Structure of human dual-specificity phosphatase 7, a potential cancer drug target

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Human dual-specificity phosphatase 7 (DUSP7/Pyst2) is a 320-residue protein that belongs to the mitogen-activated protein kinase phosphatase (MKP) subfamily of dual-specificity phosphatases. Although its precise biological function is still not fully understood, previous reports have demonstrated that DUSP7 is overexpressed in myeloid leukemia and other malignancies. Therefore, there is interest in developing DUSP7 inhibitors as potential therapeutic agents, especially for cancer. Here, the purification, crystallization and structure determination of the catalytic domain of DUSP7 (Ser141–Ser289/C232S) at 1.67 Å resolution are reported. The structure described here provides a starting point for structure-assisted inhibitor-design efforts and adds to the growing knowledge base of three-dimensional structures of the dual-specificity phosphatase family.

1. Introduction

The dual-specificity phosphatases (DUSPs) constitute a group of protein tyrosine phosphatases (PTPs) that are characterized by their ability to dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine residues (Patterson *et al.*, 2009). Within the DUSP family, the mitogen-activated protein kinase phosphatases (MKPs) represent an additional subgroup (Farooq & Zhou, 2004). MKPs contain two main domains: a conserved C-terminal catalytic domain that harbors the active site where the dephosphorylation reaction occurs (Yuvaniyama *et al.*, 1996; Stewart *et al.*, 1999) and an N-terminal rhodanese-like domain that functions as a mitogen-activated protein kinase (MAPK)-binding domain (Farooq & Zhou, 2004; Zhang *et al.*, 2011). The 320-residue human dual-specificity phosphatase 7 (DUSP7/Pyst2) is a cytosolic MKP that dephosphorylates the extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNK) and p38 kinase (Dowd *et al.*, 1998; Orlev *et al.*, 2004). Although not thoroughly characterized in the literature, DUSP7 has been shown to be overexpressed in peripheral blood mononuclear cells and bone marrow from patients diagnosed with acute leukemia (Levy-Nissenbaum, Sagi-Assif, Kapon *et al.*, 2003; Levy-Nissenbaum, Sagi-Assif, Raanani *et al.*, 2003a,b). Consequently, it has been suggested that the development of DUSP7 inhibitors may have potential therapeutic utility in the treatment of cancer (Nunes-Xavier *et al.*, 2011). Other studies have suggested, however, that DUSP7 may function as a tumor suppressor (Shin *et al.*, 1997). Therefore, DUSP7 inhibitors may be beneficial for the treatment of some forms of cancer but contraindicated for others. Animal studies examining the effects of DUSP7 deletions or ‘knock-downs’ and

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specific small-molecule inhibitors are needed to further characterize the function and therapeutic potential of DUSP7.

Although the field of protein tyrosine phosphatase drug discovery has yet to attain the level of success that has been achieved with protein kinase inhibitors, there remains significant interest in the direct inhibition of PTPs as a means of designing novel therapeutics (Ríos *et al.*, 2014; He *et al.*, 2014). The successful identification of DUSP inhibitors for progression to drug candidates, however, presents many difficult obstacles for a number of reasons (Blaskovich, 2009). In particular, the DUSP active site is usually rather shallow, a feature that is necessary for the accommodation of both phosphotyrosine and phosphoserine/phosphothreonine residues (Yuvaniyama *et al.*, 1996; Schumacher *et al.*, 2002). Additionally, the active-site pocket, which is formed by the consensus sequence (H/V)C(X₅)R(S/T) (Zhang *et al.*, 1994; Denu & Dixon, 1995), is highly conserved and exhibits a preference for negatively charged compounds which often have limited specificity, cell permeability and bioavailability. Therefore, in addition to targeting the active site of PTPs and DUSPs, an alternative approach has been to target secondary sites that extend beyond the catalytic pocket in order to achieve increased binding affinity and greater specificity (Puius *et al.*, 1997; Bahta *et al.*, 2011). This strategy has been greatly aided by the availability of high-resolution crystal structures that have facilitated the rational design of such bidentate or multidentate inhibitors (Sun *et al.*, 2003; Wu *et al.*, 2009; Kim *et al.*, 2011; Bahta *et al.*, 2011). To date, only a few DUSP7 inhibitors have been identified using virtual screening methods and a homology model of DUSP7 (Park *et al.*, 2011). Here, we present the crystal structure of the catalytic domain of DUSP7 (C232S) at 1.67 Å resolution, which provides a structural template that may be used to advance the development of DUSP7 inhibitors.

2. Materials and methods

2.1. Cloning, expression and purification of DUSP7

Efforts to crystallize the wild-type DUSP7 catalytic domain were unsuccessful. Therefore, we chose to work with a catalytically inactive form of the protein harboring the active-site C232S mutation. The catalytic domain of DUSP7 (Ser141–Ser289) was amplified from DNASU clone HsCD00434660 by the polymerase chain reaction (PCR) using the following oligodeoxyribonucleotide primers: 5′-GAG AAC CTG TAC TTC CAG AGC CAA CCA GCC TTC CCT GTC CAG ATC CTG CCC-3′ and 5′-G GGG ACC ACT TTG TAC AAG AAA GCT GGG TTA GCT TAG CCC CAG CGT CCG CTC-3′ (primer 2). The PCR product was used as the template for a second PCR amplification with primer: 5′-GGGG ACA AGT TTG TAC AAA AAA GCA GGC TCG GAG AAC CTG TAC TTC CAG GGT-3′ and primer 2. The amplicon from the second round of PCR, coding for DUSP7 (Ser141–Ser289) with an N-terminal tobacco etch virus (TEV) protease cleavage site (ENLYFQ/G), was recombined into the donor vector pDONR221 (Life Technologies, Grand Island, New York, USA) to generate the entry clone pBA2408 and its

nucleotide sequence was verified. The C232S mutation in DUSP7 was introduced by QuikChange Lightning site-directed mutagenesis (Agilent, Santa Clara, California, USA) using primers 5′-GGT GTC CTG GTG CAC TCC CTG GCA GGC ATC AGC C-3′ and 5′-GGC TGA TGC CTG CCA GGG AGT GCA CCA GGA CAC C-3′, yielding pBA2528. The C232S variant was then recombined from pBA2528 into pDEST-HisMBP (Nallamsetty *et al.*, 2005; Tropea *et al.*, 2009) to generate the expression vector pBA2529. The latter plasmid produces a His₆-MBP-DUSP7 (Ser141–Ser289/C232S) fusion protein that can be cleaved by TEV protease to give rise to DUSP7 PTP domain consisting of residues Ser141–Ser289 and containing the C232S amino-acid substitution.

The fusion protein was produced in *Escherichia coli* strain BL21-CodonPlus (DE3)-RIL (Agilent Technologies, La Jolla, California, USA). Cultures were grown to mid-log phase (OD₆₀₀ = 0.4–0.6) at 310 K in Luria broth supplemented with 100 µg ml^{−1} ampicillin, 30 µg ml^{−1} chloramphenicol and 0.2% glucose. Recombinant protein production was induced with isopropyl β-D-1-thiogalactopyranoside at a concentration of 1 mM for 4 h at 303 K. The cells were pelleted by centrifugation and stored at 193 K.

All purification steps were carried out at 277 K. 20–25 g of *E. coli* cell paste was suspended in 200 ml ice-cold 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 30 mM imidazole buffer (lysis buffer). The cells were lysed by three passes through an APV-1000 homogenizer (Invensys APV Products, Albertslund, Denmark) at 69 MPa and centrifuged at 30 000g for 30 min. The supernatant was filtered through a 0.45 µm cellulose acetate membrane and applied onto a 20 ml HisPrep column (GE Healthcare, Piscataway, New Jersey) equilibrated in lysis buffer. The column was washed to baseline with lysis buffer and the protein was eluted with a linear gradient from 30 to 250 mM imidazole. Fractions containing the fusion protein were pooled and concentrated with an Amicon stirred cell equipped with a YM30 membrane (EMD Millipore, Billerica, Massachusetts, USA). The fusion protein was digested overnight with polyhistidine-tagged TEV protease (Kapust *et al.*, 2001). The digest was diluted with 50 mM Tris–HCl pH 7.5, 150 mM NaCl to reduce the imidazole concentration to approximately 30 mM and applied onto a 20 ml HisPrep column equilibrated with lysis buffer. DUSP7 (Ser141–Ser289/C232S) was isolated in the column flowthrough and was concentrated using an Amicon stirred cell with a YM10 membrane. Dithiothreitol was added to the protein to a final concentration of 10 mM and the solution was allowed to incubate overnight. The sample was clarified by centrifugation, filtered through a 0.22 µm membrane and applied onto a HiPrep 26/60 Sephacryl S-100 HR column (GE Healthcare) equilibrated with 25 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM tris(2-carboxyethyl)phosphine buffer. Peak fractions were pooled and concentrated to 20.3 mg ml^{−1} (estimated at 280 nm using a molar extinction coefficient of 15 930 M^{−1} cm^{−1}). Aliquots were flash-frozen with liquid nitrogen and stored at 193 K prior to crystallization. The molecular weight was confirmed by electrospray ionization mass spectrometry.

Table 1

X-ray data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data-collection statistics	
X-ray source	22-BM, SER-CAT
Wavelength (Å)	1.0
Resolution (Å)	50.0–1.67 (1.70–1.67)
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 64.6, b = 64.5, c = 74.3,$ $\alpha = \gamma = 90.0, \beta = 91.7$
Total/unique reflections	262892/70511
Completeness (%)	100 (100)
R_{merge}	0.109 (0.605)
$\langle I/\sigma(I) \rangle$	14.0 (2.1)
Multiplicity	3.7 (3.6)
Refinement statistics	
Resolution (Å)	50–1.67 (1.70–1.67)
R_{work} (%)	15.5
R_{free} (%)	18.9
No. of atoms	
Protein	
Chain A	1205
Chain B	1207
Chain C	1197
Chain D	1220
Water	744
Phosphate	20
Mean B factor (Å ²)	
Protein	
Chain A	12.3
Chain B	11.7
Chain C	12.0
Chain D	12.0
Water	26.4
Phosphate	9.8
R.m.s. deviation from ideal geometry	
Bond lengths (Å)	0.009
Bond angles (°)	1.3
MolProbity validation	
Ramachandran plot	
Preferred regions (%)	97.1
Allowed regions (%)	2.9
Outliers (%)	0
MolProbity clash score	4.95 [95th percentile]
MolProbity protein geometry score	1.45 [93rd percentile]
PDB code	4y2e

2.2. Crystallization, data collection and structure determination

Initial crystallization screens were set up using the Gryphon crystallization robot (Art Robbins, Sunnyvale, California, USA). Sitting-drop vapor-diffusion experiments were conducted using IntelliPlates (Art Robbins) incubated at 292 K. Various protein-to-well-solution ratios were set up (1:1, 1:3 and 3:1) at a protein concentration of 20.3 mg ml⁻¹ using the Morpheus crystallization screen (Molecular Dimensions, Altamonte Springs, Florida, USA; Gorrec, 2009). Crystals were only obtained in condition No. 32, which consisted of 0.1 M Buffer System 2 (HEPES/MOPS pH 7.5), 0.09 M nitrate–phosphate–sulfate mixture, 37.5%(v/v) 2-methyl-2,4-pentandiol–polyethylene glycol 1000–polyethylene glycol 3350. Crystals appeared within a span of 7 d. A single crystal was retrieved directly from the crystallization drop using a LithoLoop (Molecular Dimensions) and was flash-cooled by plunging it into liquid nitrogen without any additional cryoprotectant.

X-ray diffraction data were collected remotely on SER-CAT beamline 22-BM of the Advanced Photon Source, Argonne National Laboratory using a MAR 225 CCD detector. A data set from a single crystal was collected using an X-ray wavelength of 1.0 Å, an oscillation angle of 1.0°, a crystal-to-detector distance of 150 mm and an X-ray exposure per image of 1 s. Diffraction data were processed with *HKL-3000* (Minor *et al.*, 2006). Data-collection and refinement statistics are presented in Table 1. Initial phases were obtained by molecular replacement with *MOLREP* (Vagin & Teplyakov, 2010) from the *CCP4* suite of programs (Winn *et al.*, 2011), using the structure of DUSP9/MKP-4 (PDB entry 3lj8; 78% sequence identity; Jeong *et al.*, 2011) as a search model after stripping away all non-protein atoms from the model and replacing non-identical residues with alanine. Based on the Matthews coefficient of 2.3 Å³ Da⁻¹ and solvent content of 46.8%, the asymmetric unit was estimated to contain four molecules (Kantardjieff & Rupp, 2003; Matthews, 1968). Model rebuilding and refinement were conducted with *Coot* (Emsley *et al.*, 2010) and *REFMAC5* (Murshudov *et al.*, 2011) using σ_A -weighted electron-density maps (Read, 1997). Water molecules were located using *ARP/wARP* (Langer *et al.*, 2008), manually inspected and refined with *REFMAC5*. Model validation was performed with *MolProbity* (Chen *et al.*, 2010). The coordinates and structure-factor files were deposited in the Protein Data Bank with accession code 4y2e. Structural homologs were identified using the *PDBeFold* server (Krissinel & Henrick, 2004) and structural alignments were performed using *PDEeFold* and *PyMOL* (v.1.5.0.4; Schrödinger).

3. Results and discussion

3.1. Overall structure

The structure of DUSP7 (Ser141–Ser289/C232S) was refined to a resolution of 1.67 Å with a final working R factor of 15.5% and an R_{free} of 18.9%. Structure validation using *MolProbity* indicates that 96.8% of the residues are located in the Ramachandran favored regions, with no residues flagged as outliers. Four molecules were located in the asymmetric unit, all of which share very similar structures (average r.m.s.d. of ~0.2 Å over 140 C α -atom pairs). Analysis of the protein interfaces between molecules in the asymmetric unit using the *PDBePISA* server suggests that DUSP7 exists as a monomer in solution (Krissinel & Henrick, 2007) which correlates with the gel-filtration chromatographic profile (data not shown). Chain A was selected for structural description. The catalytic domain of DUSP7 consists of a central five-stranded β -sheet that is surrounded by six α -helices (Fig. 1a). A phosphate ion occupies the active site that is formed by the phosphate-binding loop (residues 231–238) positioned between β -strand $\beta 5$ and helix $\alpha 4$. The general acid loop (D loop in DUSPs or WPD loop in the classical PTPs), located between helix $\alpha 3$ and β -strand $\beta 4$, adopts a closed conformation.

A search for related structures using the *PDBeFold* server identified the two closest structural homologs as slingshot

phosphatase 2 (PDB entry 2nta, chain A; 35% sequence identity; r.m.s.d. of 0.8 Å over 141 common C α atoms; Jung *et al.*, 2007) and the human dual-specificity phosphatase DUSP4 (MKP-2; PDB entry 3ezz, chain A; 49% sequence identity; r.m.s.d. of 0.9 Å over 142 common C α atoms; Jeong *et al.*, 2009). Interestingly, DUSP7 exhibited greater structural divergence with other Pyst-type DUSPs that share higher sequence identity such as DUSP6/Pyst1 (PDB entry 1mkp, chain A; 82% sequence identity; r.m.s.d. of 1.6 Å over 131 common C α atoms; Stewart *et al.*, 1999) and DUSP9/MKP-4 (PDB entry 3lj8, chain A; 78% sequence identity; r.m.s.d. of 1.7 Å over 132 common C α atoms; Jeong *et al.*, 2011). The superimposed structures of DUSP7 and DUSP6 reveal that the major difference between the two structures is the presence of an extra β -strand between β -strand β_4 and helix α_3 in DUSP6 as a result of an outward movement of the connecting loop (Fig. 1*b*). The electron density for the loop between strand β_4 and helix α_3 is well defined in DUSP7 (Fig. 1*c*). This loop-to-strand conformational switch is found in the structures of DUSP2, DUSP6 and DUSP9 and was predicted to also exist in DUSP7 (Stewart *et al.*, 1999; Farooq *et al.*, 2003; Jeong *et al.*, 2011, 2014). Proposed to be flexible, this region in

DUSP6 and DUSP9 has been postulated to participate in MAP kinase binding interactions. The crystal structures of the DUSPs that contain this extra β -strand also reveal that the D loop adopts an open conformation, leading to a low-activity conformation of the catalytic domain (Jeong *et al.*, 2014). In the structure of DUSP7, we do not observe such a loop-to-strand switch for the loop found between β -strand β_4 and helix α_3 , likely owing to the closed conformation of the D loop. This loop is also involved in crystal contacts with neighboring symmetry mates in the crystal, which may prevent the switch to a β -strand conformation.

3.2. Active site

The active site contains a phosphate ion that provides a snapshot of the structural interactions with active-site residues (Fig. 2*a*). The phosphate is held in place by several hydrogen bonds between the phosphate O atoms and the backbone amide N atoms of Leu233 (3.1 Å), Ala234 (3.0 Å), Gly235 (3.2 Å), Ile236 (2.9 Å), Ser237 (3.0 Å) and Arg238 (2.8 Å) (Fig. 2*b*). Additional hydrogen bonds are formed between the phosphate O2 atom and the Arg238 N ϵ^2 (2.8 Å) and NH2

Figure 1

(*a*) Stereoview of the three-dimensional structure of DUSP7 (Ser141–Ser289/C232) illustrated as green ribbons. The phosphate-binding loop (P-loop) is located between helix α_4 and strand β_5 and the general acid loop (D loop) is found between strand β_4 and helix α_3 . The bound phosphate ion is highlighted in spheres with O atoms in red and the P atom in orange. (*b*) Stereoview of the superimposed structures of DUSP7 (green ribbons) and the catalytic domain of DUSP6 (Pyst1; PDB entry 1mkp, red ribbons). (*c*) The fit of residues Leu182–Glu191 to the final $2mF_o - DF_c$ electron-density map (blue, 1.67 Å resolution, contoured at the 1σ level).

Figure 2

(a) Stereoview of the fit of the residues forming the active site of DUSP7 to the final $2mF_o - DF_c$ electron-density map (blue, 1.67 Å resolution, contoured at the 1σ level). C atoms are colored green, O atoms red, N atoms blue and the P atom orange. (b) Stereoview illustrating the interactions of the bound phosphate ion with the active-site residues of DUSP7. Hydrogen bonds are indicated by red dashes.

(2.9 Å) N atoms. The Arg238 side chain forms a cradle-like structure around the active site that contributes a favorable positive charge to stabilize the negative charge of the phosphate ion. The side-chain O γ atom of Ser232 (cysteine in the wild-type enzyme) is located about 3.4 Å from the phosphate P atom, indicating that the residue occupies an appropriate position to function as the catalytic nucleophile (Zhou *et al.*, 1994). The side chain of the conserved Asp201 residue in the D loop (residues 197–208) points towards the active-site pocket, enabling it to form a hydrogen bond between the side-chain carboxylate O δ^2 atom and the phosphate O3 atom (2.6 Å). As a result, the D loop adopts a closed conformation that primes the active site for catalysis. Because Asp201 functions as a general acid/base residue during the dephosphorylation reaction, its proper positioning with respect to the substrate is important for efficient catalysis (Denu *et al.*, 1995). Substrate binding induces a conformational change from the open to the closed form of the D loop (Schubert *et al.*, 1995). In contrast, the side chains of the analogous Asp262 and Asp259 residues in DUSP6 and DUSP9, respectively, point away from the active site, resulting in an open conformation of the D loop (Stewart *et al.*, 1999; Jeong *et al.*, 2011). The superimposed structures of DUSP7 and DUSP6 reveal significant deviations between the conformations of their phosphate-binding loops, suggesting that the active sites in DUSP6 and DUSP9 are not oriented optimally for efficient catalysis (Fig. 1b). Indeed, the isolated catalytic domain of DUSP9 was reported to exhibit low catalytic activity (Jeong *et al.*, 2011).

Analysis of the surface features surrounding the active-site region of DUSP7 reveals a relatively smooth surface with very

few significant indentations or neighboring pockets (Fig. 3). This, of course, poses a challenge for the design of multi-dentate and other inhibitors in general. There is a small positively charged pocket directly above the active-site pocket next to the Arg238 residue. To the left of the active site and directly below the Asp201 residue, there is a small negatively charged groove. A recent survey of DUSP structures has shown that surface features in the vicinity of the active-site pockets are variable (Jeong *et al.*, 2014), and it may be possible

Figure 3

The DUSP7 catalytic domain as viewed facing the active-site region with the electrostatic surface potentials mapped onto the surface. Positive charge is colored blue, negative charge red and hydrophobic regions gray.

to exploit these small differences to achieve some degree of inhibitor specificity.

4. Conclusions

In summary, we have determined the crystal structure of the C-terminal catalytic domain of DUSP7 (Ser141–Ser289/C232S) at a resolution of 1.67 Å, which adds to the growing structural knowledge base of DUSP family members. The structure is of particular interest because DUSP7 has been proposed as a drug target for certain malignancies such as leukemia. DUSP7 adopts the canonical catalytic domain fold common to many dual-specificity phosphatases but, interestingly, it differs structurally from related Pyst-type DUSPs in that it lacks an additional β -strand between strand β_4 and helix α_3 and its D loop is observed in the closed conformation. The relatively smooth surfaces adjacent to the active-site pocket underscore the challenges of designing dual-specificity phosphatase inhibitors. It remains to be seen whether differences in surface features near the active sites of DUSP family members will be sufficient to obtain specific inhibitors.

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References

- Bahta, M., Lountos, G. T., Dyas, B., Kim, S.-E., Ulrich, R. G., Waugh, D. S. & Burke, T. R. Jr (2011). *J. Med. Chem.* **54**, 2933–2943.
- Blaskovich, M. A. (2009). *Curr. Med. Chem.* **16**, 2095–2176.
- 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.
- Denu, J. M. & Dixon, J. E. (1995). *Proc. Natl Acad. Sci. USA*, **92**, 5910–5914.
- Denu, J. M., Zhou, G., Guo, Y. & Dixon, J. E. (1995). *Biochemistry*, **34**, 3396–3403.
- Dowd, S., Sneddon, A. A. & Keyse, S. M. (1998). *J. Cell Sci.* **111**, 3389–3399.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Farooq, A., Plotnikova, O., Chaturvedi, G., Yan, S., Zeng, L., Zhang, Q. & Zhou, M.-M. (2003). *Structure*, **11**, 155–164.
- Farooq, A. & Zhou, M.-M. (2004). *Cell. Signal.* **16**, 769–779.
- Gorrec, F. (2009). *J. Appl. Cryst.* **42**, 1035–1042.
- He, R.-J., Yu, Z.-H., Zhang, R.-Y. & Zhang, Z.-Y. (2014). *Acta Pharmacol. Sin.* **35**, 1227–1246.
- Jeong, D. G., Jung, S.-K., Yoon, T.-S., Woo, E.-J., Kim, J. H., Park, B. C., Ryu, S. E. & Kim, S. J. (2009). *Proteins*, **76**, 763–767.
- Jeong, D. G., Wei, C. H., Ku, B., Jeon, T. J., Chien, P. N., Kim, J. K., Park, S. Y., Hwang, H. S., Ryu, S. Y., Park, H., Kim, D. S., Kim, S. J. & Ryu, S. E. (2014). *Acta Cryst.* **D70**, 421–435.
- Jeong, D. G., Yoon, T. S., Jung, S.-K., Park, B. C., Park, H., Ryu, S. E. & Kim, S. J. (2011). *Acta Cryst.* **D67**, 25–31.
- Jung, S.-K., Jeong, D. G., Yoon, T.-S., Kim, J. H., Ryu, S. E. & Kim, S. J. (2007). *Proteins*, **68**, 408–412.
- Kantardjieff, K. A. & Rupp, B. (2003). *Protein Sci.* **12**, 1865–1871.
- Kapust, R. B., Tözsér, J., Fox, J. D., Anderson, D. E., Cherry, S., Copeland, T. D. & Waugh, D. S. (2001). *Protein Eng.* **14**, 993–1000.
- Kim, S.-E., Bahta, M., Lountos, G. T., Ulrich, R. G., Burke, T. R. & Waugh, D. S. (2011). *Acta Cryst.* **D67**, 639–645.
- Krissinel, E. & Henrick, K. (2004). *Acta Cryst.* **D60**, 2256–2268.
- Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.
- Langer, G., Cohen, S. X., Lamzin, V. S. & Perrakis, A. (2008). *Nat. Protoc.* **3**, 1171–1179.
- Levy-Nissenbaum, O., Sagi-Assif, O., Kapon, D., Hantisteanu, S., Burg, T., Raanani, P., Avigdor, A., Ben-Bassat, I. & Witz, I. P. (2003). *Oncogene*, **22**, 7649–7660.
- Levy-Nissenbaum, O., Sagi-Assif, O., Raanani, P., Avigdor, A., Ben-Bassat, I. & Witz, I. P. (2003a). *Cancer Lett.* **199**, 185–192.
- Levy-Nissenbaum, O., Sagi-Assif, O., Raanani, P., Avigdor, A., Ben-Bassat, I. & Witz, I. P. (2003b). *Methods Enzymol.* **366**, 103–113.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Minor, W., Cymborowski, M., Otwinowski, Z. & Chruszcz, M. (2006). *Acta Cryst.* **D62**, 859–866.
- 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.
- Nallamsetty, S., Austin, B. P., Penrose, K. J. & Waugh, D. S. (2005). *Protein Sci.* **14**, 2964–2971.
- Nunes-Xavier, C., Romá-Mateo, C., Ríos, P., Tárrega, C., Cejudo-Marín, R., Tabernero, L. & Pulido, R. (2011). *Anticancer Agents Med. Chem.* **11**, 109–132.
- Orlev, L. N., Ehud, B., Tamar, B. G., Orit, S. A., Yoel, K. & Witz, I. P. (2004). *Immunol. Lett.* **92**, 149–156.
- Park, H.-S., Jeon, J.-Y., Ryu, S.-E. & Kim, S.-J. (2011). *Bull. Korean Chem. Soc.* **32**, 2167–2168.
- Patterson, K. I., Brummer, T., O'Brien, P. M. & Daly, R. J. (2009). *Biochem. J.* **418**, 475–489.
- Puius, Y. A., Zhao, Y., Sullivan, M., Lawrence, D. S., Almo, S. C. & Zhang, Z.-Y. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 13420–13425.
- Read, R. J. (1997). *Methods Enzymol.* **277**, 110–128.
- Ríos, P., Nunes-Xavier, C. E., Tabernero, L., Köhn, M. & Pulido, R. (2014). *Antioxid. Redox Signal.* **20**, 2251–2273.
- Schubert, H. L., Fauman, E. B., Stuckey, J. A., Dixon, J. E. & Saper, M. A. (1995). *Protein Sci.* **4**, 1904–1913.
- Schumacher, M. A., Todd, J. L., Rice, A. E., Tanner, K. G. & Denu, J. M. (2002). *Biochemistry*, **41**, 3009–3017.
- Shin, D.-Y., Ishibashi, T., Choi, T. S., Chung, E., Chung, I. Y., Aaronson, S. A. & Bottaro, D. P. (1997). *Oncogene*, **14**, 2633–2639.
- Stewart, A. E., Dowd, S., Keyse, S. M. & McDonald, N. Q. (1999). *Nature Struct. Biol.* **6**, 174–181.
- Sun, J.-P., Fedorov, A. A., Lee, S.-Y., Guo, X.-L., Shen, K., Lawrence, D. S., Almo, S. C. & Zhang, Z.-Y. (2003). *J. Biol. Chem.* **278**, 12406–12414.
- Tropea, J. E., Cherry, S. & Waugh, D. S. (2009). *Methods Mol. Biol.* **498**, 297–307.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* **D66**, 22–25.
- Winn, M. D. et al. (2011). *Acta Cryst.* **D67**, 235–242.

- Wu, S., Vossius, S., Rahmouni, S., Miletic, A. V., Vang, T., Vazquez-Rodriguez, J., Cerignoli, F., Arimura, Y., Williams, S., Hayes, T., Moutschen, M., Vasile, S., Pellecchia, M., Mustelin, T. & Tautz, L. (2009). *J. Med. Chem.* **52**, 6716–6723.
- Yuvaniyama, J., Denu, J. M., Dixon, J. E. & Saper, M. A. (1996). *Science*, **272**, 1328–1331.
- Zhang, Y.-Y., Wu, J.-W. & Wang, Z.-X. (2011). *Sci. Signal.* **4**, ra88.
- Zhang, Z.-Y., Wang, Y., Wu, L., Fauman, E. B., Stuckey, J. A., Schubert, H. L., Saper, M. A. & Dixon, J. E. (1994). *Biochemistry*, **33**, 15266–15270.
- Zhou, G., Denu, J. M., Wu, L. & Dixon, J. E. (1994). *J. Biol. Chem.* **269**, 28084–28090.