Crystal structure of AibC, a reductase involved in alternative *de novo* isovaleryl coenzyme A biosynthesis in *Myxococcus xanthus*

Tobias Bock,^a Rolf Müller^b and Wulf Blankenfeldt^{a,c,*}

Received 19 May 2016

Accepted 8 July 2016

Edited by R. A. Pauptit, Macclesfield, England

Keywords: alternative isovaleryl coenzyme A biosynthesis; medium-chain dehydrogenase/reductase; zinc-dependence; hexahistidine tag; TEV protease-recognition sequence; *Myxococcus xanthus*.

PDB reference: AibC, 5kis

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

^aStructure and Function of Proteins, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany, ^bDepartment of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland, Saarland University, Universitäts-campus E8 1, 66123 Saarbrücken, Germany, and ^cInstitut für Biochemie und Bioinformatik, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany. *Correspondence e-mail: wulf.blankenfeldt@helmholtz-hzi.de

Isovaleryl coenzyme A (IV-CoA) performs a crucial role during development and fruiting-body formation in myxobacteria, which is reflected in the existence of a *de novo* biosynthetic pathway that is highly upregulated when leucine, the common precursor of IV-CoA, is limited. The final step in *de novo* IV-CoA biosynthesis is catalyzed by AibC, a medium-chain dehydrogenase/reductase. Here, the crystal structure of AibC from *Myxococcus xanthus* refined to 2.55 Å resolution is presented. The protein adopts two different conformations in the crystal lattice, which is a consequence of partial interaction with the purification tag. Based on this structure, it is suggested that AibC most probably uses a Zn²⁺-supported catalytic mechanism in which NADPH is preferred over NADH. Taken together, this study reveals structural details of the alternative IV-CoA-producing pathway in myxobacteria, which may serve as a base for further biotechnological research and biofuel production.

1. Introduction

Branched-chain fatty acids and lipids are important compounds for the Gram-negative bacterium *Myxococcus xanthus*, since they are utilized as signalling molecules during development and serve as membrane fluidity-maintaining substances (Hoiczky *et al.*, 2009; Downward & Toal, 1995). These molecules are produced from the common precursor isovaleryl coenzyme A (IV-CoA; Bode *et al.*, 2006; Ring *et al.*, 2006; Toal *et al.*, 1995), which is typically generated by the degradation of leucine (Michal, 1999). However, Bode and coworkers recently showed that *M. xanthus* harbours an additional, acetyl-CoA-dependent pathway to produce IV-CoA *de novo*, assigned as 'alternative isovaleryl coenzyme A biosynthesis' (aib), a route which appeared to be highly active under leucine-limiting conditions (Fig. 1a; Bode *et al.*, 2009). Further biochemical analysis revealed four proteins directly involved in aib, namely MvaS, LiuC and AibA/AibB (Fig. 1a; Bode *et al.*, 2009; Li *et al.*, 2013; Bock *et al.*, 2016). The last step in aib, the conversion of 3,3-dimethylacrylyl coenzyme A (DMA-CoA) to IV-CoA, is catalyzed by AibC. Based on its sequence, AibC was predicted to belong to the alcohol dehydrogenases (EC 1.1.1.1) as a member of the medium-chain dehydrogenases/reductase family. Preliminary characterization revealed that AibC requires NADH and seems to be metal-independent (Li *et al.*, 2013).

Alcohol dehydrogenase (ADH) family members are found in all three kingdoms of life. They possess a wide spectrum of physiological functions and participate, for instance, in the detoxification of ethanol and formaldehyde, in signalling

processes and in various metabolic pathways (Persson *et al.*, 1994; Sanghani *et al.*, 2000; Deltour *et al.*, 1999). Because of this broad functionality, these enzymes are capable of converting a large number of substrates, ranging from branched and cyclic alcohols to ketones and aldehydes (Vitale *et*

al., 2013). Another specific feature of ADHs is the stereo-selectivity of the reaction that they catalyse. This makes them perfectly suited for the biotechnological food industry, pharmaceutical and fine chemical production, especially in the case of ADH enzymes that show tolerance to heat and denaturants

Figure 1

Contribution of AibC to the alternative isovaleryl coenzyme A-biosynthesis pathway and sequence alignment of AibC and related ADHs from different phyla performed using BLAST (Altschul *et al.*, 1990). (a) The reaction catalyzed by AibC is coloured red. AcAc-CoA, acetoacetyl CoA; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MG-CoA, 3-methylglutaconyl CoA; DMA-CoA, 3,3-dimethylacrylyl CoA; IV-CoA, isovaleryl CoA. (b) Sequence alignment of myxobacterial AibC (AibC), human liver alcohol dehydrogenase (NADH-dependent; hADH; Colonna-Cesari *et al.*, 1986), cinnamyl alcohol dehydrogenase from *Arabidopsis thaliana* (NADPH-dependent; AtCAD5; Youn *et al.*, 2006), quinone oxidoreductase Rv1454c from *Mycobacterium tuberculosis* (NADPH-dependent; MtADH; Zheng *et al.*, 2015), alcohol dehydrogenase from *Pseudomonas aeruginosa* (NAD-dependent; PaADH; Levin *et al.*, 2004) and threonine 3-dehydrogenase from *Thermus thermophilus* (TtADH; RIKEN Structural Genomics/Proteomics Initiative, unpublished work). Residues involved in structural Zn²⁺ coordination are highlighted in green; those involved in coordination of the active-site Zn²⁺ are highlighted in blue. The red box indicates residues that mediate the preference between NADH and NADPH.

(Vitale *et al.*, 2013; Persson *et al.*, 1991; Radianingtyas & Wright, 2003). ADHs can be grouped into three different classes according to their size. Class I, also known as medium-chain dehydrogenases/reductases (MDRs), consist of approximately 350–390 amino acids and typically contain Zn^{2+} cations for structural and functional purposes. Furthermore, the oligomerization state of class I members differs according to their origin. Bacterial MDRs usually appear as tetramers, whereas dimers are found in higher plants and mammals (Korkhin *et al.*, 1998). Class II proteins, known as nonmetallic or short-chain enzymes, consist of 250 amino acids per chain, whereas class III enzymes possess up to 700 residues (Korkhin *et al.*, 1998).

At the structural level, MDRs are built of two domains: a C-terminal NAD/NADP-binding Rossmann-fold domain and an N-terminal catalytic domain characterized by a GroES-like fold that typically harbours a catalytic Zn^{2+} cation. However, the residues involved in Zn^{2+} coordination differ among MDR members. It has been shown that ‘constant ligands’ such as cysteine and histidine are always present, whereas ‘variable ligands’ vary between negatively charged amino acids (aspartic acid or glutamic acid) and an arginine or lysine residue that typically forms a salt bridge with the respective aspartic or glutamic acid (Meijers & Cedergren-Zeppeauer, 2006). In addition to the catalytic Zn^{2+} cation, several MDR members contain a second Zn^{2+} cation located in a loop region that is coordinated by four cysteine residues and is termed the ‘structural Zn^{2+} -binding region’ (Vitale *et al.*, 2010; Littlechild *et al.*, 2004; Baker *et al.*, 2009).

Here, we present the crystal structure of AibC refined at 2.55 Å resolution. We show that AibC contains a structural as well as an active-site Zn^{2+} cation. Furthermore, sequence and structural analysis reveal a sequence motif indicating a preference for NADPH over NADH, suggesting a Zn^{2+} - and NADPH-dependent reaction mechanism.

2. Materials and methods

2.1. Protein production

The gene coding for AibC was PCR-amplified from pET-28a-AibC using the forward and reverse primers listed in Table 1 and the PCR product was subsequently cleaved with NdeI and XhoI. The resulting fragment was inserted into NdeI/XhoI-linearized pET-19m expression vector (modified pET-19b; Novagen), leading to a TEV-protease-cleavable His₆-tagged (pET-19m-AibC) construct (Table 1). The correct insertion of *aibC* was controlled by sequencing. The expression plasmid pET-19m-AibC was freshly transformed into chemically competent *Escherichia coli* Rosetta2 pLysS (DE3) cells and subsequently used for preculture inoculation supplemented with 50 mg l⁻¹ kanamycin and 34 mg l⁻¹ chloramphenicol. Large-scale protein production was carried out in lysogeny broth (LB) including the same antibiotics. The cells were incubated at 310 K and 120 rev min⁻¹ until the optical density reached 0.6–0.8. At this point, the temperature was reduced to 293 K and heterologous gene expression was

Table 1

Protein-production information.

Source organism	<i>M. xanthus</i> DK1622
DNA source	pET-28a-AibC (Li <i>et al.</i> , 2013)
Forward primer†	ATTATCATATGAAGCCGTCGACTGCGCAGCT-TT
Reverse primer†	ATTATCTCGAGTTATCACGCTCGGGGGGCACC
Cloning vector	pET-19m
Expression vector	pET-19m (assigned as pET-19m-AibC)
Expression host	<i>E. coli</i> Rosetta2 pLysS (DE3)
Complete amino-acid sequence of the construct produced‡	MHHHHHAENLYFQ GHMKAVVLRSFGEAGNLKM-ETMPMPRPGRGVEVLLRVHACGVYHDVINRRG-NLPRTSVPAITLGHEAAGEVIEVGPDPGWKGTG-DRAATLQRMSCGDCALCRSGRNSLCKTDNRFF-GEELPGGYAQFMVAPVGGGLGRVPASLPWNEAA-TVCCCTGTAVHTVTRGKVRAGETVLITGASG-GVGLSSVQLARLDGARVIAVTSSEAKVQALKE-AGADEVIVSRGLDFASDVVRKRTQGAGVDVAIVEVGSATFDQTLKSMAPGGRVVVGNLESGMVQ-LNPLGLVIVKEILGAYATTQAEALDEALRLTA-TGGVRQFVTDVAPLAEAAKHFLENREVAGR-LVLVPPEA

† The respective restriction-enzyme recognition sites are underlined. ‡ Complete sequence of the construct produced. The underlined residues are the hexahistidine tag and TEV protease cleavage site, where | indicates the cleavage site.

induced with 500 µM IPTG for a further 20 h. The cells were harvested by centrifugation at 5000g for 10 min and flash-frozen in liquid nitrogen until needed. The cells were thawed on ice and diluted with buffer A (50 mM NaH₂PO₄ pH 7.0, 300 mM NaCl, 20 mM imidazole) and subsequently lysed using an EmulsiFlex-C3 homogenizer (AVESTIN). To remove insoluble parts the cell suspension was centrifuged for 45 min at 30 000g and 277 K. The cleared supernatant was applied onto a HisTrap chelating column (GE Healthcare Life Sciences) loaded with 100 mM nickel sulfate and equilibrated in buffer A. The column was washed with buffer A until the absorption at 280 nm reached the baseline, and the bound proteins were eluted with a three-step gradient from 12% buffer B (buffer A containing 500 mM imidazole) to 20 and 60% buffer B. Fractions containing His-tagged AibC were combined, concentrated and applied onto an S200 16/60 size-exclusion column (GE Healthcare Life Sciences) equilibrated with buffer C (50 mM HEPES pH 7.0, 100 mM NaCl). Pure protein fractions were collected, concentrated to 20 mg ml⁻¹, flash-frozen in liquid nitrogen (77 K) and stored at 193 K until needed for further experiments.

2.2. Crystallization

Initial crystallization conditions were identified using the vapour-diffusion method in a 96-well sitting-drop format. Screening was performed by mixing 0.2 µl AibC protein solution at a concentration of 15 mg ml⁻¹ and 0.2 µl reservoir solution using a dispensing robot (Zinsser Analytics) and equilibrating against 70 µl reservoir solution. Crystals appeared after 5–8 d using a precipitant consisting of 3 M NaCl, 0.1 M HEPES pH 7.5. Well diffracting crystals were obtained by mixing 1 µl AibC protein solution (15 mg ml⁻¹) and 1 µl reservoir solution consisting of 3 M NaCl, 0.1 M HEPES pH 7.4, which was equilibrated against 500 µl reservoir solution in a hanging-drop setup. Reservoir solution

Table 2
Crystallization.

Method	Hanging-drop vapour diffusion
Plate type	24-well VDXm Plate (Hampton Research)
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	15
Buffer composition of protein solution	0.1 M HEPES pH 7, 100 mM NaCl
Composition of reservoir solution	3 M NaCl, 0.1 M HEPES pH 7.4
Volume and ratio of drop	1 µl protein solution and 1 µl reservoir solution
Volume of reservoir (µl)	500

Table 3
Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	Beamline BL14.1, BESSY II
Wavelength (Å)	1.2779
Temperature (K)	100
Detector	Pilatus 6M
Crystal-to-detector distance (mm)	300
Rotation range per image (°)	0.1
Total rotation range (°)	360
Exposure time per image (s)	0.2
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	77.3, 77.4, 309.6
α , β , γ (°)	90, 90, 90
Mosaicity (°)	0.10
Resolution range (Å)	48.36–2.55 (2.62–2.55)
Total No. of reflections	800735 (60642)
No. of unique reflections	61786 (4473)
Completeness (%)	100 (100)
Anomalous completeness† (%)	100 (100)
Multiplicity	13.0 (13.6)
$\langle I/\sigma(I) \rangle$	16.60 (1.9‡)
<i>R</i> _{anom} (%)	3.8
<i>R</i> _{r.i.m.}	0.152 (1.646)
<i>R</i> _{p.i.m.}	0.042 (0.446)
Overall <i>B</i> factor from Wilson plot§ (Å ²)	54

† Calculated at 3.0 Å (anomalous resolution cutoff). ‡ The mean $\langle I/\sigma(I) \rangle$ in the outer shell is <2.0 when the resolution reaches 2.60 Å. § No anomalies were observed in the Wilson plot.

supplemented with 20%(v/v) glycerol was used as cryoprotectant (Table 2).

2.3. Data collection and processing

Since our sequence analysis predicted that AibC binds Zn²⁺, despite previous work showing that AibC is metal-independent (Li *et al.*, 2013), we collected single anomalous diffraction (SAD) data at a wavelength of 1.2779 Å on beamline BL14.1 operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II electron-storage ring (Berlin-Adlershof, Germany). These data were indexed and integrated with *XDS* (Kabsch, 2010) and scaled with *AIMLESS* (Evans & Murshudov, 2013) from the *CCP4* package (Winn *et al.*, 2011). Data-collection and processing statistics are summarized in Table 3.

2.4. Structure solution and refinement

Anomalous differences were used to locate Zn²⁺ cations and derive initial phases for preliminary model building with the *AutoSol* routine of the *PHENIX* software suite (Adams *et al.*,

Table 4
Structure solution and refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	42.94–2.55 (2.59–2.55)
Completeness (%)	100 (100)
No. of reflections, working set	58603 (2619)
No. of reflections, test set	3087 (146)
Final <i>R</i> _{cryst}	0.234 (0.3503)
Final <i>R</i> _{free}	0.269 (0.3732)
No. of non-H atoms	
Protein	9842
Ion	8
Solvent	107
Total	19596
R.m.s. deviations	
Bonds (Å)	0.002
Angles (°)	0.437
Average <i>B</i> factors (Å ²)	
Protein	87
Ion	91
Ramachandran plot	
Most favoured (%)	95.03
Allowed (%)	4.82
Disallowed (%)	0.15

al., 2010; Terwilliger *et al.*, 2009). The structure was refined using alternating steps of manual adjustment in *Coot* (Emsley *et al.*, 2010) and maximum-likelihood and TLS (translation/libration/screw motion) refinement in *PHENIX* (Adams *et al.*, 2010; Schomaker & Trueblood, 1968). *MolProbity* was used for final structure validation (Chen *et al.*, 2010). Refinement statistics are summarized in Table 4.

3. Results and discussion

Sequence alignment of AibC with related enzymes shows that AibC contains motifs that are characteristic for the binding of a structural and a catalytic Zn²⁺ cation as well as for NADPH binding, which is in line with its initial annotation as a Zn²⁺-dependent MDR (Fig. 1*b*; Bode *et al.*, 2009). In contrast to this, Li and coworkers later proposed that AibC functions as a metal-independent and NADH-utilizing enzyme, based on the finding that IV-CoA formation was observed in the presence of 1 mM NADH and 5 mM EDTA (Li *et al.*, 2013).

To resolve this contradiction, we determined the crystal structure of His₆-tagged AibC to 2.55 Å resolution using the single anomalous diffraction of Zn²⁺ cations that had been trapped during protein expression. The final structure was refined to *R* values of *R*_{cryst} = 23.4 and *R*_{free} = 26.9. AibC crystallized in space group *P*2₁2₁2₁ with four chains and eight Zn²⁺ cations in the asymmetric unit. According to the interaction surface of 2520 Å² (10% of the total surface area) analyzed using *PDBEPIA* (Krissinel & Henrick, 2007), the stable oligomeric state of AibC is a dimer. The same oligomer was observed during size-exclusion chromatography, in which AibC eluted with a retention volume characteristic of the dimeric form. Interestingly, MDR dimers are usually only found in higher plants or mammals, but not in bacteria (Korkhin *et al.*, 1998).

At the structural level, AibC resembles other members of the MDR family with its characteristic length of 345 amino

acids, two bound Zn^{2+} cations and a cofactor-binding domain and a catalytic domain separated by α -helices $\alpha 4$ – $\alpha 5$. Both the cofactor-binding and catalytic domain form a deep cleft in the central region of the protein. The cofactor-binding domain consists of α -helices $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 9$, $\alpha 10$ and $\alpha 11$ and β -strands $\beta 8$ – $\beta 14$ arranged in a Rossmann fold with six parallel β -strands surrounded by five α -helices, whereas the catalytic domain comprises α -helices $\alpha 1$ – $\alpha 4$, $\alpha 9$, $\alpha 10$ and β -strands $\beta 1$ – $\beta 7$, $\beta 15$ and $\beta 16$ (Fig. 2*a*).

We made extensive attempts to crystallize AibC without a purification tag and in the presence of DMA-CoA or potential cofactors. Although several different affinity tags, protease-cleavage sites and solubility helpers were used, only the TEV protease-cleavable His₆-tagged construct led to crystal formation, which is based on crystal contacts mediated by the purification tag. In the structure, we found one active site of each dimer to be occupied by the TEV-protease cleavage site and the His₆ tag of another symmetry mate in the crystal

Figure 2

Overall dimeric structure of AibC and crystal-contact interaction with the purification tag. (*a*) Dimeric arrangement and secondary-structure assignment of AibC. Monomers *A* and *B* are shown. The left monomer is coloured according to its domains (cofactor-binding domain, blue; catalytic domain, orange; purification tag, magenta). The second monomer is shown in grey. (*b*) Interaction with the purification tag. Monomer *A* (surface representation, magenta) and its symmetry mate from the crystal lattice (cartoon representation, grey) are shown. (*c*) Comparison of open, tag-bound monomer *A* (magenta) and closed, tag-free monomer *C* (yellow). The two most strongly differing regions are highlighted by black circles. (*d*) Close-up view of the active site (magenta) occupied by the tag (grey). All molecular representations were prepared with PyMOL (Schrödinger; <http://www.pymol.org>).

(residues -2 to -13 ; Fig. 2*b*) such that His -9^* (where $*$ indicates a symmetry mate) directly coordinates the catalytic Zn^{2+} cation (Fig. 2*d*). Further interactions between the tag and AibC involve π - π contacts of His -13 and Tyr41, backbone interactions of His -11^* and Val264, and side chain-backbone interactions of Asp -6^* and Asn50, together with hydrogen bonds between Gln -2^* and Arg24 and Glu70.

The purification tag induces two different conformations of AibC in the crystal lattice. Monomers *A* and *C* show the purification tag at the N-terminus and interact with the same chain from a symmetry mate. In contrast, monomers *B* and *D* lack electron density for the tag, and the active site is free. In addition, these two chains are much more flexible, characterized by high *B* factors and regions of uninterpretable electron density. Superposition of the two conformations reveals that chains *B* and *D* adopt a conformation in which the active site is closed, whereas monomers *A* and *C* represent a more open form. Differences between these conformations are mainly rooted in two loops connecting $\beta 11$ and $\alpha 9$ and $\beta 12$ and $\alpha 10$, located around the active site (Fig. 2*c*). However, this seems to be a crystallization artefact and a consequence of the inter-

action with the purification tag. Typically, the apo form is open and more flexible compared with the compact cofactor-bound or substrate-bound state of MDR members (Meijers & Cedergren-Zeppezauer, 2006).

Two Zn^{2+} cations are found in each monomer of AibC. The structural Zn^{2+} also found in other MDRs is coordinated by Cys92, Cys95, Cys98 and Cys106 in the typical tetrahedral geometry (Fig. 3*b*). The second Zn^{2+} cation is part of the catalytic domain; it is located in the active site and is coordinated by Cys40, His62 and Cys149. It shows the typical location and coordination observed for metals involved in MDR catalysis (Meijers & Cedergren-Zeppezauer, 2006). The binding site is further surrounded by His42 and Gln68, which do not directly interact with the active-site Zn^{2+} (Fig. 3*a*).

MDRs are known utilize both NADH and NADPH, with the preference for NADH being mediated by an aspartic acid residue and that for NADPH by the presence of a specific serine residue (Powell *et al.*, 2000; Pereira *et al.*, 2001; Youn *et al.*, 2006). AibC lacks the aspartic acid but has the typical Ser199 in a motif comprising Thr198, Ser199 and Ser200 (Figs. 1*b* and 3*c*), suggesting a preference for NADPH in line with the anabolic function of AibC in IV-CoA biosynthesis. While we did not succeed in obtaining the structure of a complex of AibC with NADH or NADPH, superimposition with the structure of the related AtCAD5 allows the identification of amino acids that are possibly involved in cofactor binding in AibC (Fig. 3*c*). According to this model, the pyrophosphate group interacts with Arg337, the backbone amide of Val179 and the side chain of Tyr41, which would have to rotate to occupy a position suitable for interaction. The adenine moiety could interact with Glu268 and the ribose group with Lys203. The carboxamide moiety probably binds to Thr153 and the backbone atoms of Ala289 and Ala291.

A critical step during ADH-catalyzed reactions is proton shuttling between the substrate and the solvent. It has been

Figure 3
 Zn^{2+} -binding and hypothetical NADPH-binding sites of AibC. Green meshes show σ_A -weighted $|F_o - F_c|$ difference electron density of Zn^{2+} cations before their incorporation into the model, displayed at 3σ . (a) Catalytic Zn^{2+} coordination site. (b) Structural Zn^{2+} coordination site. (c) Model of AibC in complex with NADPH (cyan), based on superimposition with the AtCAD5-NADPH complex (PDB entry 2cf6; Youn *et al.*, 2006). Residues potentially involved in NADPH binding are shown as sticks.

shown that the cofactor, a threonine and a histidine residue are involved in this transfer in MDRs related to AibC (Youn *et al.*, 2006; Levin *et al.*, 2004). In AtCAD5, for example, Thr49, His52 and Asp57 together with the O2' and O3' hydroxyl groups of the NADPH ribose ring are perfectly oriented to enable proton shuttling from the solvent to the substrate ligated to the catalytic Zn²⁺ (Youn *et al.*, 2006). In AibC, these residues are replaced by His42, Ile45 and Asn50, indicating a different proton-shuttle system or mechanism that will only be identifiable when the structures of potentially rearranged complexes of substrate complexes become available.

4. Conclusion

Here, we have determined the crystal structure of AibC, the terminal enzyme in *de novo* IV-CoA biosynthesis by *M. xanthus*, and have shown that it is a Zn²⁺-dependent MDR, resolving a contradiction between previous reports. AibC shows structural features that are typical of NADPH-dependent enzymes and we identified residues potentially involved in cofactor binding. Our findings provide the first structural insights into the conversion of 3,3-dimethylacrylyl coenzyme A to isovaleryl coenzyme A, the last reaction in a pathway with potential applications in biotechnology and in green energy production.

Acknowledgements

We thank the BESSY II synchrotron (Helmholtz-Zentrum Berlin, Berlin) for beamline access and support during data collection and the X-ray community at HZI in Braunschweig for data collection. TB is supported by the HZI Graduate School for Infection Research.

References

- Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). *J. Mol. Biol.* **215**, 403–410.
- Baker, P. J., Britton, K. L., Fisher, M., Esclapez, J., Pire, C., Bonete, M. J., Ferrer, J. & Rice, D. W. (2009). *Proc. Natl Acad. Sci. USA*, **106**, 779–784.
- Bock, T., Kasten, J., Müller, R. & Blankenfeldt, W. (2016). *Chembiochem*, **17**, 1257–1262.
- Bode, H. B., Ring, M. W., Schwär, G., Altmeyer, M. O., Kegler, C., Jose, I. R., Singer, M. & Müller, R. (2009). *Chembiochem*, **10**, 128–140.
- Bode, H. B., Ring, M. W., Schwär, G., Kroppenstedt, R. M., Kaiser, D. & Müller, R. (2006). *J. Bacteriol.* **188**, 6524–6528.
- 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.
- Colonna-Cesari, F., Perahia, D., Karplus, M., Eklund, H., Brändén, C. I. & Tapia, O. (1986). *J. Biol. Chem.* **261**, 15273–15280.
- Deltour, L., Foglio, M. H. & Duester, G. (1999). *J. Biol. Chem.* **274**, 16796–16801.
- Downward, J. & Toal, D. (1995). *Mol. Microbiol.* **16**, 171–175.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Evans, P. R. & Murshudov, G. N. (2013). *Acta Cryst.* **D69**, 1204–1214.
- Hoiczky, E., Ring, M. W., McHugh, C. A., Schwär, G., Bode, E., Krug, D., Altmeyer, M. O., Lu, J. Z. & Bode, H. B. (2009). *Mol. Microbiol.* **74**, 497–517.
- Kabsch, W. (2010). *Acta Cryst.* **D66**, 125–132.
- Korkhin, Y., Kalb (Gilboa), A. J., Peretz, M., Bogin, O., Burstein, Y. & Frolow, F. (1998). *J. Mol. Biol.* **278**, 967–981.
- Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.
- Levin, I., Meiri, G., Peretz, M., Burstein, Y. & Frolow, F. (2004). *Protein Sci.* **13**, 1547–1556.
- Li, Y., Luxenburger, E. & Müller, R. (2013). *Angew. Chem. Int. Ed.* **52**, 1304–1308.
- Littlechild, J. A., Guy, J. E. & Isupov, M. N. (2004). *Biochem. Soc. Trans.* **32**, 255–258.
- Meijers, R. & Cedergren-Zeppezauer, E. S. (2006). In *Handbook of Metalloproteins*, Part 8. Chichester: John Wiley & Sons. doi:10.1002/0470028637.met001.
- Michal, G. (1999). *Biochemical Pathways*. Heidelberg: Spektrum.
- Pereira, P. J. B., Macedo-Ribeiro, S., Párraga, A., Pérez-Luque, R., Cunningham, O., Darcy, K., Mantle, T. J. & Coll, M. (2001). *Nature Struct. Biol.* **8**, 215–220.
- Persson, B., Krook, M. & Jörnvall, H. (1991). *Eur. J. Biochem.* **200**, 537–543.
- Persson, B., Zigler, J. S. & Jörnvall, H. (1994). *Eur. J. Biochem.* **226**, 15–22.
- Powell, A. J., Read, J. A., Banfield, M. J., Gunn-Moore, F., Yan, S. D., Lustbader, J., Stern, A. R., Stern, D. M. & Brady, R. L. (2000). *J. Mol. Biol.* **303**, 311–327.
- Radianingtyas, H. & Wright, P. C. (2003). *FEMS Microbiol. Rev.* **27**, 593–616.
- Ring, M. W., Schwär, G., Thiel, V., Dickschat, J. S., Kroppenstedt, R. M., Schulz, S. & Bode, H. B. (2006). *J. Biol. Chem.* **281**, 36691–36700.
- Sanghani, P. C., Stone, C. L., Ray, B. D., Pindel, E. V., Hurley, T. D. & Bosron, W. F. (2000). *Biochemistry*, **39**, 10720–10729.
- Schomaker, V. & Trueblood, K. N. (1968). *Acta Cryst.* **B24**, 63–76.
- Terwilliger, T. C., Adams, P. D., Read, R. J., McCoy, A. J., Moriarty, N. W., Grosse-Kunstleve, R. W., Afonine, P. V., Zwart, P. H. & Hung, L.-W. (2009). *Acta Cryst.* **D65**, 582–601.
- Toal, D. R., Clifton, S. W., Roe, B. A. & Downard, J. (1995). *Mol. Microbiol.* **16**, 177–189.
- Vitale, A., Rosso, F., Barbarisi, A., Labella, T. & D'Auria, S. (2010). *Gene*, **461**, 26–31.
- Vitale, A., Thorne, N., Lovell, S., Battaile, K. P., Hu, X., Shen, M., D'Auria, S. & Auld, D. S. (2013). *PLoS One*, **8**, e63828.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.
- Youn, B., Camacho, R., Moinuddin, S. G. A., Lee, C., Davin, L. B., Lewis, N. G. & Kang, C. (2006). *Org. Biomol. Chem.* **4**, 1687–1697.
- Zheng, Q., Song, Y., Zhang, W., Shaw, N., Zhou, W. & Rao, Z. (2015). *FEBS J.* **282**, 2697–2707.