

which were filled with other atoms from the boron group (In and Tl) by 3 to 5 at.%. The binary convex hulls for these systems were also calculated.

From our results we compare the mechanical and energetical stability of different types of theoretical heptagonal approximants, in relation to the stable modifications of Gallium. We could draw conclusions about the mechanisms of heptagonal ordering in Gallium from the structure types decomposing during relaxation. We were also able to estimate the influence of doping on the stability of our approximants.

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**Keywords:** gallium, quasicrystal, calculation

## MS64.P01

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### A novel structure: PSPC1/NONO heterodimer, members of the DBHS protein family

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Proteins of the Drosophila Behavior and Human Splicing (DBHS) family share a common domain structure: two RNA recognition motifs (RRMs), a NONA/Paraspeckle (NOPS) domain followed by a coiled-coil region. Paraspeckle Protein Component 1 (PSPC1) and Non-POU domain-containing octamer-binding protein (NONO) are members of this family and have a highly similar sequences within the DBHS domain. PSPC1 and NONO form a heterodimer and they are co-localised to paraspeckles, a dynamic sub-ribonuclear bodies in eukaryotic cells [1]. These proteins will be recruited to paraspeckles at the point of transcription of a long non-coding RNA, called NEAT1 which forms the structural scaffold of these bodies [2,3,4]. Within paraspeckles, PSPC1 and NONO are involved in transcriptional regulation by confining RNA to the nucleus, preventing subsequent protein production [2,3,4]. However, the mechanisms at the molecular level behind these processes are still unclear. Structural analysis of PSPC1 and NONO and investigation of RNA binding partners will broaden the knowledge about their functions in transcriptional control. Here we present the first paraspeckle protein structure, the PSPC1/NONO heterodimer. This structure highlights the DBHS domain with a novel arrangement of four different RRM. Furthermore, we describe a new protein-protein interaction domain (NOPS). We also observe the first example of an anti-parallel right handed heterodimeric coiled-coil. This PSPC1/NONO structure provides us with new insights into the functions of paraspeckle assembly as well as possible RNA binding modes.

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**Keywords:** paraspeckle proteins, rna-recognition motif, coiled-coil

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### Structural basis of inhibition mechanism by carrot EDGP against endoglucanase

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Plant cell wall is composed of various polysaccharides such as cellulose, hemicellulose and pectin. Cellulose microfibrils are linked via hemicellulose. The network of cellulose-hemicellulose provides tensile strength. Carrot extracellular dermal glycoprotein (EDGP) is one of proteinous inhibitor to protect cell wall. To penetrate and use plant cell walls nutritionally, pathogen secretes cell wall degrading enzymes. These enzymes including endoglucanases, xylanase and polygalacturonases are classified into glycoside hydrolase (GH) families. EDGP shows inhibitory activity against xyloglucan specific endo- $\beta$ -1,4-glucanase (XEG) from *Aspergillus aculeatus*. XEG belongs to GH12 family. XEG specifically cleaves xyloglucan that is a major hemicellulose of dicots. Xyloglucan consists of  $\beta$ -linked glucose backbone substituted with xylose side-chains. The degradation of xyloglucan is great damage for dicotyledonous plants. Thus, inhibition of XEG by EDGP is important in plant defense system. Until now, the homologous proteins of EDGP were found in various plants. The tomato homolog (xyloglucan specific endo- $\beta$ -1,4-glucanase inhibitor protein, XEGIP) and tobacco homolog (Necturin IV, NEC4) also inhibit XEG. In contrast, the homologous protein from wheat (*Triticum aestivum* xylanase inhibitor, TAXI) inhibits GH11 xylanases. Interestingly, a soybean homolog (Basic 7S globulin, Bg7S) lacks inhibitory activity for both GH11 and GH12 enzymes.

To clarify the inhibition mechanism of EDGP against GH12 endoglucanase, we work on structure determination of EDGP and EDGP in complex with GH12 enzyme by X-ray crystallography. EDGP and the inhibition complex with FI-CMCase, which is GH12 endoglucanase from *Aspergillus aculeatus*, were successfully crystallized. Hexagonal crystal of EDGP belonged to space group  $P6_6$ , with unit cell parameters  $a = b = 130.4$ ,  $c = 44.5$  Å and  $\beta = 120^\circ$ . Monoclinic crystal of EDGP complex with FI-CMCase belonged to space group  $C2$  and unit cell parameters  $a = 169.5$ ,  $b = 143.0$ ,  $c = 63.0$  Å and  $\beta = 110.9^\circ$ . The crystal structure of EDGP was determined by SIRAS method using iodine derivative crystal, and the crystal structure of EDGP~FI-CMCase complex was determined by molecular replacement. The structure of EDGP~FI-CMCase complex reveals that Arg423 of EDGP intrudes into the active site of FI-CMCase. The arginine residue is conserved in homologous proteins that have inhibitory activity for GH12 enzymes. This work provides structural basis of inhibition mechanism by EDGP and its homologous proteins against GH12 enzymes.

**Keywords:** plant, inhibitor, structure

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### Architecture of the mediator head module

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## Poster Sessions

The concept of the cell as a collection of multi-subunit protein complexes are emerging as a cornerstone of modern biology. Transcription by RNA polymerase II (Pol II) is a prime example for this concept as it is regulated by large protein assemblies comprising many subunits, including Mediator. Structure determination of these multi-protein complexes is essential to understand gene regulation mechanism.

We have solved crystal structure of the Mediator Head module (7 subunits, 223 kDa) at 4.3 Å resolution [1]. Our Mediator Head structure reveals the striking complex assembly mechanism: the multi-helical bundle with five different Mediator subunits is formed as a single structure unit, thereby ensuring stable assembly of the Head subunits, as well as providing the binding sites for general transcription factors (GTFs) and Pol II. Such interactions could not have been determined from structures of individual subunits alone, or from analyzing pairwise small domain-domain interactions, but only by study of the multi-protein complex in its entirety.

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**Keywords:** transcription, macromolecule, complex

### MS64.P04

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**Substrate fingerprint and the structure of NADP<sup>+</sup> dependent serine dehydrogenase from *Saccharomyces cerevisiae* complexed with NADP<sup>+</sup>**

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NADP<sup>+</sup>-dependent serine dehydrogenase [EC 1.1.1.276] from *Saccharomyces cerevisiae* (YMR226C) was determined to a resolution of 2.36 Angstroms. The protein is the first structure solved of the NADP<sup>+</sup> serine 3-dehydrogenase group with the conformation of all three substrate binding loops fully resolved.

This protein contains a 5 substrate-fingerprint of AG-YTG, which is one of the five most observed substrate-fingerprints in the TGYK-SCOR family comprising over 150 members from different species of bacteria and lower eukaryotes. The binding of the cofactor and a bond between the substrate fingerprint residues Y162 and R209 stabilizes the 3<sup>rd</sup> substrate binding loop forming the binding pocket. Although all residues in the predicted 5-substrate binding fingerprint may not directly contact the substrate, the structure revealed their importance to forming the secondary shell to the binding pocket and verifying the predicted residues in clustering and characterizing members in this subfamily.

**Keywords:** NADP<sup>+</sup> dependent serine dehydrogenase, substrate fingerprint, short chain oxidoreductase

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**Transcriptional activator DmpR - combining BioCrystallography and BioInformatics**

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Our goal is to understand the molecular mechanisms by which bacteria sense and respond to environmental pollutants. The *Pseudomonas putida* DmpR protein is an aromatic compound sensor and a  $\sigma^{54}$ -dependent transcriptional regulator that belongs to the AAA<sup>+</sup> superfamily of ATPases. DmpR controls a multitude of physiological processes in response to environmental signals. Its ATPase activity is essential for the activation of the isomerisation incompetent  $\sigma^{54}$ -RNA polymerase. The evolutionary highly conserved catalytic C-domain of DmpR harbours the ATPase activity that defines the family and interacts with the transcriptional apparatus.

Using X-ray crystallography, small angle X-ray scattering, SAXS, and bioinformatics methods we elucidate the structure of the inactive dimer and the active hexameric form. In addition, by designing single point mutations and deletion mutations in the active site environment of the C-domain of DmpR we explore the contribution of certain amino acids to ATP binding and ATPase activity.

**Keywords:** AAA<sup>+</sup> ATPase, DNA binding, signalling

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**Crystal Structure of Archaeal Cambialistic Superoxide Dismutase**

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Superoxide dismutases (SODs) play a protective role against oxidative stress by catalyzing disproportionation of the superoxide anion radical to hydrogen peroxide and dioxygen. The SOD-catalyzed reaction proceeds through a redox cycle of metal ions. SODs are grouped into four classes according to their metal cofactors: Cu,Zn-SOD, Fe-SOD, Mn-SOD, and Ni-SOD. Fe- and Mn-SODs are closely related in amino acid sequences. Several SODs, which are termed cambialistic SODs, are active in the presence of either Fe or Mn. A hyperthermophilic archaeon *Aeropyrum pernix* K1 produces a cambialistic SOD (ApeSOD), with more activity in the presence of Mn than Fe. Here we present the crystal structures of ApeSOD in the apo, Mn-bound, and Fe-bound forms determined at the resolutions of 1.56, 1.35, and 1.48 Å, respectively [1].

The overall structure consisted of a homotetramer both in crystal and solution. The tetrameric assembly of ApeSOD contained significantly more intersubunit ion pairs than SOD from a thermophilic bacterium *Thermus thermophilus* (TthSOD); 24 and 4 intersubunit ion pairs were found from ApeSOD and TthSOD, respectively. In accordance with this, ApeSOD was more stable than TthSOD under organic conditions, although these enzymes showed similar thermostability in aqueous conditions. Next we focused on differences in active site structures of ApeSOD depending on binding of metal cofactors. While Mn was in trigonal bipyramidal coordination with five ligands, the Fe-bound form contained additional water and the metal was in octahedral coordination with six ligands. The additional water occupied the position of superoxide binding in the Fe-bound form. Upon binding of Fe, the OH of Tyr residue in the outer sphere of the active site shifted 1.1 Å to the central metal cofactor, whereas the shift upon Mn binding was negligible. These features are discussed in relation with lower activity of ApeSOD in the presence of Fe.