

metabolism. AT reversibly catalyzes the transamination reaction in which the alpha-amino acid of amino acid 1 is transferred to the 2-oxo acid of amino acid 2 to produce the 2-oxo-acid of amino acid 1 and amino acid 2. Thus, AT recognizes two different kinds of amino acid 1 and 2 (Dual substrate recognition). psi-BLAST search showed that TTHA0173 is a dual functional AT which works at serine and alanine metabolic pathway. TTHA0173 is serine:pyruvate AT and at the same time, alanine:glyoxylate AT. Three-dimensional structure of unliganded TTH0173 was determined by MAD method using Se-Met tSPAT at 1.45 Å resolution. TTH0173 is a homodimer and the polypeptide chain is folded into the small and large domains. The active-site pocket is formed at the subunit interface and the domain interface with the coenzyme PLP forming a Schiff base with the catalytic lysine residue. Program DALI was used to search PDB for proteins possessing structures similar to that of TTHA0173, therefore the highest Z-score was calculated to be 47.4 with sequence identity of 33% for human serine pyruvate-alanine:glyoxylate AT. The  $K_{cat}$  and  $K_d$  values of tSPAT for various amino acid and their 2-oxo acid measured. Unexpectedly, glutamate and its 2-oxo acid which have bulky side chain, were good substrate. In order to elucidate the substrate recognition mechanism, we have determined the crystal structure of complex with 2-methylalanine and 2-methyl glutamine at 1.55 and 1.50 Å resolution, respectively.

Keywords: substrate binding, transaminases, vitamin B6

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### Crystal structure of mouse sulfotransferase 2A4 (SULT2A4)

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Cytosolic sulfotransferase (SULT) sulfates specific substrates such as hormones, neurotransmitters, xenobiotics, drugs by transferring sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to hydroxyl group of substrate. It is known that SULT regulates concentration of endogenous compounds or detoxifies xenobiotics for increasing hydrophilicity of them and excreting them out of body through blood or urine. The mouse SULT2A4 has unique substrate specificity for cholic acid. To gain insight into a molecular basis for the substrate specificity, we solved the crystal structure of SULT2A4 complexed with 3'-phosphoadenosine 5'-phosphate (PAP) and cholic acid. The overall structure is similar to those of SULT enzymes and the PAP binding site is conserved, however, significant differences exist in the positions of loops Pro14-Ser20, Glu79-Ile82 and Tyr234-Gln244 in the cholic acid binding pocket. Moreover, completely conserved His, which is proposed catalytic base is not conserved in SULT2A3. However the functionary identical His residues exist in PSB (phosphosulfate binding motif) loop. The difference should need to sulfuryl transfer to 7'-OH of steroid ring position not in 3'-OH.

Keywords: crystal structure analysis, enzyme catalytic reaction mechanism, sulfotransferase

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### Unusual conformational pathways of mismatched dNTP incorporation by DNA Polβ

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Understanding how DNA polymerases control fidelity requires elucidation of the mechanisms of matched and mismatched dNTP incorporations. Little is known about the latter because mismatched complexes do not crystallize readily. In this report, we employed small-angle X-ray scattering (SAXS), X-ray crystallography and structural modeling to probe the conformations of different intermediate states of mammalian DNA polymerase β (Pol β) in its wild-type and an error-prone variant, I260Q. Our structural results indicate that the mismatched ternary complex lies in-between the open and the closed forms, but more closely resembles the open form for WT and the closed form for I260Q. On the basis of molecular modeling, this over-stabilization of mismatched ternary complex of I260Q is likely caused by formation of a hydrogen bonding network between the side chains of Gln260, Tyr296, Glu295 and Arg258, freeing up Asp192 to coordinate Mg dNTP. These results argue against recent reports suggesting that mismatched dNTP incorporations follow a conformational path distinctly different from that of matched dNTP incorporation, or that its conformational closing is a major contributor to fidelity.

Keywords: crystal structure, DNA polymerase & error-prone variant I260Q, small-angle X-ray scattering (SAXS)

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### Crystal structure of Delta1-tetrahydrocannabinolic acid synthase from *Cannabis sativa*

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Delta1-tetrahydrocannabinolic acid (THCA) synthase is the enzyme that catalyzes oxidative cyclization of cannabigerolic acid into THCA, the precursor of Delta1-tetrahydrocannabinol. In order to investigate the structure-function relationship of THCA synthase, this enzyme was overproduced in insect cells, purified and finally crystallized in 0.1 M HEPES buffer pH 7.5 containing 1.4 M sodium citrate. A single crystal suitable for X-ray diffraction measurement was obtained in 0.09 M HEPES buffer pH 7.5 containing 1.26 M sodium citrate. The crystal diffracted to 2.8 Å resolution at beamline BL41XU, SPring-8. The crystal belonged to the primitive cubic space group *P432*, with unit-cell parameters  $a = b = c = 178.2$  Å. *R* value of the structure model was 19.6%. Structure of THCA synthase was divided into two domains, and there was FAD of a coenzyme

between domains. In addition, FAD formed covalent bond in His114 and Cys176. Based on the structural information obtained above, amino acid mutations of the four ionizable residues (H292, Y417, E442 and Y484) located in the vicinity of FAD is now in progress to clarify their contribution on its enzymatic function.

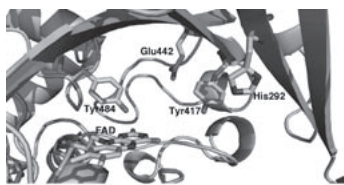


Figure. Active site of THCA synthase

Keywords: enzyme mechanics, flavoenzymes, plants

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#### Structure determination of hydrogenase maturation factor HypB from *Archaeoglobus fulgidus*

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The assembly of the [NiFe]-hydrogenases requires incorporation of Ni ions into the enzyme's metalcenter, which process requires the GTPase activity of hypB. To better understand the structure-function of hypB, we have solved the structure of apo-form of AfHypB by X-ray crystallography, crystals of AfHypB were grown using the hanging-drop-vapour-diffusion method. Protein (1 ul at 15 mg/ml) was mixed with 1 ul of reservoir solution (8% PEG4000, 0.1 M sodium acetate at pH 4.6), and equilibrated over 1ml of reservoir solution. Crystals were diffracted to  $\sim 2.4$  Å, and belong to space group  $P2_12_12_1$ , with unit cell dimensions  $a=73.14$ ,  $b=82.15$ ,  $c=68.59$  Å,  $\alpha=\beta=\gamma=90$  deg. The structure was determined by molecular replacement using GTP- $\gamma$ -S-bound form of hypB from *M. jannaschii* as a search template, and two molecules of AfHypB were found in the asymmetric units. Structure differences between the apo-form of AfHypB and GTP-bound form of hypB from *M. jannaschii* is discussed. This work is supported by RGC grants (CUHK4610/06M).

Keywords: hydrogenase maturation factor, nickel, protein interaction

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#### Production and crystallization of tomato $\beta$ -galactosidase 4

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$\beta$ -Galactosidase hydrolyzes terminal, non-reducing  $\beta$ -D-galactosyl residues from  $\beta$ -D-galactosides. Tomato  $\beta$ -galactosidase 4 (TBG4) is closely related to tomato fruit softening. We have started an X-ray crystal structure analysis of TBG4 to clarify the substrate recognition mechanism of this novel enzyme. The His-tagged recombinant proteins of TBG4 were expressed under the control of the alcohol oxidase promoter in *Pichia pastoris* and secreted into the culture medium. The recombinant TBG4 was purified by nickel affinity

chromatography and migrated as a single 78-kDa band on SDS-PAGE. The purified enzyme showed  $\beta$ -galactosidase activity and strong exo- $\beta$ -1,4-galactanase activity. The optimum pH was 4.0 and the  $K_m$  value against  $p$ -nitrophenyl- $\beta$ -D-galactopyranoside was 1.67 mM. The solution of purified TBG4 was concentrated in 20 mM sodium acetate (pH 4.0) prior to crystallization. The screening of crystallization is currently under way.

Keywords: beta-galactosidase, crystallization, solanum lycopersicum

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#### Crystal structure of *Aquifex aeolicus* PPX/GPPA in complex with ppGpp

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The crystal structure of the *Aquifex aeolicus* exopolyphosphatase/guanosine pentaphosphate phosphohydrolase in complex with the intracellular second messenger guanosine tetraphosphate was determined at 2.7 Å resolution. The hydrolytic base is identified as E119. Results from this study identify the glutamic acid residue 119 as the important hydrolytic base and establish that the dual specificity of the homologous *Escherichia coli* protein is compatible with a common active site for guanosine pentaphosphate and polyphosphate hydrolysis. This resolved a dispute originating from previous structural studies of the apo-enzymes. Distinct and different configurations between the two domains of the enzyme are associated with substrate binding. The residues R22 and R267, residing in different domains, are crucial for guanosine pentaphosphate specificity and recognition of the unique 3'-ribose phosphorylation. The orientation of the nucleotide base is somewhat different from expectations based on modeling of the related ATP-binding ASKHA protein family members.

Keywords: guanosine pentaphosphate phosphohydrolase, exopolyphosphatase, protein-ligand complex

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#### Inhibition by dTTP of the bifunctional dCTP deaminase:dUTPase from *Mycobacterium tuberculosis*

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dCTP deaminase from *Mycobacterium tuberculosis* has been proven to be a bifunctional dCTP deaminase:dUTPase. dCTP deaminase:dUTPase is a homotrimeric enzyme, closely related to the dCTP deaminases and the trimeric dUTPases. The enzyme catalyses both the deamination and the triphosphate hydrolysis