Structure of a critical metabolic enzyme: *S*-adenosylmethionine synthetase from *Cryptosporidium parvum*

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S-Adenosyl-L-methionine (AdoMet), the primary methyl donor in most biological methylation reactions, is produced from ATP and methionine in a multistep reaction catalyzed by AdoMet synthetase. The diversity of group-transfer reactions that involve AdoMet places this compound at a key crossroads in amino-acid, nucleic acid and lipid metabolism, and disruption of its synthesis has adverse consequences for all forms of life. The family of AdoMet synthetases is highly conserved, and structures of this enzyme have been determined from organisms ranging from bacteria to humans. Here, the structure of an AdoMet synthetase from the infectious parasite *Cryptosporidium parvum* has been determined as part of an effort to identify structural differences in this enzyme family that can guide the development of species-selective inhibitors. This enzyme form has a less extensive subunit interface than some previously determined structures, and contains some key structural differences from the human enzyme in an allosteric site, presenting an opportunity for the design of selective inhibitors against the AdoMet synthetase from this organism.

1. Introduction

S-Adenosyl-L-methionine (AdoMet) is a critically important biological sulfonium compound that plays a central role in many essential biochemical processes in all known organisms. This metabolite functions as the primary donor in most methylation reactions (Cheng & Blumenthal, 2000). The amino group of AdoMet is donated in the biotin biosynthetic pathway (Stoner & Eisenberg, 1975), and its ribosyl group is a precursor in the next-to-last biosynthetic step for a modified tRNA nucleoside (Van Lanen *et al.*, 2003). AdoMet is also the source of aminopropyl groups to produce diamines during polyamine biosynthesis (Fontecave *et al.*, 2004), and polyamines are essential for the growth of *Trypanosoma cruzi* (Heby *et al.*, 2007). This diversity of group-transfer reactions places AdoMet at a key nexus of amino-acid, nucleic acid and lipid metabolism. Not surprisingly, disruption of AdoMet metabolism prevents the growth of every organism that has been examined.

The only known route for AdoMet biosynthesis is catalyzed by L-methionine *S*-adenosyltransferase (AdoMet synthetase or MetK/MAT). Consistent with its critical functions, the methionine metabolic cycle feeding into the synthesis of AdoMet is tightly regulated at both the genetic and protein levels (Mato *et al.*, 1997; Cheng & Blumenthal, 2000). An extensive family of these enzymes has been identified (Sánchez-Pérez *et al.*, 2004; Kamikawa *et al.*, 2009). Sequence comparison among AdoMet synthetases selected from bacterial, archaeal and mammalian species (Zano *et al.*, 2013)

found that in addition to the high overall sequence homology in this family, the motifs that are involved in methionine, ATP and pyrophosphate binding (Sánchez-Pérez *et al.*, 2004), and the active-site catalytic amino acids (Reczkowski *et al.*, 1998), are all fully conserved. This sequence homology also extends to AdoMet synthetases from protozoal and fungal species. However, despite the highly conserved nature of the AdoMet synthetase family there are some significant sequence differences, and some known structural differences that can potentially be exploited for the identification and optimization of selective inhibitors.

Multiple structures of bacterial AdoMet synthetases have been determined, primarily from *Escherichia coli* (EcoMetK; Takusagawa, Kamitori, Misaki *et al.*, 1996; Takusagawa *et al.*, 1996; Komoto *et al.*, 2004), but also from *Mycobacterium* (Baugh *et al.*, 2015), *Burkholderia* (Baugh *et al.*, 2013) and the thermophilic archaeon *Sulfolobus* (Wang *et al.*, 2014). The only structures of AdoMet synthetases from parasitic protozoal species are from *Entamoeba histolytica* (PDB entry 3so4; Medical Structural Genomics of Pathogenic Protozoa, unpublished work) and *Cryptosporidium hominis* (ChoMetK; PDB entry 40dj; Seattle Structural Genomics Center for Infectious Diseases, unpublished work). However, detailed descriptions and analyses of these protozoal enzyme structures have not been published. Several medium-resolution structures of rat MATs (González *et al.*, 2003) and increasingly higher resolution structures of human MAT isoforms (Murray *et al.*, 2014, 2016) have provided a clearer picture of the subunit assembly and catalytic mechanism of the mammalian orthologs. The majority of AdoMet synthetase orthologs that have been examined are homotetrameric, assembling as a dimer of dimers. However, some MetK/MATs from divergent classes, such as archaeal and mammalian MAT isoforms, can exist as functional dimers in solution. Of particular interest to this study was the recent structural characterization of human liver MAT in the presence of a potent allosteric inhibitor (Quinlan *et al.*, 2017). In this structure an allosteric inhibitor was found to be bound to dimeric MAT2A, binding along the dimer–dimer interface at a site that would typically be occupied by the MAT2B dimeric regulatory subunit. This key finding suggests that targeting this allosteric site in other MetK/MATs where it is present may lead to novel, potent and selective inhibitors.

A new crystal structure of AdoMet synthetase from the infectious parasite *C. parvum* (CpaMetK) has been determined to high resolution. Structural insights from this essential target in a disease-causing organism present a promising opportunity for a structure-guided approach to the design of novel antiparasitic drugs.

2. Materials and methods

2.1. Expression and purification of AdoMet synthetase from *C. parvum*

A glycerol stock of an expression construct of AdoMet synthetase from *C. parvum* (CpaMetK) was obtained from the

Table 1
Macromolecule-production information.

Source organism	<i>C. parvum</i>
DNA source	Seattle Structural Genomics Center for Infectious Disease (SSGCID)
Forward primer	5'-CTCTACTGTGACGTCTAGTCGACAGGA GGATAATAATGGATTCTTCGAGGTTTA GTGG-3'
Reverse primer	5'-TCTGTGCTCGAAGATGTCGAGTCTAGA TTGTAAAATAATTGGCTTTTCCCAAAGA AACTC-3'
Expression vector	AVA0421 expression vector derived from pET-14b; provides a cleavable hexahistidine tag at the N-terminus of the expressed protein
Expression host	<i>E. coli</i> BL21(DE3) R3 Rosetta
Complete amino-acid sequence of the construct produced	MDSRFRSGNKSSTYDLQTTSEQLFSSSEV CSGHPDKLCDQISDAILDACLEQDPESF VACETCTKTGFIMVFGEITTKANVNYER VVRETVEKEIGYDSEKGLDYKTMVDVIK LEQQSNQIAGCVHVDKNVEDIGAGDQGM MFGYATNETKELMPLTHVLATSITRELD YIRVKGVSRRVGLRPDGAQVTVEYNC KHGVLIPKRIHTILVSVQHDENIENEER REFVLENVIKKVCPSDLMDKETRILNP SGRFTIGGPAADAGLTGRKIIIVDTYGGW GAHGGGAFSGKDATKVDKSGAYMARLVA KSIVFSGGLCSRCLVQVSYGIGIARPLSL YINTFGTAKDGYNDKLEIVNKVDFDR PGILIKQLNLKSPFKKTSNGHFGFRSE EEFLWEKPIILQ

Seattle Structural Genomics Center for Infectious Disease (SSGCID). The *metK* gene was cloned via ligation-independent cloning into an expression vector derived from pET-14b, which yields expressed protein with an N-terminal His₆ tag, and the recombinant plasmids were transformed into *E. coli* BL21(DE3) R3 Rosetta cells (Myler *et al.*, 2009; Table 1). The expression and purification of the enzyme were carried out following the provided protocol with some modifications. Starting cultures (25 ml) were grown in LB medium with the added antibiotics ampicillin (50 µg ml⁻¹) and carbenicillin (50 µg ml⁻¹) and were incubated for 16 h at 310 K. Tunair growth flasks containing 1 l freshly prepared ZYP-5052 auto-induction medium (Studier, 2005) with ampicillin and carbenicillin were inoculated with the overnight culture. The cultures were initially grown for 24 h at 298 K and the temperature was then decreased to 288 K for 72 h. The cells were harvested by centrifugation and stored at 193 K.

The frozen bacterial cell pellet (5–10 g) was resuspended in 200 ml lysis buffer (25 mM HEPES, 500 mM NaCl, 5% glycerol, 30 mM imidazole, 0.5% CHAPS, 10 mM MgCl₂, 1 mM TCEP, 250 mg ml⁻¹ AEBSE, 0.05 mg ml⁻¹ lysozyme pH 7.0). The cells were sonicated using an on/off duty cycle for 30 min to release the cellular content. In order to digest the nucleic acids, 20 µl Benzonase (5 kU, Sigma) was added to the cell suspension and incubated at room temperature for 45 min. The crude soluble lysate was pelleted by centrifugation at 15 560g for 1 h. The filtered supernatant was loaded onto a 20 ml immobilized metal-affinity chromatography (IMAC) column charged with Ni²⁺ ions and previously equilibrated with buffer A (25 mM HEPES, 500 mM NaCl, 5% glycerol, 30 mM imidazole, 5 mM β-mercaptoethanol pH 7.0). The

Table 2
Crystallization.

Method	Hanging-drop vapor diffusion
Plate type	24-well VDX
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	10
Buffer composition of protein solution	25 mM HEPES, 500 mM NaCl, 5% glycerol, 2 mM DTT
Composition of reservoir solution	25% PEG 3350, 0.2 M ammonium sulfate, 0.1 M bis-Tris pH 6.5, 5% ethylene glycol
Volume and ratio of drop	1.0 µl drop, 1:1 ratio
Volume of reservoir (µl)	600

unbound proteins were removed by washing with 20 column volumes of buffer A under the control of an ÄKTAexplorer, and the enzyme was then eluted using a linear gradient of imidazole from 30 to 250 mM imidazole over ten column volumes. The fractions containing purified AdoMet synthetase, based on UV absorbance at 280 nm and enzyme-activity measurements, were pooled and dialyzed overnight at 277 K against storage buffer (25 mM HEPES, 500 mM NaCl, 5% glycerol, 2 mM DTT). The purified enzyme was concentrated to 10 mg ml⁻¹ for crystallization screening.

2.2. Enzyme assay

The AdoMet synthetase assay was performed in standard 96-well plates (Grenier Bio-One) of 200 µl in volume using the previously published enzyme-coupled assay (Zano *et al.*, 2013). The production of NADPH was monitored at 340 nm using a SpectraMax 190 microplate reader (Molecular Devices) at room temperature. The kinetic parameters were determined by fitting the data to the kinetic equations developed by Cleland (1979).

2.3. Enzyme crystallization

Crystals of *CpaMetK* were grown using the hanging-drop vapor-diffusion method at 293 K. The initial crystallization conditions for the apoenzyme were identified using the Index screen from Hampton Research. The optimized crystallization condition was refined by using 1 µl enzyme solution mixed with 1 µl crystallization solution consisting of 25% PEG 3350, 0.2 M ammonium sulfate, 0.1 M bis-Tris pH 6.5 with 5% ethylene glycol added to the reservoir (Table 2). Well formed rectangular crystals were obtained from this condition after about one week. The crystals were cryoprotected by soaking them in reservoir solution with 20%(v/v) ethylene glycol added directly to the mother-liquor drop and then flash-cooling them in liquid nitrogen.

2.4. Data collection and processing

Crystallographic data were collected on the GM/CA 23-ID-B beamline at Argonne National Laboratory (ANL) using an EIGER 16M detector. A single data set was collected with a crystal-to-detector distance of 250 mm, a data wedge of 0.5° per frame and an exposure time of 1 s per frame. The diffraction data were integrated and scaled in the monoclinic

Table 3
Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	GM/CA 23-ID-B, ANL
Wavelength (Å)	1.03315
Temperature (K)	100
Detector	EIGER 16M
Crystal-to-detector distance (mm)	250.00
Rotation range per image (°)	0.5
Total rotation range (°)	180
Exposure time per image (s)	1.0
Space group	C2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	134.39, 62.06, 191.58
α , β , γ (°)	90, 90.65, 90
Mosaicity (°)	0.5
Resolution range (Å)	19.98–1.85 (1.92–1.85)
Total No. of reflections	230220 (22563)
No. of unique reflections	127686 (12821)
Completeness (%)	94.28 (94.92)
Multiplicity	1.8 (1.8)
$\langle I/\sigma(I) \rangle$	8.15 (1.39)†
<i>R</i> _{int}	0.077 (0.741)
Overall <i>B</i> factor from Wilson plot (Å ²)	25.3

† The $\langle I/\sigma(I) \rangle$ value in the last shell is lower than 2.0 owing to somewhat weaker data in the highest resolution shell. However, the CC_{1/2} value for this shell is 0.532, suggesting that there is still meaningful signal in the data. The $\langle I/\sigma(I) \rangle$ value is greater than 2.0 in the 1.994–1.925 Å resolution shell.

setting C2 using *iMosflm* (Battye *et al.*, 2011) and *AIMLESS* from the *CCP4* suite (Winn *et al.*, 2011).

2.5. Structure solution and refinement

The structure was determined by the molecular-replacement method using *MOLREP* (Vagin & Teplyakov, 2010) with the structure of *C. hominus* MetK (PDB entry 4odj) as the search model. The resulting model was improved using alternating rounds of manual correction in *Coot* (Emsley *et al.*, 2010) with crystallographic refinement in *REFMAC5* (Murshudov *et al.*, 2011) and *PHENIX* (Adams *et al.* 2010). Figures and buried surface area calculations were generated using *PyMOL* (DeLano, 2002). The final model was deposited in the Protein Data Bank (<http://www.rcsb.org>; Berman *et al.*, 2000) with PDB code 6c07. The data-collection and merging statistics are shown in Table 3, while the structure-refinement statistics are shown in Table 4.

3. Results and discussion

3.1. Overall structure of *C. parvum* MetK

The structure of unliganded *CpaMetK* was determined to 1.85 Å resolution with good statistics (Table 4). No outliers were identified in the Ramachandran plot, and there were no red or yellow flags in the *MolProbity* analysis (Chen *et al.*, 2010). The structure is well ordered, with the exception of 28–30 residues at the amino-terminus and 10–14 residues from flexible ‘gating-loop’ regions that are missing from the model owing to a lack of supporting electron density. The overall structure consists of a tight dimer of the native, full-length (406-amino-acid) protein (Fig. 1). The structure does not have substrates or products bound, but there are bound phosphate,

Table 4
Structure refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	19.98–1.85 (1.92–1.85)
Completeness (%)	94.3 (94.9)
σ Cutoff	1.4
No. of reflections, working set	127362 (12699)
No. of reflections, test set	6387 (592)
Final R_{cryst}	0.160 (0.278)
Final R_{free}	0.205 (0.318)
No. of non-H atoms	
Total	12645
Protein	11434
Ion	10
Ligand	0
Water	1201
Total No. of protein residues	1486
R.m.s. deviations	
Bonds (Å)	0.011
Angles (°)	1.39
Average B factors (Å ²)	
Overall	31.7
Protein	30.7
Ion	33.3
Ligand	N/A
Water	41.7
Ramachandran plot	
Favored regions (%)	98.2
Additionally allowed (%)	1.8
Outliers (%)	0

magnesium and potassium ions, as observed in previously determined ‘apo’ MAT/MetK structures.

3.2. Structural analysis of *CpaMetK*

CpaMetK assembles into a spherical homodimer (Fig. 1*b*), with the interface between the two monomers formed by a relatively flat hydrophobic surface of stacked β -sheets, with the α -helices on the exterior forming the solvent-exposed surfaces. The active site in each subunit is wedged within the homodimer interface, with the location indicated by the

presence of a pair of Mg^{2+} ions in the active site (Fig. 1). The subunit interface, as is typical for this enzyme family, is quite extensive, with a total of 4929 Å² of buried surface area. The flexible gating loop, which typically becomes ordered in the presence of substrates, is disordered and was not modeled in this structure.

The active site of the MetK/MAT enzyme is formed at the interface of two monomers; a single monomer of MetK/MAT is not catalytically active. In many species these homodimers associate to form functional tetramers with D_2 or 222 symmetry, as illustrated by *EcoMetK* (PDB entry 1p7l; Fig. 2*a*; Komoto *et al.*, 2004). Human MAT enzymes, along with many mammalian MATs, form a complex composed of a MAT2A homodimer that is enzymatically modulated by a regulatory homodimer MAT2B (PDB entry 5ugh; Fig. 2*b*; Quinlan *et al.*, 2017). This new structure of *CpaMetK* is consistent with other previously determined MetK structures in that it contains a functional homodimer with the active sites formed at the interface between the two monomers.

The *CpaMetK* homodimer interface found in this structure reveals that despite the absence of bound substrates in the active site, the catalytic pocket is well formed, with clear electron density for bound ions and catalytic residues. The active site is highly conserved in MetK/MAT enzymes and this structure is consistent with previously determined structures. Analysis using *PISA* (Krissinel & Henrick, 2007) and *PyMOL* (DeLano, 2002) revealed that the interface between the monomers within the homodimer is composed of ~230 residues and covers 4929 Å² of buried surface area.

PISA analysis was used to evaluate whether *CpaMetK* may also form a homotetramer consisting of a dimer of homodimers, similar to those found in human MAT and *EcoMetK*. In contrast to the clearly defined tetramer interface found in other MetK/MAT enzymes, the interface between the two homodimers in this structure includes only ~160 residues and

Figure 1

Structure of the *CpaMetK* dimer. The functional dimer interface is shown as a side view (left panel) and a top view (right panel), with the monomers shown in salmon and orange and the positions of the two bound Mg^{2+} ions in each monomer shown as spheres.

Figure 2
Comparison of dimer–dimer assemblies in MetK/MAT. (a) Tetrameric *EcoMetK* with bound AdoMet substrate, showing tight association of the two homodimers. (b) Human MAT2A with the inhibitor PF-9366 bound in each active site, showing a similar dimer interface to that in *EcoMetK*. (c) *CpaMetK* also forms a dimer of dimers, but with a less extensive dimer interface.

Figure 3

Structure-based alignment of MetK/MATs. The aligned amino-acid sequences of MetK/MAT from mammalian and protozoan species. The *EcoMetK* and human sequences are included for comparison, with fully conserved residues shown in red, partially conserved residues in yellow and nonconserved residues in white, and with conserved amino-acid blocks marked by bold boxes. Abbreviations are HSA, *Homo sapiens*; CPA, *Cryptosporidium parvum*; CHO, *Cryptosporidium hominis*; EHI, *Entamoeba histolytica*; ECO, *Escherichia coli*.

encompasses 1892 Å² (Fig. 2c). This is a significantly smaller interface when compared with that in *EcoMetK* (Fig. 2a), where the buried surface area for the dimer–dimer interface was calculated to be 3894 Å². The buried surface area for the dimer–dimer interface for *ChoMetK* was also calculated to be much smaller than that of *EcoMetK* at 1195 Å², suggesting that both *CpaMetK* and the closely related *ChoMetK* complexed with AdoMet and triphosphosphate (PDB entry 4odj) are likely to exist as stable homodimers in solution.

The degree of buried surface area may also contribute to the enzymatic activity of the bacterial and protozoal MetK enzymes. *EcoMetK* has the most extensive dimer–dimer interface and is also the most active, with a k_{cat}/K_m of $4.9 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, while *CpaMetK* has a minimal dimer–dimer interface and was determined to have a k_{cat}/K_m of $7.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, being only about 10% as active as the *E. coli* ortholog. In mammalian MAT/MetK enzymes the interface between the two homodimers typically functions in a regulatory capacity, with both the α and β subunits helping to create the active enzyme (Murray *et al.*, 2016). Additional studies will need to be conducted to determine the role of the quaternary structure in the activity of the protozoan MetKs.

3.3. MetK/MAT sequence comparison

The structure-based sequence alignment of three protozoan MATs from *C. parvum*, *C. hominis* and *E. histolytica*, the human MATI and MATII isozymes, rat liver MAT and the bacterial MetK from *E. coli* is shown in Fig. 3. The two *Cryptosporidium* MetKs and the two isoforms of human MAT closely resemble each other, with 99% and 84% identity,

respectively. The sequence identity to MATI ranges from 57% for *EcoMetK* to 84% for rat liver MAT, with the *Cryptosporidium* and *Entamoeba* enzymes being 58% and 64% identical to MATI, respectively. The alignment reveals the five blocks of amino acids that are fully conserved in the MAT family (Sánchez-Pérez *et al.*, 2004). The first block includes the classical sequence GHPDK for methionine binding that is found in all MAT sequences, while block III includes the dynamic active-site loop and the ATP-binding site GAGDQG. The fourth block is where two important catalytic residues, Asp187 and Lys189, are found. The longest stretch of conserved amino-acid residues is found in block V and is involved in substrate and cation binding. Notably, this block has a high glycine content (ten glycine residues). Lastly, block VII consists of residues near the C-terminus. Additional conserved blocks described in the rat liver enzyme are also evident in Fig. 3 (Sánchez-Pérez *et al.*, 2004). Block II includes the active-site residues Glu63 and Glu75 within β -sheets β_1 and β_2 , while block VI forms part of β -sheets β_{12} and β_{13} . The MetK/MAT family shares many highly conserved amino acids, including the active-site residues. Not surprisingly, all of these clusters of amino acids and key amino-acid residues were found to be conserved in *CpaMetK*.

3.4. Comparison of the new *CpaMetK* structure with the inhibitor-bound human MAT

In order to facilitate the structure-guided design of novel antiprotozoal drugs, the structure of *CpaMetK* was superimposed on the structure of human MAT2A (*HsaMAT2A*; PDB entry 5ugh) complexed with the allosteric inhibitor

Figure 4
Comparison of the *CpaMetK* dimer with the allosteric inhibitor-bound structure of human MAT2A (PDB entry 5ugh). (a) Top view of the dimer of *CpaMetK* from this study (salmon trace) superimposed on the dimer of human MAT2A (gray trace). The C α traces show good structural similarity between the two enzyme forms. (b) Close-up view of the binding site of the allosteric inhibitor PF-9366 from PDB entry 5ugh with the key side-chain differences from *CpaMetK* highlighted.

PF-9366 (Fig. 4*a*). As shown in the sequence alignment (Fig. 3), the active-site residues of both *CpaMetK* and *HsaMAT2A* include the histidine and lysine residues crucial for the proposed S_N2 reaction, which assist the nucleophilic methionyl sulfur in attacking the C5 CH_2 group of ATP (Markham *et al.*, 1987). The significant degree of conservation within the catalytic site among the MetK/MAT family members suggests that targeting areas outside of the substrate-binding pocket, such as in the dimer–dimer interface region, may provide better opportunities for the development of species-selective inhibitors.

While it is not clear that the allosteric regulatory site found in *HsaMAT2A* plays the same role in *CpaMetK*, there is a structurally related pocket that could be occupied by inhibitors (Fig. 4*b*). However, there are key differences within the PF-9366 binding site, suggesting that this specific inhibitor structure is not likely to bind to *CpaMetK*. In particular, there is an aromatic π -stacking interaction between phenylalanine (Phe333) and the inhibitor in *HsaMAT2A* that contributes to its low-nanomolar binding affinity (Fig. 4*b*). In *CpaMetK* this residue is replaced by Asn341. Another significant difference between these two forms of MetK/MAT is that *HsaMAT2A* has a Ser331 residue that helps to form the binding pocket, while *CpaMetK* has Tyr339 in the equivalent position that projects directly into the pocket and would be likely to prevent PF-9366 from binding. While PF-9366 itself is not expected to inhibit *CpaMetK*, this new enzyme structure will provide guidance for the design of inhibitors that could occupy the same pocket.

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References

Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L.-W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read,

R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C. & Zwart, P. H. (2010). *Acta Cryst.* **D66**, 213–221.

Battye, T. G. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. W. (2011). *Acta Cryst.* **D67**, 271–281.

Baugh, L., Gallagher, L. A., Patrapuvich, R., Clifton, M. C., Gardberg, A. S., Edwards, T. E., Armour, B., Begley, D. W., Dieterich, S. H., Dranow, D. M., Abendroth, J., Fairman, J. W., Fox, D. III, Staker, B. L., Phan, I., Gillespie, A., Choi, R., Nakazawa-Hewitt, S., Nguyen, M. T., Napuli, A., Barrett, L., Buchko, G. W., Stacy, R., Myler, P. J., Stewart, L. J., Manoil, C. & Van Voorhis, W. C. (2013). *PLoS One*, **8**, e53851.

Baugh, L., Phan, I., Begley, D. W., Clifton, M. C., Armour, B., Dranow, D. M., Taylor, B. M., Muruthi, M. M., Abendroth, J., Fairman, J. W., Fox, D. III, Dieterich, S. H., Staker, B. L., Gardberg, A. S., Choi, R., Hewitt, S. N., Napuli, A. J., Myers, J., Barrett, L. K., Zhang, Y., Ferrell, M., Mundt, E., Thompson, K., Tran, N., Lyons-Abbott, S., Abramov, A., Sekar, A., Serbzhinskiy, D., Lorimer, D., Buchko, G. W., Stacy, R., Stewart, L. J., Edwards, T. E., Van Voorhis, W. C. & Myler, P. J. (2015). *Tuberculosis*, **95**, 142–148.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. & Bourne, P. E. (2000). *Nucleic Acids Res.* **28**, 235–242.

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.

Cheng, X. & Blumenthal, R. M. (2000). *S-Adenosylmethionine-Dependent Methyltransferases: Structures and Functions*. Singapore: World Scientific.

Cleland, W. W. (1979). *Methods Enzymol.* **63**, 103–138.

DeLano, W. L. (2002). *PyMOL*. <http://www.pymol.org>.

Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.

Fontecave, M., Atta, M. & Mulliez, E. (2004). *Trends Biochem. Sci.* **29**, 243–249.

González, B., Pajares, M. A., Hermoso, J. A., Guillerme, D., Guillerme, G. & Sanz-Aparicio, J. (2003). *J. Mol. Biol.* **331**, 407–416.

Heby, O., Persson, L. & Rentala, M. (2007). *Amino Acids*, **33**, 359–366.

Kamikawa, R., Sanchez-Perez, G. F., Sako, Y., Roger, A. J. & Inagaki, Y. (2009). *Mol. Phylogenet. Evol.* **53**, 565–570.

Komoto, J., Yamada, T., Takata, Y., Markham, G. D. & Takusagawa, F. (2004). *Biochemistry*, **43**, 1821–1831.

Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.

Markham, G. D., Parkin, D. W., Mentch, F. & Schramm, V. L. (1987). *J. Biol. Chem.* **262**, 5609–5615.

Mato, J. M., Alvarez, L., Ortiz, P. & Pajares, M. A. (1997). *Pharmacol. Ther.* **73**, 265–280.

Murray, B., Antonyuk, S. V., Marina, A., Lu, S. C., Mato, J. M., Hasnain, S. S. & Rojas, A. L. (2016). *Proc. Natl Acad. Sci. USA*, **113**, 2104–2109.

Murray, B., Antonyuk, S. V., Marina, A., Van Liempd, S. M., Lu, S. C., Mato, J. M., Hasnain, S. S. & Rojas, A. L. (2014). *IUCrJ*, **1**, 240–249.

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.

Quinlan, C. L., Kaiser, S. E., Bolaños, B., Nowlin, D., Grantner, R., Karlicek-Bryant, S., Feng, J. L., Jenkinson, S., Freeman-Cook, K., Dann, S. G., Wang, X., Wells, P. A., Fantin, V. R., Stewart, A. E. & Grant, S. K. (2017). *Nature Chem. Biol.* **13**, 785–792.

Reczkowski, R. S., Taylor, J. C. & Markham, G. D. (1998). *Biochemistry*, **37**, 13499–13506.

Sánchez-Pérez, G. F., Bautista, J. M. & Pajares, M. A. (2004). *J. Mol. Biol.* **335**, 693–706.

Stoner, G. L. & Eisenberg, M. A. (1975). *J. Biol. Chem.* **250**, 4037–4043.

- Studier, F. W. (2005). *Protein Expr. Purif.* **41**, 207–234.
- Takusagawa, F., Kamitori, S. & Markham, G. D. (1996). *Biochemistry*, **35**, 2586–2596.
- Takusagawa, F., Kamitori, S., Misaki, S. & Markham, G. D. (1996). *J. Biol. Chem.* **271**, 136–147.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Van Lanen, S. G., Kinzie, S. D., Matthieu, S., Link, T., Culp, J. & Iwata-Reuyl, D. (2003). *J. Biol. Chem.* **278**, 10491–10499.
- Wang, F., Singh, S., Zhang, J., Huber, T. D., Helmich, K. E., Sunkara, M., Hurley, K. A., Goff, R. D., Bingman, C. A., Morris, A. J., Thorson, J. S. & Phillips, G. N. (2014). *FEBS J.* **281**, 4224–4239.
- Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G. W., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterton, E. A., Powell, H. R., Read, R. J., Vagin, A. & Wilson, K. S. (2011). *Acta Cryst.* **D67**, 235–242.
- Zano, S., Bhansali, P., Luniwal, A. & Viola, R. E. (2013). *Arch. Biochem. Biophys.* **536**, 64–71.