FA1-MS06-P01

The Crystal Structure of a Junction Between two Z-DNA Helices. Matteo de Rosa[a, b], Daniele de Sanctis[c], Ana Lucia Rosario[b], Margarida Archer[b], Alexander Rich[d], Alekos Athanasiadis[a] and Maria Armenia Carrondo[b]. [a]*IGC, Oeiras, Portugal.* [b]*ITQB, Oeiras, Portugal.* [c]*ESRF, Grenoble, France.* [d]*MIT, Cambridge, USA.*
E-mail: matteo@itqb.unl.pt; mdrosa@igc.gulbenkian.pt

The double helix of DNA or RNA when composed of purine-pyrimidine repeats, can adopt a left-handed helical structure called Z-DNA and Z-RNA respectively [1]. Such dinucleotide repeats in genomic sequences have been associated with instability leading to cancer, for reasons not entirely understood.
Adoption of the left handed conformation in just a region of a polynucleotide sequence results in the formation of conformational junctions: a B-Z junction is formed at the boundaries of the left handed helix [2], a Z-Z junction is commonly formed in sequences where the dinucleotide repeat is interrupted by single base insertions or deletions that bring neighboring left-handed helices out of phase.
We report the first crystal structure of a Z-Z junction stabilized by Zα, the Z-DNA binding domain of the RNA editing enzyme ADAR1. The junction structure consists of a single base-pair and leads to partial or full disruption of the helical stacking. The junction region allows intercalating agents to insert themselves in the otherwise resistant to intercalation left-handed helix. However, unlike a B-Z junction the bases do not become fully extruded and the stacking between the two left handed helices is not continuous [2].

[1] Rich A, Zhang S, *Nat Rev Genet*, 2003, 4, 566. [2] Ha SC, Lowenhaupt K, Rich A, Kim YG, Kim KK. C. *Nature* 2005, 437.

Keywords: protein-DNA complexes, nucleic acid topology, editing

FA1-MS06-P02

Glutathione transferases in the herbicide resistant weed black-grass (*Alopecurus myosuroides*). Stefanie Freitag-Pohl[a], Ian Cummins[b], Ehmke Pohl[a,b], Robert Edwards[a,b]. [a]*Chemistry Department Durham University, Durham UK.* [b]*School of Biological and Biomedical Sciences Durham University, Durham UK.*
E-mail: stefanie.freitag-pohl@durham.ac.uk

Glutathione transferases (GSTs) are a diverse group of soluble enzymes with dimer molecular masses of around 50 kDa. They generally catalyze the transfer of glutathione to a co-substrate containing a reactive electrophilic centre to form an *S*-glutathionylated product. Plant GSTs are predominantly expressed in the cytosol where they have evolved to perform a wide range of functional roles with glutathione as co-substrate including counteracting oxidative stress, catabolizing tyrosine and detoxifying herbicides [1].
Black grass (*Alopecurus myosuroides*), is a problem weed in wheat in Northern Europe and can prove difficult to control using herbicides due to its ability to rapidly detoxify them. Glutathione transferase *Am*GSTF1 in black-grass has been linked to herbicide resistance in this weed [2,3] and the determination of enzymatic mechanism and the structural basis of herbicide binding is important in developing improved crop protection agents.
We have crystallized *Am*GSTF1 in its apo-form and determined the structure by molecular replacement to a resolution of 2.0 Å Bragg spacing. Co-crystallization and soaking experiments are currently underway to determine the various enzyme-ligand complexes.

[1] Dixon, D.P.; Lapthorn, A.; Edwards, R., *Genome Bio* 2003 3, 3004.1. [2] Cummins, I.; Moss, S.; Cole, D.J.; Edwards, R., *Pesticide Science*, 1997, 51, 244. [3] Dixon, D.; Cummins, I.; Cole, D.J.; Edwards, R., *Current Opinion Plant Biol.* 1998, 258.

Keywords: glutathione transferase, herbicide resistance, Alopecurus myosuroides

FA1-MS06-P03

Effects of GTP on Oligomeric State of GPA1; Arabidopsis G protein alpha subunit. Burcu Kaplan-Turkoz, Anıl Akturk and Zehra Sayers. *Biological Sciences and Bioengineering,Sabanci, University Istanbul,Turkey.*
E-mail: bkaplan@sabanciuniv.edu

Heterotrimeric G proteins are mediators that transmit external signals arriving at receptor molecules to effector molecules and play a crucial role in signal transduction in mammalian and plant systems. The α subunit can bind and hydrolyze GTP and this enzymatic activity serves as an on/off switch for the heterotrimeric signalling cycle. Despite the demonstrated importance of heterotrimeric G proteins in plant development and abiotic response, there is no report on biophysical or structural characterization of any plant heterotrimeric G proteins.
We have expressed and purified A.thaliana heterotrimeric G protein subunits, GPA1 (alpha), AGB1 (beta) and AGG2 (gamma). Recombinant expression of the alpha subunit, GPA1 was achieved using yeast P.pastoris. The theoretical molecular mass of GPA1 is ~48 kDa including the 6his and myc tags. The purified protein had GTP binding and hydrolysis activity.[1,2]. Initial SAXS data analysis pointed out that GDP bound GPA1 was stable in a dimeric form. We aimed further characterization of GPA1 and investigated the effect of GTPγS on the biophysical and structural properties of protein. GTPγS is a non hydrolyzable analog of GTP and binds irreversibly to G protein alpha subunits. SAXS data was collected from both GDP and GTPγS bound monodisperse GPA1 solutions.
In the presence of GTPγS, GPA1 molecular mass was calculated as 53 kDa with a radius of gyration of 3.6 nm. The molecular mass and radius of gyration calculated for GDP bound form were 86 kDa and 3.9 nm, respectively. Comparing the results, we can conclude that GPA1 is stabilized in monomeric form when bound to GTP. This will be further analyzed by other biophysical methods such as dynamic light scattering and circular dichroism spectrapolarimetry.
In a previous study, GPA1 was shown to be present as part of high molecular mass oligomers in Arabidopsis plasma membrane fractions, which dissociated upon GTPγS addition [3].Following this, our results point out the possibility that plant heterotrimeric G proteins function through a reversible dimer-monomer conformational change upon nucleotide exchange. This study is novel in terms of contributing to understanding on how plant G protein signaling is controlled. There is only one or two G protein alpha subunits in plant

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

[1] Kaplan-Turkoz, B., PhD Thesis, Sabanci University (TR) 2009. [2] Kaplan-Turkoz, B., Ðskit, S., Sayers, Z., Acta Crystallographica Section A,2009, 65, s164. [3] Wang, S., Assmann, S.M., Fedoroff, N.V., J Biol Chem, 2008, 283(20), 13913-22.

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.*
E-mail: mikami@kais.kyoto-u.ac.jp

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*
E-mail: andrea.musacchio@ifom-ieo-campus.it

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].

[1] Santaguida S and Musacchio A, *EMBO J* 2009, 8:2511. [2] Musacchio A and Salmon ED, *Nat Rev Mol Cell Biol* 2007, 8:379

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.*
E-mail: aknagata@mail.ecc.u-tokyo.ac.jp

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.

[1] Jia M.Z., Horita S., Nagata, K., Tanokura, M., *J. Mol. Biol.*, *in press*, doi: 10.1016/j.jmb.2010.03.055.

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.*
E-mail: nogi@protein.osaka-u.ac.jp