systems, thus the inactive dimer conformation may be the keypoint in determining signaling specifity.

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Keywords: GTP-binding proteins, biophysical analysis, SAXS

FA1-MS06-P05

Crystal Structure of Protein-Glutaminase and Its Pro-Structures Similar to Enzyme-Substrate Complex. Bunzo Mikami[a], Ryota Hashizume[a] Kimihiko Mizutani[a], Nobuyuki Takahashi[a], Hiroyuki Matsubara[b], Akiko Matsunaga[b], Shotaro Yamaguchi[b]. [a]*Laboratory of Applied Structural Science, Graduate School of Agriculture, Kyoto University, Japan.* [b]*Gifu R&D Center, Amano Enzyme Inc.Japan.*
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Protein-glutaminase, which converts glutamine residues in protein or peptide to glutamic acid residues, is expected to see wide use as a new food processing enzyme. Deamidation of proteins can improve their solubility, emulsifying activity, forming activity, and other functional properties by increasing the number of negative charges. The crystal structure of mature protein-glutaminase with 185 amino acid residues was determined by the single-wavelength anomalous dispersion (SAD) method and refined at 1.15 Å. The overall structure, which consists of four helices and two antiparallel □-sheets with four and two strands, has a weak homology with the core domain of human tissue transglutaminase 2. The catalytic triad, Cys-His-Asp conserved in transglutaminases was also found in this enzyme. The important differences in the catalytic region from transglutaminase could be explained by their reaction specificity. The enzyme has large hydrophobic surface in order to interact with protein substrate. We also determined the structures of recombinant pro-enzyme and its mutant with 299 amino acid residues. It is remarkable that a short loop around Ala 47 in the pro-region covers and interacts with the active site of the mature region. Crystal structures of A47Q mutant showed that the side chain of Gln 47 interacts with the catalytic residue, Cys 156, forming a covalent bond between Cys156/Sγ and Gln47/Cδ depending on the pH of the crystallization medium. The structure of A47Q mutant provides insights into the catalytic mechanism of the enzyme which forms a covalent S-acyl intermediate before release of ammonia.

Keywords: protein-glutaminase, pro-enzyme, enzyme mechanisms

FA1-MS06-P06

How chromosomes interact with microtubules. Andrea Musacchio. *Department of Experimental Oncology, European Institute of Oncology, Milan, Italy*
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To be parted into two equal complements during mitosis, the sister chromatids need to establish secure connections with the mitotic spindle, a microtubule- and motor-based structure [1]. The point of attachment of chromosomes to spindle microtubules is named the kinetochore. This protein assembly contains up to 100 or more proteins. Our current challenge is to understand the molecular mechanism of the interaction of kinetochores with microtubules. A 10-protein assembly known as the KMN network is responsible for this interaction. We have combined different approached to gain a structural and functional understanding of the role of the KMN network in microtubule attachment. Furthermore, we are investigating the mechanisms through which the KMN network regulates feedback control mechanisms that are responsible for the fidelity of the chromosome segregation process, namely the spindle assembly checkpoint [2].

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Keywords: cell cycle proteins, chromosome dynamics, biological macromolecules

FA1-MS06-P07

An Archaeal Dim2 Homolog Forms a Ternary Complex with a/eIF2α and 16S rRNA. Koji Nagata[a], Min Ze Jia[a,b], Shoichiro Horita[a], Masaru Tanokura[a]. [a]*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan.* [b]*Institute of Biophysics, Chinese Academy of Sciences, China.*
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Dim2p is a eukaryal small ribosomal subunit RNA processing factor required for the maturation of 18S rRNA. We have shown that an archaeal homolog of Dim2p, aDim2p, forms a ternary complex with the archaeal homolog of eIF2α, a/eIF2α, and the RNA fragment that possesses the 3'-end sequence of 16S rRNA both in solution and in crystal [1]. The 2.8-Å crystal structure of the ternary complex reveals that two KH domains of the aDim2p, KH-1 and -2, are involved in binding the anti-Shine-Dalgarno (SD) core sequence (CCUCC-3') and a highly conserved adjacent sequence (5'-GGAUCA), respectively, of the target rRNA fragment. The surface plasmon resonance results show that the interaction of the aDim2p with the target rRNA fragment is very strong with a dissociation constant of 9.8×10^{-10} M and that the aDim2p has a strong nucleotide sequence preference for the 3'-end sequence of 16S rRNA. On the other hand, the aDim2p interacts with the isolated α subunit and the intact αβγ complex of the a/eIF2, irrespective of the RNA binding. These results suggest that the aDim2p is a possible archaeal pre-rRNA processing factor recognizing the 3'-end sequence (5'-GAUCACCUCC-3') of 16S rRNA, and may have multiple biological roles *in vivo* by interacting with other proteins such as the a/eIF2 and the aRio2p.

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Keywords: archaeal Dim2p, archaeal eIF2, rRNA processing

FA1-MS06-P08

Structural analysis of PDZ modules of bacterial site-2 protease. Terukazu Nogi, Junichi Takagi. *Institute for Protein Research, Osaka University, Japan.*
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Site-2 proteases (S2Ps) constitute a large family of intramembrane cleaving proteases (I-CLiPs). S2Ps are metalloproteases and widely found in many species ranging from prokaryotes to higher eukaryotes. It is known that S2Ps participate in signal transduction related to stress response and lipid metabolism. In many systems, membrane-anchored precursor of transcription factor or suppressor protein of transcription factor are identified as the physiological substrates of S2Ps. The intramembrane proteolysis of the substrate results in release of the transcription factor from the membrane and expression of stress response genes. In *Escherichia* (*E.*) *coli,* an S2P homologue, RseP, is involved in the extracytoplasmic stress response. RseP cleaves the transmembrane sequence of the type II membrane protein RseA in cooperation with DegS protease and activates the transcription factor σ^E. Similar to other site-2 proteolysis, the processing by RseP requires prior C-terminal truncation of RseA by DegS, but it remains unclear how RseP recognizes the truncated form of RseA. RseP possesses two PDZ modules in its periplasmic region and it is known that the PDZ modules of other proteins interact with the C-terminal tails of their ligands. In fact, it has recently been reported that the second PDZ module recognizes the C-terminal hydrophobic tail of RseA generated from the cleavage by DegS. However, it was also shown that point mutations to the first PDZ module have considerable effect on the substrate recognition mode of RseP. In this study, we produced and crystallized a soluble fragment of PDZ modules of RseP orthologue to analyze how the two PDZ modules are involved in the substrate recognition in the course of regulated intramembrane proteolysis.

Keywords: X-ray crystallography of proteins, molecular recognition, intramembrane proteolysis

FA1-MS06-P09

Crystal Structures of Ferrocenyl-Phosphazene Derivatives. Asli Ozturk[a], Nuran Asmafiliz[b], Tuncer Hokelek[c], Zeynel Kilic[b]. [a]*Department of Physics, Pamukkale University, Denizli, Turkey.* [b]*Department of Chemistry, Ankara University, Ankara, Turkey.* [c]*Department of Physics Engineering, Hacettepe University, Ankara, Turkey.*
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On the study of ferrocenyl-phosphazene derivatives are very limited in the literature [1]. In this study, crystal structures of two ferrocenyl-phosphazenes, $C_{30}H_{50}FeN_9P_3$, (I), and $C_{26}H_{30}Cl_4Fe_2N_5P_3$, (II), are investigated. The data have been collected with Mo K_α radiation on an Enraf-Nonius CAD-4 diffractometer. *spiro*(propane-1,3-diamino)[N-(1-ferrocenyl methyl)]-4,4,6,6-tetrapyrrolidinocyclotriphosphaza triene (I) is a *spiro*cyclic monoferrocenyl phosphazene derivative and it belongs to the space group P-1 with cell parameters a=13.475(2), b=15.041(3), c=19.666(9) Å and α=68.50(3)°, β=87.12(3)°, γ=66.32(2)°. The asymmetric unit of (I) contains two independent molecules. It has π-π contact between cyclopentadiene rings [centroid-centroid distance = 3.289(3) Å]. The C-H…N intermolecular hydrogen bonds[2] link the molecules, forming infinite one dimensional chains running approximately parallel to b axis. *spiro*(butane-1,4-diamino)[N,N'-bis(1-ferrocenyl-methyl)]-4,4,6,6-tetrachloro-cyclotriphosphazatriene (II) is a *spiro*cyclic bisferrocenyl phosphazene derivative including two ferrocenes and it belongs to the space group P-1 with cell parameters a=10.782(1), b=11.546(2), c=13.282(2) Å andα= 68.19(1)°, β=79.75(9)°, γ=88.62(1)°. It also has π-π contact between cyclopentadiene rings and the intramolecular C-H…N H bonds form a dimerization.

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Keywords: phosphazenes, hydrogen bonds, ferrocenyl-phosphazenes

FA1-MS06-P10

A novel mechanism for azoreduction. Ali Ryan[a], Nicola Laurieri[a], Chan-Ju Wang[a], Isaac Westwood[a], Edward Lowe[b], Edith Sim[a]. [a]*Department of Pharmacology, University of Oxford.* [b]*Laboratory of Molecular Biophysics, University of Oxford.*
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Soluble flavin-dependent azoreductases are a class of enzymes found in many bacteria, homologues are also found in eukaryotes. Bacterial azoreductases are of interest due to their ability to detoxify azo dyes and activate drugs targeted for the colon. As in eukaryotes the physiological role of the bacterial enzymes is thought to be in quinone detoxification. The long established mechanism for these enzymes is unable to explain their ability to reduce both quinone and azo compounds [1]. Our work focuses on three azoreductases from *P. aeruginosa* [2, 3]. The crystal structure of one of these azoreductases (paAzoR1) in complex with the inflammatory bowel disease drug balsalazide (PDB: 3LT5), lead us to propose a novel mechanism for these enzymes [4]. The structure clearly shows binding of an alternative hydrazone tautomer of balsalazide within the active site. The hydrazone tautomer contains a quinoneimine in an ideal position to be reduced by the FMN cofactor. As well as detailing the full mechanism this talk will lay out further evidence that has been collected to support it. These data have allowed the reclassification of one of these azoreductases as an NADH quinone oxidoreductase.

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Keywods: azoreductase, quinone, reaction mechanism

FA1-MS06-P11

Structure and Allosteric Effects of Activating Compounds on Protein Kinase PDK1. Jörg O Schulze[a], V Hindie[a,b], A Stroba[c], H Zhang[a], LA Lopez-Garcia[a], L Idrissova[a], S Zeuzem[a], D Hirschberg[d], F Schaeffer[b], TJ Jørgensen[d], M Engel[c], PM Alzari[b], RM Biondi[a]. [a]*Universitätsklinikum Frankfurt, Germany.* [b]*Pasteur Institute, Paris, France.* [c]*University of Saarland, Saarbrücken, Germany.* [d]*University of Southern Denmark, Odense M, Denmark.*
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