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

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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

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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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