Comparison of four different crystal forms of the *Mycobacterium tuberculosis* ESX-1 secreted protein regulator EspR

Shanti P. Gangwar, Sita R. Meena and Ajay K. Saxena*

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

Correspondence e-mail:
ajaysaxena@mail.jnu.ac.in

Received 21 January 2014
Accepted 23 February 2014

PDB reference: EspR, 4ndw

The *Mycobacterium tuberculosis* ESX-1 secreted protein regulator (EspR, Rv3849) is the key protein that delivers bacterial proteins into the host cell during mycobacterial infection. EspR binds directly to the *espACD* operon and is involved in transcriptional activation. In the current study, *M. tuberculosis* EspR has been crystallized and its X-ray structure has been determined at 3.3 Å resolution in a $P3_221$ crystal form. EspR forms a physiological dimer in the crystal. Each EspR monomer contains an N-terminal helix–turn–helix DNA-binding domain and a C-terminal dimerization domain. The EspR structure in the $P3_221$ crystal form was compared with previously determined EspR structures in $P3_2$, $P2_1$ and $P2_12_12_1$ crystal forms. Structural comparison analysis indicated that the N-terminal helix–turn–helix domain of EspR acquires a rigid structure in the four crystal forms. However, significant structural differences were observed in the C-terminal domain of EspR in the $P2_1$ crystal form when compared with the $P3_221$ and $P3_2$ crystal forms. The interaction, stabilization energy and buried surface area analysis of EspR in the four different crystal forms have provided information about the physiological dimer interface of EspR.

1. Introduction

The protein export systems of *Mycobacterium tuberculosis* are considered as new drug targets (Feltcher *et al.*, 2010). The ESX-1 protein secretion system is the principal virulence system of *M. tuberculosis*, which delivers virulence factors into host macrophages during mycobacterial infection (Stanley *et al.*, 2003; Hsu *et al.*, 2003; Pathak *et al.*, 2007). After macrophage infection, the ESX-1 secretion system is involved in innate immune modulation (Stanley *et al.*, 2003, 2007; MacGurn & Cox, 2007; Volkman *et al.*, 2004). Precise regulation of the ESX-1 secretion system during infection is essential for pathogenicity of *M. tuberculosis*.

The precise mechanism of the *M. tuberculosis* ESX-1 secretion system and its essential role in virulence are currently unknown. The ESAT-6 (early secretory antigenic target, 6 kDa), CFP-10 (culture filtrate protein, 10 kDa) and ESPS (ESX-1 secretion-associated proteins) of the ESX-1 secretion system interfere with host cell metabolism and help mycobacteria to escape phagosomal engulfment (Hsu *et al.*, 2003; Pathak *et al.*, 2007; de Jonge *et al.*, 2007; Singh *et al.*, 2003; Bitter *et al.*, 2009; Simeone *et al.*, 2009).

EspR is a DNA-binding transcriptional regulator and activates the *espACD* operon (*rv3616c–rv3614c*) directly upon macrophage infection (Fortune *et al.*, 2005; Garces *et al.*, 2010; Raghavan *et al.*, 2008). EspR is induced upon phagocytosis and activates the expression of downstream *M. tuberculosis* ESX-1 components. Deletion of EspR affects *M. tuberculosis* gene expression, including loci critical for function of the ESX-1 secretion system (Raghavan *et al.*, 2008). The EspR protein contains 132 residues with a molecular mass of ~14.7 kDa. The N-terminus of EspR harbours a helix–turn–helix DNA-binding domain, while there is a typical dimerization domain at the C-terminus. Deletion of the C-terminal ten amino acids of EspR abolishes the ESX-1 secretion activity, but did not affect the DNA affinity (Raghavan *et al.*, 2008).

In the current work, we have determined the crystal structure of *M. tuberculosis* EspR in a $P3_221$ crystal form at 3.3 Å resolution

Table 1

Intensity data-collection and refinement statistics of four EspR crystal forms.

Values in parentheses are for the highest resolution shell.

Data set	$P3_221$, SeMet	$P3_221$, native	$P3_2$	$P2_1$	$P2_12_12_1$
PDB code		4ndw	3r1f (Rosenberg <i>et al.</i> , 2011)	3qf3 (Blasco <i>et al.</i> , 2011)	3qwg (Blasco <i>et al.</i> , 2011)
Space group	$P3_221$	$P3_221$	$P3_2$	$P2_1$	$P2_12_12_1$
Wavelength (Å)	0.97833	0.97625	1.11588	1.000	1.0097
X-ray source	BM14, ESRF	BM14, ESRF	ALS, BI 8.3.1	SLS, PSI Switzerland	SLS, X06DA, Switzerland
Resolution (Å)	50–3.55 (3.61–3.55)	48–3.30 (3.48–3.30)	48.85–2.50 (2.54–2.50)	36.92–2.41 (2.56–2.41)	44.86–1.99 (2.11–1.99)
Unit-cell parameters (Å, °)	$a = b = 84.9$, $c = 129.9$	$a = b = 83.9$, $c = 130.75$	$a = b = 148.4$, $c = 129.6$	$a = 52.2$, $b = 81.7$, $c = 124.8$, $\beta = 95.8$	$a = 46.5$, $b = 55.4$, $c = 76.6$
Unique reflections	12707	8535	100727	40265	24707
Completeness	100 (100)	100 (100)	91.05 (86.9)	98.9 (96.6)	94.4 (82.6)
$R_{\text{merge}}^{\dagger}$ (%)	11.0 (38.9)	15.9 (55.3)	14.0 (73.0)	7.7 (65.1)	10.4 (38.7)
Average $I/\sigma(I)$	21.5 (5.8)	11.1 (2.6)	3.9 (1.5)	13.3 (2.6)	9.8 (3.1)
Multiplicity	11.7 (11.8)	11.8 (12.1)	2.1 (2.0)	3.2 (3.1)	3.4 (3.0)
Monomers in asymmetric unit	2	2	18	6	2
Solvent (%)	68	69	59	59	32
V_M (Å ³ Da ⁻¹)	3.88	3.91	3.02	3.0	1.81
Refinement					
Resolution (Å)		48.69–3.30	48.85–2.50	36.92–2.41	44.86–1.99
$R_{\text{work}}/R_{\text{free}}^{\ddagger}$ (%)		27/33	19.7/23.2	18/24	18.5/23.6
Protein atoms		2020	17207	6135	1441
Water atoms			19	232	132
R.m.s.d. bonds (Å)		0.009	0.010	0.008	0.007
R.m.s.d. angle (°)		1.33	1.2	0.9	0.954
Ramachandran plot					
Favoured (%)		85.5	87.8	94.2	94.3
Allowed (%)		13.8	11.7	5.8	5.7
Generous (%)		0.4	0.5	0.0	0.0
Forbidden (%)		0.0	0.0	0.0	0.0

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the i th intensity measurement of reflection hkl and $\langle I(hkl) \rangle$ is the average intensity of that reflection. $\ddagger R_{\text{work}}/R_{\text{free}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$.

(PDB entry 4ndw; two monomers in the asymmetric unit). The current EspR structure was compared with previous EspR structures in (i) a $P3_2$ crystal form (PDB entry 3r1f; 18 monomers in the asymmetric unit; Rosenberg *et al.*, 2011), (ii) a $P2_1$ crystal form (PDB entry 3qf3; six monomers in the asymmetric unit; Blasco *et al.*, 2011) and (iii) a $P2_12_12_1$ crystal form (PDB entry 3qwg; two monomers in the asymmetric unit; Blasco *et al.*, 2011). The structural comparison analysis has revealed information on the physiological dimer interface and important structural changes in EspR in different crystal forms.

2. Materials and methods

2.1. Protein expression and purification

The gene encoding EspR (Met1–Ala132) was amplified from *M. tuberculosis* H37Rv strain by polymerase chain reaction using the forward primer 5'-GATCCATATGATGAGCAGCAGTTCGCT-3' and the reverse primer 5'-CATGCTCGAGCTAAGCGTCGATCCCTCC-3'. The amplified EspR gene was digested and ligated into pET28a(+) expression vector (Novagen) using *Nde*I and *Xho*I restriction sites. The resulting EspR plasmid was transformed in *Escherichia coli* BL21(DE3) cells and the protein was expressed in the soluble fraction. The EspR was purified using Ni-NTA and size-exclusion chromatography as described in Gangwar *et al.* (2011). The recombinant EspR protein contains 152 residues [132 residues from native EspR and 20 residues from the pET28a(+) vector containing a 6×His tag and a thrombin cleavage site].

2.2. Preparation of selenomethionine derivative of EspR

The selenomethionine derivative of EspR was prepared using SelenoMethionine Base plus Nutrient Mix medium obtained from Molecular Dimensions. 30 mg of seleno-DL-methionine (Sigma–

Aldrich) was added to 1 l of the selenomethionine medium. The expression and purification of selenomethionine-substituted EspR protein were similar to those of native EspR described in Gangwar *et al.* (2011).

2.3. Crystallization and X-ray structure determination

The purified EspR was concentrated to 10–12 mg ml⁻¹ in buffer consisting of 20 mM Tris–HCl pH 7.5 for crystallization experiments. Initial crystallization screenings were performed on native and selenomethionine-substituted EspR as described in Gangwar *et al.* (2011). The selenomethionine-substituted EspR crystals were obtained in several conditions using the sitting-drop vapour-diffusion technique. The best selenomethionine-substituted EspR crystals appeared in buffer consisting of 24%(w/v) PEG 3350, 200 mM sodium malonate, 100 mM bis-tris propane pH 6.5 after 10–15 d.

For intensity data collection, the native EspR crystals were transferred into mother liquor consisting of 35%(w/v) PEG 3350, 200 mM sodium malonate, 100 mM bis-tris propane pH 6.5. These crystals were cooled directly in liquid nitrogen as 35%(w/v) PEG 3350 acts as a good cryoprotectant for data collection at cryogenic temperature. The native EspR crystal diffracted to 3.3 Å resolution and a full intensity data set was collected at 100 K on the BM14 synchrotron beamline at the ESRF, Grenoble, France. The selenomethionine-substituted EspR crystal diffracted to 3.5 Å resolution and a complete single anomalous dispersion data set (SAD) was collected at the selenium edge. The native and selenomethionine SAD data sets were processed using *DENZO* and *SCALEPACK* from the *HKL*-2000 suite (Otwinowski & Minor, 1997). The details of intensity data collection and processing are given in Table 1.

The selenium sites were identified using *SHELXD* and *SHELXE* from the *HKL2MAP* suite (Pape & Schneider, 2004). Initial phases were obtained by *AutoSol* from the *PHENIX* suite (Adams *et al.*,

Figure 1

The size-exclusion chromatogram and SDS-PAGE of purified recombinant EspR protein. The recombinant EspR elutes as a dimer from a Superdex 75 (16/60) column. The calculated molecular mass of EspR is denoted in the figure.

2010). The selenomethionine-substituted EspR model was built by *AutoBuild* from the *PHENIX* suite. The obtained EspR structure was transferred into the native data set collected at 3.3 Å resolution using *Phaser* from the *CCP4* suite (Winn *et al.*, 2011). The EspR structure was refined using the *REFMAC5* program (Murshudov *et al.*, 2011) of the *CCP4* suite and further model building was done using *Coot* (Emsley & Cowtan, 2004). Figures were generated using *PyMOL* v1.3 (DeLano, 2002).

3. Results and discussion

3.1. Structure determination

We recombinantly expressed EspR protein in *E. coli* and purified it using Ni-NTA affinity and size-exclusion chromatography as described in Gangwar *et al.* (2011). The native EspR eluted as a dimer (molecular mass of ~30 kDa) from a Superdex 75 (16/60) column (Fig. 1). The native EspR was crystallized in space group $P3_221$ and an intensity data set was collected to 3.3 Å resolution. We crystallized selenomethionine-substituted EspR in the same condition as used for native EspR. The EspR structure was determined using a single anomalous dispersion data set collected at 3.5 Å resolution using the selenium edge (Supplementary Fig. S1[†]). The obtained structure was placed in the EspR native data set collected to 3.3 Å resolution using *Phaser* from the *CCP4* suite.

The $P3_221$ form of EspR contains two monomers in the asymmetric unit packed as a functional homodimer. The $P3_2$ form of EspR contains 18 monomers in the asymmetric unit that are packed as nine closely interacting dimers (Rosenberg *et al.*, 2011). The $P2_1$ form of EspR contains six monomers in the asymmetric unit that are packed into three functional EspR dimers (Blasco *et al.*, 2011). The $P2_12_12_1$ form of the C-terminal deletion mutant of EspR contains two monomers in the asymmetric unit (Blasco *et al.*, 2011). The EspR dimer interface in the $P2_12_12_1$ form was quite different to the EspR dimer interface observed in the $P2_1$, $P3_221$ and $P3_2$ forms (Blasco *et al.*

Figure 2

Overall structure of EspR. The EspR adopts an S-shaped structure. The EspR dimer is formed through the C-terminal domain.

al., 2011). It indicates that the $P2_12_12_1$ form of the C-terminal deletion mutant of EspR lacks the key residues involved in the formation of the physiological dimer interface of EspR (Blasco *et al.*, 2011).

3.2. Overall structure

The $P3_221$ form of EspR contains two monomers in the asymmetric unit with a Matthews coefficient (V_M) of $3.91 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 69% (Matthews, 1968). In the $P3_221$ form of EspR, two monomers of EspR are arranged as a physiological dimer (Fig. 2). Analysis of the EspR dimer in the $P3_221$ form using the *PISA* server (Krissinel & Henrick, 2007) indicated that both EspR monomers contain 4217 Å^2 buried surface area and have $21.1 \text{ kcal mol}^{-1}$ free energy of dissociation. The interacting residues between two EspR monomers are shown in Fig. 3. These data indicate that the EspR dimer in the $P3_221$ form is thermodynamically stable.

Analysis of the $P3_221$ form of EspR using the *ESBRI* server (Costantini *et al.*, 2008) indicated that three salt bridges and 26 hydrophobic interactions are involved in the dimer interface (Table 2). In the $P3_221$ form of EspR, $0.2 \text{ kcal mol}^{-1}$ hydrogen-bond energy, $-16.5 \text{ kcal mol}^{-1}$ electrostatic energy and a total of $-72 \text{ kcal mol}^{-1}$ stabilizing energy were observed at the dimer interface (Table 2). The *COILCHECK* program (Alva *et al.*, 2008) identified the three side-chain hydrogen bonds at the EspR dimer interface: (i) between Glu87 ($O^{\delta 1}$) of chain A and Arg100 (NH1) of chain B, (ii) between Glu87 ($O^{\delta 1}$) of chain A and Arg100 (NH2) of chain B, and (iii) between His107 ($N^{\delta 1}$) of chain A and Thr93 ($O^{\gamma 1}$) of chain B (Fig. 3).

3.3. Conformational changes

The EspR crystal structure has been determined in four different crystal forms: (i) in a $P3_221$ form at 3.3 Å resolution (PDB entry 4ndw, current structure), (ii) in a $P3_2$ form at 2.5 Å resolution (PDB entry 3r1f; Rosenberg *et al.*, 2011), (iii) in a $P2_1$ form at 2.4 Å resolution (PDB entry 3qf3; Blasco *et al.*, 2011) and (iv) an EspR mutant in a $P2_12_12_1$ form at 1.9 Å resolution (PDB entry 3qwg; Blasco *et al.*,

[†] Supporting information has been deposited in the IUCr electronic archive (Reference: FW5444).

2011). Superposition of the EspR dimer in the $P_{3_2}2_1$ form on the P_3_2 form gave an r.m.s.d. of 0.54 Å for 239 C α atoms (Fig. 4). Superposition of the EspR dimer of the $P_{3_2}2_1$ form on the P_2_1 form gave an r.m.s.d. of 0.75 Å for 189 C α atoms; however, significant structural differences were observed in the C-terminal domain of EspR.

Hinge angles between the α_7 and α_8 helices of EspR of 102° in the $P_{3_2}2_1$ form, 101° in the P_3_2 form and 124° in the P_2_1 form were observed. Hinge angles between the α_6 and α_7 helices of EspR of 37° in the $P_{3_2}2_1$ form, 30° in the P_3_2 form and 47° in the P_2_1 form were observed (Fig. 4). These data indicate that the conformation of the C-terminal domain of EspR is identical in the $P_{3_2}2_1$ and P_3_2 forms but changed significantly in the P_2_1 form.

3.4. Dimer interface analysis

As shown in Table 3, the EspR dimer interface in the $P_{3_2}2_1$ form contains 4220 Å² of buried surface area and has 21 kcal mol⁻¹ dissociation free energy. In the P_3_2 form of EspR, 4580 Å² of buried

Figure 3
The physiological dimer interface of EspR. Residues involved in hydrogen-bonding interactions at the EspR dimer interface are shown.

Table 2
Stabilization energy and number of interactions between two monomers in the four EspR crystal forms.

	$P_{3_2}2_1$	P_3_2	P_2_1	$P_{2_1}2_12_1$
Hydrogen-bond energy (kJ mol ⁻¹)	5.9	10.7	11.2	13.9
Electrostatic energy (kJ mol ⁻¹)	-69.1	-186.3	-144.2	-39.1
Van der Waals energy (kJ mol ⁻¹)	-242.5	-296.7	-321.1	-101.1
Total stabilizing energy (kJ mol ⁻¹)	-305.7	-472.3	-454.1	-126.3
Energy per residue (kJ mol ⁻¹)	-1.2	-1.8	-1.7	-0.7
Short contacts	2	0	1	1
Hydrophobic interactions	26	24	25	0
Van der Waals pairs	4078	4337	4827	1589
Salt bridges	4	8	9	2
Hydrogen bonds	3	2	7	0
Electrostatic interactions	60	59	61	18

Table 3
Accessible surface area in the EspR dimer in the four crystal forms.

Crystal form	Total area per subunit (Å ²)	Contact area (Å ²)	Contact area (%)	Dissociation energy (kcal mol ⁻¹)
$P_{3_2}2_1$	13528.3	3975.1	29.4	21.1
P_3_2	13297.3	3905.1	29.4	18.2
P_2_1	13483.6	3961.3	29.4	22.7
$P_{2_1}2_12_1$	10131.2	2987.9	29.5	1.3

surface area and 18.8 kcal mol⁻¹ free energy of dissociation were observed at the dimer interface. In the P_2_1 form of EspR, 4540 Å² of buried surface area and 23.1 kcal mol⁻¹ free energy of dissociation were observed at the dimer interface. In the $P_{2_1}2_12_1$ form of truncated EspR, only 585 Å² of buried surface area was observed at the dimer interface. These results indicate that EspR monomers do not form a physiological dimer in the $P_{2_1}2_12_1$ crystal form and packed as a result of crystallographic packing (Blasco *et al.*, 2011). The stabilizing energy and various interactions at the EspR dimer interface are shown in Table 2. The residues interacting at the EspR dimer interface differ from one crystal form to the other, but do not affect the EspR dimer formation (Table 4).

3.5. Crystallographic contacts

Each crystal form of EspR contains distinct crystallographic contacts, which are given in Supplementary Table S1. In the EspR P_2_1

Figure 4
Superposition of EspR structures obtained in four different crystal forms. The EspR structures in the P_3_2 , P_2_1 and $P_{2_1}2_12_1$ crystal forms were superposed on that in the $P_{3_2}2_1$ crystal form.

Table 4

Interactions occurring in the EspR dimer interfaces in the four crystal forms.

Crystal form	Residue (atom)	Residue (atom)	Distance (Å ²)
<i>P</i> ₃ ₂ ₁	Glu87 (O ^{e1})	Arg100 (NH1)	2.7
	Glu87 (O ^{e1})	Arg100 (NH2)	3.1
	His107 (N ^{δ2})	Thr93 (O ^{γ1})	3.7
	His107 (N ^{ε1})	Asp96 (O ^{δ1})	3.5
<i>P</i> ₃ ₂	Glu87 (O ^{e1})	Arg100 (NH1)	3.4
	Glu87 (O ^{e1})	Arg100 (NH2)	2.9
	Glu87 (O ^{e2})	Arg100 (NH1)	3.2
	Arg100 (NH2)	Glu87 (O ^{e1})	3.4
	Arg105 (N ^ε)	Asp122 (O ^{δ1})	2.9
	His107 (N ^{δ1})	Gln89 (N ^{δ1})	3.4
<i>P</i> ₂ ₁	Asp122 (O ^{δ2})	Arg105 (O ^{δ2})	3.2
	Arg101 (NH1)	Glu88 (O ^{e1})	2.9
	Arg101 (NH1)	Glu88 (O ^{e2})	3.3
	Arg101 (NH2)	Glu88 (O ^{e2})	3.2
	Gln116 (O ^{e1})	Arg102 (NH2)	3.1
	Asp123 (O ^{δ1})	Arg106 (N ^ε)	2.8
<i>P</i> ₂ ₁ ₂ ₁	Asp123 (O ^{δ1})	Arg100 (NH2)	2.7
	Asp132 (O ^{δ2})	Arg106 (NH2)	2.8
	Thr5 (O ^{γ1})	Asn53 (N ^{δ2})	3.1
	Ser51 (O ^γ)	Asn11 (N ^{δ2})	3.1
	Asn53 (N ^{δ2})	Thr5 (O ^{γ1})	3.3

crystal form, two intermolecular crystal contacts, Arg54–Asn11 and Arg54–Asp15, were observed in the N-terminal domain of EspR. In the EspR *P*₃₂₁ crystal form, the Arg120, Glu123, Leu124, Arg126 and Ala127 residues of the EspR monomer interact with their symmetry mates. In the *P*₃₂ form, no residues of the EspR monomers are involved in crystallographic contacts; however, the structure aligns very well with the *P*₃₂₁ crystal form. These results indicate that crystal contacts do not induce conformational change in the C-terminal domain of EspR in the *P*₂₁ crystal form, which is quite different from the observed structures in the *P*₃₂ and *P*₃₂₁ crystal forms.

In the current study, we have determined the structure of EspR in the *P*₃₂₁ crystal form and compared it with the structures of EspR in the *P*₃₂, *P*₂₁ and *P*₂₁₂₁ crystal forms. These results indicate that the N-terminal domain of EspR acquires a rigid structure and significant structural changes are observed in the C-terminal domain of EspR. The crystallographic contact analysis in various EspR crystal forms indicates that they are not involved in inducing structural change in the C-terminal domain of EspR. In the *P*₃₂ and *P*₂₁ crystal forms, high atomic fluctuations and structural changes are observed in the C-terminal domain of various EspR subunits in the asymmetric unit (Blasco *et al.*, 2011). The α6–α8 helices of the C-terminal domain of EspR form tight intermolecular interactions and are involved in homodimer formation, which is essential for activation of the *espACD* operon and EsxA secretion. However, the orientations of these helices are different in the various EspR crystal forms, which may form divergent oligomeric arrangements of EspR upon DNA binding.

The current work was supported by grants from UGC Networking, Capacity Buildup and DST Purse. The authors thank the staff members of the X-ray diffraction facility of AIRF, Jawaharlal Nehru University, India and the staff of the DBT-BM14 beamline at the ESRF, Grenoble, France for their help in intensity data collection and processing.

References

- Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
- Alva, V., Syamala Devi, D. P. & Sowdhamini, R. (2008). *Protein Pept. Lett.* **15**, 33–38.
- Bitter, W. *et al.* (2009). *PLoS Pathog.* **5**, e1000507.
- Blasco, B., Stenta, M., Alonso-Sarduy, L., Dietler, G., Peraro, M. D., Cole, S. T. & Pojer, F. (2011). *Mol. Microbiol.* **82**, 251–264.
- Costantini, S., Colonna, G. & Facchiano, A. M. (2008). *Bioinformatics*, **3**, 137–138.
- DeLano, W. L. (2002). *PyMOL*. <http://www.pymol.org>.
- Emsley, P. & Cowtan, K. (2004). *Acta Cryst.* **D60**, 2126–2132.
- Feltcher, M. E., Sullivan, J. T. & Braunstein, M. (2010). *Future Microbiol.* **5**, 1581–1597.
- Fortune, S. M., Jaeger, A., Sarracino, D. A., Chase, M. R., Sassetti, C. M., Sherman, D. R., Bloom, B. R. & Rubin, E. J. (2005). *Proc. Natl Acad. Sci. USA*, **102**, 10676–10681.
- Gangwar, S. P., Meena, S. R. & Saxena, A. K. (2011). *Acta Cryst.* **F67**, 83–86.
- Garces, A., Atmakuri, K., Chase, M. R., Woodworth, J. S., Krastins, B., Rothchild, A. C., Ramsdell, T. L., Lopez, M. F., Behar, S. M., Sarracino, D. A. & Fortune, S. M. (2010). *PLoS Pathog.* **6**, e1000957.
- Hsu, T. *et al.* (2003). *Proc. Natl Acad. Sci. USA*, **100**, 12420–12425.
- Jonge, M. I. de, Pehau-Arnaudet, G., Fretz, M. M., Romain, F., Bottai, D., Brodin, P., Honoré, N., Marchal, G., Jiskoot, W., England, P., Cole, S. T. & Brosch, R. (2007). *J. Bacteriol.* **189**, 6028–6034.
- Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.
- MacGurn, J. A. & Cox, J. S. (2007). *Infect. Immun.* **75**, 2668–2678.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). *Acta Cryst.* **D67**, 355–367.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Pape, T. & Schneider, T. R. (2004). *J. Appl. Cryst.* **37**, 843–844.
- Pathak, S. K., Basu, S., Basu, K. K., Banerjee, A., Pathak, S., Bhattacharyya, A., Kaisho, T., Kundu, M. & Basu, J. (2007). *Nature Immunol.* **8**, 610–618.
- Raghavan, S., Manzanillo, P., Chan, K., Dovey, C. & Cox, J. S. (2008). *Nature (London)*, **454**, 717–721.
- Rosenberg, O. S., Dovey, C., Tempesta, M., Robbins, R. A., Finer-Moore, J. S., Stroud, R. M. & Cox, J. S. (2011). *Proc. Natl Acad. Sci. USA*, **108**, 13450–13455.
- Simeone, R., Bottai, D. & Brosch, R. (2009). *Curr. Opin. Microbiol.* **12**, 4–10.
- Singh, B., Singh, G., Trajkovic, V. & Sharma, P. (2003). *Clin. Exp. Immunol.* **134**, 70–77.
- Stanley, S. A., Johndrow, J. E., Manzanillo, P. & Cox, J. S. (2007). *J. Immunol.* **178**, 3143–3152.
- Stanley, S. A., Raghavan, S., Hwang, W. W. & Cox, J. S. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 13001–13006.
- Volkman, H. E., Clay, H., Beery, D., Chang, J. C. W., Sherman, D. R. & Ramakrishnan, L. (2004). *PLoS Biol.* **2**, e367.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.