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Structural basis for inhibiting human tankyrases

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Poly (ADP-ribose) polymerases (PARPs) are enzymes that catalyze a covalent post-translational modification of proteins. PARPs attach ADP-ribose units from NAD^+ to glutamate and lysine residues of the target molecule or to the growing chain of poly (ADP-ribose). These enzymes are involved in important cellular functions like DNA damage detection and repair, transcriptional regulation, intracellular trafficking, chromatin modification and cell death.

Tankyrases form a subfamily of human PARPs. They are also known as TRF1 (telomeric repeat binding factor)-interacting ankyrin related ADP ribose polymerases 1 & 2 [1]. Tankyrases are multidomain proteins having four distinct characteristics domains: HPS domain with an unknown function, 24 ankyrin repeats involved in the protein-protein interactions, SAM (sterile-alpha motif) domain required for multimerization of tankyrase and a C-terminal PARP domain catalyzing poly(ADP- ribose) polymerization.

Tankyrases are potential drug targets for cancer and therefore efforts are ongoing within industry and academia to find potent inhibitors for it. The most evident benefits for inhibiting tankyrase are due to its functions Wnt signaling and in telomere homeostasis. Both of these are frequently misregulated in cancers leading to excessive Wnt signaling and subsequently accumulation of β-catenin, and elongated telomeres of cancer cells. Recently potent inhibitors for tankyrase were reported providing basis for development of drugs against tankyrases [2], [3].

We present a crystal structure of tankyrase 2 in complex with a highly potent and selective inhibitor. Most PARP inhibitors utilize the binding site of nicotinamide of NAD^+. This is the first reported experimental complex structure of a PARP inhibitor not occupying that site. The structure reveals the plasticity of the NAD^+ binding loop and it will create new strategies for inhibitor development for PARPs and especially for tankyrases.

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"Crystal structure of the CBS domain pair of human CNNM4 transporter"

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This work describes the crystal structure of the CBS-pair regulatory domain of the human ancient domain protein 4 (ACDP4), also known as CNNM4 [2]. ACDP proteins represent the least-studied members of the eight different types of magnesium transporters that have been identified in mammals to date [1]. In humans the ACDP family includes four members: CNNM1–4 [6]. CNNM1 acts as a cytosolic copper chaperone and has been associated with urofacial syndrome; CNNM2, encoding a basolateral protein required for renal Mg2+ handling is mutated in dominant Hypomagnesemia. Interestingly, mutations in the CNNM4 gene have clinical consequences that are limited to retinal function and biomineralization and are considered to be the cause of Jalili syndrome, which consists of autosomal recessive cone-rod dystrophy and amelogenesis imperfecta [3], [4], [5]. The truncated protein was overexpressed, purified and crystallized in the orthorhombic space group C222. The crystals diffracted X-rays to 3.6 Å resolution using synchrotron radiation.

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Keywords: ion transporter, CBS domain, Jalili syndrome

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Structural studies of aspergillus fumigatus UDP-galactopyranose mutase

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Drug resistant pathogens are a serious health problem, creating an urgent need for alternative antibiotics and antibiotic drug targets. One such target is UDP-galactopyranose mutase (UGM). UGM is a flavoprotein that catalyzes the interconversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf); a precursor for the construction of Galf containing oligosaccharides. Galf is an essential component of the cell wall in bacteria, fungi and the cell surface matrix of protozoan parasites and appears to be essential for survival and virulence. Gene knock-out studies using Mycobacterium smegmatis (a model for M. tuberculosis) revealed that UGM is essential for the survival of the bacteria. A recent study in the protozoan parasite Leishmania major has shown that a UGM-- strain is attenuated towards virulence [1]. The L.major knock-out strain is deficient in Galf-containing lipophosphoglycans (LPG) and expresses truncated glycoinositol-phospholipids (GIPLs). Furthermore, mice inoculated with the UGM- mutant didn't develop lesion and are completely avirulent, indicating the importance of UGM for pathogenic survival and proliferation in mammals [1]. Work has also been done on A. niger and A. nidulans showing that Galf is important in Aspergillus. Deletion of the UGM gene dramatically attenuates virulence in Aspergillus. Since Galf is unique and UGM is not found in humans UGM is an interesting target for novel structure based drug design.

We are focusing on UGMs from eukaryotic pathogens from the fungus Aspergillus fumigatus (causes acute pneumonia and aspergillosis) and the protozoan parasite L. major (causes leishmaniasis). The eukaryotic enzymes share 51% sequence identity, but share only about 18% amino acid sequence identity to the prokaryotic UGMs [2]. We have solved the structure of L. major UGM (LmUGM) and used this structure to solve the Aspergillus fumigatus UGM (AfUGM) structure with and without substrate UDPgalp and inhibitor UDP. Despite low sequence identity with known prokaryotic UGMs the overall fold

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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Solution of the structure of the TNF-TNFR2 complex

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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.

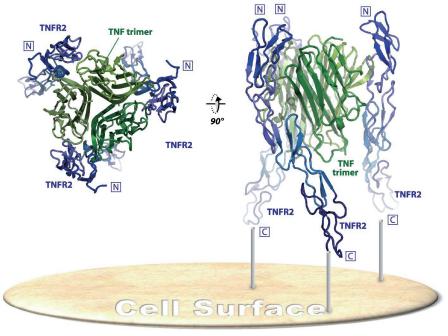

Fig. The structure of the TNF-TNFR2 complex (PDB:3ALQ)

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

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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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The role of glutathione transferases in herbicide resistant black grass weeds

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