

**FA1-MS06-P01**

**The Crystal Structure of a Junction Between two Z-DNA Helices.** Matteo de Rosa<sup>a,b</sup>, Daniele de Sanctis<sup>c</sup>, Ana Lucia Rosario<sup>b</sup>, Margarida Archer<sup>b</sup>, Alexander Rich<sup>d</sup>, Alekos Athanasiadis<sup>a</sup> and Maria Armenia Carrondo<sup>b</sup>. <sup>a</sup>IGC, Oeiras, Portugal. <sup>b</sup>ITQB, Oeiras, Portugal. <sup>c</sup>ESRF, Grenoble, France. <sup>d</sup>MIT, Cambridge, USA.

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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  $Z\alpha$ , 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. *Nature* 2005, 437.

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**FA1-MS06-P02**

**Glutathione transferases in the herbicide resistant weed black-grass (*Alopecurus myosuroides*).** Stefanie Freitag-Pohl<sup>a</sup>, Ian Cummins<sup>b</sup>, Ehmke Pohl<sup>a,b</sup>, Robert Edwards<sup>a,b</sup>. <sup>a</sup>Chemistry Department Durham University, Durham UK. <sup>b</sup>School of Biological and Biomedical Sciences Durham University, Durham UK.

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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 *AmGSTF1* 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 *AmGSTF1* 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.; Laphorn, 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.

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**FA1-MS06-P03**

**Effects of GTP on Oligomeric State of GPA1; Arabidopsis G protein alpha subunit.** Burcu Kaplan-Turkoz, Anil Akturk and Zehra Sayers. *Biological Sciences and Bioengineering, Sabanci, University Istanbul, Turkey.*

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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  $\alpha$  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 GTP $\gamma$ S on the biophysical and structural properties of protein. GTP $\gamma$ S is a non hydrolyzable analog of GTP and binds irreversibly to G protein alpha subunits. SAXS data was collected from both GDP and GTP $\gamma$ S bound monodisperse GPA1 solutions.

In the presence of GTP $\gamma$ S, 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 spectropolarimetry.

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 GTP $\gamma$ S 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