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**Keywords: endonuclease, catalytic mechanism, fold**

## MS93.P47

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**Non-catalytic domain of invertases: the key for oligomerization and specificity.**

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Fructans are polymeric sugars derived from sucrose. They are most interesting because of their physiological characteristics, such as preventing colon cancer and dental caries, selectively stimulating the growth of bifidobacteria and lactobacilli, decreasing total cholesterol and triacylglycerol lipids in blood serum and promoting the absorption of calcium and magnesium ions. Therefore, the enzymes involved in fructans processing attract great biotechnological attention for the production of functional foods and pharmaceuticals. In particular, a detailed knowledge of the molecular mechanisms involved in substrate recognition, transfructosylating efficiency and product specificity of the enzymes used as catalyst for these processes is essential.

We have solved the crystal structure of three invertases from yeast. First, the *Schwanniomyces occidentalis* Invertase, complexed with long substrates, revealed for the first time that the ancillary domain plays a direct role in oligomerization and substrate binding [1], which is a unique feature that shed light on the molecular mechanism regulating specificity within the GH32 enzymes from eukariota. We report also the *Phaffia rhodozyma* Invertase structure [2], an atypical highly glycosylated enzyme, with an unique insertion in the sequence of the ß-sandwich that folds over the catalytic domain and is involved in a new oligomerization pattern conferring high stability to the enzyme. Finnally, we have studied the *Saccharomyces cerevisiae* Invertase, an enzyme reported to adopt different aggregation states upon changes in the environment. The crystal structure revealed a sophisticated mechanism of molecular interaction between subunits that form higher aggregates throughout further involvement of the ancillary domains. Our results assign a direct catalytic role to the supplementary ß-sandwich domain of these enzimes, the first time that such a role has been observed within GH32 enzimes.

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**Keywords: invertases, enzymatic specificity, crystal structure.**

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**Structural and functional studies of *Staphylococcus aureus* Pyruvate Carboxylase**

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Pyruvate carboxylase (PC) catalyzes the ATP-dependent transformation of pyruvate to oxaloacetate, which marks the first step in gluconeogenesis. Oxaloacetate is also an important intermediate in the tricarboxylic acid (TCA) cycle. Therefore, PC is considered an essential enzyme in intermediary metabolism. The structural architecture of PC consists of four domains, the biotin carboxylase (BC) domain, the carboxyltransferase (CT) domain, the biotin carboxyl carrier protein (BCCP) domain, and a novel PC tetramerization (PT) domain. It belongs to a group of biotin dependent enzymes where the biotin is covalently bound to BCCP, which swings between the distinct active sites on the BC and CT domains to carry out the catalysis. The recently discovered PT domain is essential for keeping the tetramer intact and mutations in this domain disrupt the tetramer in both human and *Staphylococcus aureus* PC. In terms of regulation, it is known that PCs from vertebrate sources are highly activated by acetyl-CoA, while PCs from prokaryotes have varying degrees of dependency on acetyl-CoA.

Structural and biochemical studies performed on the BC domain of this important enzyme will be presented. Through mutagenesis studies of four key residues that reside in the BC dimer interface of *Staphylococcus aureus* PC, we found that the BC dimer is very stable. Most of the mutations eliminated enzymatic activity, as well as disrupted the normal tetrameric state of PC in solution. This indicates that PC has a strong tendency to self-associate in its active, tetrameric form, especially at high concentrations of protein.

**Keywords: enzyme, biotin-dependent carboxylase, metabolism**

## MS93.P49

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**Crystal structure of the catalytic domain of multidomain PHB depolymerase**

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Polyhydroxyalkanoate (PHA) is a naturally occurring biodegradable biopolyester which is synthesized and degraded by microbes. PHA represents various physical properties depending on the chemical structure of monomer units, and it draws attention as an environment-friendly material. PHA is synthesized and stored as an energy-storage material in bacterial cells, and stabilized in an amorphous state, coated with fatty acids and proteins. This native PHA changes its structure to a partially crystallized state (denatured PHA) by removal of associated fatty acids and proteins. Specific PHA depolymerases acting on each types of PHA are known. Extracellular depolymerases are able to act on denatured PHA but not on native PHA, whereas intracellular depolymerases have the opposite specificity.

Most of known extracellular depolymerases act only on denatured PHA with short-chain-length monomer units (mainly *R*-3-hydroxybutyrate), so they are often called polyhydroxybutyrate (PHB) depolymerase. They are typically composed of three domains: catalytic, linker and PHB-binding domains. We have succeeded in determination of the crystal structure of the catalytic domain of an extracellular PHB depolymerase from *Ralstonia pickettii* T1 at 2.4 Å resolution by Au-SAD method, and refined its coordinates to 2.1 Å resolution. Crystals of the enzyme belonged to monoclinic space group $P2_1$, with cell dimensions $a$ = 67.3 Å, $b$ = 150.7 Å, $c$ = 78.3 Å, β = 93.1˚, and contained four polypeptides in the asymmetric unit. Crystals of a gold derivative were obtained by quick-soaking using a solution containing $KAuCl_4$. Diffraction data were collected with X-ray of the

peak wavelength of gold, and processed with HKL2000. Phasing, model building and refinement were conducted using PHENIX, REFMAC5, COOT and other programs of CCP4. Electron densities for the catalytic domain of the enzyme were clearly obtained, but those for the linker and PHB-binding domains were not, in spite that there is remaining space for these domains to be packed in the crystal lattice.

The overall structure of the catalytic domain represented an α/β hydrolase fold which is often observed for the large superfamily of esterases including lipases and carboxypeptidases. A cervice was formed on the surface of the protein, at the bottom of which are located catalytic triad residues Ser-166, Asp-241 and His-300. The structure was compared with that of a fungal single-domain depolymerase with circular permuted polypeptide connectivity [1]. These two enzymes differ in the product composition. The main product is dimer of *R*-3-hydroxybutyrate for the bacterial enzyme, whereas it is monomer for the fungal enzyme. Structures of loop regions around the active site were different between the two enzymes. This may differentiate the mode of interactions with monomer units of the substrate polymer for these enzymes, which may explain the difference in the product composition.

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**Keywords: biodegradable polyester, esterase, crystallography**

## MS93.P50

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**Intermediates along the NAD$^+$ cyclisation reaction pathway of ADP-ribosyl cyclase**

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Calcium is a ubiquitous, versatile intracellular signal regulating numerous cellular processes [1]. The versatility of $Ca^{2+}$ signalling emerges from the use of an extensive repertoire of signalling components known as a $Ca^{2+}$ signalling toolkit that can be assembled in combinations to create signals with different spatial and temporal profiles. Mobilisation of intracellular $Ca^{2+}$ stores into the cytoplasm is mediated through three structurally divergent messengers, one of them being cyclic ADP-ribose (cADPR) [2].

cADPR acts on the ryanodine receptor to elicit $Ca^{2+}$ release. it is ubiquitous across species and is an endogenous modulator of $Ca^{2+}$-induced $Ca^{2+}$ release thereby regulating a wide range of physiological processes [3]. cADPR is a cyclic nucleotide converted from NAD$^+$ by multifunctional enzymes of the ADP-ribosyl cyclase family [4]. *Aplysia* ADP-ribosyl cyclase (*Aplysia* cyclase) was the first enzyme identified to catalyse the cyclisation of NAD$^+$ to cADPR, with the release of nicotinamide . *Aplysia* cyclase is a 30kDa protein initially isolated from *Aplysia* ovotestis [5]. It is also found to be present in the neurons of the *Aplysia* buccal ganglion, where production of cADPR can enhance the evoked synaptic transmission [6]. Recently, it has been shown that *Aplysia* cyclase translocates from the cytosol into the nucleus upon depolarisation of *Aplysia* neurons, providing a mechanism for selective and specific activation of the nuclear $Ca^{2+}$ store in neurons offering versatility for the neurons to respond to a wide range of stimuli [7].

Cyclisation of NAD$^+$ into cADPR involves a two step reaction, the elimination of the nicotinamide ring and the cyclisation of the intermediate resulting in the covalent attachment of the adenine ring to the anomeric carbon of the terminal ribose. Cyclisation of NAD$^+$ results in the linkage of the adenine and terminal ribose moieties established via the N1-position of the adenine ring, while cyclisation of NAD$^+$ analogues resulted in the covalent attachment to the terminal ribose via the N7 position of the purine ring of these analogues [8].

In this study, we have determined the structures of wildtype *Aplysia* cyclase complexed with its substrates, NAD$^+$ and NGD$^+$, and its products cADPR and cGDPR. In addition, we were also able to capture the reaction intermediates of the cyclisation reaction either by controlling the soaking time of the substrate or with the use of substrate analogues. Taken together, we are able to obtain snapshots of the cyclisation process of the dinucleotide resulting in either N1 or N7 linkage of the purine ring to the terminal ribose.

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**Keywords: enzyme, catalysis, mechanism**

## MS93.P51

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**Sulfolobus solfataricus adenine phosphoribosyltransferase**

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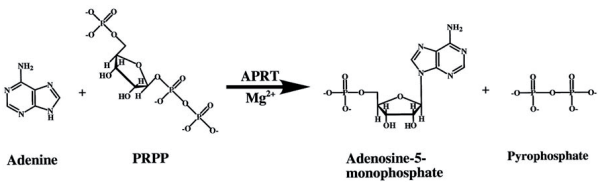
Phosphoribosyltransferases (PRTases) are a group of enzymes that catalyze the formation of nucleotide 5´-monophosphates as essential precursors in the synthesis DNA or RNA. **PRTases** all use a common substrate, 5-phosphoribosyl-α-1-pyrophosphate (PRPP), and in the presence of $Mg^{2+}$ they transfer nucleobases to C1 of the ribose 5´-phosphate moiety of PRPP to form nucleotide 5´-monophosphates under the release of pyrophosphate ($P_2O_7^{4-}$). PRTases are involved in both the *de novo* biosynthesis of nucleotides, e.g. orotate PRTase (OPRTase) and in the salvage pathways, e.g. uracil PRTase (UPRTase), adenine PRTase (APRTase) and hypoxantine-guanine-xanthine PRTases (HGXPRTases; often with mixed specificity). PRTases share a common domain fold (type 1) which defines the specificity for PRPP and a variable domain for recognition of the various nucleobases.




**Adenine PRTase** structures are known for eukaryotes and bacteria, and details of their active sites have been mapped out. **APRTase** from the **thermophile archaean *Sulfolobus solfataricus*** is an enzyme with unexpected properties: The substrate binding order is reversed with adenine binding first followed by PRPP. It has a double pH optimum and is potently inhibited by AMP and ADP. Phosphate $PO_4^{3-}$ seems to facilitate adenine binding.

Sequentially, ***Ss*APRTase** does not resemble other APRTases but merely HGXPRTases from eukaryotes, bacteria and archaea. The closest sequence homologues in PDB (1nul [1] & 1vdm [2]) used for molecular replacement share about 31 % sequence identity for a 143/210 residue stretch of the sequence. The remaining sequence does