# Advanced Research Center for Beam Science – Structural Molecular Biology –

http://www.scl.kyoto-u.ac.jp/~fujii/indexE.html

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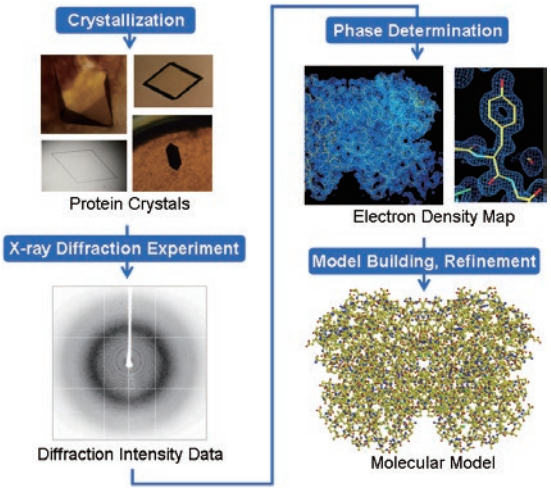
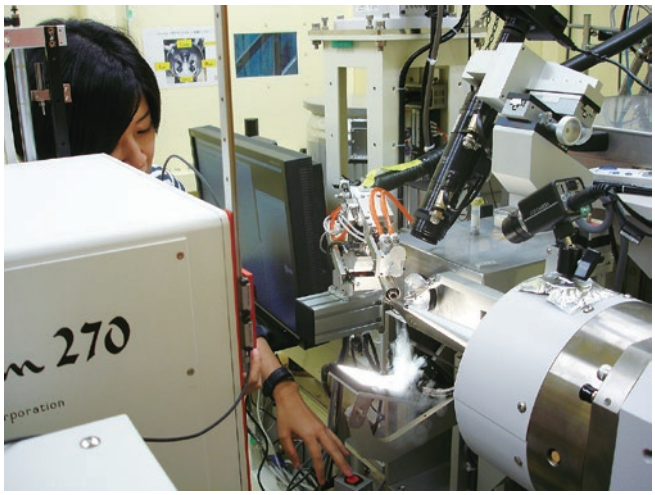
## Scope of Research


**KEYWORDS**
Crystal
X-ray Crystallographic Analysis
Structural Biology
Protein Crystallography
Structure and Function


Our laboratory analyzes the electronic state of atomic or molecular structure in materials, which are obtained from diffraction images and spectra observed by X-ray irradiation of these substances, respectively, to elucidate the relationships between the structure and functions or physical properties. Our main themes are (1) high-resolution experimental and theoretical studies on the natural width of elements in materials, (2) the development in the measurements of the diagram lines in soft X-rays region, (3) structural determination of novel protein molecules and their complexes, and (4) studies on the structural basis of functions, physical properties, and intermolecular interactions.



## Selected Publications


Fujii, T.; Sato, A.; Okamoto, Y.; Yamauchi, T.; Kato, S.; Yoshida, M.; Oikawa, T.; Hata, Y., The Crystal Structure of Maleylacetate Reductase from *Rhizobium* sp. Strain MTP-10005 Provides Insights into the Reaction Mechanism of Enzymes in Its Original Family, *Proteins: Structure, Function, and Bioinformatics*, **84,** 1029-1042 (2016).

Fujii, T.; Yamauchi, T.; Ishiyama, M.; Gogami, Y.; Oikawa, T.; Hata, Y., Crystallographic Studies of Aspartate Racemase from *Lactobacillus sakei* NBRC 15893, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **71,** 1012-1016 (2015).

Fujii, T.; Goda, Y.; Yoshida, M.; Oikawa, T.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Maleylacetate Reductase from *Rhizobium* sp. Strain MTP-10005, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **64,** 737-739 (2008).

Fujii, T.; Oikawa, T.; Muraoka, I.; Soda, K.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Tetrameric Malate Dehydrogenase from the Novel Antarctic Psychrophile *Flavobacterium frigidimaris* KUC-1, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **63,** 983-986 (2007).

Fujii, T.; Sakai, H.; Kawata, Y.; Hata, Y., Crystal Structure of Thermostable Aspartase from *Bacillus* sp. YM55-1: Structure-based Exploration of Functional Sites in the Aspartase Family, *J. Mol. Biol.*, **328,** 635-654 (2003).

## Structural Features and Low-temperature Adaptation of Aspartate Racemase

Amino-acid racemases are responsible for the racemization of amino acids and can be mainly grouped into two families, pyridoxal 5'-phosphate (PLP) dependent and PLP independent. Aspartate racemase (AspR) catalyzes the interconversion between L- and D-aspartate and belongs to the PLP-independent racemase group. The enzyme is thought to employ a two-base mechanism to catalyze both the directions of racemization and utilize two cysteine residues as the conjugated catalytic acid and base in the catalytic reaction. The only crystal structure of the PLP-independent amino-acid racemase is now available from a hyperthermophilic archaeon. To elucidate the structure and the low-temperature adaptation of the racemase group, we determined the crystal structures of AspR from *Lactobacillus sakei* NBRC 15893 (LsAspR), which works in the low-to-medium temperature range, and for comparison AspR from *Thermococcus litoralis* DSM 5473 (TlAspR), which has the maximum activity at 95 °C.

LsAspR and TlAspR were crystallized at 20 °C by the sitting-drop vapour-diffusion method using a precipitant solution of 25% (v/v) PEG-MME 550, 5% (v/v) 2-propanol and 0.1 M sodium acetate pH 4.8 and a precipitant solution of 24% (w/v) PEG1500, 0.2 M L-proline and 0.1 M HEPES pH 7.5, respectively. Diffraction experiments were performed at beamlines NE-3A of Photon Factory AR, and BL-5A of Photon Factory, Tsukuba, Japan. The crystals were flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected at a wavelength of 1.000 Å using Quantum 270 and 315r CCD detectors. The crystals belonged to space groups $P3_121$ with unit cell parameters of $a = b = 105.5$ Å and $c = 96.5$ Å, and $P2_12_11$ with unit cell parameters of $a = 90.26$, $b = 125.78$, $c = 40.64$ Å, respectively. The structures of LsAspR and TlAspR were determined by molecular replacement and refined at 2.6 Å resolution ($R = 23.8\%$, $R_{free} = 31.6\%$) and 2.0 Å resolution ($R = 18.7\%$, $R_{free} = 25.0\%$), respectively.

Both LsAspR and TlAspR molecules are homodimers with molecular two-fold axis. The subunit of each enzyme molecule comprises the N-terminal and C-terminal domains. In each domain, a central four-stranded parallel β-sheet is flanked by six α-helices. The molecules are formed mainly by intersubunit interactions between the N-terminal α-helices and intersubunit hydrogen-bonds between the N-terminal β-sheets in the dimer interface. The active-site cleft exists between both the domains. The spatial arrangement of the strictly conserved cysteine residues in the cleft reveals the Cys residues involved in the enzymatic catalysis: Cys84 and Cys196 of LsAspR and Cys83 and Cys194 of TlAspR.

A structural comparison of LsAspR and TlAspR reveals structural factors probably involved in thermostability of AspR. The molecular volume, intersubunit interaction, and the number of ion pairs suggest that the LsAspR molecule is more loose than that of TlAspR (Fig. 1). Most characteristic difference at the dimer interface between LsAspR and TlAspR is the interactions between α1 helices (Fig. 2). Moreover, π-π interaction between indole rings of Trp74 is found at the dimer interface of TlAspR (Fig. 3). In LsAspR, Leu75 corresponds to Trp74 in TlAspR and does not make an intersubunit interaction.

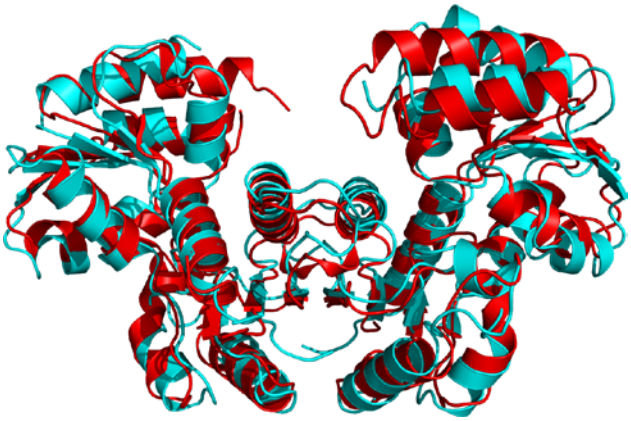


**Figure 1.** Superposition of LsAspR and TlAspR dimers. Cyan, LsAspR; Red, TlAspR.

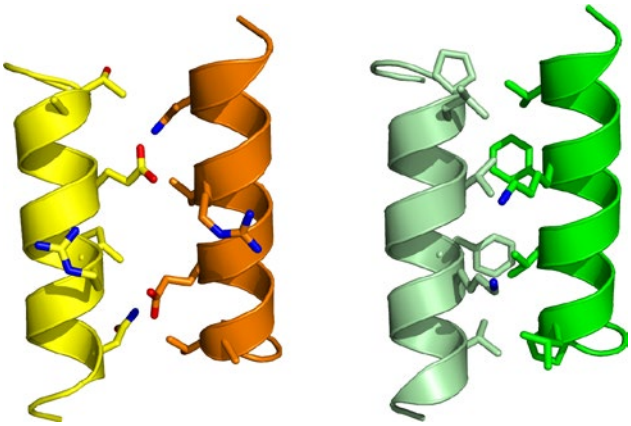


**Figure 2.** Interactions between α1 helices in dimer interface. (Left panel) LsAspR. (Right panel) TlAspR.

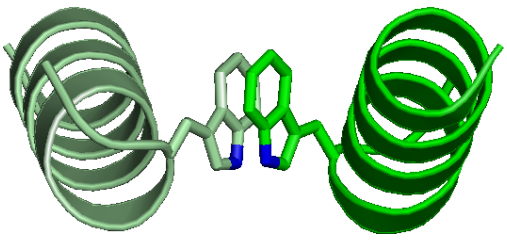


**Figure 3.** π-π Interaction between indole rings of Trp74 at dimer interface of TlAspR.