Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Preliminary X-ray crystallographic analysis of ornithine acetyltransferase (Rv1653) from *Mycobacterium tuberculosis*

R. Sankaranarayanan,‡ C. R. Garen,‡ M. M. Cherney, M. Yuan, C. Lee and M. N. G. James*

Protein Structure and Function Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

‡ These authors contributed equally.

Correspondence e-mail: michael.james@ualberta.ca

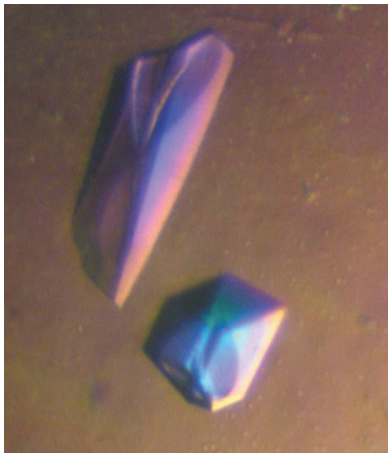
Received 19 November 2008
Accepted 5 January 2009

The gene product of open reading frame Rv1653 from *Mycobacterium tuberculosis* is annotated as encoding a probable ornithine acetyltransferase (OATase; EC 2.3.1.35), an enzyme that catalyzes two steps in the arginine-biosynthesis pathway. It transfers an acetyl group from *N*-acetylornithine to L-glutamate to produce *N*-acetylglutamate and L-ornithine. Rv1653 was crystallized using the sitting-drop vapour-diffusion method. The native crystals diffracted to a resolution of 1.7 Å and belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 60.1$, $b = 99.7$, $c = 155.3$ Å. The preliminary X-ray study showed the presence of a dimer in the asymmetric unit of the crystals, which had a Matthews coefficient V_M of 2.8 Å^3 Da^{-1}.

1. Introduction

Tuberculosis (TB) is a highly contagious and chronic respiratory disease caused by the human pathogen *Mycobacterium tuberculosis* (*Mtb*). Despite the existence of a somewhat effective cocktail treatment to cure this disease, noncompliant use of these drugs in chemotherapy has given rise to multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), which are virtually incurable with any of the presently available drugs (Dorman & Chaisson, 2007). TB is a leading killer among HIV-infected people and, according to the World Health Organization (WHO), a quarter of a million TB deaths are HIV-related. There were about 9.1 million new cases of TB and 1.7 million people died from this disease during 2006 alone (http://www.who.int/tb/publications/global_report/2008/pdf/summary.pdf). The Tuberculosis Structural Genomics Consortium (TBSGC) was set up in 2000 with the primary goal of providing a structural basis for the development of new and effective antimycobacterial therapeutics (Goulding *et al.*, 2002; Smith & Sacchettini, 2003; Terwilliger *et al.*, 2003; Murillo *et al.*, 2007).

Here, we report the crystallization and preliminary X-ray crystallographic analysis of the gene product from open reading frame Rv1653. This gene was annotated as an *argJ* gene that encodes ornithine acetyltransferase (OATase; EC 2.3.1.35) in *Mtb* (Cole *et al.*, 1998). This enzyme also has the alternative name glutamate *N*-acetyltransferase. OATase is involved in the acetyl-recycling process in the arginine-biosynthesis pathway in prokaryotes (Cunin *et al.*, 1986; Xu *et al.*, 2007). The enzyme catalyzes two consecutive reactions. In the first reaction, it removes the acetyl group from *N*-acetylornithine to produce L-ornithine. In the second step, the acetylated enzyme transfers the acetyl group to L-glutamate to produce *N*-acetylglutamate (Marc *et al.*, 2000; Elkins *et al.*, 2005). Extensive biochemical analyses of OATases from thermophilic bacteria and yeast have been carried out (Liu *et al.*, 1995; Crabeel *et al.*, 1997; Abadjieva *et al.*, 2000; Marc *et al.*, 2000, 2001). However, the recent structural analysis of OATase from *Streptomyces clavuligerus* is the only structural study that has been reported to date (Elkins *et al.*, 2005). In addition, the coordinates of the structure of OATase from *Bacillus halodurans* have been deposited in the Protein Data Bank (PDB code 1vra; Joint Centre for Structural Genomics, unpublished work). Rv1653 has been identified as one of the essential genes required for



the optimal growth of *Mtb* (Sassetti *et al.*, 2003). Recently, a different *N*-acetylglutamate synthase (EC 2.3.1.1) gene has been identified as an *argA* gene (Rv2747) in *Mtb* (Errey & Blanchard, 2005). The protein encoded by Rv2747 is a small protein with a molecular mass

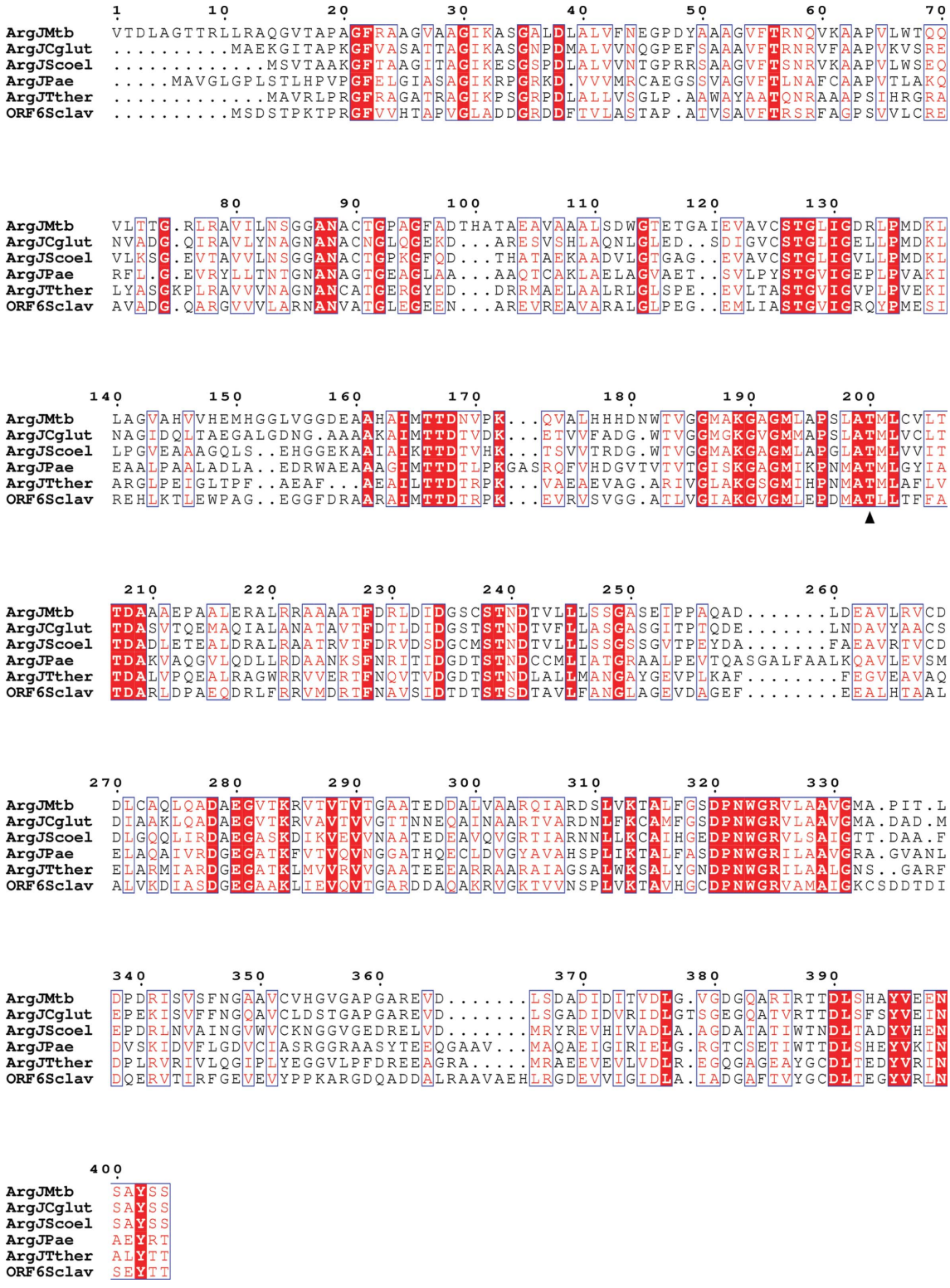


Figure 1
Sequence alignment of Rv1653 of *Mtb* with the proteins encoded by *argJ* and ORF6 genes from other bacteria: ArgJMtb, ArgJ from *M. tuberculosis*; ArgJCglut, ArgJ from *Corynebacterium glutamicum* (Q59280); ArgJScoel, ArgJ from *Streptomyces coelicolor* (Q8CK24); ArgJPae, ArgJ from *Pseudomonas aeruginosa* (Q9HWO4); ArgJTther, ArgJ from *Thermus thermophilus* (P96137); ORF6Scalv, ORF6 gene from *S. clavuligerus* (PDB 1vz6). The active-site threonine is indicated by a black triangle. Sequence alignment was performed using the program *ClustalW* (Thompson *et al.*, 1994) and the figure was generated using the program *ESPript* (Gouet *et al.*, 1999). The gene accession numbers are given in parentheses.

Table 1
Crystal parameters and data-collection statistics for the native crystal of Rv1653.

Values in parentheses are for the highest resolution shell.

Crystal parameters	
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a	60.1
b	99.7
c	155.3
No. of molecules in the unit cell (*Z*)	8
Data collection	
Temperature (K)	100
Detector	ADSC Q315
Wavelength (Å)	1.115872
Resolution (Å)	50.0–1.7 (1.76–1.70)
Unique reflections	98245 (9834)
Multiplicity	3.5 (3.5)
Mosaicity (°)	0.5–0.9
$\langle I/\sigma(I)\rangle$	13.4 (4.1)
Completeness (%)	95.6 (96.5)
$R_{\rm merge}$† (%)	7.7 (32.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th observation of reflection *hkl* and $\langle I(hkl)\rangle$ is the weighted average intensity of all observations *i* of reflection *hkl*.

of 19 kDa; it consists of 174 amino-acid residues. Rv2747 transfers an acetyl group from acetyl-CoA to L-glutamate. Sequence alignment of Rv1653 from *Mtb* with the protein sequences of six other bacterial genes has been carried out (Fig. 1). All of them contain a strictly conserved N-terminal nucleophile (Ntn) threonine residue (Thr200 in *Mtb*).

2. Materials and methods

2.1. Cloning, expression and purification

A genomic template DNA from *Mtb* strain H37Rv was derived from a bacterial artificial chromosome (BAC) library generated at L'Institut Pasteur (Brosch *et al.*, 1998; Gordon *et al.*, 1999). The Rv1653 open reading frame (ORF) was amplified using the polymerase chain reaction (PCR) with GoTaq Green Polymerase (Promega). Directional cloning of the ORF into the Gateway cloning system (Invitrogen) was achieved using the primer pair Rv1653F (5′-**GGGACAAGTTTGTACAAAAAAGCAGGCTCC***GAAAACCTGTATTTTCAGGGG*GTGACCGACCTGGCCGGCACCACC-3′) and Rv1653R (5′-**GGGACCACTTTGTACAAGAAAGCTGGGT**CTCATGAGCTGTAGGCCGAGTCTCTTCG-3′). Nucleotides in bold denote the recombination sites and those in italics show an engineered tobacco etch virus protease site, while those in regular type are the gene-specific sequences for Rv1653. The Rv1653 ORF encoding residues 1–404 was cloned into an expression plasmid with an amino-terminal glutathione *S*-transferase (GST) fusion tag (pDEST-15, Invitrogen). The resulting plasmid, pGST-1653, was confirmed by DNA-sequence analysis of the inserted ORF (DNA Core Facility, Department of Biochemistry, University of Alberta, Canada).

The GST-Rv1653 fusion protein was expressed in *Escherichia coli* BL21(DE3) pLysS cells (Novagen). A 2 l LB culture supplemented with 100 µg ml^{-1} ampicillin and 34 µg ml^{-1} chloramphenicol was incubated at 310 K until the $OD_{600\,nm}$ reached 0.8. The growth temperature was then shifted to 295 K and the culture was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) in a single dose to a final concentration of 0.5 m*M*. After overnight incubation, the cells were harvested by centrifugation at 7000*g* for 15 min at 277 K and the resulting bacterial pellets were resuspended in buffer *A* [1× phosphate-buffered saline (PBS), 5 m*M* β-mercaptoethanol pH 7.4 containing 1 m*M* EDTA, 100 µ*M* PMSF, 0.25 mg ml^{-1} hen egg-white lysozyme (Sigma) and a Complete EDTA-free protease-inhibitor tablet (Roche)] and then frozen. Thawed samples were subjected to ultrasonication and then passed through an EmulsiFlex-C5 high-pressure homogenizer (Avestin). The lysate was cleared by centrifugation (30 min, 20 000*g*) and the supernatant was loaded onto a 5 ml GSTrap glutathione Sepharose column (GE Healthcare) pre-equilibrated with 1× PBS. The GST-Rv1653 fusion protein was competitively eluted from the column with 15 m*M* glutathione in 50 m*M* Tris–HCl pH 8.0. Fractions containing recombinant protein were pooled and allowed to autoprocess at 277 K and were then subjected to TEV cleavage. Complete proteolysis yielded full-length GST and the pair of peptides resulting from the Ntn self-cleavage activity of Rv1653 as described. After thorough dialysis against 1× PBS, the cleaved protein mixture was once again loaded onto a 5 ml GSTrap column and the flowthrough fractions containing Rv1653 were dialyzed against 25 m*M* Tris–HCl pH 7.4, 350 m*M* NaCl and concentrated using a 10 kDa molecular-weight cutoff Amicon Ultra Centrifugal Concentrator (Millipore) for loading onto a HiLoad 16/60 Superdex 75pg size-exclusion chromatography (SEC) column for final purification of the recombinant protein. SEC fractions containing recombinant Rv1653 were concentrated once again as above and then dialyzed to change the buffer to 2.5 m*M* HEPES pH 7.5. The maximum final protein concentration achieved was 13.5 mg ml^{-1}. All steps of purification were performed at 277 K and the result of each step was monitored by 16% SDS–PAGE.

2.2. Crystallization

The initial crystallization condition for Rv1653 crystals was obtained using a Hydra I robot (Robbins Scientific Corporation) that utilizes the sitting-drop vapour-diffusion method in 96-well plates (IntelliPlates, Hampton Research). Crystallization was performed by mixing equal volumes (0.4 µl) of protein solution and reservoir solution. A protein concentration of 13.5 mg ml^{-1} in 2.5 m*M* HEPES buffer pH 7.5 was used. Orthorhombic crystals (Fig. 2) grew in approximately 2 d from Index Screen condition No. E11, which contains 0.02 *M* $MgCl_2$, 22% polyacrylic acid 5100 and 0.1 *M* HEPES pH 7.5.

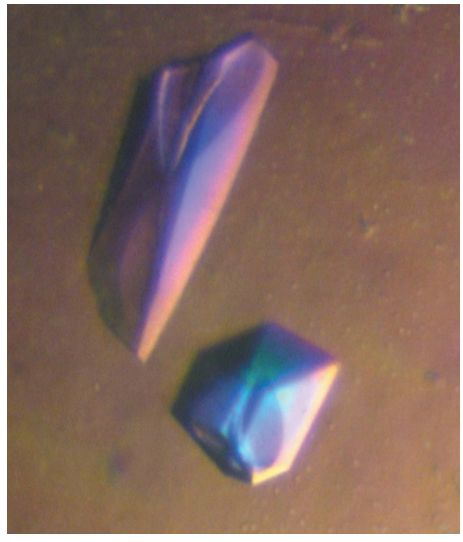


Figure 2
Orthorhombic crystals of Rv1653.

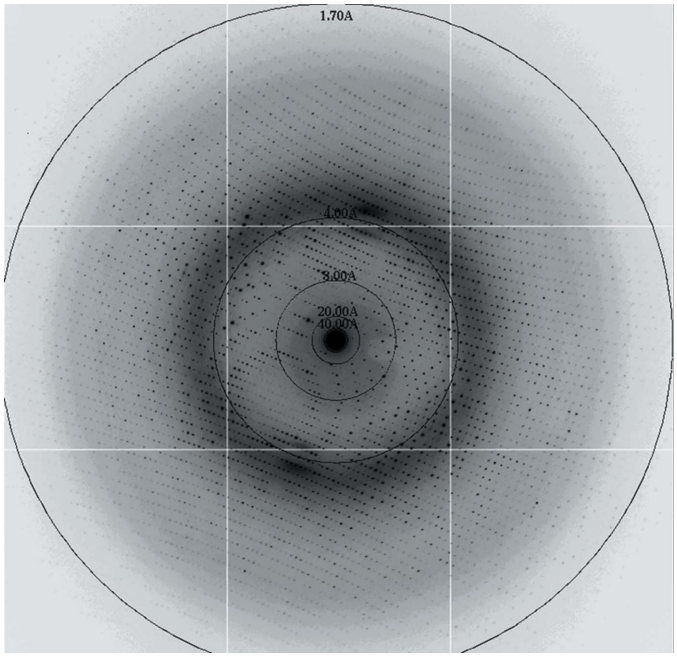


Figure 3
X-ray diffraction-pattern frame collected from Rv1653 crystals. The pattern displays a maximum resolution of 1.7 Å and space group $P2_12_12_1$.

2.3. Data collection and processing

Crystals for synchrotron data collection were first rinsed in cryoprotectant (30% glycerol in reservoir solution) and then flash-cooled in liquid nitrogen. Native data sets were collected on synchrotron beamline 8.3.1 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory, USA (Fig. 3). The raw data were indexed, integrated and scaled using the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results

The crystal parameters and data-collection statistics for the native crystal of Rv1653 are summarized in Table 1. The monomer of Rv1653 consists of 404 amino acids with a molecular mass of 41 kDa. The Matthews coefficient V_M of 2.8 Å^3 Da^{-1} (Matthews, 1968; Kantardjieff & Rupp, 2003) suggested the presence of two molecules per asymmetric unit and a solvent content of 56.5%. The structure of Rv1653 was solved by the molecular-replacement program *Phaser* (McCoy *et al.*, 2005) as incorporated in the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). The monomer of the OATase encoded by the ORF6 gene from *S. clavuligerus* (PDB code 1vz6) was used as a template model. Calculations performed using *Phaser* led to an acceptable solution, with *Z* scores of TF*Z* = 16.5 and RF*Z* = 4.2 and a log-likelihood gain of 241 for a dimer in the asymmetric unit. OATase from *S. clavuligerus* has been reported to be an N-terminal nucleophile (Ntn) enzyme (Elkins *et al.*, 2005). The detailed structural analysis will be published elsewhere.

X-ray diffraction data were collected on beamline 8.3.1 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory under agreements with the Alberta Synchrotron Institute (ASI). The ALS is supported by the National Institutes of Health and operated by the Department of Energy. Beamline 8.3.1 was funded by the National Science Foundation, University of California and Henry Wheeler. The ASI synchrotron-access program in Alberta is supported by grants from the Alberta Science and Research Authority (ASRA), the Alberta Heritage Foundation for Medical Research (AHFMR), the Alberta Ingenuity Fund and Western Economic Diversification of the Federal Government of Canada. Research in the laboratory of MNGJ is supported by the Alberta Heritage Foundation for Medical Research (AHFMR) and the Canadian Institutes of Health Research (CIHR).

References

Abadjieva, A., Hilven, P., Pauwels, K. & Crabeel, M. (2000). *J. Biol. Chem.* **275**, 11361–11367.
Brosch, R., Gordon, S. V., Billault, A., Garnier, T., Eiglmeier, K., Soravito, C., Barrell, B. G. & Cole, S. T. (1998). *Infect. Immun.* **66**, 2221–2229.
Cole, S. T. *et al.* (1998). *Nature (London)*, **393**, 537–544.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Crabeel, M., Abadjieva, A., Hilven, P., Dessimpelaere, J. & Soetens, O. (1997). *Eur. J. Biochem.* **250**, 232–241.
Cunin, R., Glansdorff, N., Piérard, A. & Stalon, V. (1986). *Microbiol. Rev.* **50**, 314–352.
Dorman, S. E. & Chaisson, R. E. (2007). *Nature Med.* **13**, 295–298.
Elkins, J. M., Kershaw, N. J. & Schofield, C. J. (2005). *Biochem. J.* **385**, 565–573.
Errey, J. C. & Blanchard, J. S. (2005). *J. Bacteriol.* **187**, 3039–3044.
Gordon, S. V., Brosch, R., Billault, A., Garnier, T., Eiglmeier, K. & Cole, S. T. (1999). *Mol. Microbiol.* **32**, 643–655.
Gouet, P., Courcelle, E., Stuart, D. I. & Métoz, F. (1999). *Bioinformatics*, **15**, 305–308.
Goulding, C. W. *et al.* (2002). *Curr. Drug Targets Infect. Disord.* **2**, 121–141.
Kantardjieff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
Liu, Y., Van Heeswijck, R., Høj, P. & Hoogenraad, N. (1995). *Eur. J. Biochem.* **228**, 291–296.
Marc, F., Weigel, P., Legrain, C., Almeras, Y., Santrot, M., Glansdorff, M. & Sakanyan, V. (2000). *Eur. J. Biochem.* **267**, 5217–5226.
Marc, F., Weigel, P., Legrain, C., Glansdorff, N. & Sakanyan, V. (2001). *J. Biol. Chem.* **276**, 25404–25410.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C. & Read, R. J. (2005). *Acta Cryst.* D**61**, 458–464.
Murillo, A. C. *et al.* (2007). *Infect. Dis. Drug Targets.* **7**, 127–139.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sassetti, C. M., Boyd, D. H. & Rubin, E. J. (2003). *Mol. Microbiol.* **48**, 77–84.
Smith, C. V. & Sacchettini, J. C. (2003). *Curr. Opin. Struct. Biol.* **13**, 658–664.
Terwilliger, T. C. *et al.* (2003). *Tuberculosis (Edinb.)*, **83**, 223–249.
Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). *Nucleic Acids Res.* **22**, 4673–4680.
Xu, Y., Labedan, B. & Glansdorff, N. (2007). *Microbiol. Mol. Biol. Rev.* **71**, 36–47.