml), carbenicillin (50 μ g/ml), and chloramphenicol (34 μ g/ml) for ~18 hours at 37 °C. The protein were expressed in 2L of

ZYP-5052 auto-induction media [11] in a LEX bioreactor. After 24 hours at 25 °C the temperature was reduced to 15 °C for another 60 hours. The samples were centrifuged at $4000 \times g$ for 20 minutes at 4 °C. Cell paste was flash frozen and stored at -80 °C.

The cells were re-suspended 6:1 v/w in 20 mM HEPES pH 7.4, 300 mM NaCl, 5% v/v glycerol, 0.5% w/v CHAPS, 30 mM imidazole, 10 mM MgCl₂, 3 mM β-mercaptoethanol, protease inhibitor cocktail tablets (Roche), and 0.05 mg/mL lysozyme at 4 °C and disrupted on ice for 15 minutes with a Branson Digital 450D Sonifier (70% amplitude, with alternating cycles of five seconds of pulse-on and ten seconds of pulse-off). The cell debris was incubated with 20 µl of Benzonase nuclease at room temperature for 40 minutes. The lysate was clarified by centrifugation with a Sorvall RC5 at 10,000 RPM for 60 min at 4 °C in a F14S Rotor (Thermo Fisher). Clarified solution was syringe filtered through a 0.45 µm cellulose acetate filter (Corning Life Sciences, Lowell, MA). Tagged protein was purified by IMAC using a HisTrap FF 5 ml column (GE Biosciences, Piscataway, NJ) and an ÄKTA FPLC system (GE Biosciences) equilibrated with binding buffer (20 mM HEPES, pH 7.0, 300 mM NaCl, 5% glycerol, 30 mM imidazole, 1 mM TCEP), and eluted with binding buffer supplemented with 500 mM imidazole.

For the *M. smegmatis* and *M. thermoresistibile* orthologs but not *M. tuberculosis* Rv1496, the N-terminal affinity tag was removed with 3C protease [12]. The samples were dialyzed overnight at 4 °C against cleavage buffer (20 mM HEPES pH 7.6, 500 mM NaCl, 5% Glycerol, 1 mM TCEP) and purified by a subtractive nickel affinity chromatography. For all three target protein samples, the protein was then concentrated and purified by size exclusion chromatography (SEC) using a Superdex 75 26/60 column (GE Biosciences) equilibrated in SEC buffer (20 mM HEPES, pH 7.0, 300 mM NaCl, 5% glycerol and 1 mM TCEP). The elution profiles were consistent with each protein being a dimer in solution. After purity analysis by SDS-PAGE, the pooled fractions were concentrated and flash-frozen in liquid nitrogen. Samples were stored at -80 °C until used in crystallization experiments.

Crystallization and data collection

Nucleotides were obtained from Sigma-Aldrich. Sparse matrix crystallization screens were set up in 96-well sitting drop vapor diffusion format at 16 °C with an equal volume of protein (28.7 mg/mL for *M. smegmatis*, 42.5 mg/mL for *M. thermoresistibile*, and 22 mg/mL for *M. tuberculosis*; 0.4 µL) and precipitant against reservoir (80 µL). For the *M. tuberculosis* sample set up in the presence of GDP, crystals appeared after several days out of the JCSG+ screen condition D3 (50% PEG 200, 0.1 M Na/K phosphate pH 6.2, 0.2 M NaCl). Based on this condition, a gradient optimization screen was designed using the Rigaku Reagents E-Wizard optimization software. Co-crystallization experiments for *M. tuberculosis* Rv1496 were set up in the presence of 2 mM GDP analogs in the JCSG+ D3 focus screen, yielding several single large crystals within a few days. Crystals of the *M. smegmatis* ortholog of Rv1496 were obtained within a week from the JCSG+ screen condition F6 (0.1 % Bicine pH 9.0, 10% MPD). Crystals of the *M. thermoresistibile* ortholog of Rv1496 were obtained within two weeks from the PACT screen condition B10 (0.2 M MgCl₂, 0.1 M MES pH 6.0, 20% PEG 6000).

A crystal of *M. tuberculosis* Rv1496 grown in the presence of GDP was harvested, vitrified in liquid nitrogen, and a 2.45 Å resolution data set was collected at 100 K using a Rigaku 007-HF X-ray generator with VariMax optics and a Saturn 944 detector (Table 1). Crystals of *M. tuberculosis* Rv1496 grown in the presence of GDP analogs were harvested, vitrified in liquid nitrogen, and data sets were collected at the Advanced Light Source (ALS). A crystal of the *M. smegmatis* ortholog of Rv1496 was harvested, vitrified in liquid nitrogen, and a 2.3 Å resolution data set was collected at the ALS beamline 5.0.2 with an ADSC Q315R detector. A crystal of the *M. thermoresistible* ortholog of Rv1496 was cryo-protected with reservoir supplemented with 2 mM GDP and 25% ethylene glycol, harvested, vitrified in liquid nitrogen, and a 2.4 Å resolution data set was collected at the ALS beamline 5.0.1. The data were reduced with XDS/XSCALE [13].

Structure solution and refinement

The structure of *M. tuberculosis* Rv1496 grown in the presence of GDP was solved by molecular replacement using the *Methylobacterium extorquens* MeaB crystal structure [5] as a search model in Molrep [14] from the CCP4 suite [15]. The final model was obtained after numerous iterative rounds of refinement in REFMAC [16] and manual re-building in COOT [17]. The structures of the *M. smegmatis* and *M. thermoresistible* orthologs were solved by molecular replacement using the *Mtb* Rv1496 monomer as a search model in Phaser [18].

Structure validation and deposit

The structures were assessed and corrected for geometry and fitness using Molprobrity [19]. The atomic coordinates and structure factors are available in the Protein Data Bank under accession codes 3MD0, 3NXS, 3TK1, 3P32, and 4GT1.

Results and Discussion

Rv1496 crystallization

M. tuberculosis Rv1496 crystallized in many conditions from several sparse matrix screens, yielding thin needle clusters that despite extensive optimization efforts did not diffract to better than 7 Å resolution. To obtain structures of potential MeaB/MMAA homologs from *mycobacteria*, we pursued two rescue strategies in parallel. First, we attempted co-crystallization with GDP. Second, orthologs were selected from closely related *Mycobacterium* species such as *M. marinum*, *M. smegmatis*, and *M. thermoresistible*. Both rescue strategies resulted in successful structure determination.

To test whether the mycobacterial proteins bind GTP similar to MeaB [5] and MMAA [6], we attempted co-crystallization with GDP. A 2.45 Å resolution data set was collected and the structure of *Mtb* Rv1496 (Table 1) was solved by molecular replacement using the protein only model of MeaB [5] (58% sequence identity over 312 residues). Like MeaB, *Mtb* Rv1496 exhibits a dimeric quaternary structure in which each protomer consists of an N-terminal α-helical domain followed by a G protein domain and a C-terminal α-helical dimerization domain (Figure 2a). Overall, Rv1496 is similar to both the GDP-bound structures of MeaB (r.m.s.d. 2.13 Å over 292 similar C_α atoms) and MMAA (r.m.s.d. 1.87 Å over 228 similar C_α atoms) with the largest changes (r.m.s.d. >3 Å) taking place in the

positioning of the C-terminal α -helical dimerization domain relative to the central G-domain (Figure 2).

Rv1496 binds GDP via hydrogen bonds, one salt bridge, and van der Waals interactions (Figure 3a). The GDP β -phosphate is recognized by the side chain of K71 and backbone amides of G68, G70 and K71. The α -phosphate is recognized by the backbone amide and side chain oxygen of T73. The nucleobase forms hydrophobic packing interactions with the side chains of K205 and V245 while forming two hydrogen bonds through its Watson-Crick face (N1 and N2) with the side chain of D207. Like the ribose ring itself, the sugar edge is solvent exposed and not in direct contact with *Mtb* Rv1496. In general, the GDP binding interactions appear similar to that previously observed for MeaB [5] and MMAA [6].

Rv1496 ortholog-GDP crystal structures

Concurrent with ligand co-crystallization experiments, we entered Rv1496 orthologs from closely related species into the SSGCID structure determination pipeline [20,21]. The ortholog strategy has proven successful previously [22]. We targeted both the commonly used model organism *M. smegmatis* [23] as well as the thermophilic *M. thermoresistible* [24]. Both targets are highly homologous (78-80% sequence identity) to Rv1496.

Co-crystallization with GDP resulted in co-crystal structures of both the *M. smegmatis* and *M. thermoresistible* orthologs (Table 1). In each case the structures were highly similar to the *Mtb* structure overall (r.m.s.d. 1.00 Å for *M. smegmatis* over 291 similar C $_{\alpha}$ atoms and 1.21 Å for *M. thermoresistible* over 276 similar C $_{\alpha}$ atoms; Figure 2b). The GDP binding sites for *Mtb* Rv1496 and its *M. smegmatis* and *M. thermoresistible* orthologs were highly similar (Figure 3). All residues in contact with GDP were identical in *M. smegmatis*. *M. thermoresistible* contained a single amino acid difference at L239 which was a V245 in *Mtb* Rv1496. Despite sequence conservation, two minor structural differences were observed. The side chain of K71 only interacted with the GDP β phosphate in the *Mtb* Rv1496 structure, and S66 in *M. thermoresistible* did not appear to interact with the GDP β phosphate, whereas the equivalent residue did in *Mtb* Rv1496 (S72) and *M. smegmatis* (S68). Thus, in this example both the ortholog and ligand co-crystallization approaches were viable strategies for obtaining crystal structures of the *Mtb* or highly similar ortholog structures.

Rv1496 nucleotide specificity

We attempted to examine the nucleotide specificity through differential scanning fluorescence. In the apo form, Rv1496 and its mycobacterial orthologs exhibit a two-phase melting profile (data not shown), presumably reflecting the homodimeric structure and complicating the analysis of the profiles in the presence of compounds. Therefore, the nucleotide specificity of Rv1496 was examined through co-crystallization with a number of nucleotide diphosphates and triphosphates. Rv1496 co-crystallized in the presence of GTP under the same conditions as GDP, and a 1.9 Å resolution structure was obtained (Table 1). Examination of the ligand electron density showed clear evidence for GDP rather than GTP (Figure 3b), indicating that Rv1496 may have hydrolyzed GTP to GDP during the course of the crystallization experiment. Co-crystallization with 2'-deoxyguanosine diphosphate, 2' -

dGDP resulted in a 2.0 Å resolution structure (Table 1) that was nearly identical to the GDP complex, as expected because the 2'-hydroxyl of GDP is solvent exposed and does not interact with the protein. Co-crystallization with GppNHp (GMPPNP), inosine triphosphate, 7-methylguanosine diphosphate or 2-Methylthioadenosine diphosphate resulted in ~2 Å resolution data sets in the same crystal form, but failed to result in suitable electron density for the full ligand and surrounding protein atoms (data not shown). In contrast, co-crystallization with ADP or ATP failed to yield crystals under these conditions, but yielded crystals similar to the apo protein with diffraction no better than 8 Å resolution. Since we were unable to obtain an ADP or ATP bound structure, it is difficult to predict how adenosine-based compounds would fit into the G-site binding pocket. In principle, the nucleobase of ATP could move out to form hydrogen bonds with D207 through N1 and N6, although this would significantly diminish the packing interactions with K205 and especially V245 without additional structural movement.

Active site conservation

Although the global fold can be used to infer homology, in depth analysis of active site pockets is important for understanding function. Several methods exist for this kind of analysis using discrete points, however, the ICM method, which utilizes continuous pharmacophoric atomic property fields (APF) [25,26] was shown in an independent comparative study to display the best in terms of molecular alignment and docking accuracy [27]. We obtained the software and applied this analysis method to the GDP-bound crystal structures of Rv1496, its mycobacterial orthologs from *M. smegmatis* and *M. thermoresistible*, MMAA from humans, and MeaB from *M. extorquens*. The pre-normalized pseudo-energies for the APF superpositions are shown in Table 2, and the normalized pocket similarity values are shown in Table 3 along with the overall sequence identity between pairs. There is a clear trend between the overall sequence identity of a pair and the similarity of the active site pocket. Rv1496 and all of its mycobacterial orthologs have higher active site similarity based on either atomic property fields or active site sequence identity in comparison with MeaB than MeaB has to its human homolog MMAA.

P and switch loop conservation

In general, GTPases have a P-loop and two switch loops which tend to be highly conserved within the protein family. The P-loop of *M. extorquens* MeaB is completely conserved with Rv1496 and the other two *Mycobacterium* orthologs (Rv1496 residues 65-73, GVPGVGKST). The switch I loop is also highly conserved between *M. extorquens* MeaB and Rv1496 and its orthologs with two conservative amino acid differences (Rv1496 residues 95-111 DPSS[T/V]R[T/S]GGSILGDKTRM). The switch I loop is disordered in all of these GDP-bound Rv1496 and *Mycobacterium* ortholog structures, which is consistent with the GDP-bound structure of MeaB (Figure 4). Only in the GMPPNP bound structure of MeaB does this loop appear ordered (PDB ID 4JYB) [28]. The switch II loop is also completely conserved in sequence (Rv1496 residues 157-165 ETVGVGQSE), although structurally there is some mobility of this loop between the different structures [29]. A switch III loop was recently described that is specific to MeaB and other MMAA orthologs and exhibits different conformations in MeaB depending on the bound ligand [28]. Rv1496 and its mycobacterial orthologs are highly conserved in this family-specific switch III loop

(Rv1496 residues 184-192 GD[E/Q]LQGIKK). This loop is ordered in all three *Mycobacterium* structures as well as in the MeaB structures (Figure 4c), but disordered in the MMAA structure. However, as described previously, this region exhibits conformational plasticity [28], and this appears consistent with the different *Mycobacterium* structures presented here which also exhibit variations in the conformation of this region despite high sequence conservation. Given that this loop interacts with both the switch II loop and the other protomer in the dimer, the observed conformational plasticity may be a requirement for functionality. As detailed for MeaB, mutations in this switch III loop region affect MCM turnover and repair, GAP function, AdoCbl loading, and can lead to methylmalonic aciduria in humans [28]. Given the high sequence homology and similar structural plasticity, we suspect that mutations in this region will similarly affect Rv1496 function in mycobacteria.

Summary

Crystal structures of *M. tuberculosis* Rv1496 and its orthologs from *M. smegmatis* and *M. thermoresistible* reveal a similar overall fold, GDP-binding and switch loop conformations as MeaB and MMAA GTPases. Based on these results, the original annotation of Rv1496 and its mycobacterial homologs as LAO ATPases is likely incorrect, although final confirmation would need to be derived from enzymatic data. Based on the structural data presented here, Rv1496 and its mycobacterial orthologs are likely MMAA/MeaB homologs and as such likely interact with mycobacterial MCM homologous proteins involved in glyoxylate regulation much in the same way as has been observed previously for MeaB/MCM.

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Abbreviations

ALS	Advanced Light Source
APF	atomic property force
ASU	asymmetric unit
GTP	guanosine 5' triphosphate
IMAC	immobilized metal ion affinity chromatography
MCM	methylmalonyl-CoA-mutase
MMAA	methylmalonyl aciduria associated protein A
PDB	Protein Data Bank
SBM	sleeping beauty mutase
SSGCID	Seattle Structural Genomics Center for Infectious Disease

Figure 1.

Mutliple sequence alignment of *Mtb* Rv1496 (MytuD.00200.a, PDB ID 3MD0) and its orthologs in *M. smegmatis* (MysmA.00200.b, PDB ID 3NXS), *M. thermoresistibile* (MythA.00200.a, PDB ID 3TK1), *Methylobacterium extorquens* (MeaB, PDB ID 2QM7 [5]), *Homo sapiens* (MMAA, PDB ID 2WWW [6]), and *Escherichia coli* (ArgK/YgfD, PDB ID 2P67, no primary citation).

Figure 2.

Crystal structure of *Mtb* Rv1496 exhibits a homodimer with an N-terminal α -helical domain, a central G-domain bound to GDP and a C-terminal α -helical dimerization domain (a). The GDP-bound *Mtb* Rv1496 crystal structure overlaid with those of its orthologs (b) from *M. smegmatis* (Msm) shown in yellow ribbons and *M. thermoresistibile* (Mth) shown in orange ribbons. The *Mtb* Rv1496 crystal structure overlaid with MeaB from *M. extorquens* (PDB ID 2QM7) shown in cyan ribbons (c). The biologically relevant dimer is shown for each structure. The *Mtb* Rv1496 crystal structure overlaid with MMAA from *Homo sapiens* (PDB ID 2WWW) shown in magenta ribbons (d); due to differences in the biological dimer detailed previously for human versus bacterial MMAA/MeaB proteins [6], we are only showing the monomer.

Figure 3.

Recognition of GDP (a) or GTP (b) by *M. tuberculosis* Rv1496 and GDP by the Rv1496 orthologs from *M. smegmatis* (c) or *M. thermoresistible* (d). For *Mtb* Rv1496-GDP the $|F_o| - |F_c|$ map is shown in green mesh contoured at 3.0σ prior to modelling GDP. For *Mtb* Rv1496 crystals grown in the presence of GTP, the $2|F_o| - |F_c|$ map is shown in blue mesh contoured at 1.0σ . Electron density was only present for the α and β phosphates of “GTP” and the model was otherwise nearly identical to the GDP bound structure, leading us to hypothesize that Rv1496 may have hydrolyzed the GDP to GTP. Only one amino acid differs between all three species, V245 in *Mtb* is V241 in *Msm* yet L239 in *Mth* (labelled in red).

Figure 4.

Examination of the conformational plasticity of the switch III loop in MeaB and its mycobacterial orthologs. (a) The *Mth* Rv1496 homodimer is shown as colored in Figure 2, but with the addition of the P-loop colored in red, the switch I loop colored in orange, the switch II loop colored in purple, and the switch III loop colored in blue. Close up (b) and overlay (c) of the P- and switch loops of MeaB, *Mtb* Rv1496 and its *Msm* and *Mth* orthologs, demonstrating structural rigidity of the GDP recognizing P-loop, but structural plasticity of the three switch loops.

Table 1

Data-collection and refinement statistics

Protein species	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>	<i>M. smegmatis</i>	<i>M. thermoresistible</i>
Ligand entering trials	GDP	GTP	2'-dGTP	GDP	GDP
Crystal Parameters					
Space group	C222 ₁	C222 ₁	C222 ₁	P2 ₁ 2 ₁ 2	P2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 65.5, <i>b</i> = 187.4, <i>c</i> = 66.2	<i>a</i> = 65.5, <i>b</i> = 187.4, <i>c</i> = 66.2	<i>a</i> = 65.4, <i>b</i> = 187.4, <i>c</i> = 66.3	<i>a</i> = 78.6, <i>b</i> = 91.5, <i>c</i> = 52.5	<i>a</i> = 42.4, <i>b</i> = 106.4, <i>c</i> = 134.0
Matthews coefficient (Å ³ /Da)	2.63	2.67	2.66	2.91	2.12
Solvent content (%)	53.3	53.9	53.8	57.7	41.9
Data collection					
X-ray source	Rigaku 007-HF	ALS 5.0.1	ALS 5.0.2	ALS 5.0.2	ALS 5.0.1
Wavelength (Å)	1.5418	0.97946	0.99990	1.0000	0.9
Resolution range (Å)	50-2.45 (2.50-2.45)	50-1.9 (1.95-1.90)	50-2.1 (2.14-2.10)	50-2.3 (2.36-2.30)	50-2.4 (2.46-2.40)
R _{merge}	0.072 (0.573)	0.046 (0.571)	0.082 (0.538)	0.054 (0.489)	0.078 (0.519)
No. unique reflections	15,339 (1090)	32,993 (2442)	24,210 (1771)	18,658 (1325)	23,270 (1743)
Multiplicity	6.8 (4.6)	8.7 (8.7)	6.0 (6.1)	8.7 (9.1)	5.3 (5.2)
Completeness (%)	99.4 (97.2)	99.8 (99.4)	99.7 (100)	98.2 (97.9)	94.8 (97.8)
Mean I/σ(I)	20.5 (2.7)	27.6 (3.9)	14.2 (3.7)	22.9 (4.3)	17.1 (3.0)
Refinement residuals					
R _{work}	0.211 (0.270)	0.199 (0.324)	0.191 (0.221)	0.209 (0.245)	0.230 (0.289)
R _{free}	0.251 (0.467)	0.223 (0.410)	0.227 (0.253)	0.231 (0.299)	0.277 (0.335)
No. reflections	15,268 (1,018)	32,897 (2,285)	28,336 (1,910)	18,502 (1,247)	23,269 (1,422)
No. reflections R _{free} set	780 (64)	1,670 (112)	1,431 (128)	943 (69)	2,369 (175)
Completeness (%)	99.0 (96.9)	99.5 (99.0)	100 (100)	97.8 (97.0)	94.8 (97.6)
Model Quality					
R.m.s.d. bonds (Å)	0.015	0.015	0.013	0.015	0.012
R.m.s.d. angles (°)	1.556	1.406	1.514	1.501	1.492
Residues in favored region (%)	97.6	98.7	100	98.7	99.7
Residues in allowed region (%)	100	100	100	100	100
Molprobability score (percentile)	1.91 (96 th)	1.48 (96 th)	1.15 (100 th)	1.65 (98 th)	1.88 (96 th)
Mean B factor (Å ²)	45.7	40.8	38.3	56.9	37.4

Protein species	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>	<i>M. tuberculosis</i>	<i>M. smegmatis</i>	<i>M. thermoresistible</i>
Nucleotide <i>B</i> factor (Å ²)	48.8	43.8	38.1	65.5	41.2
Model Contents					
Protomers in ASU	1	1	1	1	2
Protein atoms	2214	2255	2203	2283	4435
Hetero atoms	32	48	47	29	57
Waters	80	140	140	90	107
PDB ID	3MD0	3P32	4GT1	3NXS	3TK1

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Table 2

Pseudo-energies for APF superpositions (pre-normalization)

Protein species	<i>M. tuberculosis</i>	<i>M. smegmatis</i>	<i>M. thermoresistible</i>	<i>H. sapiens</i>	<i>M. extorquens</i>
<i>M. tuberculosis</i> (A) ^b	-557.0				
<i>M. smegmatis</i> (A)	-442.0	-515.7			
<i>M. thermoresistible</i> (B)	-430.3	-458.5	-496.5		
<i>H. sapiens</i> (D)	-397.1	-367.8	-360.3	-515.1	
<i>M. extorquens</i> (B)	-479.7	-433.5	-448.4	-403.0	-528.8

^a PDB entries 3MD0, 3NXS, 3TK1, 2WVW, and 2QM7 were used for *M. tuberculosis*, *M. smegmatis*, *M. thermoresistible*, *H. sapiens*, and *M. extorquens*, respectively.

^b Chain from PDB file

Table 3
Normalized pocket similarity values, PS(APF), with overall amino acid identity in parenthesis^a

Protein species	<i>M. tuberculosis</i>	<i>M. smegmatis</i>	<i>M. thermoresistible</i>	<i>H. sapiens</i>
<i>M. smegmatis</i> (A) ^b	82.5% (80%)			
<i>M. thermoresistible</i> (B)	81.8% (79%)	90.6% (83%)		
<i>H. sapiens</i> (D)	74.1% (50%)	71.4% (47%)	71.3 % (46%)	
<i>M. extorquen</i> (B)	88.4% (57%)	83.0% (56%)	87.5% (57%)	77.2% (47%)

^aPDB entries 3MD0, 3NXS, 3TK1, 2WWW, and 2QM7 were used for *M. tuberculosis*, *M. smegmatis*, *M. thermoresistible*, *H. sapiens*, and *M. extorquens*, respectively.

^bChain from PDB file