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Structure of Cytokinin-specific Binding Protein in Complex with Plant Hormone

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The high-resolution (1.2 Å) crystal structure a cytokinin-specific binding protein from mung bean (VrCSBP) complexed with zeatin reveals that the protein, structurally resembles plant pathogenesis related proteins of class 10 (PR-10), despite a low sequence conservation (below 20%). The four VrCSBP molecules present in the asymmetric unit assemble into two dimers. Between the concave face of the molecular β-sheet and the C-terminal helix, a binding pocket is formed where the zeatin molecules are located. Surprisingly, in three (out of the four) binding pockets two zeatin molecules are found, with excellent definition in the electron density maps. In one of the binding sites (observed also in the forth, single-site, VrCSBP molecule), the ligand molecules, located deep in the cavity, have identical conformation and hydrogen-bonding pattern. In the second binding site, at the entrance to the internal cavity, the ligand molecules show variable, but clearly defined, binding modes.

Keywords: protein-ligand complexes, plant hormones, high resolution X-ray structures macromolecules

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Modular Assembly of the Cellulosome Revealed by X-ray Crystallography

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Clostridium thermocellum is an anaerobic bacterium isolated from hot springs which converts hemicellulose into ethanol. These microorganisms express multienzyme complexes dedicated to the degradation of the plant cell wall. These complexes (cellulosomes) are composed of modules assembled by an integrating protein (scaffoldin), composed of several type I cohesins, which bind type I dockerins. A type II dockerin of the scaffoldin binds to a type II cohesin and anchors the whole complex to the cell. Other modules named Carbohydrate Binding Modules (CBM), are responsible for adherence to the substrate.

The crystal structure of type I cohesin-dockerin complex was solved to 2.2 Å and revealed for the first time how protein-protein recognition is achieved in the complex [1]. The 2.5 Å crystal structure of the type II cohesion, solved by MIR/MAD will be described. Subtle differences between type I and type II cohesins give insight into the structural determinants of cohesin-dockerin specificity. We will also report the 1.98 Å structure (MAD-SeMet) of the family 11 CBM belonging to a cellulosomal enzyme. The structure of the CBM11 reveals a concave side that forms a potential carbohydrate binding cleft [2].

[1] Carvalho A.L., et al., *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 13809-14. [2] Carvalho A.L., et al., *J. Biol: Chem.*, 2004, **279**, 34785-93.

Keywords: cellulosome, cohesin, carbohydrate binding module

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Structural Studies of Thioredoxins and Associated Inhibitor Based Complexes

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The thioredoxin redox system is ubiquitous in all living cells and is used as a sophisticated mechanism for maintaining an intracellular reduced state. The redox proteins are also known to be important in a multitude of biological functions, including controlling cell cycle regulation, and studies in various human malignancies and cell lines in *vitro* have shown an up regulation of thioredoxin, demonstrating a definite link between thioredoxin and cancer [1], [2].

There are currently two novel heteroaromatic quinol inhibitors under development at the Cancer Research Laboratories of the University of Nottingham. These inhibitors are thought to have a novel mode of action leading to an irreversible binding of the inhibitor to the active site, thus irreparably inactivating the protein.

The research group has obtained the crystal structures of *Tuberculosis Bacterium* and human thioredoxins. By studying the crystal structure of thioredoxin-inhibitor complex it will be possible to apply structure-activity relationships and thus enable the research group to not only understand how these quinols block the activity of thioredoxin, but also to develop these drugs with the intention of improving their affinity for the binding site.

[1] Arrigo A.P., *Free Radical Biol. and Med.*, 1999, **27 (9/10)** 936. [2] Soini Y., et al., *Clinical Can. Res*., **7**, 1750.

Keywords: thioredoxin, cancer, heteroaromatic quinols

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A Dramatic Side Chain Movement in Adrenaline-Synthesising PNMT: Implications for Drug Design

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Phenylethanolamine N-methyltransferase (PNMT) catalyses the methylation of noradrenaline to form adrenaline using S-adenosyl-L-methionine as the methyl donor. Adrenaline is produced in the adrenal medulla (hormone), and in selected neurons in the CNS (neurotransmitter). The role of adrenaline in the CNS is poorly understood, though it has been implicated in blood pressure control and Alzheimer's disease.

Classic inhibitors of PNMT also act on the α2-adrenoreceptor, or are unable to cross the blood brain barrier. Therefore we are using the crystal structure of PNMT to design potent selective CNS-active PNMT inhibitors. The structure of PNMT with 7-SO_2NH_2-THIQ[1] revealed room in the binding pocket for bulkier 7 substituents so these were designed and tested for PNMT inhibition. Some inhibited with high potency despite predicted steric clashes. A co-crystal structure revealed a dramatic conformational change in a lysine residue to accommodate the substituent, indicating that drug design strategies must address large conformational changes at active sites.

[1] Martin J.L., Begun J., McLeish M.J., Caine J.M., Grunewald G.L., *Structure*, 2001, **9**, 1.

Keywords: enzyme inhibitor drug design, structure-based drug design, protein flexibility

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Structural Studies of Glutathione S-transferase Inhibitors – A Promising Target for Anti-cancer Drug Design

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Glutathione S-Transferases (GSTs), phase II detoxification enzymes, primarily function to detoxify unwanted toxic compounds in the cell [1]. They are, however, overexpressed in many cancers and shown to be deleterious to cancer chemotherapy's success by reacting with certain anti-cancer drugs. GSTs, therefore, have been identified