Iker Oyenarte,^{a‡} Tomas
Majtan,^{b‡} June Ereño,^a
María Angeles Corral-
Rodríguez,^a Jan P. Kraus^b and
Luis Alfonso Martínez-Cruz^{a*}

^aStructural Biology Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia, Edificio 800, Derio, Bizkaia 48160, Spain, and ^bDepartment of Pediatrics, University of Colorado School of Medicine, Aurora, CO 80045, USA

‡ These authors contributed equally to this work.

Correspondence e-mail:
amartinez@cicbiogune.es

Received 13 July 2012
Accepted 29 August 2012

Purification, crystallization and preliminary crystallographic analysis of human cystathionine β -synthase

Human cystathionine β -synthase (CBS) is a pyridoxal-5'-phosphate-dependent hemeprotein, whose catalytic activity is regulated by *S*-adenosylmethionine. CBS catalyzes the β -replacement reaction of homocysteine (Hcy) with serine to yield cystathionine. CBS is a key regulator of plasma levels of the thrombogenic Hcy and deficiency in CBS is the single most common cause of homocystinuria, an inherited metabolic disorder of sulfur amino acids. The properties of CBS enzymes, such as domain organization, oligomerization degree or regulatory mechanisms, are not conserved across the eukaryotes. The current body of knowledge is insufficient to understand these differences and their impact on CBS function and physiology. To overcome this deficiency, we have addressed the crystallization and preliminary crystallographic analysis of a protein construct (*hCBS*_{516–525}) that contains the full-length CBS from *Homo sapiens* (*hCBS*) and just lacks amino-acid residues 516–525, which are located in a disordered loop. The human enzyme yielded crystals belonging to space group *I*222, with unit-cell parameters $a = 124.98$, $b = 136.33$, $c = 169.83$ Å and diffracting X-rays to a resolution of 3.0 Å. The crystal structure appears to contain two molecules in the asymmetric unit which presumably correspond to a dimeric form of the enzyme.

1. Introduction

Cystathionine β -synthase (CBS, EC 4.2.1.22) lies at the critical branch point of sulfur amino-acid metabolism, where homocysteine, a toxic metabolite of the methionine cycle, is diverted into the transsulfuration pathway (Mudd *et al.*, 2001). In this pathway, CBS catalyzes the condensation of homocysteine and serine to form cystathionine (Fig. 1). Deficiency in CBS activity, mainly caused by the presence of missense mutations in the CBS gene, is the most common cause of hyperhomocysteinemia and homocystinuria (Mudd *et al.*, 2001; Mudd, 2011). More than 160 pathogenic mutations in the CBS gene have been described so far (<http://medschool.ucdenver.edu/krauslab>). The CBS-deficient homocystinuria is an inherited metabolic disorder clinically characterized by dislocated optic lenses, various vascular manifestations, skeletal deformities, connective tissue defects and mental retardation (Mudd *et al.*, 2001). In addition, even a slightly elevated plasma level of homocysteine is an independent risk factor for cardiovascular and neurovascular diseases, dementia and cognitive impairment (Mudd *et al.*, 1982; Welch & Loscalzo, 1998; Seshadri *et al.*, 2002; Folin *et al.*, 2005).

Human CBS is a fascinating pyridoxal-5'-phosphate (PLP)-dependent hemeprotein with a complex domain structure and regulatory mechanism (reviewed in Miles & Kraus, 2004; Singh *et al.*, 2007). The enzyme contains four identical 63 kDa subunits each consisting of an N-terminal heme-binding region, a central PLP-containing catalytic core and a C-terminal regulatory region. Although the exact role of heme in CBS remains enigmatic, it is thought to function in redox sensing and/or the enzyme's folding, assembly or stability (Janosik, Oliveriusová *et al.*, 2001; Singh *et al.*, 2007; Majtan *et al.*, 2010). The fold of the central catalytic core is very conserved and analogous to other PLP-dependent enzymes in the fold-type-II family, such as *O*-acetylserine-sulfhydrylases (Christen & Mehta, 2001; Meier *et al.*, 2001). The C-terminal regulatory domain encompasses a tandem of CBS domains, which bind *S*-adenosylmethionine (AdoMet), the CBS allosteric activator (Bateman, 1997;

© 2012 International Union of Crystallography
All rights reserved

Figure 1

Scheme depicting the CBS-catalyzed reaction. CBS catalyzes the β -replacement reaction of homocysteine with serine to yield cystathionine, using pyridoxal-5'-phosphate (PLP) and heme b as cofactors and S-adenosylmethionine (AdoMet) as allosteric activator.

Kery *et al.*, 1998). In addition to AdoMet, autoinhibition imposed by CBS domains can be relieved by the presence of a missense mutation in the CBS domain, by partial thermal denaturation of the enzyme or by a complete removal of this region (Kery *et al.*, 1998; Janosik, Kery *et al.*, 2001; Maclean *et al.*, 2002). Moreover, the regulatory domain is essential for tetramerization, since its removal yields a 45 kDa truncated dimer (Kery *et al.*, 1998).

Interestingly, the domain architecture, the oligomerization degree and the regulatory mechanisms of CBS enzymes are not conserved across the eukaryotes. The heme-binding domain is absent in CBS enzymes from lower eukaryotes, such as *Saccharomyces cerevisiae*,

Trypanosoma cruzi or *Caenorhabditis elegans* (Maclean *et al.*, 2000; Nozaki *et al.*, 2001; Vozdek *et al.*, 2012) contrasting with its presence among higher eukaryotes, such as insects, rodents and mammals (Meier *et al.*, 2001; Koutmos *et al.*, 2010). The regulatory domain is present in a majority of CBS enzymes with a few exceptions, such as *T. cruzi* and *C. elegans* CBS (Nozaki *et al.*, 2001; Vozdek *et al.*, 2012); however, only mammalian CBS enzymes appear to bind and thus be regulated by AdoMet (Maclean *et al.*, 2000; Janosik, Kery *et al.*, 2001; Koutmos *et al.*, 2010). The majority of CBS enzymes ranging from yeast to human form native homotetramers, but insect CBS enzymes appear to exist as homodimers (Koutmos *et al.*, 2010).

To address these differences among the CBS enzymes, structural information is required in order to understand the molecular mechanisms and structural determinants of CBS regulation by AdoMet. At present, the structural data on CBS are limited to a truncated form of the human enzyme (Meier *et al.*, 2001; Taoka *et al.*, 2002) and more recently to the full-length CBS from *Drosophila melanogaster* (Koutmos *et al.*, 2010). Since both enzymes are highly active AdoMet-unresponsive dimers, none of these structures can sufficiently address and explain the complex regulatory mechanism mediated by AdoMet or tetramerization of CBS enzymes. To overcome this lack of structural information, we have crystallized and performed a preliminary analysis of an optimized protein construct (*hCBS*_{516–525}) that contains the full-length CBS from *Homo sapiens* (*hCBS*) and just lacks amino acid residues 516–525 (Fig. 2). The *hCBS*_{516–525} protein has been crystallized in the absence of AdoMet; thus it corresponds to the 'basal' or 'resting' form of the enzyme. To our knowledge, these data provide the first basis for a crystallographic analysis of the human CBS enzyme and should help us comprehend the regulatory role played by the C-terminal domain as well as the effect of some of the pathogenic mutations.

2. Materials and methods

2.1. Cloning of *hCBS*_{516–525}

The human CBS DNA coding sequence in the previously prepared pET28-C-*hCBS* construct (Majtan & Kraus, 2012) was optimized using the OptimumGene codon optimization technology (GenScript)

Figure 2

Sequence alignment of the CBS domain pair region of cystathionine β -synthases from *Apis mellifera* (AmCBS), *Drosophila melanogaster* (DmCBS) and *Homo sapiens* (hCBS). The Uniprot codes of AmCBS, DmCBS and hCBS are Q2V0C9, Q9VRD9 and P35520, respectively. Most common pathological mutations in hCBS are indicated in red along the sequence. Sequence alignment was done with *ClustalW* (Larkin *et al.*, 2007) and represented with *CINEMA* (Parry-Smith *et al.*, 1998).

in order to enhance protein expression in the *Escherichia coli* host. Subsequently, the identified loop in the CBS1 domain (516-IQYHSTGKSS-525) was deleted by using the QuickChange II XL mutagenesis kit (Agilent). The mutagenesis primers were as follows: forward (5'-GGTTGTGCACGAACAGcagcgccaaatgtct) and reverse (5'-agaccattggcgctgCTGTTCGTGCACAACC). Template DNA strands were digested by *DpnI* (NEB Biolabs) and the reaction mixture was transformed into *E. coli* XL10-GOLD competent cells (Agilent). Plasmid DNAs from several colonies were sequenced in order to confirm the deletion. The verified plasmid was transformed into expression host *E. coli* BL21-GOLD (DE3) (Agilent).

2.2. Expression and purification of recombinant *hCBS*_{516–525}

Preparation of recombinant *hCBS*_{516–525} followed essentially the protocol that we developed for various human CBS constructs carrying the 6xHis tag (Majtan & Kraus, 2012) with a few modifications. Briefly, bacterial cells were grown (303 K, 275 rev min^{−1}) in six 2.8 l baffled Fernbach flasks containing 1 l of LB medium supplemented with 0.001% thiamine-HCl, 0.0025% pyridoxine-HCl, 0.1 mM ferric chloride, 0.3 mM δ -aminolevulinic acid and 30 μ g ml^{−1} kanamycin. When the cell density reached $A_{600} \approx 0.8$, the *hCBS*_{516–525} expression was induced by adding IPTG to a final concentration of 1 mM. After induction, the cells continued to grow overnight and were then harvested by centrifugation at 9000g for 7 min at 277 K. The cell pellet was washed with 1 $\times$ PBS (phosphate-buffered saline) and kept at 193 K before processing.

The cell pellet was resuspended in a lysis buffer [50 mM sodium phosphate pH 7.4, 300 mM NaCl, 0.1 mM PLP and protease inhibitor cocktail VII (A.G. Scientific)] at a 1:5(w/v) ratio using a homogenizer. Resuspended cells were treated with 2 mg ml^{−1} lysozyme for 1.25 h at 277 K prior to sonication (eight cycles of 2 min at 50% duty; Misonix S-3000, Qsonica). The cell lysate was clarified by centrifugation at 58 000g for 30 min at 277 K. The supernatant was loaded on a 50 ml TALON column (Clontech) equilibrated in 50 mM sodium phosphate pH 7.4, 300 mM NaCl. The bound protein was subsequently washed with at least five column volumes of wash buffer (50 mM

sodium phosphate, pH 7.4, 300 mM NaCl, 15 mM imidazole) and then eluted with 200 mM imidazole in the wash buffer. The TALON eluate was immediately desalted on a Sephadex G-25 (GE Healthcare) column (3.2 cm inner diameter $\times$ 22 cm) and the buffer was exchanged with the DEAE Sepharose loading buffer (15 mM potassium phosphate pH 7.2, 1 mM EDTA, 1 mM dithiothreitol (DTT), 10% ethylene glycol). The sample was loaded onto a DEAE Sepharose (GE Healthcare) column (2.5 cm inner diameter $\times$ 8.0 cm) and washed with five column volumes of the DEAE Sepharose loading buffer containing 50 mM potassium phosphate followed by 300 mM potassium phosphate in the DEAE Sepharose loading/wash buffer in order to elute the *hCBS* Δ 516–525. The DEAE Sepharose eluate was buffer-exchanged into a final buffer (20 mM HEPES pH 7.4, 1 mM Tris(2-carboxyethyl)phosphine (TCEP) on a Sephadex G-25 column and subsequently concentrated using an ultrafiltration device (Amicon) equipped with a YM-30 (Millipore) membrane. Finally, after 30 min spin at 21 000g, 277 K, the enzyme, which was confirmed to be active and responsive to AdoMet stimulation (*i.e.* behaves like wt-*hCBS*), was aliquoted, flash-frozen in liquid nitrogen and stored at 193 K.

2.3. Crystallization

Crystallization trials were carried out by the vapor-diffusion technique in a sitting-drop format with 96-well MRC crystallization plates using a variety of commercially available screens [Crystal Screen HT, Index HT (Hampton Research), JCSG Core Suites I–IV (Qiagen), JCSG+ Suite, PACT Premier HT-96 (Molecular Dimensions)]. Screening plates were set up in the high-throughput crystallization facility at CIC bioGUNE and incubated at a constant temperature of 293 K. Drops consisted of 200 nl protein solution mixed with 200 nl precipitant solution and the reservoir volume was 50 μ l; the protein concentration was $\sim$ 27 mg ml^{−1}. An initial crystallization hit yielding microcrystalline precipitate and/or tiny needle-like crystals was obtained in 1 M sodium acetate, at physiological pH. These needles were crushed manually using a microspatula (Hampton Research) and were used as microseeds. The sample containing the seeds was diluted with a stabilizing solution containing 0.6 M sodium acetate, 0.1 M HEPES pH 7.5 to an end volume of 100 μ l to obtain the seed-stock. Improved crystals of *hCBS*_{516–525} (Fig. 3) were subsequently obtained by refining the successful condition in a hanging-drop format using 24-well VDX plates (Hampton Research) with drops consisting of 0.6 μ l protein with 0.4 μ l precipitant solution (1.0 M sodium acetate, 0.1 M HEPES pH 7.5) and 0.2 μ l of the seed-stock solution equilibrated over a reservoir volume of 0.5 ml and incubated at a constant temperature of 293 K. The diffraction properties of the crystals were examined at beamlines ID23-1 or ID29 of the ESRF, Grenoble.

2.4. Preliminary crystallographic analysis

The diffraction quality of the crystals was firstly evaluated at room temperature in the absence of cryoprotectants. Then, and after testing a wide variety of cryoprotectants including alcohols, sucrose, agarose, silicone oils or paratone, the crystals were transferred to a crystallization buffer containing 1.0 M sodium acetate, 0.1 M HEPES pH 7.5 and 22% glycerol for a few seconds before being flash-cooled by directly immersing into the liquid nitrogen at 93 K. Crystals were mounted for X-ray data collection using either Cryoloops (Hampton Research) or MicroMount loops (MiTeGen). Data sets were collected at beamline ID23-1 of the ESRF, Grenoble ($\lambda = 0.9793$ Å). A total of 125 diffraction images over a range of 125° could be collected from the best crystal before severe crystal decay was

Figure 3

Crystals of full-length human CBS. Crystals grown in 1.0 M sodium acetate, 0.1 M HEPES pH 7.5 belong to space group *I*222 and diffracted X-rays to 3.0 Å. The red colour of the crystals reveals the presence of the bound heme *b* group. The approximate dimensions of the crystals are 100 $\times$ 200 $\times$ 200 μ m.

Table 1Data-processing statistics for *h*CBS crystals.

Values in parentheses are for the outer-resolution shell. Data sets were collected at beamline ID23-1 of the ESRF, Grenoble.

Beamline	ID23.1
Wavelength (Å)	0.9793
Resolution range (Å)	50–3.0
Total number of reflections	97414 (15150)
No. of unique reflections	26649 (4185)
Space group	<i>I</i> 222
Unit-cell parameters (Å)	<i>a</i> = 124.98, <i>b</i> = 136.33, <i>c</i> = 169.83
CC _{1/2} (%)†	99.6 (66.8)
Mosaicity (°)	0.9
Completeness (%)	91.6 (90.0)
Redundancy	3.7 (3.6)
<i>R</i> _{merge} ‡	10.4% (77.6%)
Mean <i>I</i> /σ(<i>I</i>)	11.6 (2.2)
Wilson <i>B</i> value (Å ²)	116.9

† CC_{1/2} indicates the percentage of correlation significant at the 0.1% level between intensities from random half data sets (Karplus & Diederichs, 2012). ‡ $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th observation of reflection *hkl* and $\langle I(hkl) \rangle$ is the weighted average intensity for all observations *i* of reflection *hkl*.

observed. Diffraction data were processed using *HKL-2000* (Otwinowski & Minor, 1997) or *XDS* (Kabsch, 2010). Preliminary analysis of the data sets was performed using the *CCP4* program suite (Winn *et al.*, 2011). The plot of the self-rotation function at *k* = 180° was calculated with *MOLREP* (Vagin & Teplyakov, 2010) (Fig. 5). The data-collection statistics are summarized in Table 1.

3. Results and discussion

First attempts to crystallize human CBS were carried out with the wild-type enzyme in the presence or absence of its allosteric regulator AdoMet. However, all efforts were unsuccessful despite testing more

than 10 000 different conditions, which included all available commercial screens as well as in-house-developed recipes. Alternatively, and based on careful analyses of sequence and structural alignments of *h*CBS versus *Dm*CBS (Koutmos *et al.*, 2010) and *Am*CBS (Oyenarte *et al.*, 2012), we engineered a protein construct (*h*CBS_{516–525}) that lacks amino-acid residues 516–525, which presumably form a disordered loop that may impede crystal growth (Fig. 2). Accordingly, we explored potential crystallization conditions of the *h*CBS_{516–525} construct using the screens formerly mentioned. Growing crystals of *h*CBS_{516–525} required a large amount of sample (*ca* 115 mg of pure protein was used in this work) and a significant experimental effort. After 3–4 d, microcrystalline precipitates and/or tiny needle-shaped crystals appeared in a low (*ca* 5%) fraction of the drops. The nucleation ratio could only be slightly improved to ~10% after using the obtained needles as seeds for further crystallization experiments. The microseeding strategy allowed us to grow polyhedral crystals in *ca* 10–15% of the drops after 2–3 d of seeding (Fig. 3). Among all crystals tested (about 200), only 5% showed suitable diffraction properties (~3–3.5 Å resolution) on the ESRF beamlines. Native crystals of *h*CBS diffract to 3.0 Å resolution (Fig. 4, Table 1) and belong to space group *I*222 (unit-cell parameters *a* = 124.98, *b* = 136.33, *c* = 169.83 Å). One, two or three molecules within the asymmetric unit gave a Matthews coefficient of 5.96, 2.98 and 1.99 Å³ Da^{−1}, and a solvent content of 79, 59 and 38%, respectively (Matthews, 1968). Considering the fragility of the *I*222 crystals and their limited diffraction power, we estimated that two molecules in the asymmetric unit was the most probable value. In support of this, the corresponding plot of the self-rotation function at *k* = 180° (Fig. 5) showed three twofold axes parallel to the *X*, *Y* and *Z* axes, suggesting that the two molecules are related by a twofold axis and probably form a dimer. Interestingly, a dimeric species does not reflect the expected tetrameric state of the full-length *h*CBS which

Figure 4

Representative X-ray diffraction data frame from the *h*CBS crystals, recorded on the ESRF beamline ID23-1. The crystals were exposed for 20 s per image over a 0.85° simple rotation range. Resolution circles are included for clarity.

Figure 5

Plot of the self-rotation function at *k* = 180° of the *I*222 *h*CBS crystals. The presence of three perpendicular twofold axes which are parallel to the *X*, *Y* and *Z* axes, respectively, suggests the presence of two molecules in the asymmetric unit which probably form a dimer.

contains the C-terminal regulatory domain (truncation of the CBS motif pair of *hCBS* results in a tetramer-to-dimer conversion of the enzyme) (Miles & Kraus, 2004). Accordingly, the ongoing structure determination will help to determine whether the traditionally proposed tetramer of *hCBS* is in fact an association of two dimers related by a twofold symmetry axis.

We thank Dr Alexander Popov at beamline ID23.1 of the ESRF for his valuable support during synchrotron data collection and the staff of beamline ID29 for excellent technical assistance. We also thank Dr Adriana Rojas for maintenance of the in-house X-ray equipment. This work has been supported by Postdoctoral Fellowship 0920079G from the American Heart Association (to TM), by the National Institutes of Health grant No. HL065217, by the American Heart Association Grant In-Aid 09GRNT2110159, by a grant from the Jerome Lejeune Foundation (all to JPK) and by grants from Departamento de Educación, Universidades e Investigación del Gobierno Vasco (PI2010-17), Departamento de Industria, Innovación, Comercio y Turismo del Gobierno Vasco (ETORTEK IE05-147, IE07-202), Diputación Foral de Bizkaia (Exp. 7/13/08/2006/11 and 7/13/08/2005/14) and Ministerio Español de Ciencia y Tecnología (MICINN) (BFU2010-17857) (all to LAMC).

References

- Bateman, A. (1997). *Trends Biochem. Sci.* **22**, 12–13.
- Christen, P. & Mehta, P. K. (2001). *Chem. Rec.* **1**, 436–447.
- Folin, M., Baiguera, S., Gallucci, M., Conconi, M. T., Di Liddo, R., Zanardo, A. & Parnigotto, P. P. (2005). *Biogerontology*, **6**, 255–260.
- Janosik, M., Kery, V., Gaustadnes, M., Maclean, K. N. & Kraus, J. P. (2001). *Biochemistry*, **40**, 10625–10633.
- Janosik, M., Oliveriusová, J., Janosiková, B., Sokolová, J., Kraus, E., Kraus, J. P. & Kozich, V. (2001). *Am. J. Hum. Genet.* **68**, 1506–1513.
- Kabsch, W. (2010). *Acta Cryst.* **D66**, 125–132.
- Karplus, P. A. & Diederichs, K. (2012). *Science*, **336**, 1030–1033.
- Kery, V., Poneleit, L. & Kraus, J. P. (1998). *Arch. Biochem. Biophys.* **355**, 222–232.
- Koutmos, M., Kabil, O., Smith, J. L. & Banerjee, R. (2010). *Proc. Natl Acad. Sci. USA*, **107**, 20958–20963.
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. (2007). *Bioinformatics*, **23**, 2947–2948.
- Maclean, K. N., Gaustadnes, M., Oliveriusová, J., Janosik, M., Kraus, E., Kozich, V., Kery, V., Skovby, F., Rüdiger, N., Ingerslev, J., Stabler, S. P., Allen, R. H. & Kraus, J. P. (2002). *Hum. Mutat.* **19**, 641–655.
- Maclean, K. N., Janosik, M., Oliveriusová, J., Kery, V. & Kraus, J. P. (2000). *J. Inorg. Biochem.* **81**, 161–171.
- Majtan, T. & Kraus, J. P. (2012). *Protein Expr. Purif.* **82**, 317–324.
- Majtan, T., Liu, L., Carpenter, J. F. & Kraus, J. P. (2010). *J. Biol. Chem.* **285**, 15866–15873.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Meier, M., Janosik, M., Kery, V., Kraus, J. P. & Burkhard, P. (2001). *EMBO J.* **20**, 3910–3916.
- Miles, E. W. & Kraus, J. P. (2004). *J. Biol. Chem.* **279**, 29871–29874.
- Mudd, S. H. (2011). *Am. J. Med. Genet. C. Semin. Med. Genet.* **157**, 3–32.
- Mudd, S. H., Havlik, R., Levy, H. L., McKusick, V. A. & Feinleib, M. (1982). *Am. J. Hum. Genet.* **34**, 1018–1021.
- Mudd, S. H., Levy, H. L. & Kraus, J. P. (2001). *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed., edited by C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, B. Childs, K. Kinzler & B. Vogelstein, pp. 2007–2056. New York: McGraw-Hill.
- Nozaki, T., Shigeta, Y., Saito-Nakano, Y., Imada, M. & Kruger, W. D. (2001). *J. Biol. Chem.* **276**, 6516–6523.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Oyenarte, I., Majtan, T., Ereño, J., Corral-Rodríguez, M. A., Klaudiny, J., Majtan, J., Kraus, J. P. & Martínez-Cruz, L. A. (2012). *Acta Cryst.* **F68**, 1323–1328.
- Parry-Smith, D. J., Payne, A. W., Michie, A. D. & Attwood, T. K. (1998). *Gene*, **221**, GC57–63.
- Seshadri, S., Beiser, A., Selhub, J., Jacques, P. F., Rosenberg, I. H., D'Agostino, R. B., Wilson, P. W. & Wolf, P. A. (2002). *N. Engl. J. Med.* **346**, 476–483.
- Singh, S., Madzalan, P. & Banerjee, R. (2007). *Nat. Prod. Rep.* **24**, 631–639.
- Taoka, S., Lepore, B. W., Kabil, O., Ojha, S., Ringe, D. & Banerjee, R. (2002). *Biochemistry*, **41**, 10454–10461.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Vozdek, R., Hnízda, A., Krijt, J., Kostrouchová, M. & Kožich, V. (2012). *Biochem. J.* **443**, 535–547.
- Welch, G. N. & Loscalzo, J. (1998). *N. Engl. J. Med.* **338**, 1042–1050.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.