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Plant Glutathione S-transferases (GSTs) are a highly diverse superfamily of abundant soluble proteins with largely unknown physiological roles. These enzymes generally catalyse the transfer of glutathione to various co-substrates containing an electrophilic centre. Plant GSTs have been shown to play a critical role in the detoxification of xenobiotic compounds such as herbicides by conjugating these compounds to glutathione [1].

Black Grass (*Alopecurus myosuroides, Am*) is a problem weed in cereal crop production in wide parts of the northern hemisphere due to its ability to develop multi-herbicide resistance. The high level of resistance has been linked to the up-regulation of one certain member of the GST superfamily, namely, *Am*GSTF1 [2]. In order to further elucidate the molecular mechanism of glutathione conjugation and detoxification we have determined the crystal structures of *Am*GSTF1 in two different modifications. Diffraction data to a resolution of better than 2.0 Angstrom were collected at the Swiss Light Source protein crystallography beam lines X06SA and X10SA [3]. The structure was solved by molecular replacement using PHASER [4] and refined with Refmac [5].

The crystal structure shows the family GST fold with the active site blocked by interaction with a symmetry-related cysteine mimicking the glutathione-substrate. Co-crystallisation experiments with various substrate analogues are currently underway with the ultimate goal of unravelling the enzymatic mechanism.

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Crystallographic studies of thioredoxin-interacting protein

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Thioredoxin-interacting protein (TXNIP) is a binding partner of thioredoxin (TRX) and acts as a negative regulator of TRX function [1, 2]. TXNIP expression is robustly induced under a variety of stress stimuli including high glucose, heat shock, UV, H_2O_2 and mechanical stress, while the expression and protein levels of TRX remain the same or down-regulated. The overall consequence of the elevated levels of TXNIP and the subsequent TXNIP-TRX association is an inhibition of the many biological activities of TRX and cellular oxidative stress. Elevated TXNIP expression and the resulting cellular consequences have been demonstrated to contribute to the pathologies of diabetes and cardiovascular disease [3]. More recently, TXNIP has been shown to be directly involved in glucose and lipid metabolism [4], and has been identified as a binding partner and an activator of the inflammasome [5]. Many studies support the hypothesis that disrupting the interaction between TXNIP and TRX may be therapeutically beneficial in conditions such as diabetes and cardiovascular disease [6, 7]. Given the pivotal role in a number of important biological pathways and its potential as a drug target, the high-resolution structure of TXNIP would be of great value.

Based on primary sequence, TXNIP is remotely (~10% sequence identity) related to β-arrestins, which include the visual arrestins. While overall structure of TXNIP is predicted to be similar to that of β-arrestins, some features of β-arrestins appear not to be present in TXNIP.

In order to pursue the crystallographic studies of human TXNIP, we have identified an expression system that allows us to produce large amounts of pure protein. Thus far, we have crystallized the N-terminal domain of TXNIP. The crystals belong to a monoclinic space group $P2_1$ with cell parameters a=79, b=179, c=88 Å, b=113°. A complete data set was collected using an ADSC Q210 detector on the MX1 beamline at the Australian synchrotron. The calculated Matthews coefficient (V_M) of 31.16 $Å^3Da^{-1}$ for the asymmetric unit indicates the possible presence of at least eight to as many as twelve molecules per asymmetric unit with the solvent content ranging from 50-70%. A native Patterson map ruled out any translational symmetry present in the crystals. However, several two-fold axes perpendicular and also possibly along the crystallographic axis are detected by a self-rotation function calculated to various resolution ranges. The crystal structures of several β-arrestins as well as theoretical models of TXNIP are available for use in molecular replacement.

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Crystallographic and SAXS studies of cancer-relevant forms of Galectin-3

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Galectins are a family of carbohydrate binding proteins which all possess at least one conserved carbohydrate recognition domain (CRD) [1]. CRDs of several galectins have been structurally characterized and all contain a single β-galactoside binding site. Over the last decade a considerable body of evidence has accumulated implicating Galectin-3 in cancer progression [2]. Galectin-3 is unique among other galectins in that it contains a non-CRD N-terminal domain of unknown structure. This domain harbors a functional cleavage site (Ala62-Tyr63) that serves as a substrate for the matrix metalloproteinases (MMP) gelatinases MMP-2 and -9 [3]. These MMPs are well known to facilitate cancer dissemination. Our immunohistochemical studies using archival human breast cancer specimens and antibodies specific for cleaved and non-cleaved Galectin-3 showed that while Galectin-3 is abundant in both low-and high-grade human breast cancers, it is almost all cleaved in high-grade lesions. Furthermore, we have demonstrated that addition of

exogenous recombinant 'truncated' Galectin-3 (analogous to the MMP-processed form), but not full length Galectin-3, dramatically increases migration of the human breast cancer cell line BT-549. These results suggest that the MMP-cleaved Galectin-3 and the resulting structural changes are responsible for pro-metastatic properties of Galectin-3. We have obtained crystallographic data for CRD of Galectin-3 in complex with a pentasaccharide to which Galectin-3 has higher binding affinity compared to other galectin family members. This structural information may be utilized in the design of Galectin-3 specific inhibitors targeting the carbohydrate-binding site. We have also explored the structural differences resulting from MMP cleavage of Galectin-3 using SAXS.

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Hinge-loop mutation can be used to control domain swapping of human cystatin C

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Human cystatin C (hCC) is a low molecular mass protein (120 amino acid residues, 13,343 Da) that belongs to a family of single chain, reversible inhibitors of papain-like (C1 family) and legumain-related (C13 family) cysteine proteases [1]. In pathophysiological processes, which nature of is not understood, hCC is codeposited in the amyloid plaques of Alzheimer's disease or Down's syndrome. The amyloidogenic properties of HCC are greatly increased in a naturally occurring L68Q variant, resulting in fatal cerebral amyloid angiopathy in early adult life [2]. At physiological conditions wild-type hCC is a monomeric protein, but under crystallization conditions (pH 4.8) forms a domain-swapped dimer [3]. The dimerization process is facilitated by the presence in the hCC structure of a flexible region created by the loop L1 (55-59, QIVAG) connecting protein subdomains undergoing the exchange process. This loop is the only part of hCC which undergoes significant structural changes during the dimerization process and, according to experimental [4], [5] and theoretical [6], [7] studies, these changes are driven by the conformational constraints attributed to the located near the top of the loop Val residue (Val57 for hCC).

With the aim to check implications of grater or decreased stability of this loop on dimerization and aggregation propensity of human cystatin C, we designed and constructed hCC L1 mutants with Val57 residue replaced by Asp, Asn [8], Gly (residues favored in this position of β-turns) or Pro, respectively. By applying this rational mutagenesis approach we were able to obtain hCC variants stable in the monomeric form both in solution and in the crystal (V57G, Figure 1), monomeric in solution but dimeric in the crystal (V57D) and dimeric in solution and oligomeric in the crystal (V57P). The results of structural studies of hCC L1 mutants will be presented.

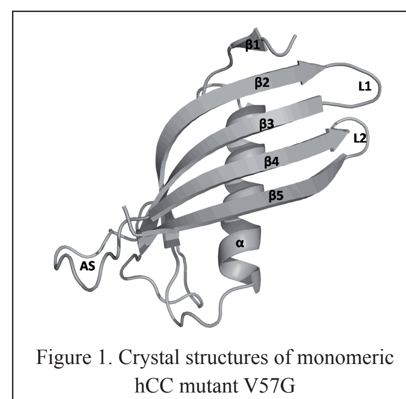

Figure 1. Crystal structures of monomeric hCC mutant V57G

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How group A streptococcus alpha-enolase interacts with human plasmin(ogen)

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The bacterium *Streptococcus pyogenes* causes a wide range of diseases in humans. More commonly known as Group A Streptococcus (GAS), this pathogen spans a huge spectrum of clinical conditions; from trivial to life threatening. Very common, non-invasive diseases like pyoderma and pharyngitis aside, this is also a causative agent of toxic-shock like syndrome, rheumatic heart disease, and necrotizing fasciitis. A prerequisite for these invasive conditions is that GAS has to migrate to and proliferate in areas of the body that are normally sterile.

It has been shown that the ability of GAS, and other bacteria, to interact with human plasminogen, a zymogen that circulates in our plasma at high concentrations, plays a major role in the invasive process. Plasmin, the active form of plasminogen, is a broad spectrum serine protease implicated in breaking down extracellular matrix and blood clots. The conversion of plasminogen to plasmin is normally under tight control; however this is subverted by GAS which activates this conversion by secreting the virulence determinant streptokinase. By forming a stable complex with either plasminogen or plasmin, streptokinase makes either complex display plasmin activity. GAS can bind the plasmin(ogen)-streptokinase complex to its surface via three known receptors; one of which is the streptococcal surface enolase (SEN).

SEN contains 435 residues per polymer and takes the form of an octameric ring, with a central 4-fold symmetry axis, and is found on the surface of GAS. Recent work [1] has focused on exploring the proposed plasmin(ogen) binding motifs in both wild type SEN, and in a range of point mutated residues. Choice of mutants was aided by creating an