significantly modify the structural properties and organization of the protein. The crystal structure of TmPrxNtrC40S has been determined for the oxidized form and for the reduced form in presence of hydrogen sulfite, as well as two crystalline forms of the TmPrx module.

The full-length protein TmPrxNtr, which contains a FMN prosthetic group, displays nitroreductase, peroxidase and quinone, flavine, chromate and iron reductase activities using either NADH or NADPH as a donor. The mutation of the catalytic cysteine of the Prx module (TmPrxNtrC40S) does not alter the catalytic efficiency of the protein suggesting that the activity measured does not require the Prx module. Both modules TmPrx and TmNtr have peroxidase activity but the oxidized enzyme is not regenerated in the same way. NAD(P)H is the direct reductant for TmNtr while TmPrx is regenerated *in vitro* via glutaredoxins or thioredoxins. In addition TmPrx is able to reduce H_2O_2 and COOH whereas TMPrxNtr only reduces H_2O_2.

The TmPrxNtrC40S enzyme crystallizes as a dimer, the active form of the protein. The Ntr module of one monomer is in contact with the Ntr and Prx modules of the other one but no contact is observed between the two Prx modules. On the contrary, in the crystalline forms of the isolated domain, two TmPrx molecules are linked by an intermolecular disulfide bond. In TmPrxNtrC40S, the FMN prosthetic groups bind in deep pockets at the dimer interface and interact with elements of both monomers. An ion sulphate is positioned by hydrogen bonding to the catalytic site of the Prx modules. In the reduced TmPrxNtrC40S crystal, one sulphate is replaced by a bisulfite introducing structural modifications to the associated Prx module.

Altogether, the results indicate that the TmPrx and TmNtr modules apparently function independently, each possessing its own peroxidase activity, with the TmNtr module having diaphorase activity linked to the FMN moiety versus a broad range of substrates. This raises the question of their fusion which is specifically found in thermotogales.

Keywords: hybrid-flavoenzyme, reductase, X-ray

MS93.P45

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Hpy188I-DNA structures - snapshots of the GIY-YIG nuclease mediated catalysis

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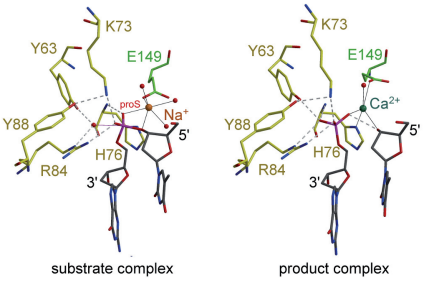
GIY-YIG nucleases are present in all kingdoms of life and have very diverse roles. Well characterized functions include transposon migration, flap cutting and Holliday junction resolution in eukaryotes and nucleotide excision repair in prokaryotes. Despite many roles of GIY-YIG nucleases, until very recently no GIY-YIG domain has ever been crystallized in complex with target DNA. Therefore, many questions about the catalytic mechanism of the GIY-YIG module were unresolved.

We have obtained Hpy188I crystal structures in ternary substrate and product complexes with DNA and metal ion in the active site [1]. Our structures suggest that GIY-YIG nucleases catalyze DNA hydrolysis by a single substitution reaction. They are consistent with a previous proposal that a tyrosine residue (which we expect to occur in the phenolate form) acts as a general base for the attacking water molecule. In contrast to the earlier hypotheses, our data identify the general base with the GIY and not the YIG tyrosine. A conserved glutamate residue (Glu149 provided in trans in Hpy188I) anchors a single metal cation in the active site. This metal ion contacts the phosphate proS oxygen atom and the leaving group 3'-oxygen atom, presumably to facilitate its departure. Taken together, our data reveal striking analogy in the absence of homology between GIY-YIG and ββα-Me nucleases. Our mechanistic conclusions agree with those for Eco29kI restriction endonuclease [2].



[1] M. Sokolowska, H. Czapinska, M. Bochtler, *Nucleic Acids Res.* **2011,** *39*, 1554-1564. [2] A.N. Mak, A.R. Lambert, B.L. Stoddard, *Structure* **2010**, *18*, 1321-1331.

Keywords: GIY-YIG nuclease, mechanism, restriction enzyme

MS93.P46

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Structural variability of type II restriction endonucleases

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Type II restriction endonucleases are very diverse in sequence and have therefore long been considered as phylogenetically unrelated. This picture changed when crystal structures revealed that the frequently used enzymes all belong to the PD-(D/E)XK class of nucleases, which use a two metal ion mechanism to catalyze DNA hydrolysis. We have systematically studied restriction endonucleases that cleave DNA with unusual staggers. This property turns out to "select" for structurally novel enzymes. Almost simultaneously with Prof. Barry Stoddard, we have solved the first structures of ββα-Me and GIY-YIG restriction endonucleases, in our case with Hpy99I and Hpy188I as the model enzymes [1], [2], [3]. The structures reveal interesting mechanistic analogies between the new groups of restriction endonucleases and "classic" PD-(D/E)XK enzymes. Surprisingly, the metal ion B, which promotes the departure of the 3'-OH leaving group, and is "optional" in at least some of the PD-(D/E)XK nucleases is conserved in both ββα-Me and GIY-YIG nucleases. In contrast, the metal ion A, which is obligatory in PD-(D/E)XK enzymes and activates the nucleophilic water molecule is absent from both new nuclease families. The attacking water molecule is instead activated by a histidine or a tyrosine residue (we presume the latter to be present in the phenolate form).

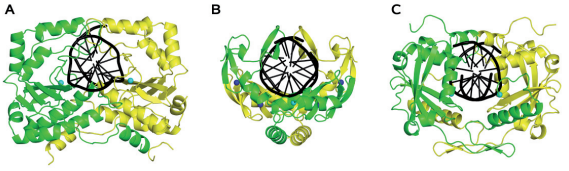

Fig. 1: PD-(D/E)XK endonuclease ThaI (**A**), ββα-Me endonuclease Hpy99I (without N-terminal domain) (**B**) and GIY-YIG nuclease Hpy188I (**C**). All three enzymes are functional dimers and recognize palindromic (symmetric) or pseudopalindromic (approximately symmetric) target sites.

[1] M. Sokolowska, H. Czapinska, M. Bochtler, *Nucleic Acid Res* **2011,** *39*, 1554-1564. [2] M. Sokolowska, H. Czapinska, M. Bochtler, *Nucleic Acid Res.* **2009,** *37*, 3799-3810. [3] M. Firczuk, M. Wojciechowski, H. Czapinska, M. Bochtler, *Nucleic Acid Res*. **2011,** *39*, 744-754.

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.

[1] M. Álvaro-Benito *et al. J. Biol. Chem.* **2010,** *285(18),* 13930-13941. [2] A. Polo *et al*. *Acta Crystallog.* **2010,** *F66,* 1441-1444.

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