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Expression, purification, crystallization and preliminary X-ray crystallographic analysis of human histidine triad nucleotide-binding protein 2 (hHINT2)

Histidine triad nucleotide-binding protein 2 (HINT2) is a mitochondrial adenosine phosphoramidase mainly expressed in the pancreas, liver and adrenal gland. HINT2 possibly plays a role in apoptosis, as well as being involved in steroid biosynthesis, hepatic lipid metabolism and regulation of hepatic mitochondria function. The expression level of HINT2 is significantly down-regulated in hepatocellular carcinoma patients. To date, endogenous substrates for this enzyme, as well as the three-dimensional structure of human HINT2, are unknown. In this study, human HINT2 was cloned, overexpressed in *Escherichia coli* and purified. Crystallization was performed at 278 K using PEG 4000 as the main precipitant; the crystals, which belonged to the tetragonal space group $P4_12_12$ with unit-cell parameters $a = b = 76.38$, $c = 133.25$ Å, diffracted to 2.83 Å resolution. Assuming two molecules in the asymmetric unit, the Matthews coefficient and the solvent content were calculated to be 2.63 Å³ Da⁻¹ and 53.27%, respectively.

1. Introduction

Histidine triad nucleotide-binding protein 2 (HINT2) belongs to a histidine triad (HIT) protein superfamily consisting primarily of mononucleotide and dinucleotide hydrolases and nucleotide transferases that have a characteristic C-terminal active-site motif HXHXHXX, where X is a hydrophobic residue (Brenner, 2002). HINT homologues are present in a wide variety of organisms including metazoa (Bieganski *et al.*, 2002; Parks *et al.*, 2004; Wu *et al.*, 2010), plants (Guranowski *et al.*, 2011), fungi (Korsisaari *et al.*, 2003) and bacteria (Chou *et al.*, 2005; Bardaweel *et al.*, 2010). HINTs are described as the most conserved members of the HIT superfamily with nucleotide hydrolase activity. The strongly conserved sequences suggest their fundamental role in cell metabolism.

The functions of HINT1 are well characterized, *e.g.* as a nucleoside- and nucleotide-binding protein (Lima *et al.*, 1996; Brenner *et al.*, 1997), as an enzyme with activity as an aminoacyl-adenylate hydrolase (Chou & Wagner, 2007; Chou *et al.*, 2007), a nucleoside-5'-*O*-phosphoramidate phosphoramidase (Bieganski *et al.*, 2002; Murakami *et al.*, 2010) and a desulfurase of 5'-*O*-phosphorothioylated nucleosides (Krakowiak *et al.*, 2007; Ozga, 2010). HINT1 is involved in transcription regulation (Lee *et al.*, 2004; Lee & Razin, 2004; Weiske & Huber, 2006; Cen *et al.*, 2009; Wang *et al.*, 2009) and cell-cycle control (Weiske & Huber, 2005). HINT1 plays a role in psychiatric disorders such as schizophrenia, bipolar disorder, major depression and anxiety (Guang *et al.*, 2004; Vawter *et al.*, 2004; Barbier *et al.*, 2007; Elashoff *et al.*, 2007; Chen *et al.*, 2008; Barbier & Wang, 2009; Liu *et al.*, 2008; Varadarajulu *et al.*, 2011). Recent studies suggest that mammalian HINT1 modulates apoptosis in cancer cells and is a potential tumour suppressor (Su *et al.*, 2003; Li *et al.*, 2006; Weiske & Huber, 2006; Wang *et al.*, 2007, 2009; Hsieh *et al.*, 2009; Zhang *et al.*, 2009; Zambelli *et al.*, 2010). The crystal structure of HINT1 has been known for 17 years (Lima *et al.*, 1996). HINT1 was initially described as an inhibitor of protein kinase C (Pearson *et al.*, 1990) and further as PKCI (protein kinase C-interacting; Klein *et al.*, 1998). Finally, the 'HINT' name was coined after structural analysis performed by Brenner *et al.* (1997). Deposited structures display

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HINT1 in the apo state (Lima *et al.*, 1996) or as complexes with selected ligands: substrate analogues or products of their hydrolysis (Lima *et al.*, 1997; Krakowiak *et al.*, 2007; Bardaweel *et al.*, 2010). To date, the highest registered data resolution was 1.38 Å for human (Dolot *et al.*, 2012) and 1.10 Å for rabbit (Dolot *et al.*, 2011) HINT1 homologues. In contrast to HINT1, data concerning HINT2 protein are very limited.

HINT2 is a mammalian-specific 16.9 kDa nuclear-encoded mitochondrial HINT hydrolase that is predominantly expressed in the liver, pancreas and adrenal gland, and is localized in the mitochondrial matrix. The HINT2 gene (GenBank AF356515) at human 9p13.2 encodes a 163 amino-acid polypeptide with a 35 amino-acid N-terminal extension when aligned with HINT1, which corresponds to a predicted mitochondrial import signal (Claros & Vincens, 1996).

Over the range of 128 alignable residues, HINT2 is 61% identical to HINT1 (Fig. 1). Based on the high homology of HINT2 and HINT1, HINT2 was tested for adenosine phosphoramidase activity using a model compound AMP-pNA. The measured activity, characterized by the k_{cat}/K_m of HINT2, was tenfold higher than that of HINT1 (Bieganski *et al.*, 2002). The mutation of H149A in the HIT motif of HINT2 resulted in the loss of adenosine phosphoramidase activity (Martin *et al.*, 2006). HINT2 protein sensitizes cells to apoptosis and is down-regulated in hepatocellular carcinoma (Martin *et al.*, 2006). HINT2 also plays a role in biosynthesis of steroid compounds (*e.g.* pregnenolone) in H295R adrenocortical cells. Silencing of HINT2 with specific siRNA gives a reduced steroidogenic response, while overexpression of HINT2 did not change the pregnenolone secretion. Similar effects were observed for the mitochondrial calcium signal.

Figure 1

Multiple sequence alignment of the HINT homologues selected for the MR search, performed using the *PRALINE* server (Simossis & Heringa, 2005). Secondary-structure prediction was calculated using *DSSP* (Kabsch & Sander, 1983) and *PSIPRED* (Jones, 1999).

These findings suggest that HINT2 is required for an optimal steroidogenic response to both Ca^{2+} -dependent and Ca^{2+} -independent agonists but its role is not rate limiting (Lenglet *et al.*, 2008). The recent studies of Martin and coworkers demonstrate that HINT2 contributes to the normal function of hepatic mitochondria. Positive regulation of lipid metabolism, mitochondrial respiration, glucose tolerance and response to fasting can be partially explained by a modulation of the extent of acetylation of selected mitochondrial proteins (Martin *et al.*, 2013).

In the present study, we report the crystallization and preliminary X-ray diffraction studies of human histidine triad nucleotide-binding protein 2 (hHINT2), a mammalian-specific mitochondria-located HINT homologue. The three-dimensional structure, which is being determined by molecular replacement, will provide a molecular insight into the HINT2 structure and its possible function.

2. Materials and methods

2.1. Cloning of hHINT2

The mRNA fraction was isolated from HEK293T cells using TriPure Isolation Reagent (Roche). HINT2 cDNA was amplified using the forward primer 5'-TCTTCTCCATGGCGGCAGCCGTGGTGCT and the reverse primer 5'-TCTTCTCTCGAGTCAACCTGGAGGCCACTGGAG in a one-step RT-PCR (reverse transcriptase polymerase chain reaction). The PCR product was digested with *Nco*I and *Xho*I restriction enzymes (EURx). Restriction sites for *Nco*I and *Xho*I are indicated in bold and italics, respectively. The PCR product was purified and cloned into a pHAT2 expression vector (EMBL Heidelberg, Germany). Positive clones of *Escherichia coli* TOP10 cells (Invitrogen) were analysed by colony PCR and the insert-containing vectors were sequenced to identify vectors containing insert (CoreLab, Medical University of Łódź, Poland).

2.2. Expression and purification

The *E. coli* BL21 (DE3) Star competent cells were transformed with the pHAT2/hHINT2 construct to obtain the hHINT2 protein

with a hexahistidine tag (MSHHHHHHS) at the N-terminus. Fresh Luria-Bertani (LB) medium (containing $35 \mu\text{g ml}^{-1}$ of kanamycin) was inoculated with the overnight culture and cells were grown at 310 K, with shaking at 200 rev min^{-1} . When the optical density measured at a wavelength of 600 nm (OD_{600}) reached 0.6, the culture was chilled on ice, induced by the addition of 1 mM IPTG and then incubated at 295 K with shaking at 200 rev min^{-1} overnight. The cells were harvested by centrifugation, resuspended in buffer A (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole pH 5.3) and disrupted by sonication on ice ($20 \times 10 \text{ s}$ intervals). The cell lysates were centrifuged at 12 000g for 30 min and the collected supernatant was gently agitated with Ni-NTA agarose resin (Qiagen) for 1 h. The resin with bound protein was applied onto a column, washed with buffer B (50 mM Tris-HCl, 300 mM NaCl, 50 mM imidazole pH 6.5) and the hHINT2 recombinant protein was eluted with buffer C (50 mM Tris-HCl, 300 mM NaCl, 250 mM imidazole pH 7.5). The eluted fraction containing protein was dialysed against buffer consisting of 150 mM NaCl, 25 mM Tris-HCl pH 7.5. The purity of the protein was judged by SDS-PAGE analysis (Fig. 2). The protein identity was confirmed by peptide mass fingerprinting on an ESI Waters Q-TOF Premier Micromass electrospray spectrometer after tryptic digestion (CoreLab, Medical University of Łódź, Poland). Finally, the protein was concentrated to 5 mg ml^{-1} using Amicon centrifugal filters (Millipore) ($<10 \text{ kDa}$ cutoff).

Figure 2
SDS-PAGE showing purification on a Ni^{2+} -affinity column. Lane 1, supernatant of sonicated His₆-hHINT2 just before loading onto the Ni^{2+} -affinity column. Lane 2, the purified His₆-hHINT2 after washing from the column. This fraction was concentrated and used for crystallization trials.

Figure 3
(a) Crystals grown in 30% (w/v) PEG 4000, 0.1 M HEPES pH 7.5, 0.2 M CaCl_2 . The approximate dimensions of the crystals are $50 \times 20 \times 20 \mu\text{m}$. (b) Crystals grown in 25% (w/v) PEG 4000, 0.1 M Tris-HCl pH 8.5, 0.2 M CaCl_2 . The approximate dimensions of the crystals are $100 \times 50 \times 50 \mu\text{m}$. These crystals were used for diffraction experiments.

Table 1

Crystallographic parameters and data-collection statistics.

Values in parentheses are for the outermost resolution shell.

X-ray source	Beamline BL14.1, BESSY
Wavelength (Å)	0.91841
Detector	Rayonics MX-225
Crystal-to-detector distance (mm)	340
Oscillation width (°)	1.0
Temperature (K)	100
No. of frames	120
Space group	<i>P</i> 4 ₁ 2 ₁ 2
Unit-cell parameters	
<i>a</i> = <i>b</i> (Å)	76.38
<i>c</i> (Å)	133.25
Total No. of reflections	91871 (7535)
Unique reflections	9888 (934)
Completeness (%)	99.7 (98.1)
Resolution (Å)	41.96–2.83 (2.93–2.83)
<i>R</i> _{merge} [†]	0.108 (0.590)
Multiplicity	9.3 (8.1)
Mosaicity (°)	0.26
Wilson <i>B</i> factor (Å ²)	41.3
<i>I</i> / <i>σ</i> (<i>I</i>)	18.2 (4.2)

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an individual measurement of the reflection and $\langle I(hkl) \rangle$ is the mean intensity of the reflection.

2.3. Crystallization

Screening of crystallization conditions was carried out by mixing 1 µl protein solution and 1 µl reservoir solution in 24-well VDX plates (Hampton Research) using the hanging-drop method. The crystallization screening kits JBScreen Classic 1–10 (Jena Biosciences) were used for crystallization trials. The initial His₆-hHINT2 crystals appeared using solutions from JBScreen Classic 2 consisting of polyethylene glycol 4000 as the main precipitant. After approximately 2 months, growth of the most promising crystals was observed

Figure 4

X-ray diffraction image of hHINT2 crystal. Resolution rings from the centre outwards correspond to 40.00, 20.00, 8.00, 4.00 and 2.83 Å. This figure was prepared using ADXV (<http://www.scripps.edu/~arvai/adxv/>).

in two conditions: C4 [25% (w/v) PEG 4000, 0.1 M Tris–HCl pH 8.5, 0.2 M CaCl₂; Fig. 3a] and D2 [30% (w/v) PEG 4000, 0.1 M HEPES pH 7.5, 0.2 M CaCl₂; Fig. 3b]. All crystallization trials were performed at 278 K. Single crystals of His₆-hHINT2 obtained in condition C4, with the shape of tetragonal bipyramids, were used for further diffraction experiments. The further optimization of crystallization conditions is in progress.

2.4. X-ray data collection and processing

Diffraction data were collected using a single crystal at a wavelength of 0.91841 Å at 100 K on beamline BL14.1 at the Berliner Elektronenspeicherring-Gesellschaft für Synchrotronstrahlung (BESSY), Berlin, equipped with a Rayonics MX-225 detector (Mueller *et al.*, 2012). Crystals of HINT2 did not need cryoprotectant, but had to be mounted on a very thin film from the crystallization buffer. The excess liquid was removed by gently touching the mounting loop to the plate surface and the crystal was then flash-cooled directly in the N₂ stream. A complete data set was collected to 2.83 Å resolution (Fig. 4). The oscillation angle was 1°. The data were processed, integrated and scaled with XDS (Kabsch, 2010) and SCALA (Evans, 2006) from the CCP4 suite (Winn *et al.*, 2011). The data-collection and processing statistics are given in Table 1.

3. Results and discussion

Full-length hHINT2 was successfully cloned and expressed in *E. coli* and purified to homogeneity with an N-terminal His₆ tag. The obtained crystal belonged to the tetragonal space group *P*4₁2₁2, with unit-cell parameters *a* = *b* = 76.38, *c* = 133.25 Å. The overall *R*_{merge} was 10.8% and it was 59.0% in the outer shell. The Matthews coefficient *V*_M (Matthews, 1968) was 2.63 Å³ Da^{−1}, corresponding to a solvent content of approximately 53% and suggesting the presence of two hHINT2 molecules per asymmetric unit.

A preliminary structure of the hHINT2 model was constructed with BALBES, a molecular-replacement (MR) pipeline (Long *et al.*, 2008). 21 structures from the PDB were found to have above 15% similarity to a given hHINT2 sequence and BALBES, and five of them were used for further calculations: PDB entries 1kpf (human HINT1; 59.5% similarity; Lima *et al.*, 1997), 3o1c (rabbit HINT1; 60.0% similarity; Ozga *et al.*, 2010), 3n1s (*E. coli* HINT; 47.6% similarity; Bardaweel *et al.*, 2010), 3oj7 (HIT family protein from *Entamoeba histolytica*; 40.2% similarity; Seattle Structural Genomics Center for Infectious Disease, unpublished work) and 1xqu (HIT family hydrolase from *Clostridium thermocellum* Cth-393; 38.8% similarity; Southeast Collaboratory for Structural Genomics, unpublished work). The solution based on the 3o1c model, with the highest quality factor *Q* = 0.579 and a correct solution probability of 92.29%, showed two molecules in the asymmetric unit, shifted by a translation vector. Final model building by restrained refinement using REFMAC5 (Murshudov *et al.*, 2011) alternating with manual refinement using Coot (Emsley *et al.*, 2010) is in progress.

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References

- Barbier, E. & Wang, J. B. (2009). *BMC Neurosci.* **10**, 132.
- Barbier, E., Zapata, A., Oh, E., Liu, Q., Zhu, F., Undie, A., Shippenberg, T. & Wang, J. B. (2007). *Neuropsychopharmacology*, **32**, 1774–1782.
- Bardaweel, S., Pace, J., Chou, T.-F., Cody, V. & Wagner, C. R. (2010). *J. Mol. Biol.* **404**, 627–638.

- Bieganski, P., Garrison, P. N., Hodawadekar, S. C., Faye, G., Barnes, L. D. & Brenner, C. (2002). *J. Biol. Chem.* **277**, 10852–10860.
- Brenner, C. (2002). *Biochemistry*, **41**, 9003–9014.
- Brenner, C., Garrison, P., Gilmour, J., Peisach, D., Ringe, D., Petsko, G. A. & Lowenstein, J. M. (1997). *Nature Struct. Biol.* **4**, 231–238.
- Cen, B., Li, H. & Weinstein, I. B. (2009). *J. Biol. Chem.* **284**, 5265–5276.
- Chen, Q., Wang, X., O'Neill, F. A., Walsh, D., Kendler, K. S. & Chen, X. (2008). *Schizophr. Res.* **106**, 200–207.
- Chou, T.-F., Bieganski, P., Shilinski, K., Cheng, J., Brenner, C. & Wagner, C. R. (2005). *J. Biol. Chem.* **280**, 15356–15361.
- Chou, T.-F., Tikh, I. B., Horta, B. A. C., Ghosh, B., De Alencastro, R. B. & Wagner, C. R. (2007). *J. Biol. Chem.* **282**, 15137–15147.
- Chou, T.-F. & Wagner, C. R. (2007). *J. Biol. Chem.* **282**, 4719–4727.
- Claros, M. G. & Vincens, P. (1996). *Eur. J. Biochem.* **241**, 779–786.
- Dolot, R., Ozga, M., Krakowiak, A. & Nawrot, B. (2011). *Acta Cryst.* **D67**, 601–607.
- Dolot, R., Ozga, M., Włodarczyk, A., Krakowiak, A. & Nawrot, B. (2012). *Acta Cryst.* **F68**, 883–888.
- Elashoff, M., Higgs, B. W., Yolken, R. H., Knable, M. B., Weis, S., Webster, M. J., Barci, B. M. & Torrey, E. F. (2007). *J. Mol. Neurosci.* **31**, 221–243.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Evans, P. (2006). *Acta Cryst.* **D62**, 72–82.
- Guang, W., Wang, H., Su, T., Weinstein, I. B. & Wang, J. B. (2004). *Mol. Pharmacol.* **66**, 1285–1292.
- Guranowski, A., Wojdyla, A. M., Rydzik, A. M., Stepinski, J. & Jemielity, J. (2011). *Acta Biochim. Pol.* **58**, 131–136.
- Hsieh, S.-Y., Hsu, C.-Y., He, J.-R., Liu, C.-L., Lo, S.-J., Chen, Y.-C. & Huang, H.-Y. (2009). *J. Proteome Res.* **8**, 3977–3986.
- Jones, D. T. (1999). *J. Mol. Biol.* **292**, 195–202.
- Kabsch, W. (2010). *Acta Cryst.* **D66**, 125–132.
- Kabsch, W. & Sander, C. (1983). *Biopolymers*, **22**, 2577–2637.
- Klein, M. G., Yao, Y., Slosberg, E. D., Lima, C. D., Doki, Y. & Weinstein, I. B. (1998). *Exp. Cell Res.* **244**, 26–32.
- Korsisaari, N., Rossi, D. J., Luukko, K., Huebner, K., Henkemeyer, M. & Mäkelä, T. P. (2003). *Mol. Cell. Biol.* **23**, 3929–3935.
- Krakowiak, A., Kaczmarek, R., Baraniak, J., Wiczorek, M. & Stec, W. J. (2007). *Chem. Commun.* **21**, 2163–2165.
- Lee, Y.-N., Nechushtan, H., Figov, N. & Razin, E. (2004). *Immunity*, **20**, 145–151.
- Lee, Y.-N. & Razin, E. (2004). *Mol. Cell. Biol.* **25**, 8904–8912.
- Lenglet, S., Antigny, F., Vetterli, L., Dufour, J. F. & Rossier, M. F. (2008). *Endocrinology*, **149**, 5461–5469.
- Li, H., Zhang, Y., Su, T., Santella, R. M. & Weinstein, I. B. (2006). *Oncogene*, **25**, 713–721.
- Lima, C. D., Klein, M. G. & Hendrickson, W. A. (1997). *Science*, **278**, 286–290.
- Lima, C. D., Klein, M. G., Weinstein, I. B. & Hendrickson, W. A. (1996). *Proc. Natl Acad. Sci.* **93**, 5357–5362.
- Liu, Q., Puche, A. C. & Wang, J. B. (2008). *Neurochem. Res.* **33**, 1263–1276.
- Long, F., Vagin, A. A., Young, P. & Murshudov, G. N. (2008). *Acta Cryst.* **D64**, 125–132.
- Martin, J., Magnino, F., Schmidt, K., Piguet, A.-C., Lee, J.-S., Semela, D., St-Pierre, M. V., Ziemiecki, A., Cassio, D., Brenner, C., Thorgeirsson, S. S. & Dufour, J.-F. (2006). *Gastroenterology*, **130**, 2179–2188.
- Martin, J., Maurhofer, O., Bellance, N., Benard, G., Graber, F., Hahn, D., Galinier, A., Hora, C., Gupta, A., Ferrand, G., Hoppeler, H., Rossignol, R., Dufour, J.-F. & St-Pierre, M. V. (2013). *Hepatology*, **57**, 2037–2048.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Mueller, U., Darowski, N., Fuchs, M. R., Förster, R., Hellmig, M., Paithankar, K. S., Pühringer, S., Steffien, M., Zocher, G. & Weiss, M. S. (2012). *J. Synchrotron Rad.* **19**, 442–449.
- Murakami, E., Tolstykh, T., Bao, H., Niu, C., Steuer, H. M., Bao, D., Chang, W., Espiritu, C., Bansal, S., Lam, A. M., Otto, M. J., Sofia, M. J. & Furman, P. A. (2010). *J. Biol. Chem.* **285**, 34337–34347.
- 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.
- Ozga, M. (2010). *Postępy Biochem.* **56**, 55–60.
- Ozga, M., Dolot, R., Janicka, M., Kaczmarek, R. & Krakowiak, A. (2010). *J. Biol. Chem.* **285**, 40809–40818.
- Parks, K. P., Seidle, H., Wright, N., Sperry, J. B., Bieganski, P., Howitz, K., Wright, D. L. & Brenner, C. (2004). *Physiol. Genomics*, **20**, 12–14.
- Pearson, J. D., DeWald, D. B., Mathews, W. R., Mozier, N. M., Zürcher-Neely, H. A., Heinrikson, R. L., Morris, M. A., McCubbin, W. D., McDonald, J. R., Fraser, E. D., Kay, C. M. & Walsh, M. P. (1990). *J. Biol. Chem.* **265**, 4583–4591.
- Simossis, V. A. & Heringa, J. (2005). *Nucleic Acids Res.* **33**, W289–W294.
- Su, T., Suzui, M., Wang, L., Lin, C.-S., Xing, W.-Q. & Weinstein, I. B. (2003). *Proc. Natl Acad. Sci.* **100**, 7824–7829.
- Varadarajulu, J., Lebar, M., Krishnamoorthy, G., Habelt, S., Lu, J., Weinstein, I. B., Li, H., Holsboer, F., Turck, C. W. & Touma, C. (2011). *Behav. Brain Res.* **220**, 305–311.
- Vawter, M. P., Weickert, C. S., Ferran, E., Matsumoto, M., Overman, K., Hyde, T. M., Weinberger, D. R., Bunney, W. E. & Kleinman, J. E. (2004). *Neurochem. Res.* **29**, 1245–1255.
- Wang, L., Li, H., Zhang, Y., Santella, R. M. & Weinstein, I. B. (2009). *Int. J. Cancer*, **124**, 1526–1534.
- Wang, L., Zhang, Y., Li, H., Xu, Z., Santella, R. M. & Weinstein, I. B. (2007). *Cancer Res.* **67**, 4700–4708.
- Weiske, J. & Huber, O. (2005). *J. Cell Sci.* **118**, 3117–3129.
- Weiske, J. & Huber, O. (2006). *J. Biol. Chem.* **281**, 27356–27366.
- Winn, M. D. et al. (2011). *Acta Cryst.* **D67**, 235–242.
- Wu, L., Wu, X., Deng, H. & Huang, Y. (2010). *Dev. Comput. Immunol.* **34**, 76–83.
- Zambelli, D., Zuntini, M., Nardi, F., Manara, M. C., Serra, M., Landuzzi, L., Lollini, P. L., Ferrari, S., Alberghini, M., Lombart-Bosch, A., Piccolo, E., Iacobelli, S., Picci, P. & Scotlandi, K. (2010). *Int. J. Cancer*, **126**, 41–52.
- Zhang, Y.-J., Li, H., Wu, H.-C., Shen, J., Wang, L., Yu, M.-W., Lee, P.-H., Weinstein, I. B. & Santella, R. M. (2009). *Cancer Lett.* **275**, 277–284.