Purification, crystallization and preliminary X-ray diffraction analysis of pyridoxal kinase from *Plasmodium falciparum* (PfPdxK)

Thales Kronenberger,^{a‡} Sergey Lunev,^{b‡} Carsten Wrenger^a and Matthew R. Groves^{b*}

^aUnit for Drug Discovery, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, Avenida Professor Lineu Prestes 1374, São Paulo-SP 05508-000, Brazil, and ^bDepartment of Drug Design, Groningen Research Institute of Pharmacy (GRIP), Rijksuniversiteit Groningen (RUG), Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

‡ These authors contributed equally to this article.

Correspondence e-mail: m.r.groves@rug.nl

Received 6 August 2014

Accepted 2 September 2014

Pyridoxal kinases (PdxK) catalyze the phosphorylation of vitamin B₆ precursors. Thus, these enzymes are an essential part of many metabolic processes in all organisms. The protozoan parasite *Plasmodium falciparum* (the main causative agent of Malaria tropica) possesses a unique *de novo* B₆-biosynthesis pathway in addition to a interconversion pathway based on the activity of plasmodial PdxK (PfPdxK). The role of PdxK in B₆ salvage has prompted previous authors to suggest PdxK as a promising target for structure-based antimalarial drug design. Here, the expression, purification, crystallization and preliminary X-ray diffraction analysis of PfPdxK are reported. PfPdxK crystals have been grown in space group *P*2₁, with unit-cell parameters *a* = 52.7, *b* = 62.0, *c* = 93.7 Å, β = 95°. A data set has been collected to 2 Å resolution and an initial molecular-replacement solution is described.

1. Introduction

Malaria remains a major health problem, as approximately half of the population of the world live in regions where it is transmitted (*i.e.* in most parts of the tropics and subtropics). Malaria is caused by infection with one of several species of the genus *Plasmodium*, of which *P. falciparum* is responsible for the most severe and lethal form (Guerin *et al.*, 2002). In 2012 *Plasmodium* infected over 200 million people and killed at least 600 000 people worldwide (World Health Organization, 2013). The actual number of deaths and infections is likely to be higher, as in many cases the data are unavailable or undocumented. There is no effective vaccine available against malaria and the parasite rapidly develops resistance to drugs used for its treatment, making the identification of new drug targets important for global health (Olliaro *et al.*, 2001; Bhattacharai *et al.*, 2007; White, 2008). New antimalarial agents that provide higher efficacy and less toxicity are urgently required.

A member of the ribokinase superfamily, pyridoxal kinase (PdxK) functions in the vitamin B₆-salvage pathway (Yang *et al.*, 1996) and catalyzes the phosphorylation of pyridoxal, pyridoxine and pyridoxamine into precursors of the active form of B₆ (pyridoxal 5-phosphate, PLP; Müller *et al.*, 2010). This active form is essential for both prokaryotes and eukaryotes as it is involved in a variety of metabolic processes as a cofactor, as well as in combating oxidative stress caused by singlet oxygen (Knöckel *et al.*, 2012; Kronenberger *et al.*, 2013). For example, PLP is an essential cofactor in the activity of plasmodial aspartate aminotransferase (PfAspAT), which has been suggested to be a drug target as it bridges both carbon metabolism and nucleotide biosynthesis (Jain *et al.*, 2010; Wrenger *et al.*, 2011, 2012). Unlike mammalian cells, *P. falciparum* has a functional pathway for *de novo* B₆ biosynthesis (Wrenger *et al.*, 2005), which has already been validated as a drug target (Reeksting *et al.*, 2013). Additionally, the parasite possesses an interconversion pathway based on PdxK, which has already been exploited for pro-drug discovery (Müller *et al.*, 2009).

Pyridoxal kinases have been structurally characterized in several organisms, including *Homo sapiens* (Musayev *et al.*, 2007), *Escherichia coli* (Safo *et al.*, 2006), *Bacillus subtilis* (Park *et al.*, 2004),

Table 1
Macromolecule-production information.

The *Sac*II (forward primer) and *Hind*III (reverse primer) cleavage sites are underlined and the six-His tag is underlined and shown in italics both in the reverse primer and in the complete construct sequence.

Source organism	<i>P. falciparum</i>
DNA source	<i>P. falciparum</i> 3D7 cDNA
Plasmid ID	PF3D7_0616000
Forward primer	5'-GCGCCCGCGGTATGAAGAAGGAAATATTATCTCC-3'
Reverse primer	5'-GCGCAAGCTTCTAATGATGATGATGATGATGCCAAAAA-TCCAAAAAAACAGGCTCTTCTTAATTAATAATC-3'
Expression vector	pASK-IBA3 (IBA Lifesciences)
Expression host	<i>E. coli</i> Rosetta 2 (DE3)
Complete amino-acid sequence of the construct produced	MGDRGMKKENIIISIQSVDFGCGNNIAAFVRRRHGHIPKIL-SQVFDGFCGNIIAFAVRRRHGHIPKILNTVQYYSKFKHS-VELNSQVEVDIILSEYNKQDEFMNDSNIYFLTGYIKNAECV-DMVTKNILELRRKRKIHGKGSNDNGMNGHMNGHMNGHMN-GHMNGHMNGHMNGHMNGHMNGHMNGHMNGHMNGHTNGHMN-GHMNDHMNGHMNGHTNDHMNGHTNDHMNGHTNDHMNGHTN-DHMNGHTNDHMNDHMNDHMNGHTNSHTGLTNGHMD-EPNGEHPYRLMNSNELKSSHQIIPQKGQIHEKMDLKNIL-TISQGRKKDEELYFIENIINLNLWVCDPVMGDNGRLYVD-ERVVSYKKAIEYVDIITPNQYETELLCGKINEEKDVLIK-CLDVLHLKGKIVIIITSVNYNFDKDLHFLYVSFFNNKNKI-VYFKYKILKIHFCNFGSGDLFSCLLSFIVKQKGNILHIIS-KVLNIVQNVIKNSLTGLLELNIIENQDIIASDGLINDILIK-EEPVFFGHHHHHH
Length of the construct (amino acids)	508
Molecular weight of the construct (kDa)	58.5
Ab _{50,1%} (1 mg ml ⁻¹) of the construct	0.48
Complete amino-acid sequence of wild-type <i>Pf</i> PdXK	MKKENIIISIQSVDFGCGNNIAAFVRRRHGHIPKILNTVQYYSKFHSGVELNSQVEVDIILSEYNKQDEFMNDSNIYFLTGYIKNAECVDMVTKNILELRRKRKIHGKGSNDNGMNGHMNGHMNGHMNGHMNGHMNGHMNGHMNGHMNGHTNGHMNGHMNDHMNGHTNDHMNGHTNDHMNGHTNDHMNGHTNDHMNGHTNSHTHGLTNGHMDPENGEHPYRLMNSNELKSSHQIIPQKGQIHEKMDLKNILITISQGRKKDEELYFIENIINLNLWVCDPVMGDNGRLYVDERVVSYKKAIEYVDIITPNQYETELLCGKINEEKDVIKCLDVLHLKGKIVIIITSVNYNFDKDLHFLYVSFFNNKNKIVYFKYKILKIHFCNFGSGDLFSCLLSFIVKQKGNILHIISKVLNIVQNVIKNSLTGLLELNIIENQDIIASDGLINDILIKEEPVFF
Length of the wild-type <i>Pf</i> PdXK (amino acids)	497
Molecular weight of wild-type <i>Pf</i> PdXK (kDa)	57.2
Ab _{50,1%} (1 mg ml ⁻¹) of wild-type <i>Pf</i> PdXK	0.49

Arabidopsis thaliana (Lum *et al.*, 2002) and the parasite *Trypanosoma brucei* (Scott & Phillips, 1997). PdxK has been proven to be essential for growth of *T. brucei*, as knockout parasites were unable to survive in PLP-free medium (Jones *et al.*, 2012).

The availability of a crystal structure of the plasmodial PdxK (*PfPdxK*) is essential for the dissection of the unique vitamin B₆ pathway of *P. falciparum* (Wrenger *et al.*, 2005) and for its validation as a drug target in the treatment of malaria. It will provide new details of the mechanism of the B₆ interconversion pathway and can also be used in comparative studies with PdxK homologues from other organisms. *PfPdxK* is a polypeptide of 497 amino acids with a predicted molecular mass of 57.2 kDa (Wrenger *et al.*, 2005; PlasmoDB PF3D7_0616000). Sequence analysis of wild-type *PfPdxK* shows that it contains a 205-amino-acid insert (109–314) that has no structural homologues. The 292 amino acids N- and C-terminal to this insert (51% of the sequence) can be aligned with the *H. sapiens* PdxK (GenBank accession O00764) with 32% identity, resulting in an overall identity of 16%. The structural and functional differences could be used in the design of selective antimalarials.

Here, we report the expression, purification, crystallization and preliminary X-ray diffraction characterization of PfPdxK.

2. Materials and methods

2.1. Macromolecule production

2.1.1. Cloning of *PfPdxK*. The *PdxK* gene of *P. falciparum* was amplified by polymerase chain reaction (PCR) using *P. falciparum* 3D7 cDNA (PF3D7_0616000) as template and sequence-specific sense (5'-GCGCCCGCGGTATGAAGAAGGAAAATATTATC-TCC-3') and antisense (5'-GCGCAAGCTTCTAATGATGATGATGATGATGTCCAAAAA AACAGGCTCTTCTTTAATTAAAA-TATC-3') oligonucleotides containing *Sac*II and *Hind*III restriction sites, respectively (Table 1). The PCR was performed with *Pfu* polymerase using the following conditions: one cycle of 367 K for 7 min followed by 35 cycles of 1 min at 367 K, 1.5 min at 315 K and 2 min at 341 K, according to Wrenger *et al.* (2005). The PCR fragments were cloned into pASK-IBA3 vector (IBA Lifesciences) previously digested with *Sac*II and *Hind*III and the nucleotide sequence was determined by automated sequencing (Seqlab, Germany). Nucleotide-sequence and protein-sequence analyses were performed using *Gene Runner* (<http://www.generunner.net/>). The final construct consisted of full-length *PfPdxK* with the additional amino acids MGDRG at the N-terminus and GHHHHHH (six-His tag) at the C-terminus (Table 1).

2.1.2. Expression of *PfPdxK*. *PfPdxK* was recombinantly expressed in *E. coli* Rosetta 2 (DE3) cells (Novagen) using the expression plasmid pASK-IBA3-*PfPdxK*, which contains the open reading frame for the *PfPdxK* gene fused to a C-terminal His tag to facilitate purification. Transformed *E. coli* Rosetta 2 (DE3) cells were propagated in 21 selective medium (LB in the presence of 50 $\mu\text{g ml}^{-1}$ ampicillin, 35 $\mu\text{g ml}^{-1}$ chloramphenicol, 4 mM MgCl_2) at 310 K in 2 l baffled Erlenmeyer flasks (Nalgene) and induced at an OD_{600} of 0.6 with 200 ng ml^{-1} anhydrotetracycline (AHT). After induction, the temperature of the culture was lowered to 291 K and the cells were incubated overnight. They were subsequently harvested by centrifugation at 10 000g for 30 min.

2.1.3. Purification of PfPdxK. The bacterial pellet was resuspended in 30 ml lysis buffer [100 mM Tris pH 8.0, 300 mM NaCl, 20 mM imidazole, 5% (v/v) glycerol, 0.1 mM β -mercaptoethanol (BME)]. The cells were lysed on ice by sonication and the homogenate was clarified by centrifugation at 45 000g for 60 min. It was not necessary to supplement the lysis buffer with lysozyme as sonication was sufficiently effective.

The supernatant containing the soluble His-tagged protein was filtered using a 0.45 μm filter (Whatman) and incubated for 10 min with 1.0 ml Ni-NTA affinity resin (Ni-NTA Agarose, Protino, Macherey Nagel, Germany) at room temperature. Recombinant protein was purified employing the C-terminal His tag according to the manufacturer's recommendations. The following steps were also performed at room temperature as no significant protein degradation was observed. The resin was poured into a gravity-flow column (Bio-Rad) and washed with 50 ml lysis buffer (as defined above) followed by 10 ml wash buffer [100 mM Tris pH 8.0, 300 mM NaCl, 50 mM imidazole, 5%(v/v) glycerol, 3 mM BME]. The protein was eluted with elution buffer [50 mM Tris pH 8.0, 250 mM NaCl, 0.3 M imidazole, 5%(v/v) glycerol, 3 mM BME].

The eluate was collected and concentrated to a volume of 1 ml and applied onto a HiLoad 16/60 Superdex 75 column (GE Healthcare) previously equilibrated with 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) pH 6.5, 100 mM NaCl, 100 mM ammonium nitrate, 5% (v/v) glycerol, 2 mM BME. The gel-filtration buffer was chosen based upon a Thermofluor-based stability assay (Nettleship *et al.*, 2008; Ericsson *et al.*, 2006) as previous crystallization attempts resulted in crystals unsuitable for diffraction experiments. Thermofluor data

Table 2
Crystallization.

Method	Hanging drop
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	10
Buffer composition of protein solution	10 mM MES pH 6.5, 0.1 M NaCl, 0.1 M ammonium nitrate, 5% (v/v) glycerol, 2 mM BME
Composition of reservoir solution	0.1 M HEPES pH 7.75, 0.2 M CaCl ₂ , 31% (v/v) PEG 400, 5% (v/v) glycerol
Volume and ratio of drop (nl)	200, 1:1
Volume of reservoir (ml)	1
Crystal size (maximal dimension) (μm)	100

were collected on a CFX96 Real-Time System (Bio-Rad). SYPRO Orange (Invitrogen) was added to the protein sample (concentrated to 2 mg ml⁻¹) at 1:500 dilution. Experiments were composed of 45 μl of the buffer component to be screened and 5 μl of the protein/dye solution. Inflection points in graphs of relative fluorescence units (RFU) against temperature were determined manually and used as an indicator of the sample thermal stability in the buffer component screened. Comparisons were made against a control sample containing only water. The final purified protein eluted as a single peak. This peak was pooled and concentrated using a Spin-X UF concentration unit with a 10 kDa cutoff (Corning). The final protein concentration was determined to be 10 mg ml⁻¹ based upon its theoretical absorbance at 280 nm [$Abs_{0.1\%}(1\text{ mg ml}^{-1}) = 0.48$; Gasteiger *et al.*, 2005; <http://web.expasy.org/protparam/>]. The protein was immediately used in crystallization trials. The protein purity was estimated to be better than 95% (Fig. 1*a*) as assessed by Coomassie Brilliant Blue-stained SDS-PAGE (Laemmli, 1970). The final yield of purified PfPdxK was approximately 5 mg per litre of culture.

2.2. Crystallization

Concentrated protein was submitted to the EMBL Hamburg high-throughput crystallization facility (Mueller-Dieckmann, 2006) for

Table 3
Data-collection and processing statistics for PfPdxK.

Values in parentheses are for the outer shell.	
Diffraction source	Beamline X12, EMBL Hamburg
Wavelength (Å)	1
Temperature (K)	100
Detector	MAR CCD 245
Crystal-to-detector distance (mm)	180
Beam size (μm)	200 × 200
Rotation range per image (°)	0.2
No. of images	600
Exposure time per image (s)	36
Space group	<i>P</i> 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	52.7, 62.0, 93.7
α, β, γ (°)	90, 95, 90
Multiplicity	2.55 (2.42)
Mosaicity (°)	0.39
Resolution range (Å)	20.0–2.03 (2.15–2.03)
Total No. of reflections	94957 (14956)
No. of unique reflections	37282 (6193)
Completeness (%)	95.5 (95.0)
<i>I</i> / <i>σ</i> (<i>I</i>)	13.66 (2.02)
<i>R</i> _{merge} † (%)	4.2 (51.7)
<i>R</i> _{r.i.m.} (%)	5.3 (66.1)
Overall <i>B</i> factor from Wilson plot (Å ²)	23.7

† *R*_{merge} is defined as $\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where *I_i*(*hkl*) is the *i*th intensity measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the average intensity from multiple observations.

initial screening against The Classics and Classics II Suites (Qiagen) sparse-matrix screening kits. All crystallization was performed at 293 K using equal volumes (200 nl) of protein solution and crystallization reagent.

Small crystals were observed in multiple conditions. Manual hanging-drop optimization screens were performed around these conditions in order to reproduce and improve the crystal size by varying the precipitant concentration, buffer and pH. Optimized trials were performed using the hanging-drop method, in which 1 μl of protein solution at a concentration of 10 mg ml⁻¹ was mixed with

Figure 1
(*a*) 8% SDS-PAGE of the purified PfPdxK. Sample was boiled in SDS-loading buffer prior to loading and the gel was stained with Coomassie Blue. Left lane, PfPdxK sample. Right lane, unstained protein marker (Thermo Scientific; labelled in kDa). (*b*) Thermal shift assay results for PfPdxK. When exposed to sodium chloride (*i*), BME (*ii*), ammonium chloride (*iii*) and MES pH 6.5 (*iv*) PfPdxK samples showed positive shift in thermal stability.

an equal amount of reservoir solution and equilibrated over 1 ml of the reservoir solution at 293 K. Diffraction-quality crystals were obtained in 0.1 M HEPES pH 7.75, 0.2 M CaCl_2 , 31% (v/v) PEG 400, 5% (v/v) glycerol (Table 2, Fig. 2a). Crystals appeared after 24 h and were suitable for diffraction studies after one week.

2.3. Data collection and processing

2.3.1. Diffraction analysis. *PfPdxK* crystals were directly flash-cooled to 100 K in the nitrogen stream at the beamline. After initial analysis using *BEST* (Popov & Bourenkov, 2003; Bourenkov & Popov, 2006) a 2 Å data set was collected on beamline X12 at the EMBL, DESY, Hamburg using a beam size of $200 \times 200 \mu\text{m}$. Data-collection parameters are indicated in Table 3. The space group of the *PfPdxK* crystals was determined to be $P2_1$, with unit-cell parameters $a = 52.7$, $b = 62.0$, $c = 93.7$ Å, $\alpha = 90$, $\beta = 95$, $\gamma = 90^\circ$. Based on these unit-cell parameters, a single *PfPdxK* molecule is expected in the asymmetric unit with a Matthews coefficient of $2.87 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 57% (Matthews, 1968). Data were reduced using *XDS/XSCALE* (Kabsch, 2010a,b). Data were merged using *AIMLESS* (Evans & Murshudov, 2013). An R_{free} set (Brünger, 1992) for use in subsequent structure refinement and validation was created using 5% of the reflections selected at random. The data set was examined for indications of twinning, but no statistically significant twinning could be found.

2.3.2. Molecular replacement. Molecular replacement was performed using the *BALBES* pipeline (Long *et al.*, 2008). Automated database searching within *BALBES* provided a number of homologous structures for use as molecular-replacement models. An initial solution was found using the coordinates of sheep brain PdxK (PDB entry 1rfu; Li *et al.*, 2004) as a search model. This solution was

found using the 1rfu structure sequence modified for molecular replacement to match the sequence of *PfPdxK*, with a Z-score of 4.6 and R and R_{free} factors of 0.546 and 0.546, respectively. Amino acids of the search model (PDB entry 1rfu) that were identical to the *PfPdxK* sequence were left unmodified, while non-identical amino acids were truncated to the last common atom, with the chemical identity of the atom changed to match the *PfPdxK* sequence when necessary. Initial restrained refinement was performed within the *BALBES* automated pipeline, using *REFMAC5* (Murshudov *et al.*, 2011). This resulted in a model containing the aligned portions of *PfPdxK* (amino acids 5–103 and 310–485), with R and R_{free} factors of 0.482 and 0.506, respectively (Figs. 3a and 3b).

3. Results and discussion

We have reported the availability of *PfPdxK* crystals suitable for diffraction analysis. The initial crystallization conditions for *PfPdxK* were obtained using the EMBL high-throughput crystallization facility (Mueller-Dieckmann, 2006) and were subsequently refined using manual hanging-drop screens. Small crystals (5–10 μm) were obtained when *PfPdxK* was concentrated in a buffer composed of 10 mM Tris pH 8.0, 150 mM NaCl prior to crystallization screening. However, the small crystal size resulted in poor diffraction (8–10 Å resolution) unsuitable for structure determination. An important step was the optimization of the gel-filtration buffer using the Thermo-fluor method (Ericsson *et al.*, 2006; Nettleship *et al.*, 2008). As shown in Fig. 1(b), *PfPdxK* shows increased thermal stability in the presence of β -mercaptoethanol (+5 K), ammonium chloride (+7 K) and MES pH 6.5 (+2 K) with respect to a water control. Thermofluor analysis also indicated that 100 mM NaCl was the optimal ionic strength of

Figure 2

(a) Diffraction-quality single *PfPdxK* crystals grown in 0.1 M HEPES pH 7.75, 0.2 M CaCl_2 , 31% (v/v) PEG 400, 5% (v/v) glycerol. (b) Example of the diffraction frame obtained from a *PfPdxK* crystal on beamline X12 at the EMBL, Hamburg. The edge of the detector corresponds to a resolution of 2 Å. The corresponding beamline parameters are given in Table 3.

Figure 3

(a) A diagram showing the preliminary model of the *PfPdxK* asymmetric unit. The molecular-replacement solution is shown in red and symmetry-related molecules are shown in green. (b) A diagram showing the preliminary $2F_o - F_c$ electron-density map contoured at 1.4σ . The map was calculated with weighted phases from *REFMAC5* (Murshudov *et al.*, 2011) following restrained refinement of the molecular-replacement solution. These diagrams were created using *PyMOL* (v1.5.0.4; Schrödinger).

NaCl, with higher concentration of NaCl resulting in thermal destabilization. Optimized *PfPdxK* crystals suitable for diffraction measurements were obtained from the protein concentrated to 10 mg ml^{-1} in gel-filtration buffer in a 1:1 ratio with 0.1 M HEPES pH 7.75, 0.2 M CaCl_2 , 31%(v/v) PEG 400, 5%(v/v) glycerol. The optimized crystals used for data collection had maximal dimensions of $100 \mu\text{m}$ (Fig. 2a) and diffracted to $2 \text{ \AA}$ resolution on beamline X12 at the EMBL, Hamburg. The data-collection and processing statistics are shown in Table 3. While the data quality in the highest resolution bin is relatively poor ($R_{\text{merge}} = 51.7\%$), sufficient signal exists for the use of this data in refinement based on maximum-likelihood algorithms [$\langle I/\sigma(I) \rangle = 2.03$; Murshudov *et al.*, 1997]. An example diffraction pattern is shown in Fig. 2(b). Initial molecular-replacement trials were performed in *BALBES* (Long *et al.*, 2008) using *REFMAC5* (Murshudov *et al.*, 2011) and a potential solution was found using the coordinates of PdxK from sheep brain (Li *et al.*, 2004; PDB entry 1rfu), which showed 14% sequence identity overall (Figs. 3a and 3b). There is a large insertion (104–309; 205 amino acids) in the *PfPdxK* sequence that has no structural homologue. While the remaining 303 amino acids of *PfPdxK* have a reasonable homology (28% identity) with 1rfu, we believe that this unmodelled portion may explain why the molecular-replacement solution and initial *R* factors are worse than might have been anticipated. However, the structure solution and refinement of *PfPdxK* are in progress and will be reported elsewhere.

The determined structure is likely to aid in dissecting the plasmodial vitamin B₆ metabolism and further antimalarial drug design.

This work was financially supported by grant Nos. 2009/54325-2, 2011/13706-3 and 2013/10288-1 from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and CAPES/Nuffic (MALAR-ASP 068 - 053). X-ray data were collected on beamline X12 at the EMBL, Hamburg. We would like to thank Tryfon

Zarganis-Tzitzikas and Alanod Al-Qahtani for critical reading of the manuscript, Doris Jahn and Christoph Hermes for support in beam-line maintenance and operation. This paper is dedicated to the memory of Dr C. F. Groves.

References

- Bhattarai, A., Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., Al-Mafazy, A. W., Ramsan, M., Rotllant, G., Gerstenmaier, J. F., Molteni, F., Abdulla, S., Montgomery, S. M., Kaneko, A. & Björkman, A. (2007). *PLoS Med.* **4**, e309.
- Bourenkov, G. P. & Popov, A. N. (2006). *Acta Cryst.* **D62**, 58–64.
- Brünger, A. T. (1992). *Nature (London)*, **355**, 472–475.
- Ericsson, U. B., Hallberg, B. M., DeTitta, G. T., Dekker, N. & Nordlund, P. (2006). *Anal. Biochem.* **357**, 289–298.
- Evans, P. R. & Murshudov, G. N. (2013). *Acta Cryst.* **D69**, 1204–1214.
- Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D. & Bairoch, A. (2005). *The Proteomics Protein Handbook*, edited by J. M. Walker, pp. 571–607. Totowa: Humana Press.
- Guerin, P. J., Olliaro, P., Nosten, F., Druilhe, P., Laxminarayan, R., Binka, F., Kilama, W. L., Ford, N. & White, N. J. (2002). *Lancet Infect. Dis.* **2**, 564–573.
- Jain, R., Jordanova, R., Müller, I. B., Wrenger, C. & Groves, M. R. (2010). *Acta Cryst.* **F66**, 409–412.
- Jones, D. C., Alphe, M. S., Wyllie, S. & Fairlamb, A. H. (2012). *Mol. Microbiol.* **86**, 51–64.
- Kabsch, W. (2010a). *Acta Cryst.* **D66**, 125–132.
- Kabsch, W. (2010b). *Acta Cryst.* **D66**, 133–144.
- Knöckel, J., Müller, I. B., Butzloff, S., Bergmann, B., Walter, R. D. & Wrenger, C. (2012). *Biochem. J.* **443**, 397–405.
- Kronenberg, T., Schettler, I. & Wrenger, C. (2013). *Future Med. Chem.* **5**, 769–779.
- Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
- Li, M.-H., Kwok, F., Chang, W.-R., Liu, S.-Q., Lo, S. C. L., Zhang, J.-P., Jiang, T. & Liang, D.-C. (2004). *J. Biol. Chem.* **279**, 17459–17465.
- Long, F., Vagin, A. A., Young, P. & Murshudov, G. N. (2008). *Acta Cryst.* **D64**, 125–132.
- Lum, H.-K., Kwok, F. & Lo, S. C. (2002). *Planta*, **215**, 870–879.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Mueller-Dieckmann, J. (2006). *Acta Cryst.* **D62**, 1446–1452.

- Müller, I. B., Bergmann, B., Groves, M. R., Couto, I., Amaral, L., Begley, T. P., Walter, R. D. & Wrenger, C. (2009). *PLoS One*, **4**, e7656.
- Müller, I. B., Hyde, J. E. & Wrenger, C. (2010). *Trends Parasitol.* **26**, 35–43.
- 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. D* **67**, 355–367.
- Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* **D53**, 240–255.
- Musayev, F. N., di Salvo, M. L., Ko, T.-P., Gandhi, A. K., Goswami, A., Schirch, V. & Safo, M. K. (2007). *Protein Sci.* **16**, 2184–2194.
- Nettlehip, J. E., Brown, J., Groves, M. R. & Geerlof, A. (2008). *Methods Mol. Biol.* **426**, 299–318.
- Olliaro, P., Taylor, W. R. & Rigal, J. (2001). *Trop. Med. Int. Health*, **6**, 922–927.
- Park, J.-H., Burns, K., Kinsland, C. & Begley, T. P. (2004). *J. Bacteriol.* **186**, 1571–1573.
- Popov, A. N. & Bourenkov, G. P. (2003). *Acta Cryst.* **D59**, 1145–1153.
- Reeksting, S. B., Müller, I. B., Burger, P. B., Burgos, E. S., Salmon, L., Louw, A. I., Birkholtz, L. M. & Wrenger, C. (2013). *Biochem. J.* **449**, 175–187.
- Safo, M. K., Musayev, F. N., di Salvo, M. L., Hunt, S., Claude, J. B. & Schirch, V. (2006). *J. Bacteriol.* **188**, 4542–4552.
- Scott, T. C. & Phillips, M. A. (1997). *Mol. Biochem. Parasitol.* **88**, 1–11.
- White, N. J. (2008). *Malar. J.* **7**, S8.
- World Health Organization (2013). *World Malaria Report 2013*. Geneva: World Health Organization. http://www.who.int/malaria/publications/world_malaria_report_2013/en/.
- Wrenger, C., Eschbach, M. L., Müller, I. B., Warnecke, D. & Walter, R. D. (2005). *J. Biol. Chem.* **280**, 5242–5248.
- Wrenger, C., Müller, I. B., Schifferdecker, A. J., Jain, R., Jordanova, R. & Groves, M. R. (2011). *J. Mol. Biol.* **405**, 956–971.
- Wrenger, C., Müller, I. B., Silber, A. M., Jordanova, R., Lamzin, V. S. & Groves, M. R. (2012). *Curr. Drug Metab.* **13**, 332–336.
- Yang, Y., Zhao, G. & Winkler, M. E. (1996). *FEMS Microbiol. Lett.* **141**, 89–95.