nicks. Members of this ubiquitous family of structure-specific endonucleases function as dimers and require divalent cations for cleavage. We have solved the crystal structures of cruciform cutting enzyme 1 (CCE1) from *Candida glabrata* and Holliday junction cutting enzyme (Hjc) from the hyperthermophile archaeon *Archaeoglobus fulgidus* [1] at 3 and 1.7Å resolution, respectively. They represent two structurally distinct resolvase families with the same biological function, but exhibiting clearly different substrate specificities. Co-crystallization of these proteins with selected DNA substrates is under way.

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MS03 P04

The Protease State of the Heat-Shock Protein DegP from Escherichia coli Tobias Krojer, Justyna Sawa, Fuhrmann Jakob, Gazda Linn, Kirk Rebecca, Kurt Juliane, Nussbaumer Barbara, Schmidt Bastian and Tim Clausen, *Research Institute of Molecular Pathology (IMP), Dr. Bohrgasse 7, A-1030, Vienna, Austria.*
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Keywords: DegP, protease, chaperone

DegP (a.k.a. HtrA, protease Do) is a heat-shock protein which is localized in the periplasm of *Escherichia coli*. It is an essential, ATP-independent protein quality control factor and represents the only known protein that can alternate between the two antagonistic activities of a **protease** and a **chaperone** in a temperature-dependent manner [1]. The mature protein consist of a chymotrypsin-like serine protease domain and two consecutive, C-terminal PDZ domains. DegP is present as a hexamer in solution and belongs to the diverse family of cage-forming proteases. The active sites are shielded from the environment in an inner cavity, to prevent the unwanted degradation of functional proteins. The crystal structure of the chaperone conformation of DegP revealed that substrate degradation at low temperatures is additionally abolished by a distorted and blocked active site [2].
Here we present the crystal structure of DegP in complex with the small molecule inhibitor diisopropyl fluorophosphates (DFP). DFP did not lock the active sites in the canonical conformation of chymotrypsin-like serine proteases. The inhibitor is rather bound in an atypical conformation, which was never observed before in serine proteases, again underlining the remarkable flexibility of the active site. Furthermore, time-dependent analysis of degradation products by HPLC chromatography revealed that DegP acts as a processive protease, similar to other cage-forming proteases like the proteasome or ClpP. Analysis of degradation products of DegP by mass spectrometry confirmed that DegP has a clear preference for aliphatic residues in the P1 site. Furthermore, the product spectrum shows a normal distribution with a mean peptide length of 13 to 15 residues.
In summary, our results suggest that the proteolytic active sites of DegP exhibit an unusual degree of flexibility, which may be necessary to allow the efficient degradation of a wide variety of different, misfolded proteins before those become harmful for the cell. The observed product length distribution and the processive degradation of substrate proteins refer to yet unidentified trans-acting determinants which are essential for substrate binding and degradation.

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MS03 P05

Identity of Ions in the Second Site of Subtilisins
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Keywords: subtilisin, serine protease, calcium binding

Serine proteases classified under the EC number 3.4.21.62 – subtilisins - have been extensively studied by x-ray crystallography with focus on the relationship between metal binding and stability. These α/β hydrolases with broad specificity typically bind one or more calcium ions and in some cases other, mostly monovalent, ions [1]. Subtilisins BPN´/NOVO, Carlsberg or Savinase were studied systematically as for their stability related to metal binding since 1969. Two typical sites - the strong calcium binding site (Ca-I, formerly named site A) and the weak calcium binding site (Ca-II, formerly named site B) were identified. Stability of subtilisin BPN' depends on the presence of site Ca-I whereas the stabilization effect of site Ca-II depends on the concentration of ions in the solvent [2]. Site Ca-II is accompanied by a closely related site Na-II only 2.6 Å away, with high affinity for sodium. Our detailed analysis of the contents of sites Ca-II and Na-II as present in the published structures leads to the conclusion that in some cases their contents were misinterpreted. We have reclassified the typical metal binding sites of subtilisins and subtilisin-like proteases and proposed the most likely identities of the ions and atoms bound in sites Ca-II and Na-II in the publicly available structures.
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MS03 P06

The Enolase in the Methionine Salvage Pathway: Crystal Structure and Function. A.Fedorov, H.Imker, E.Fedorov, J.Gerlt, S. Almo. *Department of Biochemistry, AECOM, New York,USA.* E-mail:fedorov@aecom.yu.edu

Keywords:Enolase, RuBisCO, Crystal structure

D-Ribulose1,5-biphosphate carboxylase/oxygenase (RuBisCO), the most abundant enzyme, is the paradigm member of the recently recognized mechanistically diverse RuBisCO superfamily. The heterofunctional homologue of RuBisCO found in Geobacillus kaustophilus catalyze the enolization of 2,3-diketo-5-methylthiopentane 1-phosphate in the methionine salvage pathway. Because the RuBisCO and the enolase – catalyzed reactions differ we sought to

establish structure-function relationships for the enolase reaction. The crystal structures of the activated enolase (carboxylated on Lys 173) and enolase liganded with Mg and 2,3-diketohexane, a stable alternate substrate, were determined. The stereochemical course of the reaction catalyzed by the enolase was determined using stereospecifically deuterated samples of an alternate substrate. On the basis of these experiments we conclude that the enolase, the functionally divergent member of the RuBisCO superfamily uses the same structural strategy as RuBisCO for stabilizing the enolate anion intermediate , but the proton abstraction is catalyzed by a different general base.

MS03 P07

Structural Study of Selenocysteine Lyase Rie Omi[a], Suguru Kurokawa[a], Hisaaki Mihara[a], Tatsuo Kurihara[a], Nobuyoshi Esaki[a], Ken Hirotsu,[b] Ikuko Miyahara[b], [a]*Institute of Chemical Research, Kyoto University, Physics,* [b]*Graduate School of Science, Osaka City University, Japan.*
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Keywords: enzyme activity mechanism, vitamin B6, substrate binding

Selenocysteine lyase (SCL), which is a pyridoxal 5'-phosphate (PLP) dependent enzyme, catalyzes the β-elimination of L-selenocysteine to yield L-alanine and selenium. SCL is specific for L-selenocysteine and has no activity for L-cystein, therefore it is known as the key enzyme in the specific selenium-delivery pathway for selenoprotein synthesis. In order to elucidate the strict discrimination between selenium and sulfur by SCL, we have determined the three-dimensional structures of native SCL and L-cysteine complexat 1.8 Å and 1.9 Å, respectivery.
Overall and active site structure of SCL is similar to those of cysteine desulfurase which catalyze the same type of reaction as SCL but act on both cysteine and selenocysteine. Why SCL does not show activity on cysteine? In the case of SCL/L-cysteine complex structure, not the amino group of L-cysteine but the thiol group is located close to the C4A of PLP in the active site. This indicates that L-cysteine is incorporated into the active site but can not make an external aldimine with PLP. The mechanism for discrimination between selenium and sulfur will be presented on the basis of the structural comparison between SCL and cysteine desulfulase;

MS03 P08

Crystal Structure Of A Dead-End Complex With Two Isopentenyl Diphosphate Molecules Sheds Light On Substrate Recognition By Human FPPS Jean-Michel Rondeau, Emmanuelle Bourgier, Sylvie Lehmann and Wolfgang Jahnke, *Discovery Technologies, Novartis Institutes for Biomedical Research, CH-4002, Basel, Switzerland.* E-mail: jeanmichel.rondeau@novartis.com

Keywords: structural enzymology, drug mechanism, activity and mechanism of enzymes

Farnesyl diphosphate synthase (FPPS) catalyses the "head-to-tail" condensation between a homoallylic diphosphate, isopentenyl diphosphate (IPP), and an allylic diphosphate, dimethylallyl diphosphate (DMAPP) or geranyl diphosphate (GPP). Early biochemical studies have shown the existence of distinct allylic and homoallylic binding sites, and the dependence of allylic substrate binding on divalent metal ions [1]. Furthermore, the FPPS reaction has been shown to follow an ordered mechanism, with the allylic substrate binding first to the enzyme [2]. However, it is not clear how the enzyme is able to differentiate, at the molecular level, between its two, structurally highly similar, substrates, IPP and DMAPP. In the absence of DMAPP, binding of up to two IPP molecules per FPPS active site has been observed. Moreover, substrate inhibition by high IPP concentrations has been reported [1], also indicating that the allylic binding site does not fully discriminate between DMAPP and IPP. We have crystallized the FPPS ternary complex with two IPP molecules and determined its X-ray structure at 1.70Å resolution. Our data reveal that IPP adopts an energetically less favorable conformation in the allylic site, with the C(1)-C(2)-C(3)-C(4) dihedral angle taking an intermediate value (22°) between that expected for DMAPP (0°) and that observed for IPP in the homoallylic binding site (51°). Hence, discrimination between the C5-isoprenoid substrates appears to be mainly achieved through steric fit and shape recognition.

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MS03 P09

PglD – an acetyl transferase from *Campylobacter jejuni*
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Keywords: Crystallography, Acetyl transferase, CoA

PglD is a protein which is part of an N-linked protein glycosylation system in *Campylobacter jejuni*. The glycan produced by this system was identified to be GalNAc-α1,4-GalNAc-α1,4-[Glcβ1,3-]GalNAc-α1,4-GalNAc-α1,4-GalNAc-α1,3-Bac-β1,*N*-Asn-Xaa, where Bac is bacillosamine, 2,4-diacetamido-2,4,6-trideoxyglucopyranose [1]. PglD has been predicted to be an acetyltransferase which is involved in the biosynthesis of bacillosamine.
Through protein crystallography the atomic structure of PglD reveals that it is a trimer composed of three identical subunits that are related by a crystallographic three-fold rotation axis. Each subunit is composed of 2 domains: an N-terminal domain which is formed by residues 2-70 and the C-terminal domain that stretches from residues 71 to 195. The N-terminal domain consists of two α helices and three parallel β sheets. What is most striking about the C-terminal is that it folds into a left-handed parallel β helix (LβH). The chain in this domain is composed of 6 coils that are wound, in a left handed sense, around the surface of an equilateral prism. Enzymes that contain this fold are placed into a superfamily termed hexapeptide acyltransferases.
PglD adds the acetyl group from acetyl CoA to UDP-4-amino-4,6-dideoxy-α-DGlcNac to make the product bacillosamine, the first reagent required in the glycosylation system [2]. PglD was crystallised with the presence of CoA in the active site which is located along a crevice that is present between the interface of two adjacent monomers. The interactions of CoA with PglD will be discussed further on the poster.