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.*
E-mail: aslio@pau.edu.tr

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.*
E-mail: ali.ryan@pharm.ox.ac.uk

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.*
E-mail: schulze@med.uni-frankfurt.de

Protein phosphorylation transduces a large set of intracellular signals. One mechanism by which phosphorylation mediates signal transduction is by prompting conformational changes in the target protein or interacting proteins. Previous work described an allosteric site mediating phosphorylation-dependent activation of AGC protein kinases. The AGC kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) is activated by the docking of a phosphorylated motif from substrates.
PDK1 is a central component of the growth factor and insulin signaling pathways. PDK1 is responsible for the stimulus-dependent phosphorylation and activation of many AGC kinases like Akt/PKB, S6K, RSK and SGK. Thus, our studies on PDK1 are relevant both for the growth factor/cancer field (PDK1 is a validated drug target for cancer treatment) and for insulin/diabetes research. PDK1 is also required for the constitutive phosphorylation of the activation loop of other protein kinases, such as all 12 protein kinase C (PKC) isoforms and the protein kinase C-related protein kinases (PRKs). In all, at least 23 protein kinases are known to be phosphorylated by PDK1.
We present the crystal structure[1] of PDK1 bound to a rationally developed low-molecular-weight activator[2] and describe the conformational changes induced by small compounds in the crystal and in solution using a fluorescence-based assay and deuterium exchange experiments. Our results indicate that the binding of the compound produces local changes at the target site, the PIF binding pocket, and also allosteric changes at the ATP binding site and the activation loop. Altogether, we present molecular details of the allosteric changes induced by small compounds that trigger the activation of PDK1 through mimicry of phosphorylation-dependent conformational changes.

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Keywords: protein kinases, allostery, phosphorylation

FA1-MS06-P12

Structural investigation of antidepressant drug and dipalmitoyl phosphatidylcholine interactions by Small Angle X-Ray Scattering method. Dilek Yonar[a], Elif Hilal Soylu[b], M. Maral Sunnetcioglu[a], Semra Ide[a]. [a]*Hacettepe University, Faculty of Engineering, Dept. of Physics Engineering, 06800, Beytepe, Ankara, Turkey.* [b]*Karadeniz Technical University, Faculty of Science and Literature, Dept. of Physics, 61080, Trabzon, Turkey.*
E-mail: ddogan@hacettepe.edu.tr

In this work, interaction of antidepressant drugs clomipramine hydrochloride and trazadone hydrochloride with dipalmitoyl phosphatidylcholine (DPPC) liposomes with/without cholesterol have been investigated. On these samples EPR spin labeling studies were performed recently [1, 2]. To receive further structural information on membrane thickness and the change of it by drug incorporation Small Angle X-Ray Scattering (SAXS) profiles have been recorded. The components were mixed in a wide capillary and left for 1 h at room temperature. The resulting complex was centrifuged into a narrow X-ray capillary. SAXS experiment was carried out with a Hecus SWAXS camera (Hecus X-Ray Systems Graz-Austria). X-ray generator operating at 50 kV and 50 mA with Cu anode (λ= 1.54 Å) in q range of 0.008 – 1.22 (Å^{-1}). Measurements were made by a step –scanning procedure and in the fixed time mode, with a sampling time 300 s for each step.
SAXS scans were also carried out from 15 to 55 ^{o}C by using an external temperature control unit causing temperature increasing in 2 ^{o}C to investigate thermal effects on the structure of drug incorporated and pure liposomes.
In the result of these studies, the changing in the lamellar repeat distances of DPPC have been determined after the incorporation of three drug compounds. The observed increase in the bilayer thickness which may allow to PBS and drug compound locations was in the range of 12-15 Å. As known as, these interactions occur by four effective types of forces defined as van der Waals, electrostatic, hydration, and undulation forces between DPPC bilayers, and during the incorporation of drug molecule into the DPPC structure [3]. The possible 3-D molecular conformations have been also investigated by using molecular mechanic calculations to explain the interaction of the drug molecules with DPPC. The results have been compared and discussed by using the related literature knowledge [4, 5].

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Keywords: liposomes, antidepressants, SAXS

FA1-MS06-P13

Drug-stabilized and drug-free complexes of topo IV from *S. pneumoniae* shed light on the mechanisms of reversible DNA scission and selective drug resistance. Ivan Laponogov[a,b], Xiao-Su Pan[b], Dennis A. Veselkov[a], Katherine McAuley[c], L. Mark Fisher[b], Mark R. Sanderson[a]. [a]*Randall Division of Cell and Molecular Biophysics, King's College London, 3rd Floor New Hunt's House, Guy's Campus, University of London, London, UK.* [b]*Molecular Genetics Group, Molecular and Metabolic Signalling Centre, Division of Basic Medical Sciences, St. George's, University of London, London, UK.* [c]*Diamond Light Source, Didcot, Oxford, UK.*
E-mail: mark.sanderson@kcl.ac.uk, lfisher@sgul.ac.uk, ivan.laponogov@kcl.ac.uk

Topoisomerase IV belongs to the type II class of DNA topoisomerases, which are responsible for changing and stabilizing DNA supercoiling and are also involved in chromosome segregation in prokaryotes. Topo IIs are essential enzymes for bacterial replication and are targeted by antibacterial drugs such as quinolones or diones. They change DNA topology by forming a transient covalent cleavage complex with a gate-DNA (G-segment) duplex and transporting the second duplex (T-segment) though a double-stranded break in the formed protein-DNA gate. Although the biological importance of these enzymes is well known,