STRUCTURAL BIOLOGY
COMMUNICATIONS

ISSN 2053-230X

Cloning, expression, purification, crystallization and X-ray diffraction analysis of dihydrodipicolinate synthase from the human pathogenic bacterium *Bartonella henselae* strain Houston-1 at 2.1 Å resolution

Kubra F. Naqvi,^a Bart L. Staker,^{b,c} Renwick C. J. Dobson,^d Dmitry Serbzhinskiy,^{b,c} Banumathi Sankaran,^e Peter J. Myler^{b,c,f,g} and André O. Hudson^{a*}

Received 6 October 2015
Accepted 2 December 2015

Edited by T. C. Terwilliger, Los Alamos National Laboratory, USA

Keywords: *Bartonella henselae*; dihydrodipicolinate synthase; diaminopimelate; lysine biosynthesis; cat-scratch disease.

PDB reference: dihydrodipicolinate synthase, 3si9

Supporting information: this article has supporting information at journals.iucr.org/f

^aThomas H. Gosnell School of Life Sciences, Rochester Institute of Technology, 85 Lomb Memorial Drive, Rochester, NY 14623-5603, USA, ^bSeattle Structural Genomics Center for Infectious Disease, USA, ^cCenter for Infectious Disease Research, 307 Westlake Avenue North, Suite 500, Seattle, WA 98109, USA, ^dBiomolecular Interaction Centre, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand, ^eBerkeley Center for Structural Biology, Ernest Orlando Lawrence Berkeley National Laboratory, USA, ^fDepartment of Global Health, University of Washington, Seattle, WA 98195, USA, and ^gDepartment of Biomedical Informatics and Health Education, University of Washington, Seattle, WA 98195, USA. *Correspondence e-mail: aohsbi@rit.edu

The enzyme dihydrodipicolinate synthase catalyzes the committed step in the synthesis of diaminopimelate and lysine to facilitate peptidoglycan and protein synthesis. Dihydrodipicolinate synthase catalyzes the condensation of L-aspartate 4-semialdehyde and pyruvate to synthesize L-2,3-dihydrodipicolinate. Here, the cloning, expression, purification, crystallization and X-ray diffraction analysis of dihydrodipicolinate synthase from the pathogenic bacterium *Bartonella henselae*, the causative bacterium of cat-scratch disease, are presented. Protein crystals were grown in conditions consisting of 20% (w/v) PEG 4000, 100 mM sodium citrate tribasic pH 5.5 and were shown to diffract to ~2.10 Å resolution. They belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 79.96$, $b = 106.33$, $c = 136.25$ Å. The final R values were $R_{\text{r.i.m.}} = 0.098$, $R_{\text{work}} = 0.183$, $R_{\text{free}} = 0.233$.

1. Introduction

Enzymes involved in the bacterial synthesis of diaminopimelate/lysine (DAP/Lys) are attractive targets for the development of bactericidal compounds, especially from bacteria that are deemed to be pathogenic. The Gram-negative bacterium *Bartonella henselae* is the causative agent of cat-scratch disease (CSD; Mazur-Melewska *et al.*, 2015). There are 25 species in the genus and 50% of the species have been implicated in CSD (Breitschwerdt & Kordick, 2000). The reservoirs of *B. henselae* include, but are not limited to, cats, guinea pigs and rabbits; the majority of CSD infections are transmitted by cats based on a study in Poland that showed positive IgG antibodies for the bacterium in 50–90% of cats tested (Podsiadly *et al.*, 2002). The symptoms of CSD can include skin inflammation, fever, lymphadenopathy, various eye disorders, encephalitis and endocarditis (Mazur-Melewska *et al.*, 2015).

There are four variants of the diaminopimelate DAP/Lys pathway in bacteria. Using L-aspartate (Asp) as the starting molecule, all four variants share the common enzymatic steps for the synthesis of tetrahydrodipicolinate (THDP) from

© 2016 International Union of Crystallography

Asp (Hudson *et al.*, 2006). The synthesis of the penultimate compound *meso*-diaminopimelate (*m*-DAP) from THDP defines the uniqueness of the variants. In most bacteria, acetylated or succinylated (acyl) intermediates are used in the conversion of THDP to L,L-diaminopimelate (L,L-DAP) employing the enzymes DapD, DapC and DapE (Fig. 1). In another variant, a few bacterial lineages employ the enzyme *meso*-diaminopimelate (*m*-DAP) dehydrogenase (Ddh), which catalyzes the synthesis of *m*-DAP from THDP in a single reaction, circumventing the acylase, aminotransferase, deacylase and epimerase enzymatic steps used in the acyl pathway (Fig. 1). A novel pathway was recently discovered in plants and subsequently in certain bacterial lineages that

employs the enzyme L,L-DAP aminotransferase (DapL). DapL utilizes the acid glutamate as the amino donor and THDP as the amino acceptor to synthesize L,L-DAP in a single step circumventing the acylase, aminotransferase and deacylase reactions that are present in the acyl pathway (Hudson *et al.*, 2006, 2008; McCoy *et al.*, 2006; Nachar *et al.*, 2012). The conversion of *m*-DAP to lysine by the enzyme *m*-DAP decarboxylase (LysA) is the final step in the pathway and is common to all four variants (Fig. 1).

The enzyme dihydrodipicolinate synthase (DapA; EC 4.2.1.52) catalyzes the committed step in the DAP/Lys pathway in bacteria. The enzyme is responsible for the synthesis of dihydrodipicolinate *via* a condensation reaction with the

Figure 1

Variants of the diaminopimelate/lysine-synthesis pathways in bacteria. (a) The synthesis of THDP from aspartate, (b) the acyl pathway, (c) the *meso*-diaminopimelate dehydrogenase (Ddh) pathway and (d) the diaminopimelate aminotransferase (DapL) pathway. Abbreviations are as follows: LysC, aspartate kinase; Asd, aspartate semialdehyde dehydrogenase; DapA, dihydrodipicolinate synthase; DapB, dihydrodipicolinate reductase; DapD, 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate *N*-acyltransferase; DapC, acyl-diaminopimelate aminotransferase; DapE, acyl-diaminopimelate deacylase; Ddh, *meso*-diaminopimelate dehydrogenase; DapL, L,L-diaminopimelate aminotransferase; DapF, diaminopimelate epimerase; LysA, *meso*-diaminopimelate decarboxylase; MurE, UDP-*N*-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase; LysU, lysine-tRNA synthetase.

Figure 2

The reaction catalyzed by dihydrodipicolinate synthase (DapA) showing the condensation of L-aspartate 4-semialdehyde and pyruvate to form L-2,3-dihydrodipicolinate.

substrates pyruvate and L-aspartate 4-semialdehyde to synthesize L-2,3-dihydrodipicolinate (Fig. 2). Based on the fact that the gene is essential in bacteria and is a putative target for antibiotic development, there have been numerous studies regarding the structure of DapA from a number of pathogenic bacteria such as *Escherichia coli*, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacteriodes thetaiotaomicron*, which is an endosymbiont of the human gut (Dobson *et al.*, 2005; Kefala *et al.*, 2008; Devenish *et al.*, 2009; Kaur *et al.*, 2011; Burgess *et al.*, 2008; Girish *et al.*, 2008; Mank *et al.*, 2015). Although there has been a move to change the name of the enzyme to 4-hydroxy-tetrahydrodipicolinate synthase, we will use the more common enzyme name here (Bochaute *et al.*, 2013).

The *de novo* biosynthesis of DAP/Lys is absent in all animals, particularly humans, since their genomes do not contain the machinery necessary for the synthesis of DAP/Lys. As such, Lys is deemed to be an essential amino acid and must be acquired through dietary means for protein synthesis. In addition, in bacteria, the penultimate intermediate *m*-DAP serves as a cross-linking amino acid in the synthesis of peptidoglycan (PG) in most Gram-negative bacteria, and Lys serves the same role in most Gram-positive bacteria (Hutton *et al.*, 2007). Therefore, since Lys and DAP are necessary for both PG and protein synthesis, the inhibition of enzymes associated

with this pathway will lead to cell death *via* lysis as a result of the osmotic pressure from the lack of PG (Cox, 1996; Baizman *et al.*, 2000). The lack of protein synthesis would also lead to a bactericidal or bacteriostatic effect since Lys is one of the 20 ubiquitous proteogenic amino acids (Triassi *et al.*, 2014). Therefore, enzymes associated with the DAP/Lys pathway are attractive targets for the development of antibiotics, herbicides and algacides. As such, the macromolecular structure of DapA from pathogenic bacteria affords the opportunity for the identification and or development of compounds that are deemed to be bactericidal. Here, we present the cloning, expression, purification, crystallization and X-ray diffraction analysis of dihydrodipicolinate synthase from the human pathogenic bacterium *B. henselae* strain Houston-1 (*BhDapA*), the causative agent of cat-scratch disease.

2. Materials and methods

2.1. Amplification and cloning of DapA from *B. henselae*

Cloning, expression and purification were conducted as part of the Seattle Structural Genomics Center for Infectious Disease (SSGCID) following standard protocols described previously (Myler *et al.*, 2009; Stacy *et al.*, 2011; Serbzhinskiy *et al.*, 2015; Bryan *et al.*, 2011; Choi *et al.*, 2011). The full-length

Figure 3

SDS-PAGE analysis of purified recombinant DapA. (a) Size-exclusion chromatography (SEC) chromatogram of *BhDapA* from the final purification column showing a large peak with a small shoulder. (b) SDS-PAGE gel of peak fractions. Fractions 21–26 were pooled, concentrated to 25.2 mg ml⁻¹ and used for crystallization trials. The proteins fractions were resolved on a gradient SDS-PAGE gel (4–20%) and stained using Coomassie Blue for visualization.

open reading frame (ORF) encoding amino acids 1–294 (UniProt Q6G468) was PCR-amplified from *B. henselae* strain Houston-1 genomic DNA (ATCC 49882D-5). The ORF was amplified using the following primer sequences: *Bh*-dapA-F, 5'-GGGTCCTGGTTCGATGCTCAAGGGAGCTGTGACCG-3', and *Bh*-dapA-R, 5'-CTTGTTCTGCTGCTGTTTATTATTCTTTAAGCAAACCCGCATGGTA-3'. The ORF was cloned into the ligation-independent cloning (LIC) expression vector pAVA0421 encoding a cleavable 6×His fusion tag followed by the human rhinovirus 3C protease-cleavage sequence (MAHHHHHHMGTLEAQTQGGPGS) followed by the ORF (Aslanidis & de Jong, 1990). The human rhinovirus 3C protease-cleavage site is between the glutamine and glycine residues that are underlined. Plasmid DNA was transformed into chemically competent *E. coli* BL21 (DE3) R3 Rosetta cells. The plasmid containing His-*Bh*-dapA was tested for expression and 2 l of culture were grown using auto-induction medium in a LEX Bioreactor (Epiphyte Three Inc.) as described previously (Studier, 2005; Choi *et al.*, 2011).

2.2. Protein production and purification

His-tagged *Bh*DapA was purified in a four-step protocol consisting of an Ni²⁺-affinity chromatography (IMAC) step, cleavage of the N-terminal His tag with 3C protease, removal of the cleaved His tag by passage over a second Ni²⁺-affinity chromatography column and size-exclusion chromatography (SEC). All chromatographic steps were performed on an ÄKTApurifier 10 (GE Healthcare) using automated IMAC and SEC programs according to previously described procedures (Serbzhinskiy *et al.*, 2015; Bryan *et al.*, 2011). Bacterial pellets were thawed into 200 ml lysis buffer consisting of 25 mM HEPES pH 7.0, 500 mM NaCl, 5% glycerol, 0.5% CHAPS, 30 mM imidazole, 10 mM MgCl₂, 1 mM TCEP, 250 µg ml⁻¹ AEBSEF, 0.025% sodium azide and were lysed by sonication. Benzonase (20 µl, 25 units µl⁻¹) was added and the crude lysate was mixed at room temperature for 45 min. The lysate was then clarified by centrifugation at 10 000 rev min⁻¹ for 1 h using a Sorvall centrifuge (Thermo Scientific). The clarified supernatant was then passed over an Ni-NTA HisTrap FF 5 ml column (GE Healthcare) with a mobile phase consisting of loading buffer composed of 25 mM HEPES pH 7.0, 500 mM NaCl, 5% glycerol, 30 mM imidazole, 1 mM TCEP, 0.025% sodium azide. The column was washed with 20 column volumes (CV) of loading buffer and was eluted with loading buffer plus 250 mM imidazole in a linear gradient over 7 CV. His-MBP-3C protease was added to the eluted protein at a ratio of 1:50 to cleave the His tag. The protein was then transferred into a dialysis bag and dialyzed overnight at 4°C in 1 l of 3C dialysis buffer consisting of 25 mM HEPES pH 7.5, 200 mM NaCl, 5% glycerol, 1 mM TCEP. After overnight dialysis, the concentrations of imidazole and sodium chloride in the reaction mixture were adjusted to 30 and 500 mM, respectively, and the dialysate was passed over a second Ni²⁺-affinity column to remove His-MBP-3C protease, uncleaved protein and cleaved His tag. The cleaved protein was recovered in the flowthrough and concentrated prior to loading

Table 1

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	Beamline 5.0.1, ALS
Wavelength (Å)	0.9774
Temperature (K)	100.0
Detector	ADSC Quantum 315r CCD
Crystal-to-detector distance (mm)	250
Rotation range per image (°)	1
Total rotation range (°)	150
Exposure time per image (s)	10
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	79.96, 106.33, 136.25
Mosaicity (°)	0.86
Resolution range (Å)	50.00–2.10 (2.14–2.10)
Total No. of reflections	417245 (20757)
No. of unique reflections	68289 (3414)
Completeness (%)	99.8 (100.0)
Multiplicity	6.1 (6.1)
$\langle I/\sigma(I) \rangle$	18.3 (3.5)
$R_{\text{r.i.m.}}$ [†]	0.098 (0.457)
Overall <i>B</i> factor from Wilson plot (Å ²)	29.0

[†] Estimated $R_{\text{r.i.m.}} = R_{\text{merge}}[N/(N-1)]^{1/2}$, where *N* is the data multiplicity.

onto the final SEC column. A SEC column (Superdex 75, GE Healthcare) was equilibrated with running buffer composed of 20 mM HEPES pH 7, 0.3 M NaCl, 5% glycerol, 1 mM TCEP. The SEC chromatogram showed one predominant peak which was separated from a 70 kDa impurity (Fig. 3). The retention time of the primary peak was used to estimate the molecular weight by comparison with known standards (data not shown). The estimated molecular weight was consistent with a tetrameric assembly in buffer solution. The peak fractions were collected and analyzed for the presence of the protein of interest using SDS-PAGE in a 4–20% gradient gel with Tris-MOPS running buffer for 40 min at 175 V. The peak fractions were pooled and concentrated to 25.2 mg ml⁻¹ using an Amicon (Millipore) purification system (Fig. 3). Aliquots of 100 µl were cryocooled in liquid nitrogen and stored at –80°C until use for crystallization.

2.3. Crystallization and data collection

Purified *Bh*DapA was screened for crystallization in 96-well sitting-drop plates against the Wizard Classic 1 & 2 and Wizard Classic 3 & 4 crystal screens (Rigaku Reagents). Equal volumes of protein solution (0.4 µl) and precipitant solution were set up at 293 K against reservoir (80 µl) in sitting-drop vapor-diffusion format. The final crystallization precipitant was Wizard Classic 3 & 4 condition B2 composed of 20% PEG 4000, 100 mM sodium citrate tribasic pH 5.5. The crystals were cryoprotected in crystallant plus 25% 1,2-ethanediol and were cryocooled by dipping them into liquid nitrogen. Data were collected under the Collaborative Crystallography program of the Berkeley Center for Structural Biology at the Advanced Light Source, Berkeley National Laboratory. Data were collected at 100°C on Advanced Light Source beamline 5.0.1 using an ADSC Quantum 315 CCD detector with 1° oscillations at a wavelength of 0.9774 Å. Data were reduced with *HKL*-2000 (Otwinowski & Minor, 1997). Raw X-ray diffraction images are available at the Integrated Resource for

Reproducibility in Macromolecular Crystallography at <http://www.proteindiffraction.org>.

2.4. Structure solution and refinement

The structure was solved by molecular replacement with *Phaser* (McCoy *et al.*, 2007) from the *CCP4* suite of programs (Winn *et al.*, 2011) using PDB entry 2rfg (B. S. Kang, M. H. Kim, G. H. Kim & K. J. Kim, unpublished work) as a search model. The structure was refined using iterative cycles of *REFMAC5* (Murshudov *et al.*, 2011) followed by manual rebuilding of the structure using *Coot* (Emsley *et al.*, 2010). The quality of all of the structures was checked using *MolProbity* (Chen *et al.*, 2010). Data-collection and data-reduction and refinement statistics are shown in Tables 1 and 2, respectively. The structure was refined to a resolution of

Figure 4
Electron density showing the placement of the 1,2-ethanediol solvent molecule. The molecule is highlighted by the yellow arrow.

Figure 5
Three-dimensional view of *BhDapA* (PDB entry 3si9) showing the tetrameric structure. The individual monomeric units are shown by the color scheme, with monomer *A* in red, monomer *B* in yellow, monomer *C* in green and monomer *D* in blue. The yellow arrow shows the ligand 1,2-ethanediol. The dimeric and tetrameric assembly interfaces are highlighted with black arrows.

Table 2

Structure solution and refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	50.00–2.10 (2.15–2.10)
Completeness (%)	99.4 (97.2)
σ Cutoff	$F > 0.000\sigma(F)$
No. of reflections, working set	67983 (4586)
No. of reflections, test set	3445 (247)
Final R_{cryst}	0.183 (0.227)
Final R_{free}	0.233 (0.300)
No. of non-H atoms	
Protein	8672
Ligand	36
Water	560
Total	9268
R.m.s. deviations	
Bonds (Å)	0.014
Angles (°)	1.440
Average B factors (Å ²)	
Protein	28.6
Ligand	39.7
Water	33.9
Ramachandran plot	
Most favored (%)	97.7
Outliers (%)	0

2.1 Å. Unambiguous electron density was visible for the placement of 1,2-ethanediol, which was used as a cryoprotectant during cryocooling of the crystals (Fig. 4). Structure figures were analyzed and prepared using *PyMOL* (v.1.5; Schrödinger) and *PISA* (Krissinel & Henrick, 2007). *PISA* analysis predicts a stable tetrameric assembly composed of four monomers, *A*, *B*, *C* and *D*, with a buried surface of 10 384 Å². Stable dimers are predicted to be formed by molecules *A* and *C* and molecules *B* and *D*. The average buried surface of the *AC* and *BD* dimers was 3420 Å² (Fig. 5; dimer interface), whereas the average buried surface of the *AB* and *CD* dimers was 2335 Å² (Fig. 5; tetramer interface). Coordinates and structure factors have been deposited with the Protein Data Bank (PDB; <http://www.rcsb.org>) as entry 3si9.

3. Results and discussion

The DapA ortholog from *B. henselae* strain Houston-1 was successfully cloned, expressed and purified to homogeneity using a four-step purification protocol to a final concentration of 25.2 mg ml^{−1}. The purity of the enzyme was assessed by size-exclusion chromatography (SEC) and SDS-PAGE analysis (Fig. 3) and was suitable for crystallization trials.

Purified *BhDapA* was screened for crystallization in 96-well sitting-drop plates. The structure was subsequently refined to a resolution of 2.1 Å. The coordinates have been deposited in the PDB as entry 3si9. The three-dimensional structure of *BhDapA* is tetrameric in nature, consisting of four monomeric units that interact *via* a putative ‘dimer interface’ and a ‘tetramer assembly’ interface (Fig. 5). This is consistent with the structures of DapA orthologs from the bacteria *E. coli* (Blickling & Knäblein, 1997; Dobson *et al.*, 2005; Mirwaldt *et al.*, 1995), *Bacillus anthracis* (Blagova *et al.*, 2006) and *Thermotoga maritima* (Pearce *et al.*, 2006) in addition to the

eukaryotic orthologs from the plant species *Nicotiana sylvestris* (Blickling *et al.*, 1997) and *Vitis vinifera* (Atkinson *et al.*, 2012). Like the *E. coli* ortholog, each monomer of *BhDapA* consists of two domains in which each polypeptide possesses a $(\beta/\alpha)_8$ -barrel structure connected to a predominantly α -helical C-terminal domain (Mirwaldt *et al.*, 1995; Fig. 6). The arrangement of the monomeric units forms a large cavity in the center where the two dyads connect (Fig. 6). The structural features of the large cavity are conserved in bacterial orthologs but not in plant orthologs, where the dyads interact in a 'back-to-back' orientation that prevents cavity formation, as shown in the structures from *N. sylvestris* and *V. vinifera* (Atkinson *et al.*, 2012). A common feature of the bacterial enzyme is the presence of a conserved lysine residue in the active site. This lysine residue is involved in the catalytic mechanism of the enzyme through the formation of an enamine linkage *via* a Schiff base to the substrate (pyruvate)

and the catalytic triad (Dobson *et al.*, 2004). The conserved lysine residue at position 166 in the structure (PDB entry 3si9) is shown in Fig. 6. The structure of the DapA ortholog from *Agrobacterium tumefaciens* (*AtDapA*) has been solved (PDB entries 4i7w, 4i7v and 4i7u; S. C. Atkinson, C. Dogovski, R. C. J. Dobson & M. A. Perugini, unpublished work) with a pyruvate covalently linked to the conserved lysine residue involved in the Schiff-base reaction to facilitate substrate catalysis. As such, the active site is clearly defined in the *A. tumefaciens* structures. An overlay of *BhDapA* with the *A. tumefaciens* ortholog clearly shows the conserved lysine residue at position 166, highlighting the active site of *BhDapA* (Fig. 7).

In summary, we present the cloning, purification, crystallization and X-ray diffraction analysis of DapA from the pathogenic bacterium *B. henselae* strain Houston-1. The structural information gathered from the characterization of

Figure 6

Graphical representation of the secondary features of *BhDapA* (PDB entry 3si9). (a) depicts the various α -helices and β -barrels that comprise one of the four polypeptides. The black arrow highlights the methionine residue that is indicative of the translation start site and the black circle highlights the conserved lysine residue in the active site of the enzyme that is involved in catalysis at position 166. (b) Graphical key depicting the secondary-structural features of the enzyme.

Figure 7
Comparative overlay of *BhDapA* (PDB entry 3si9; green) on *AtDapA* (PDB entry 4i7w; blue) bound to pyruvate (black arrow) depicting the active site of *BhDapA* by highlighting the conserved lysine residue at position 166 (yellow arrow) involved in the Schiff-base reaction involved in substrate catalysis.

DapA from this bacterium may be used to identify specific differences between bacterial orthologs of this diaminopimelate- and lysine-biosynthetic enzyme. This information has the potential to be useful in the design or identification of compounds that possess bactericidal activity.

Acknowledgements

AOH and KFN acknowledge the Thomas H. Gosnell School of Life Sciences for ongoing support of research in the Hudson laboratory. AOH acknowledges the College of Science Dean's Research Initiation Grant (D-RIG) Program at the Rochester Institute of Technology for support of this work. This project has been funded in part with Federal funds from the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Department of Health and Human Services (HHS) under Contract Nos. HHSN272200700057C and HHSN272201200025C. The Berkeley Center for Structural Biology is supported in part by the NIH, National Institute of General Medical Sciences (NIGMS) and the Howard Hughes Medical Institute (HHMI). The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences of the US Department of Energy (DOE) under Contract No. DE-AC02-05CH11231.

References

Aslanidis, C. & de Jong, P. J. (1990). *Nucleic Acids Res.* **18**, 6069–6074.
Atkinson, S. C., Dogovski, C., Downton, M. T., Pearce, F. G., Reboul, C. F., Buckle, A. M., Gerrard, J. A., Dobson, R. C. J., Wagner, J. & Perugini, M. A. (2012). *PLoS One*, **7**, e38318.
Baizman, E. R., Branstrom, A. A., Longley, C. B., Allanson, N., Sofia, M. J., Gange, D. & Goldman, R. C. (2000). *Microbiology*, **146**, 3129–3140.
Blagova, E., Levnikov, V., Milioti, N., Fogg, M. J., Kallioma, A. K., Brannigan, J. A., Wilson, K. S. & Wilkinson, A. J. (2006). *Proteins*, **62**, 297–301.

Blickling, S., Beisel, H. G., Bozic, D., Knäblein, J., Laber, B. & Huber, R. (1997). *J. Mol. Biol.* **274**, 608–621.
Blickling, S. & Knäblein, J. (1997). *Biol. Chem.* **378**, 207–210.
Breitschwerdt, E. B. & Kordick, D. L. (2000). *Clin. Microbiol. Rev.* **13**, 428–438.
Bryan, C. M., Bhandari, J., Napuli, A. J., Leibly, D. J., Choi, R., Kelley, A., Van Voorhis, W. C., Edwards, T. E. & Stewart, L. J. (2011). *Acta Cryst.* **F67**, 1010–1014.
Burgess, B. R., Dobson, R. C. J., Bailey, M. F., Atkinson, S. C., Griffin, M. D. W., Jameson, G. B., Parker, M. W., Gerrard, J. A. & Perugini, M. A. (2008). *J. Biol. Chem.* **283**, 27598–27603.
Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S. & Richardson, D. C. (2010). *Acta Cryst.* **D66**, 12–21.
Choi, R., Kelley, A., Leibly, D., Nakazawa Hewitt, S., Napuli, A. & Van Voorhis, W. (2011). *Acta Cryst.* **F67**, 998–1005.
Cox, R. J. (1996). *Nat. Prod. Rep.* **13**, 29–43.
Devenish, S. R. A., Huisman, F. H. A., Parker, E. J., Hadfield, A. T. & Gerrard, J. A. (2009). *Biochim. Biophys. Acta*, **1794**, 1168–1174.
Dobson, R. C. J., Devenish, S. R. A., Turner, L. A., Clifford, V. R., Pearce, F. G., Jameson, G. B. & Gerrard, J. A. (2005). *Biochemistry*, **44**, 13007–13013.
Dobson, R. C. J., Valegård, K. & Gerrard, J. A. (2004). *J. Mol. Biol.* **338**, 329–339.
Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
Girish, T. S., Sharma, E. & Gopal, B. (2008). *FEBS Lett.* **582**, 2923–2930.
Hudson, A. O., Gilvarg, C. & Leustek, T. (2008). *J. Bacteriol.* **190**, 3256–3263.
Hudson, A. O., Singh, B. K., Leustek, T. & Gilvarg, C. (2006). *Plant Physiol.* **140**, 292–301.
Hutton, C. A., Perugini, M. A. & Gerrard, J. A. (2007). *Mol. Biosyst.* **3**, 458–465.
Kaur, N., Gautam, A., Kumar, S., Singh, A., Singh, N., Sharma, S., Sharma, R., Tewari, R. & Singh, T. P. (2011). *Int. J. Biol. Macromol.* **48**, 779–787.
Kefala, G., Evans, G. L., Griffin, M. D. W., Devenish, S. R. A., Pearce, F. G., Perugini, M. A., Gerrard, J. A., Weiss, M. S. & Dobson, R. C. J. (2008). *Biochem. J.* **411**, 351–360.
Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.
Mank, N., Arnette, A., Klapper, V., Offermann, L. & Chruszcz, M. (2015). *Acta Cryst.* **F71**, 449–454.
Mazur-Melewska, K., Mania, A., Kemnitz, P., Figlerowicz, M. & Słuzewski, W. (2015). *Postepy Dermatol. Alergol.* **32**, 216–220.
McCoy, A. J., Adams, N. E., Hudson, A. O., Gilvarg, C., Leustek, T. & Maurelli, A. T. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 17909–17914.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Mirwaldt, C., Korndörfer, I. & Huber, R. (1995). *J. Mol. Biol.* **246**, 227–239.
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.
Myler, P. J., Stacy, R., Stewart, L., Staker, B. L., Van Voorhis, W. C., Varani, G. & Buchko, G. W. (2009). *Infect. Disord. Drug Targets*, **9**, 493–506.
Nachar, V. R., Savka, F. C., McGroty, S. E., Donovan, K. A., North, R. A., Dobson, R. C. J., Buckley, L. J. & Hudson, A. O. (2012). *Front. Microbiol.* **3**, 183.
Otwinski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pearce, F. G., Perugini, M. A., McKerchar, H. J. & Gerrard, J. A. (2006). *Biochem. J.* **400**, 359–366.
Podsiadły, E., Sokołowska, E. & Tylewska-Wierzbanowska, S. (2002). *Przegl. Epidemiol.* **56**, 399–407.
Serbzhinskiy, D. A., Clifton, M. C., Sankaran, B., Staker, B. L.,

- Edwards, T. E. & Myler, P. J. (2015). *Acta Cryst.* **F71**, 594–599.
- Stacy, R., Begley, D. W., Phan, I., Staker, B. L., Van Voorhis, W. C., Varani, G., Buchko, G. W., Stewart, L. J. & Myler, P. J. (2011). *Acta Cryst.* **F67**, 979–984.
- Studier, F. W. (2005). *Protein Expr. Purif.* **41**, 207–234.
- Triassi, A. J., Wheatley, M. S., Savka, M. A., Gan, H. M., Dobson, R. C. J. & Hudson, A. O. (2014). *Front. Microbiol.* **5**, 509.
- Van Bochaute, P., Novoa, A., Ballet, S., Rognes, S. E. & Angenon, G. (2013). *Physiol. Plant.* **149**, 449–460.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.