for both prokaryotic and eukaryotic enzymes is largely conserved. However there are several structural differences between prokaryotic UGM and eukaryotic AfUGM mainly because of five additional inserts in AfUGM, two of with play an important role in tetramerization. Comparing the un-complexed, native AfUGM structure and the ligand-bound AfUGM structure (both oxidized and reduced FAD) allowed us to address structural changes (loop movements) that play an important role in substrate binding and redox state. Structural binding studies on AfUGM revealed a unique substrate binding mechanism significantly different from proUGMs. This has helped us in identifying important conserved residues in AfUGM substrate binding and recognition. Based on the crystal structure we have made mutants of important active site residues.

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Keywords: crystallography, eukUGM, mutagenesis

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Solution of the structure of the TNF-TNFR2 complex

Yohei Mukai,[a,b] Teruya Nakamura,[c] Mai Yoshikawa,[a,b] Yasuo Yoshioka,[b,d] Shin-ichi Tsunoda,[a,b] Shinsaku Nakagawa,[b] Yuriko Yamagata,[c] Yasuo Tsutsumi,[a,b] [a]*Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Osaka (Japan).* [b]*Graduate School of Pharmaceutical Sciences, Osaka University, Osaka (Japan).* [c]*Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto (Japan).* [d]*Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka (Japan).* E-mail: yohe@phs.osaka-u.ac.jp, ytsutsumi@phs.osaka-u.ac.jp

Tumor necrosis factor (TNF) is an inflammatory cytokine that has important roles in various immune responses, which are mediated through its two receptors, TNF receptor 1 (TNFR1) and TNFR2. Antibody-based therapy against TNF is used clinically to treat several chronic autoimmune diseases; however, such treatment sometimes results in serious side effects, which are thought to be caused by the blocking of signals from both TNFRs. Therefore, knowledge of the structural basis for the recognition of TNF by each receptor would be invaluable in designing TNFR-selective drugs. Here, we solved the 3.0 angstrom resolution structure of the TNF-TNFR2 complex, which provided insight into the molecular recognition of TNF by TNFR2. Comparison to the known TNFR1 structure highlighted several differences between the ligand-binding interfaces of the two receptors. Additionally, we also demonstrated that TNF-TNFR2 formed aggregates on the surface of cells, which may be required for signal initiation. These results may contribute to the design of therapeutics for autoimmune diseases.

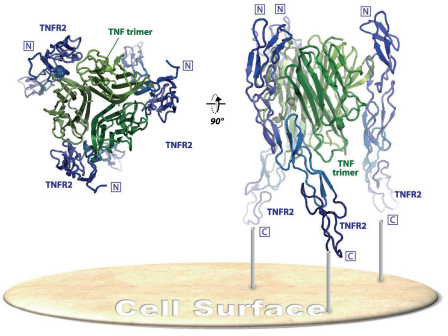

Fig. The structure of the TNF-TNFR2 complex (PDB:3ALQ)

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Keywords: cytokine, receptor, complex

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The structure of BRMS1 nuclear export signal reveals a hexamer of coiled coils

Mercedes Spínola-Amilibia,[a] José Rivera,[b] Miguel Ortiz-Lombardía,[c] Antonio Romero,[a] José L. Neira,[d,e] Jerónimo Bravo,[f] [a]*Centro de Investigaciones Biológicas, CSIC, Madrid, (Spain).* [b]*Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, (Spain).* [c]*Architecture et Fonction des Macromolécules Biologiques, UMR6098, CNRS, Universités d'Aix-Marseille I & II, (France).* [d]*Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, (Spain).* [e]*Instituto de Biocomputación y Física de Sistemas Complejos, Zaragoza, (Spain).* [f]*Instituto de Biomedicina de Valencia (IBV-CSIC), Valencia (Spain).* E-mail: mspinola@cib.csic.es.

We present here the first structural report of Breast Cancer Metastasis Suppressor 1 (BRMS1), a member of the metastasis suppressor proteins group, which during recent years have drawn much attention since they suppress metastasis without affecting the growth of the primary tumour [1]. The relevance of the predicted N-terminal coiled coil on the molecular recognition of some of the BRMS1 partners [2, 3], on its cellular localization [4] and on the role of BRMS1 biological functions such as transcriptional repression [3], prompted us to characterize its three-dimensional structure by X-Ray crystallography.

The structure of BRMS1 N-terminal region, reveals that residues 51 to 98 form an antiparallel coiled coil motif, and also that it has the capability of homo-oligomerizing in a hexameric conformation, by forming a trimer of coiled coil dimers. We have also performed hydrodynamic experiments that strongly supported the prevalence in solution of this quaternary structure for $BRMS1_{51\text{-}98}$.

This work explores the structural features of BRMS1 N-terminal region to help clarifying the role of this area in the context of the full-length protein. Our crystallographic and biophysical results suggest that the biological function of BRMS1 may be affected by its ability to promote molecular clustering through its N-terminal coiled coil region.

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Keywords: BRMS1, coiled coil domain, X-Ray Structure.

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The role of glutathione transferases in herbicide resistant black grass weeds

Ehmke Pohl,[a,b] Stefanie Freitag-Pohl,[a] Ian Cummins,[b] Robert Edwards,[c] [a]*Department of Chemistry, Durham University, Durham, (UK).* [b]*School of Biological and Biomedical Sciences, Durham*

University, (UK). [c]*Food and Environment Research Agency, Sand Hutton, York, (UK).* E-mail: ehmke.pohl@durham.ac.uk

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

Galina Polekhina,[a] Shie Foong Kok,[a] David Benjamin Ascher,[b] Mark Waltham,[b] [a]*Monash Institute of Medical research, Monash University, Clayton, VIC, (Australia).* [b]*St.Vincent's Institute of Medical Research, Fitzroy, VIC, (Australia).* E-mail: Galina.Polekhina@monash.edu

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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Keywords: crystallization, diabetes, drug target

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

Mark Waltham,[a] Shie F Kok,[a,b] Matthew Wilce,[c] Erik W Thomspon,[a] Galina Polekhina,[b] [a]*St. Vincent's Institute of Medical Research, Melbourne, VIC, (Australia).* [b]*Monash Institute of Medical Research, Monash University, Clayton, VIC, (Australia).* [c]*Monash University, Clayton, VIC, (Australia).* E-mail: mwaltham@svi.edu.au

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