stitute in Řež u Prahy. The results were plotted as inverse pole figures calculated by Harris method [2, 3]. The hydrides were documented on metallographic polished sections in UJP PRAHA.

### Changes of texture and reorientation of hydrides

Temperatures corresponding to VVER operation temperature or higher, combined with constant tensile stress, cause creep leading to increased formation of zirconium crystallites, which rotate their bases in a direction perpendicular to tangential direction (TD). This represents conditions favourable for hydride precipitation in the radial direction (RD), which dramatically reduce tube wall resistance to rupture. However, this effect was observed during experiments with open tubes without internal overpressure. Under real operation conditions in a reactor, the overpressure of gasses inside the fuel rod contributes to the deformation of cladding tubes. Results of Rogozyanov et al. [4] suggest that in standard operation conditions of VVER, the effect of axial tensile stress slightly dominate over the internal overpressure effects. The final deformation is approx. 80 x smaller than the deformation resulting from performed experiments and resulting texture changes will have only small influence on the orientation of hydrides. Distinctive unfavourable changes in hydride orientation can be expected only during accident of cask/container accompained by temperature rise and break of the cask/container.

The orientation of hydrides did not changed continuously: hydride orientation is usually random and directional alignment was found only in extremely deformed tubes, where the pole density ($p'$) of plane (100) in AD exceeded the boundary which lies in the interval 6,9 – 8,6.

### New texture

In cladding tubes exposed to 700 °C and constant tensile stress of 10 MPa for 184 h (sample G), a new (not yet described in literature) texture appeared: the highest pole density ($p'$) in TD was found for (101) pyramid followed by $p'$ for (100) prism. This texture can be explained by {111} twinning, as only this twin can face both (101) and (100) planes perpendicular to TD. Moreover, only in this sample the (110) prism shows the highest $p'$ in AD of all of the observed planes in this sample, while the (100) prism has the lowest $p'$ compared to all samples. High $p'$ of the (110) prism and the occurrence of {111} twinning prove recrystallisation. Reversible phase transformation of a significant amount of -Zr - -Zr probably contributes to the formation of this new texture.

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**S11**

## STRUCTURE AND FUNCTION OF BACTERIAL NUCLEASES

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**Keywords**: nucleases, protein crystallography, single-wavelength anomalous dispersion

### Abstract

Nucleases are a broad group of enzymes which controls hydrolysis of phosphodiester bonds in nucleic acids. The reaction is used in wide spectrum of biological processes, which is in correlation with number of different structures and reaction mechanisms. Nucleases play their role in DNA replication, transcription from DNA to RNA, nucleic acid's repairs, apoptotic processes and controlled cell death or in degradation of nucleic acids as a nutrition source. The reaction mechanisms are possible to characterise with respect to reaction centre constitution, presence of metal ions, deprotonated water or typical amino-acid residues as serine, thyrosine or histidine. One of the bacterial nucleases was successfully crystallized and diffraction data were collected. A phase problem solution is in progress.

### Introduction

Nucleases are a group of enzymes responsible for cleavage of DNA and RNA. The reaction is involved in various biological processes: DNA replication, recombination, reparation processes, nucleic acids (NA) degradation, programmed cell death, etc. Different requirements on nucleases function leads to structural and reaction mechanisms diversity. As nucleic acids are an essential compound of living beings, their degradation is fatal. Therefore, production and function of nucleases is strongly regulated in cells.

Small bacterial nuclease (SBN) was chosen for further studies, with respect to structure solution and reaction mechanism determination by X-ray crystallography.

## Theory and experiment

### Mechanism of phosphodiester bond cleavage

Nuclease catalyses disruption of one of the P – O bonds connecting units of nucleic acids. The cleavage of phosphodiester bond is a general acid-base catalysis, where a base activates a nucleofil by deprotonation and an acid stabilizes final product by protonation. When activated nucleofil is close enough to phosphate group, a highly charged intermediate is formed, where phosphorus forms 5 covalent bonds. Finally, the scissile bond breaks.

The nucleofile, initiated by deprotonation, is mostly a water molecule or a hydroxyl group of serine or thyrosine, which leads to a covalent compound of protein and nucleic acid. In this case, the complex is dissociated in the second step. Moreover, 3'-end of nucleic acid can act as a nucleofil, resulting in reordering of nucleic-acid chains (e.g. splicing) [5].

### Bacterial nucleases

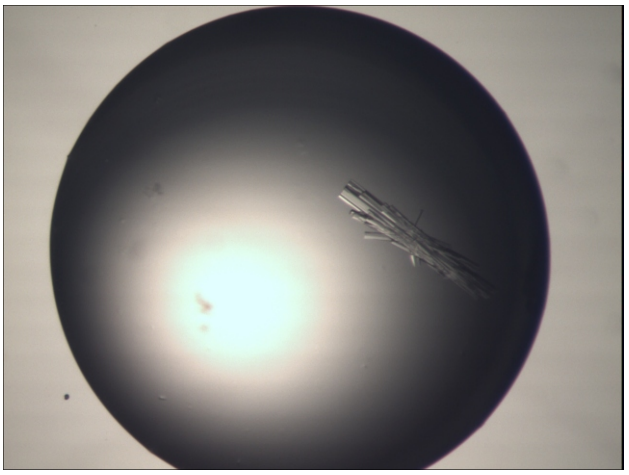
The protein sequence database UniProt contains 8996 sequences (291,462 unrevised; 28th June 2013) of bacterial nucleases but there are only 645 known structures (Protein Data Bank; 28th June 2013).

Nucleases can be classified by several criteria. The primary criterion is the position of the cleavage: exonucleases remove nucleotides from NA ends and endonucleases disassemble chains to longer products. According to substrate, DNases and RNases differs in sugar specificity, moreover, nucleases can be specific to single-stranded (ss) or double-stranded (ds) nucleic acids. Some nucleases prefer cleavage of given sequence of nucleotides. Number of nucleases fulfils several options in the criteria, for example degrading nucleases show only weak substrate specificity.

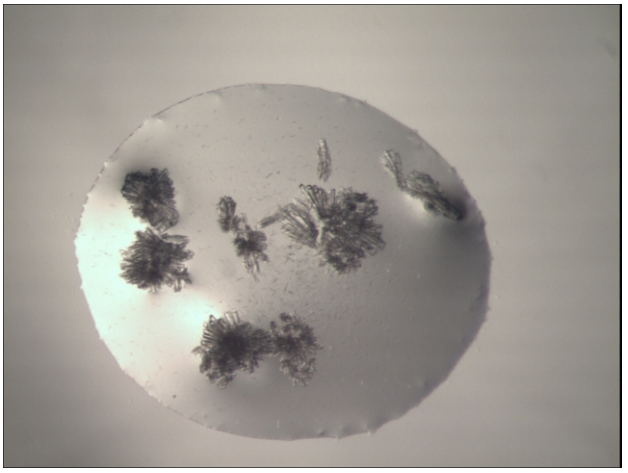
Detailed classification can be done on the basis of reaction mechanisms. An overview of nucleases with known structure was published by Yang, 2011 [5].

### Crystallization and diffraction measurements of SBN

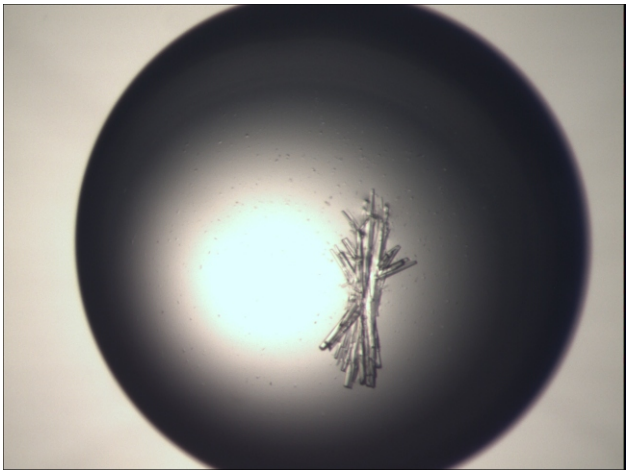
Crystallization of the small bacterial nuclease (SBN) was performed with hanging drop method. An initial screening



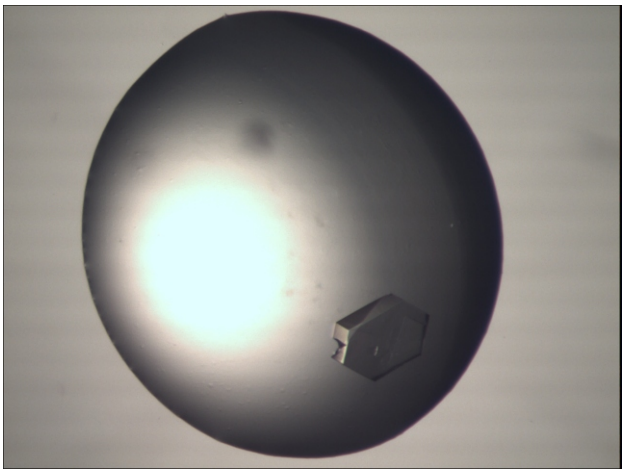
1.6 M ammonium sulphate
0.1 M Tris pH 8.5



2,2 M ammonium sulphate
0.1 M sodium acetate pH 4.6



2.0 M ammonium sulphate
0.1 M sodium acetate pH 4.6



2.2 M ammonium sulphate
0.1 M Tris pH 8.5

**Figure 1:** Examples of crystals of small bacterial nuclease grown in solution with ammonium sulphate.

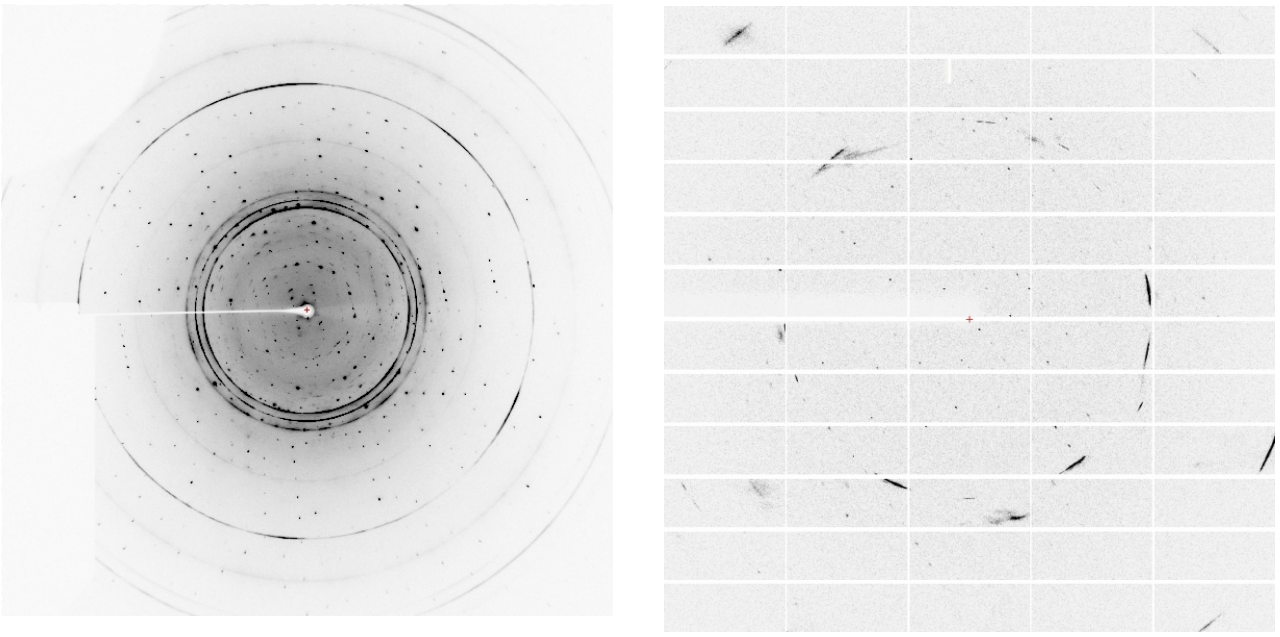


**Figure 2:** Chosen diffraction frames from dataset measured on crystal with hexagonal morphology (left panel) and needle-like morphology (right panel).

was held in crystallization plates, where wells were covered by glass cover clips and sealed by silicon grease. Initial hits occurred in the screening set Index, Hampton Research, solutions number 2 and 6 with high concentration of ammonium sulphate. Further optimization consisted of changing of concentration of ammonium sulphate.

Crystals were fished with nylon loops and vitrified in liquid nitrogen at 77 K. High concentration of salt in crystallization solution serves as a cryoprotectant. Chosen crystals were soaked in ammonium iodide.

Diffraction experiments were performed at synchrotron source BESSY II in Berlin, beamline 14.1 and 14.2 [4], using a MAR Mosaic CCD 225 or PILATUS 6M detector and mini-kappa goniometer. The wavelength of X-ray was 1.9 Å to improve anomalous scattering from sulphurs and iodines. The diffraction data were processed in XDS [2,3].

## Results and discussion

Crystals of SBN grow in concentrations of ammonium sulphate in interval from 1.6 M to 2.4 M (Fig. 1). The crystals usually form clusters of needles of length in order of hundreds of micrometers and width in order of tens of micrometers. The needle-like morphology is conserved across various conditions. In one case, a monocrystal with hexagonal shape appeared (Fig. 1; top right).

Sample images of diffraction data collected on these crystals are on Fig. 2. The data often show diffraction of several lattices and in few cases powder diffraction of water, mainly caused by ice on the surface of loop. Data with high resolution limit are usually available. However, high resolution data were not collected yet because of geometry limits of the experiment at wavelength 1.9 Å. The long wavelength experiment was chosen to maximise anomalous scattering of sulphur and iodine atoms for phase problem solution.

The data collected on the crystal with hexagonal morphology and the needle-like crystal soaked in ammonium iodide were processed in XDS (tab 1.)

Individual frames measured on the crystal with the hexagonal morphology shows both strong ice-rings and multiple crystal lattices. However, it is possible to index and integrate reflections on the major lattice. After indexing and analysis of systematic absences, space group $P2_12_12_1$ was determined with cell parameters $a$ = 47.6 Å, $b$ = 54.1 Å, $c$ = 32.5 Å. A signal to noise ratio ($I$/ ($I$)) is high even in high resolution shell and anomalous signal exceeds $I$/ ($I$) value of 1 with high correlation coefficient. Low completeness in high resolution is caused by shadows of cryo-stream nozzle and beamstop holder. Nevertheless, the experimental phasing by SAD was not successful by now.

In the case of the needle-like crystal, the -axis was set to 45° to put the crystal in more general position in the incident beam. Total 3,240° of rotation by -axis was collected to get high enough redundancy as expected space group $P1$ was confirmed. Anomalous scattering was observed, but search for anomalous scatterers and phasing was not successful.

## Conclusion

The small bacterial nuclease was successfully crystallized, but the crystals with high symmetry were not reproduced. Several datasets were collected, but the phasing and structure solution is without any results until now. The experimental phasing on the basis of the anomalous scattering on sulphurs is a demanding technique, because differences between Friedel pairs are close to experimental errors, therefore the measurement has to be optimized for this type of the experiment. The way for improvement of the results could be an inverse beam method or sophisticated usage of -geometry for “true redundancy” measurements [1].

**Table 1:** Parameters and statistics of datasets collected on crystals with hexagonal and needle-like morphology. Numbers in brackets represent the highest resolution shell.

| Crystal morphology | Hexagonal | Needle-like |
|---|---|---|
| X-ray source | BESSY II, BL 14.2 | BESSY II, BL 14.1 |
| Detector | MAR Mosaic CCD 225 | PILATUS 6M |
| Wavelength (Å) | 1.9 | 1.9 |
| Detector distance (mm) | 70 | 140 |
| Number of frames | 2,160 | 32,400 |
| Exposure time per 1 degree (s) | 0.8 | 1.5 |
| Oscillation angle (°) | 1 | 0.1 |
| Space group | $P2_12_12_1$ | $P1$ |
| Unit cell parameters (Å) | $a$ = 47.6, $b$ = 54.1, $c$ = 32.5 | $a$ = 22.8, $b$ = 48.8, $c$ = 51.1<br>= 105.6°, = 95.1°, = 89.9° |
| Resolution limits (Å) | 47.0 – 1.85 (1.89 – 1.85) | 47.0 – 1.98 (2.03 – 1.98) |
| Number of observed reflections | 503,131 (7,842) | 367,755 (10,283) |
| Number of unique reflections | 6,893 (248) | 13,373 (625) |
| Overall redundancy | 73.0 (31.6) | 27.5 (16.5) |
| Completeness (%) | 88.1 (54.1) | 90.4 (59.1) |
| Average $I/\ (I)$ | 115.2 (29.5) | 17.5 (5.7) |
| $R_{sym}$ | 0.04 (0.10) | 0.20 (0.68) |

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S12

# THE IMPORTANCE IS IN DETAILS - ISOLATION AND CRYSTALLIZATION OF PSII FROM HIGHER PLANTS WITH DIFFERENT CONTAINS OF DETERGENT

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Photosystem II (PSII) is a multisubunit pigment-protein complex that catalyses electron transfer from water to the plastoquinone pool with concomitant evolution of oxygen. PSII consists of around 25 different types of protein subunits which are organized into two structurally distinct parts: core complex (D1,D2, CP47,CP43, intrinsic and extrinsic proteins, small proteins of unknown function) and peripheral antenna (light-harvesting complex II (LHCII) proteins).

For isolation of PSII from higher plants were selected model organisms, such as tobacco, spinach, peas, haricot and soy. In our experiments we tried two different plants – spinach *Spinacia oleracea* and haricot *Phaseolus vulgaris*. Growing hydroponic plant under controlled conditions and optimization of reproducible purification protocol of homogeneous sample suitable for crystallization is the main aim of our project. We changed purification protocol and used new methodology without using of detergent TRITON, which could be problematic for PSII complex crystallization. We got preliminary results – microcrystals, which require the optimization of conditions, using different crystallization technics. It was also shown that the type of detergent and conditions of solubilization of thylakoids membranes are critical steps for crystallization, which should be carefully chosen.

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S13

# CRYSTALLIZATION OF THE MEMBERS OF MULTISTEP SIGNALING SYSTEM FROM *ARABIDOPSIS THALIANA*

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In higher plants multistep signalling system (MSS) play a major role in cytokinin reception and contribute to ethylene signal transduction. Some components of MSS take part in osmosensing, megagametogenesis and flowering promotion in plants. Therefore it is important to investigate mechanisms of functioning of MSS for deeper knowledge about higher plants vital activities. Multistep signalling systems are composed of hybrid histidine kinases (HKs) that perceive a signal, histidine-containing phosphotransfer proteins (HPs) that mediate the phosphate signal downstream to their corresponding response regulators (RRs), which usually play a role of transcription factors. One of HKs, CKI1, was suggested to participate in female gametophyte development in *Arabidopsis thaliana*. CKI1 has specificity in interactions with HPs, in particular it has affinity in binding to AHP2 and AHP3 [1].

The main aim of this project is structural characterization of the interactions among *Arabidopsis* MSS members what will allow us to get inside into details of its functioning.

For this purpose crystallization of protein complexes with further X-ray diffraction analysis are used.

In the research major attention is embedded to the complexes of receiver domain of histidine kinase ($CKI1_{RD}$) with *Arabidopsis* histidine-containing phosphotransfer proteins AHP2 and AHP3.

Screening of crystallization conditions was performed by means of different crystallization screens. Some hits were found for complexes of $CKI1_{RD}$ and AHP2 (in different concentrations) in Morpheus screen. All promising conditions contain MPD, PEG 1000 and PEG 3350, but different buffers and ligands. Obtained crystals are 3D, quite large, but too fragile. X-ray diffraction analysis for testing of crystals will follow.

Through X-ray diffraction analysis structures of complexes CKI1 with AHPs are expected to be solved.

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**S14**

# STRUCTURAL CHARACTERIZATION OF THE MEMBERS OF THE MULTISTEP SIGNALING SYSTEM FROM *A. THALIANA*

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## Introduction

Multistep signaling system (or multistep phosphorelay) plays very important role in sensing/response mechanisms in higher plants. It consists of three components: a sensory histidine kinase, which catalyses the autophosphorilation of a His residue and subsequently transfers the phosphoryl group to Asp residue of its internal receiver domain. Then, the signal is passed downstream to a His residue of histidine phosphotransfer protein (Hpt) and finally delivered to an Asp residue of receiver domain of the corresponding response regulator, which subsequently interacts with target proteins or acts as a transcription factors [1,2]. In *Arabidopsis*, MSP signaling regulates various key processes, such as osmosensing, hormone signaling and gametogenesis [3]. *Arabidopsis* genome encodes 8 histidine kinases and 5 canonic Hpt (AHP1-AHP5) [4]. The goal of this work is to get insights into structural determinants of AHP-mediated signaling by the example of receiver domain of histidine kinase 1 (CKI1, PDB code 3MMN) and AHP2.

## Experimental section

To achieve our goal AHP2 gene was cloned and transformed into *E. coli* expression strain. The protein was expressed and purified in 2 steps: by metal chelate affinity chromatography followed by anion-exchange chromatography. Initial screening of crystallization conditions was performed by sitting drop vapor diffusion method using different commercial screens. Optimization of the discovered conditions included the screening of pH, incubation temperature, type and concentration of the precipitant. The optimal buffer composition for crystallization was determined using a thermal shift assay. Diffraction data were collected on BL14.2 at the BESSY II storage ring and processed with XDSAPP. Structure of AHP2 was solved with SIRAS protocol, using anomalous signal of the Lutetium. Substructure solution, phasing, density modification and initial model building was carried out using the programs SHELXC/D/E *via* the graphical user interface HKL2MAP. Side chains were assigned using the autobuild/refine protocol in BUCCANEER. An anomalous difference Fourier electron density map was calculated using CCP4 program. Structure refinement was carried out using REFMAC5 and iterated with manual model building in COOT.

Protein-protein rigid body docking with AHP2 and CKI1rd was performed by GRAMM-X web server followed by molecular dynamic simulations using GROMACS software. The simulation was performed at 300K for 100ns.

## Results and discussion

The HPt AHP2 was cloned, overexpressed and purified to >95 % purity. The initial crystallization conditions for AHP2 were identified in condition No. 20 of Crystal Screen 2 (Hampton Research) consisting of 0.1 M MES buffer pH 6.5 and 1.6 M $MgSO_4$. Small tetragonal crystals were grown in 2 days at 298 K, but they did not diffract X-rays beyond 6Å resolution. To enhance the crystallization behaviour, AHP2 was transferred to the buffer optimal for its thermodynamic stability prior to crystallization based on the results of the thermal shift assay. As the most stabilizing buffer for AHP2 50 mM imidazole, pH 8.0 supplemented with 5 mM DTT was identified. The protein was

then transferred to this buffer prior to crystallization and simultaneously, the crystallization temperature was lowered to 4°C. This optimization resulted in formation of crystals with maximum dimensions of approximately 0.35 0.2 0.1 mm. which diffracted up to 2.5 Å and show significant anisotropic behaviour.

The structure of AHP2 was solved using SIRAS protocol using anomalous signal of the Lutetium. AHP2 protein represents α-helical bundle, comprising of four short and two long helices. Short helices form a central core of the protein with the conserved His residue carrying the phosporyl group situated in the middle of the third α-helix.

In order to gain insight into the structural features, underlying AHP2-CKI1rd interaction, molecular-dynamics simulations were carried out. Simulations were performed for 100 ns and show the stability of protein-protein complex. The key residues, responsible for the AHP2-CKI1rd interaction, were identified and reveal strong protein-protein binding. The analysis of the obtained data is currently in progress.

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**S15**

# CRYSTALLOGRAPHIC STUDY OF HALOALKANE DEHALOGENASE DpcA FROM *PSYCHROBACTER CRYOHALOLENTIS* K5

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### Introduction

Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are microbial enzymes that catalyze the hydrolytic conversion of halogenated aliphatic compounds to their corresponding alcohols [1, 2], which is the hydrolytic dehalogenation accomplished by these enzymes is one of the most important steps in the biodegradation of 1-halo-n-alkanes and -dihalo-n-alkanes, serious halogenated pollutants [3]. HLDs have a broad substrate specificity [4] and a high enantioselectivity [5], which makes these enzymes applicable in bioremediation [6], in biosensing [7,8], biocatalysis [5, 9], cellular imaging, and protein analysis [10, 11] . Understanding of the structural bases of the enzyme extremophilicity allows for the construction of HLD variants with improved activity and stability at low and high temperatures and thus enlarges their applicability in environmental and biosynthetic applications.

### Experimental details

A novel HLD enzyme, DpcA, exhibiting unique temperature profiles with exceptionally high activities at low temperature, isolated from Gram-negative psychrophilic bacteria *Psychrobacter cryohalolentis* K5 [12] was crystallized by sitting-drop and hanging-drop vapour-diffusion techniques. Crystallization drops were

prepared by mixing 2 μl of protein solution at the concentration 10 mg $ml^{-1}$ in 50 mM Tris–HCl buffer pH 7.5 and 1 μl precipitant solution plus 0.3–0.6 μl of 0.1 M L-proline. Diffraction data were collected at the beamline 14.2, Helmholtz-Zentrum Berlin (HZB) (Germany) at the BESSY II electron storage ring, detector Rayonics MX-225 CCD [13] at wavelengths of 0.978 Å. All diffraction experiments were carried out in a liquid-nitrogen stream at 100 K using a Cryojet XTL system (Oxford Instruments). The diffraction data for DpcA were indexed,