Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray analysis of a putative sensor histidine kinase domain: the C-terminal domain of HksP4 from *Aquifex aeolicus* VF5

Shoichiro Horita,[a]‡ Yosuke Yamanaka,[a]‡ Akihiro Yamamura,[a] Akitoshi Okada,[a] Jiro Nakayama,[b] Koji Nagata[a] and Masaru Tanokura[a]*

[a]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, and [b]Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581, Japan

‡ These authors contributed equally to this work.

Correspondence e-mail: amtanok@mail.ecc.u-tokyo.ac.jp

Received 1 April 2011
Accepted 15 May 2011

The histidine kinase domain of the cytoplasmic protein HksP4 from the hyperthermophilic bacterium *Aquifex aeolicus* VF5, located in the C-terminal half of the protein, was expressed, purified and crystallized. Diffraction-quality crystals were obtained in the presence of adenosine triphosphate (ATP) or adenosine 5′-(β,γ-imido)triphosphate (AMPPNP) by the sitting-drop vapour-diffusion method using PEG 3350 as the precipitant. The crystals obtained in the presence of ATP and AMPPNP diffracted X-rays to 3.1 and 2.9 Å resolution, respectively, on BL-5A at Photon Factory (Ibaraki, Japan) and were found to belong to the same space group $P2_12_12_1$, with unit-cell parameters a = 80.2, b = 105.5, c = 122.0 Å and a = 81.5, b = 105.5, c = 130.9 Å, respectively. Their Matthews coefficients (V_M = 2.74 and 2.51 Å^3 Da^{-1}, respectively) indicated that both crystals contained four protein molecules per asymmetric unit.

1. Introduction

Phosphorylation-mediated signal transduction to transfer stimuli is common to nearly all living cells. In higher eukaryotes serine, threonine and tyrosine residues serve as substrates for phosphorylation, whereas in prokaryotes and lower eukaryotes histidine residues serve as substrates for phosphorylation, which is catalyzed by histidine kinases (Marina *et al.*, 2001). Prokaryotes, yeasts and plants have two-component regulatory systems that allow them to sense and respond to many different environmental changes, including changes in temperature, osmolarity, chemoattractants and pH. The systems typically consist of a membrane-bound or cytoplasmic histidine kinase that senses a specific environmental stimulus and a corresponding response regulator that mediates the cellular response, mostly through differential expression of target genes (Stock *et al.*, 2000). Signal transduction occurs through the transfer of phosphate groups from adenosine triphosphate (ATP) to a specific histidine residue in the histidine kinase (autophosphorylation). Subsequently, the histidine kinase catalyzes the transfer of the phosphate group on the phosphorylated histidine residue to an aspartic acid residue in the response regulator, which causes a change in the conformation of the response regulator and leads to the stimulation or repression of expression of target gene(s).

Almost all histidine kinases exist as a part (domain) of a protein and can be divided into two subdomains: a histidine-containing phosphotransfer domain (which also serves as a dimerization domain) and an ATP-binding catalytic domain. To date, ten crystal structures have been reported, including four whole histidine kinase domains (CheA, Bilwes *et al.*, 1999, 2001; TM0853, Marina *et al.*, 2005; KinB, Bick *et al.*, 2009; ThkA, Yamada *et al.*, 2009), one histidine-containing phosphotransfer domain (EnvZ, Tomomori *et al.*, 1999) and five ATP-binding catalytic domains (EnvZ, Tanaka *et al.*, 1998; PhoQ, Marina *et al.*, 2001, Guarnieri *et al.*, 2008; NRII, Song *et al.*, 2004; QseC, Xie *et al.*, 2010; PrrB, E. Nowak, S. Panjikar & P. Tucker, unpublished work). Here, we report the crystallization and preliminary X-ray diffraction analysis of the histidine kinase domain of the cytoplasmic protein HksP4 from the hyperthermophilic bacterium

Aquifex aeolicus VF5. The HksP4 protein, which consists of 339 amino-acid residues, is composed of a signal-sensing PAS (Per–Arnt–Sim) domain (Ponting & Aravind, 1997) and a histidine kinase domain in its N- and C-terminal halves, respectively.

The histidine kinase domain of HksP4, hereafter referred to as AaHK, consists of 230 amino-acid residues and shows 33 and 24% sequence identity to the histidine kinase domains of TM0853 from *Thermotoga maritima* and KinB from *Geobacillus stearothermophilus*, respectively (Fig. 1), the crystal structures of which have already been solved (Marina *et al.*, 2005; Bick *et al.*, 2009; Casino *et al.*, 2009). Here, we describe the overexpression, purification, crystallization and preliminary X-ray diffraction analysis of AaHK.

2. Materials and methods

2.1. Overproduction and purification of AaHK

The gene encoding the histidine kinase domain of HksP4 (Protein ID AAC06560.1) derived from *A. aeolicus* VF5 (AaHK; gi:6626248) was amplified by PCR and cloned into the *Nde*I/*Bam*HI site of pET-26b(+) vector (Novagen). The PCR primers used were 5′-CGCGGCCG**CATATG**AAAAACAATAAAGAACGACAAGGAAA-3′ (including an *Nde*I site, shown in bold) and 5′-CGC**GGATCC**TTATTAAGAGAGGGGAAGGTGCACTTCA-3′ (including a *Bam*HI site, shown in bold). The DNA sequence of the AaHK-encoding region of the resulting plasmid was verified. AaHK without any tag was overexpressed in *Escherichia coli* KRX (Promega) grown in 1 l TB medium at 310 K. Protein expression was induced by the addition of α-L-rhamnose to a final concentration of 0.1%(*w*/*v*) when the OD_{600} reached ∼0.6 and the culture was continued for a further 15 h at 310 K. Harvested cells were resuspended in 20 m*M* MES–NaOH pH 6.0, 100 m*M* NaCl and 0.5 m*M* EDTA and then disrupted by sonication on ice. The lysate was centrifuged at 13 000*g* at 277 K for 60 min to obtain the soluble fraction. The following purification procedures were performed at room temperature (∼293 K) unless stated otherwise. After heating the soluble fraction to 353 K for 30 min, it was centrifuged at 40 000*g* at 277 K for 30 min to remove the aggregated heat-labile proteins. The supernatant was loaded onto a column of Blue Sepharose 6 Fast Flow (GE Healthcare) equilibrated with the same buffer as above. The column was washed with the same buffer to remove nonspecifically bound proteins and the bound AaHK was then eluted with a linear gradient of 100–1000 m*M* NaCl in 20 m*M* MES–NaOH pH 6.0 and 0.5 m*M* EDTA. The eluate was desalted by dialysis against 10 m*M* Tris–HCl pH 8.0 at 277 K. The desalted AaHK was further purified by anion-exchange chromatography using a HiTrap DEAE FF column (GE Healthcare) with a linear gradient of 0–1 *M* NaCl in 10 m*M* Tris–HCl pH 8.0 followed by size-exclusion chromatography using a Superdex 200 HR 10/30 column (GE Healthcare) equilibrated with 10 m*M* Tris–HCl pH 8.0. The purity of AaHK was assessed by 12% SDS–PAGE. The gel was stained with Coomassie Brilliant Blue R-250. The purified AaHK was then concentrated to approximately 10 mg ml^{-1} using a 20 ml Vivaspin concentrator (10 kDa cutoff, Vivascience). Protein concentrations were determined based on the calculated molar extinction coefficient (ε_{280} = 8940 M^{-1} cm^{-1}) and the absorbance at 280 nm obtained with a Nano Drop ND-1000 (Thermo Scientific).

2.2. Crystallization, data collection and preliminary X-ray analysis

Initial crystallization screening was performed using commercially available kits, namely Crystal Screen HT, Index HT (Hampton Research) and Wizard Screens I and II (Emerald BioSystems), at 293 K by high-throughput crystallization screening using 96-well Intelli-Plates (Art Robbins). Before crystallization, aliquots of concentrated AaHK solution (approximately 10 mg ml^{-1}) were supplemented with final concentrations of 5 m*M* ATP and 5 m*M* $MgCl_2$ or final concentrations of 5 m*M* adenosine 5′-(β,γ-imido)triphosphate (AMPPNP) and 5 m*M* $MgCl_2$. All crystallization droplets were set up manually. A sitting drop was prepared by mixing 1.0 µl protein solution and 1.0 µl reservoir solution and was equilibrated against

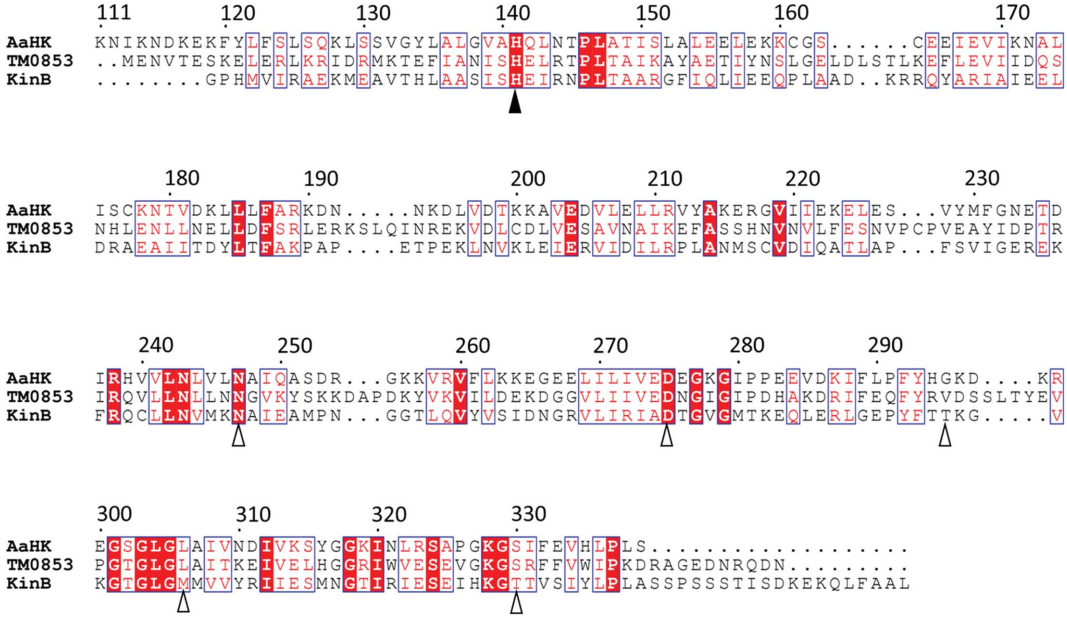


Figure 1
Sequence alignment of AaHK homologues. In AaHK, the black and white triangles represent the putative histidine residue to be phosphorylated in the histidine-containing phosphotransfer domain and the amino-acid residues that putatively interact with ATP in the ATP-binding catalytic domain, respectively. The above-mentioned residues are predicted based on the sequence alignment obtained with *ClustalW* (Thompson *et al.*, 1994) and on interaction analysis of the structures of the TM0853–ADP and KinB–ADP complexes using the *PISA* server (Krissinel & Henrick, 2007). This figure was prepared with *ESPript* (Gouet *et al.*, 1999).

70 µl reservoir solution. Crystallization conditions were improved to obtain crystals of better quality by two-dimensional grid optimization of pH and PEG 3350 concentration using 24-well Cryschem Plates (Hampton Research). A sitting drop was prepared by mixing 1.0 µl protein solution and 1.0 µl reservoir solution and was equilibrated against 400 µl reservoir solution at 293 K. Crystallization conditions were further optimized using Additive Screen HT (Hampton Research). The reservoir solution was prepared by mixing 63 µl optimized reservoir solution (1.1-fold concentration) and 7 µl of each additive solution (tenfold concentration). A sitting drop was prepared by mixing 1.0 µl protein solution and 1.0 µl reservoir solution and was equilibrated against 70 µl reservoir solution at 293 K using a 96-well Intelli-Plate (Art Robbins). The final crystallization conditions were thus determined. Finally, a sitting drop was prepared by mixing 1.0 µl protein solution and 1.0 µl reservoir solution and was equilibrated against 400 µl reservoir solution containing the appropriate additive at 293 K using a 24-well Cryschem Plate (Hampton Research). Crystals were picked up in a mounting loop and frozen in a cold nitrogen-gas stream. X-ray diffraction experiments were performed on beamlines NW-12A and BL-5A of Photon Factory (Ibaraki, Japan). The best diffraction data were obtained on beamline BL-5A as follows: 360 images were collected with a wavelength of 1.0000 Å, a crystal-to-detector distance of 257.63 mm, a rotation angle of 0.5° and an exposure time of 2.5 s per image using an ADSC Quantum 210 detector. The data were indexed and scaled using the program package *XDS* (Kabsch, 2010).

3. Results and discussion

AaHK was expressed in *E. coli* KRX (Promega) and was purified by heat treatment (353 K, 30 min) followed by three steps of column chromatography using Blue Sepharose 6 Fast Flow, HiTrap DEAE FF and Superdex 200 HR 10/30 (GE Healthcare). The SDS–PAGE gel of purified AaHK is shown in Fig. 2. Approximately 12.8 mg purified AaHK was obtained per litre of *E. coli* culture. Three types of AaHK sample containing approximately 10 mg ml^{-1} AaHK were used for crystallization experiments: (i) without any nucleotide, (ii) with 5 m*M* ATP–Mg and (iii) with 5 m*M* AMPPNP–Mg. Several reservoir solutions containing PEG 3350 as the precipitant gave protein crystals. In addition, sodium thiocyanate was shown to be effective as an additive. Finally, diffraction-quality crystals were obtained using a reservoir solution consisting of 0.2 *M* HEPES–NaOH pH 8.1, 20%(*w*/*v*) PEG 3350 and 0.2 *M* sodium thiocyanate in the presence of 5 m*M* ATP–Mg and also using a reservoir solution consisting of 0.2 *M* Tris–HCl pH 7.5, 20%(*w*/*v*) PEG 3350 and 0.2 *M* sodium thiocyanate in the presence of 5 m*M* AMPPNP–Mg. Diffraction-quality crystals were obtained in the presence of ATP–Mg or AMPPNP–Mg but not in the absence of ATP or its analogue. The crystals grown in the presence of ATP–Mg typically grew to final dimensions of 0.5 × 0.1 × 0.08 mm in two weeks at 293 K (Fig. 3*a*) and diffracted X-rays to

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Nucleotide used for crystallization	ATP–Mg	AMPPNP–Mg
X-ray source	Photon Factory BL-5A	Photon Factory BL-5A
Wavelength (Å)	1.0000	1.0000
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 81.5, b = 105.5, c = 130.9	a = 80.2, b = 105.5, c = 122.0
Solvent content (%)	55.2	51.1
Resolution range (Å)	20.0–3.10 (3.18–3.10)	20.0–2.90 (2.98–2.90)
No. of observed reflections	296541 (18185)	333336 (24435)
No. of unique reflections	20817 (1444)	23348 (1713)
Data completeness (%)	98.6 (94.4)	99.4 (99.1)
$R_{\rm merge}$†	0.095 (0.406)	0.085 (0.662)
Multiplicity	14.2 (12.6)	14.3 (14.3)
$\langle I/\sigma(I)\rangle$	22.79 (6.31)	24.56 (4.52)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

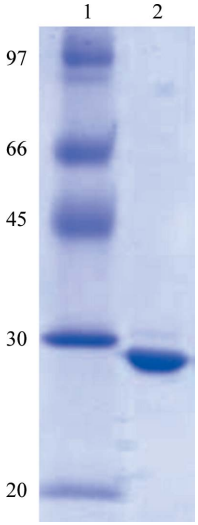
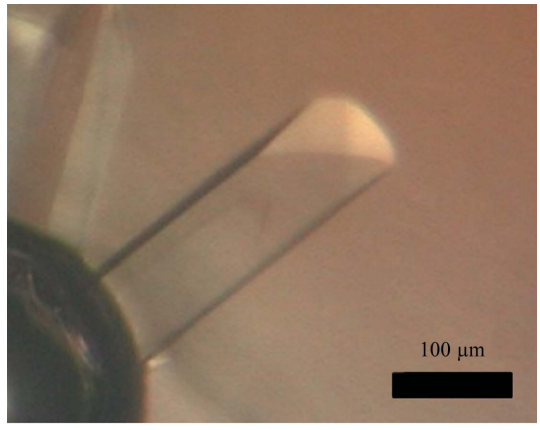
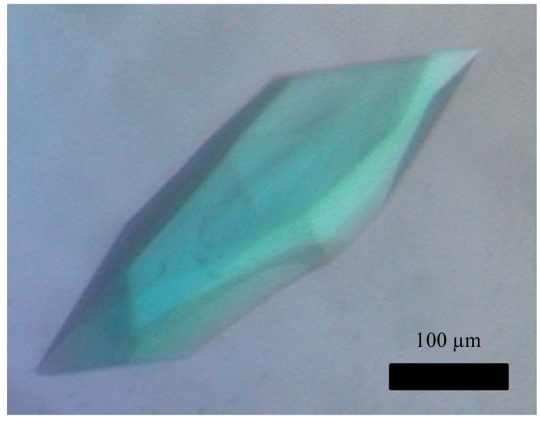


Figure 2
SDS–PAGE of purified AaHK. Lane 1, molecular-mass markers (kDa); lane 2, purified AaHK sample.



(*a*)



(*b*)

Figure 3
Diffraction-quality crystal(s) of AaHK obtained in the presence of ATP–Mg (*a*) or AMPPNP–Mg (*b*).

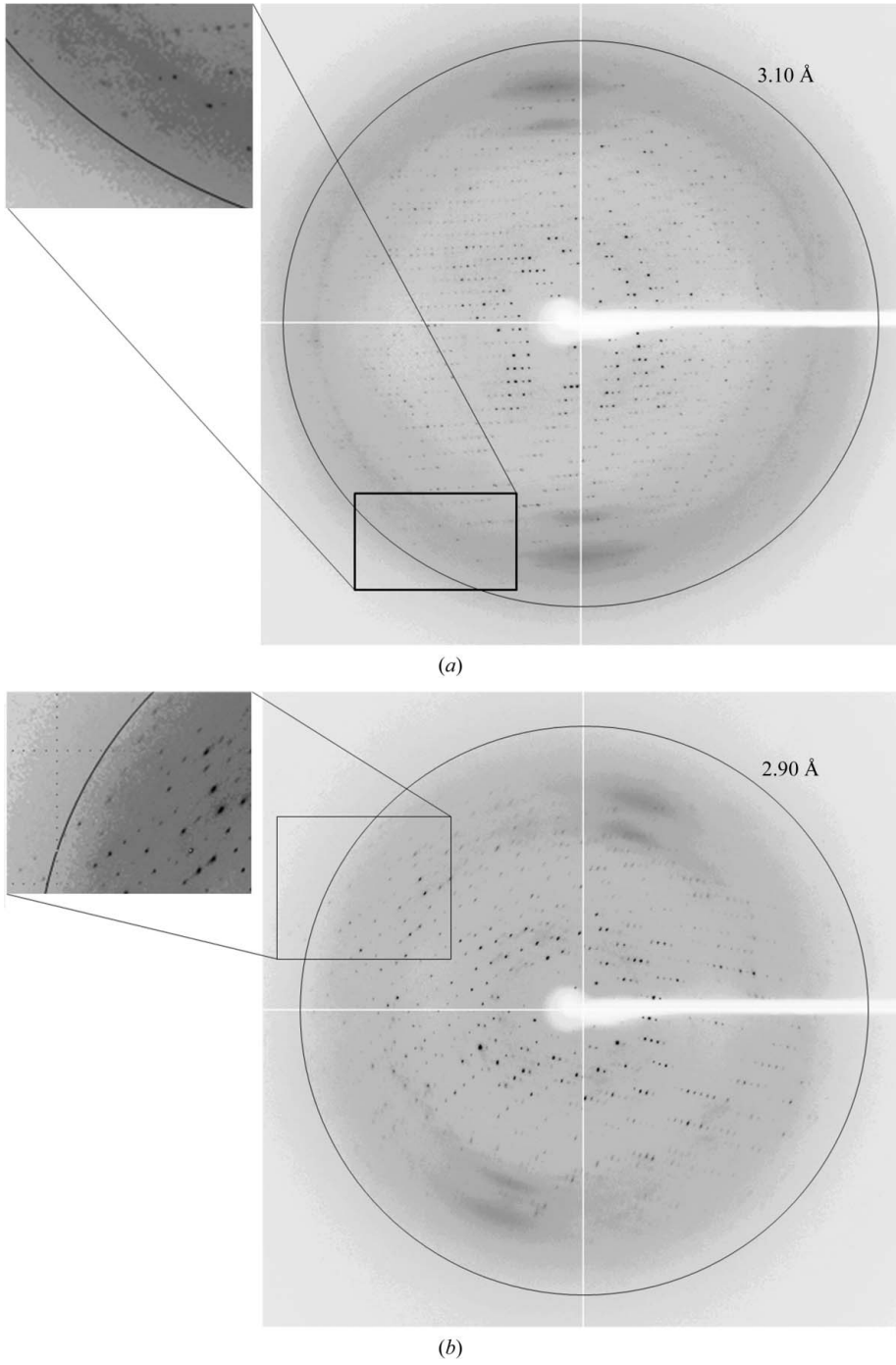


Figure 4
Diffraction images of AaHK crystals obtained in the presence of ATP–Mg (*a*) and AMPPNP–Mg (*b*).

3.1 Å resolution on beamline BL-5A of the Photon Factory (Ibaraki, Japan; Fig. 4*a*); in contrast, the crystals grown in the presence of AMPPNP–Mg typically grew to final dimensions of 0.3 × 0.06 × 0.05 mm in two weeks at 293 K (Fig. 3*b*) and diffracted X-rays to 2.9 Å resolution on the same beamline (Fig. 4*b*). The data-collection statistics are summarized in Table 1. Despite their different shapes, the crystals belonged to the same space group and had almost identical unit-cell parameters. Their Matthews coefficients (V_M = 2.74 and 2.51 Å^3 Da^{-1}; Matthews, 1968) indicated that the crystals contained four AaHK molecules per asymmetric unit, with solvent contents of 55.2 and 51.1%, respectively. Structure determination of AaHK in these crystals is currently under way by the molecular-replacement method using the atomic coordinates of TM0853 from *T. maritima* (33% identical in sequence; PDB entry 2c2a; Marina *et al.*, 2005) and KinB from *G. stearothermophilus* (24% identical in sequence; PDB entry 3d36; Bick *et al.*, 2009) as search models.

The synchrotron-radiation experiments were performed on the Photon Factory beamlines NW-12A and BL-5A with the approval of the Photon Factory, KEK (Proposal Nos. 2008G-666 and 2008S2-001). This work was partly supported by grants-in-aid for scientific research from the Ministry of Education, Culture, Sport and Technology of Japan (16087203 to KN and 21380061 to JN) and by the Targeted

Proteins Research Program (TPRP) of the Ministry of Education, Culture, Sports, Science and Technology of Japan (to MT).

References

Bick, M. J., Lamour, V., Rajashankar, K. R., Gordiyenko, Y., Robinson, C. V. & Darst, S. A. (2009). *J. Mol. Biol.* **386**, 163–177.
Bilwes, A. M., Alex, L. A., Crane, B. R. & Simon, M. I. (1999). *Cell*, **96**, 131–141.
Bilwes, A. M., Quezada, C. M., Croal, L. R., Crane, B. R. & Simon, M. I. (2001). *Nature Struct. Biol.* **8**, 353–360.
Casino, P., Rubio, V. & Marina, A. (2009). *Cell*, **139**, 325–336.
Gouet, P., Courcelle, E., Stuart, D. I. & Métoz, F. (1999). *Bioinformatics*, **15**, 305–308.
Guarnieri, M. T., Zhang, L., Shen, J. & Zhao, R. (2008). *J. Mol. Biol.* **379**, 82–93.
Kabsch, W. (2010). *Acta Cryst.* D**66**, 125–132.
Krissinel, E. & Henrick, K. (2007). *J. Mol. Biol.* **372**, 774–797.
Marina, A., Mott, C., Auyzenberg, A., Hendrickson, W. A. & Waldburger, C. D. (2001). *J. Biol. Chem.* **276**, 41182–41190.
Marina, A., Waldburger, C. D. & Hendrickson, W. A. (2005). *EMBO J.* **24**, 4247–4259.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Ponting, C. P. & Aravind, L. (1997). *Curr. Biol.* **7**, R674–R677.
Song, Y., Peisach, D., Pioszak, A. A., Xu, Z. & Ninfa, A. J. (2004). *Biochemistry*, **43**, 6670–6678.
Stock, A. M., Robinson, V. L. & Goudreau, P. N. (2000). *Annu. Rev. Biochem.* **69**, 183–215.
Tanaka, T., Saha, S. K., Tomomori, C., Ishima, R., Liu, D., Tong, K. I., Park, H., Dutta, R., Qin, L., Swindells, M. B., Yamazaki, T., Ono, A. M., Kainosho, M., Inouye, M. & Ikura, M. (1998). *Nature (London)*, **396**, 88–92.
Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). *Nucleic Acids Res.* **22**, 4673–4680.
Tomomori, C., Tanaka, T., Dutta, R., Park, H., Saha, S. K., Zhu, Y., Ishima, R., Liu, D., Tong, K. I., Kurokawa, H., Qian, H., Inouye, M. & Ikura, M. (1999). *Nature Struct. Biol.* **6**, 729–734.
Xie, W., Dickson, C., Kwiatkowski, W. & Choe, S. (2010). *Protein Pept. Lett.* **17**, 1383–1391.
Yamada, S., Sugimoto, H., Kobayashi, M., Ohno, A., Nakamura, H. & Shiro, Y. (2009). *Cell*, **17**, 1333–1344.