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NTPDases of microbial pathogens

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Nucleoside triphosphate diphosphohydrolases (NTPDase) catalyze the hydrolysis of extracellular nucleotides. These enzymes are found in mammals as well as in microbial pathogens where they are thought to modulate the host-response to an infection by hydrolysis of host ATP. We aim to determine crystal structures of microbial NTPDases to use them for structure-guided drug design.

For the production of NTPDases from Legionella pneumophila, Schistosoma mansoni, Toxoplasma gondii, Trichomonas vaginalis and Trypanosoma cruzi we have established E. coli expression systems. NTPDases of Toxoplasma gondii and Trichomonas vaginalis were expressed as insoluble inclusion bodies. Using rapid dilution systems it was possible to refold the denatured protein to an active form. NTPDase from Legionella pneumophila was expressed in soluble form by secretion to the periplasm. Subsequently the proteins were purified by chromatographic methods and applied to crystallization screens. We were able to determine the crystal structures by different phasing methods, namely SAD and MIRAS. Furthermore, the proteins could be crystallized in complex with substrate analogs. These crystal structures provide insight into the domain motion of the enzyme and broaden our knowledge about the catalytic mechanism and substrate specificity (as well as promiscuity) already derived from the crystal structure of rat NTPDase2.

Keywords: Nucleotide, Purinergic signaling, Ultra.

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Structural studies of the NTF2-like domain of G3BP1

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In the search for new drug targets in cancer treatment, Ras GTPase Activating protein SH3 Binding Protein 1 (G3BP1) has proven to be a potential candidate [1], [2], [3]. Through binding to RasGAP, G3BP1 is able to regulate the Ras signaling pathway and hereby induce uncontrolled cell growth. However, knowledge about the mechanism is limited.

We have solved the crystal structure of the N-terminal Nuclear Transport Factor 2-like (NTF2-like) domain of G3BP1 to 3.7 Å in spacegroup P6(3)22 [4]. The structure was refined using deformable elastic network (DEN) restraints and a Rfree value of 0.28 was obtained. The asymmetric unit contains one molecule with a cone-like shape comprised of three alpha-helices and five beta-strands forming a beta-sheet. The NTF2-like domain forms a dimer with the beta-sheets as interface and shows high overall structural similarity with other Nuclear Transport Factor 2 domains from various organisms. However, when comparing the NTF2 binding sites for nucleoporins and Ran*GDP with equivalent sites at the NTF2-like domain of G3BP1 there are differences in e.g. hydrophobicity and loop conformations.

The crystal structure of the G3BP1 NTF2-like domain enables structure-based drug design and provides clues about the mechanism of G3BP1 function.

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A structure-guided approach to inhibition of HGF/SF – MET interactions

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Hepatocyte Growth Factor/Scatter Factor (HGF/SF) and its receptor MET play key roles in tumour invasion [1]. HGF/SF is secreted by cells in the tumour stroma and causes tumour cells expressing the MET receptor to dissociate from neighbouring cells. Both proteins have now emerged as appealing targets for therapy against metastatic cancer. HGF/SF has a domain structure and proteolytic mechanism of activation similar to those of the blood proteinase precursor plasminogen. Biochemical, biophysical and structural investigations of individual domains [2] and extensive mutagenesis studies [1] have revealed that HGF/SF binds MET through a high affinity binding site in the NK1 domain and a low affinity binding site in the SPH domain.

NK1 is a natural splice variant of HGF/SF and experiments in transgenic mice have shown that NK1 behaves *in vivo* as a real receptor agonist [3]. NK1 is a monomer in solution, but crystallises as a head-to-tail dimer. The reappearance of this dimer in different crystallographic environments, as well as in complexes with heparin, suggests that inter-protomer interactions in the dimer are specific and that the dimer might be the biologically active form of NK1. NK1 forms a dimer in solution in the presence of heparin and can only activate MET in the presence of heparin [4]. Key residues have been identified by structural analysis of wtNK1 dimer interface.

Another approach to block activation of MET is to identify low molecular weight molecules that inhibit the interaction of HGF/SF with the MET receptor. Fragment-based drug discovery provides an alternative approach to traditional high-throughput chemical screening, by first using biophysical and computational approaches to identify low molecular weight, drug-like fragments that bind to the target and then growing or cross-linking these into lead-like molecules. Fragment libraries, which comply with the rule of three, are now readily available and can be used to explore chemical space in an efficient way. However, very few studies have been made on protein-protein interfaces.

Here we present initial results that have been obtained by protein engineering of NK1 in order to inhibit dimerisation and produce antagonists of MET activation. We will report studies of NK1-MET active complex formation using surface plasmon resonance (SPR) and X-ray crystallography. Fragment binding is further explored by means of nuclear magnetic resonance (NMR), isothermal titration calorimetry (ITC), fluorescence-based thermal shift assay, SPR and X-ray analysis.

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The crystal structure of APPL2 BAR-PH domain

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APPL (Adaptor protein containing PH domain, PTB domain and leucine zipper motif) is an adaptor protein with two isoforms APPL1 and APPL2. As a scaffold protein, the interaction of APPL with Akt2 [1], small GTPases [2], FSHR, and AdipoR [3] are associated with the roles in cell proliferation, cell cycle control, metabolism/insulin sensing [4], [5], relaying signal from endosome and transcription by activating AMPK related signaling pathway [6]. As a protein communication hub, APPL provdes a mechanism for spatial regulation of signaling transmission and processing. However, it has also been shown that APPL2 plays opposing functions from APPL1 and there have been emerging evidences that BAR-PH domain of APPL1 and APPL2 can interact with each other to form heterodimer *in vivo*.

The structure of APPL2 BAR-PH domain has been solved to resolution of 3.2 Å using X-ray crystallography. The dimer exhibits a canonical four-helical fold of conformation, just like the shape of crescent or banana, which is implicated in the assocication with curved membranes. The difference between APPL1 BP domain and APPL2 BP domain lays in the curvature of the crescents. The curvature radius increases to ~70 Å, in sharp contrast with ~55 Å of APPL1 BP dimer's [7]. It has been proposed that curvature radius of BAR family protein corresponds to specific type of endosome to which it binds. Also, the protein surface shows different electrostatic property. Thus it is reasonable to conclude that the structure of APPL2 BAR-PH domain may be implicated in alteration of endosome assocation specificity, also in the change of the profile of spatial regulation of signaling transmission.

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Regulation of an *Arabidopsis Thaliana* potassium channel

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Under abiotic stress, plant cells use calcium signaling pathways to activate certain ion channels and give a correct response. Potassium (K+) is an essential ion for plant development. Plants living under low K+ conditions often adapt their K+ uptake through a recently discovered calcium signaling pathway which mobilizes potassium channel in roots. Under K+ deficiency, a calcium sensor activates a kinase that in turn phosphorylates and activates the K+ channel. When K+ levels are restored, a phosphatase dephosphorylates and inactivates the channel.

We are carrying out structural studies (crystallographic and ITC experiments) with this K+ channel and its binding partners to understand at molecular level how K+ uptake is regulated under stress conditions.

Keywords: potassium channel, abiotic stress

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Structure-based mutagenesis studies of human type II Hsp40, Hdj1

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Hsp40 is a co-chaperone of Hsp70 that correctly folds polypeptides in non-native forms. Hsp40 is divided into two domains: the N-terminal DnaJ-like domain and the C-terminal domain. During protein refolding, DnaJ-like domain of about 8 kDa stimulates the ATPase activity of Hsp70. C-terminal domain of 21 kDa interacts with non-native polypeptides and also with the C-terminal region of Hsp70, and delivers the polypeptides to Hsp70. In this study aiming at elucidation of co-chaperone mechanism of Hsp40, we determined the crystal structures of the C-terminal domain of human type II Hsp40, Hdj1, complxed with the C-terminal octapeptide of human Hsp70, GPTIEEVD. Furthermore, we carried out mutagenesis studies on Hdj1 to identify the surface regions responsible for the cochaperone activity.

The crystal structure of the C-terminal domain of Hdj1 in complex with the octapeptide was refined at 1.9 Å resolution. Based on the refined structure, we designed Hdj1 single-point mutants at the peptide-binding sites. The Hdj1 mutants were expressed in *E. coli* and purified with cation-exchange chromatography. Cochaperone activities of wild-type Hdj1 and its mutants were measured using a luciferase refolding assay. Furthermore, the crystal structures of the mutants were determined.

C-terminal domain of Hdj1 is a twisted, horseshoe-shaped homo-dimer. The protomer consists of 11 β-strands and 3 α-helices and is folded into elongated globular domains I and II, and a C-terminal helix region related to dimerization. The octapeptides are located in two distinct sites of domain I, sites 1 and 2, of each protomer. In the site 1, the octapeptide forms an anti-parallel β-sheet with β2 strand of domain I. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 181, Lys 182 and Lys 184 of Hdj1. The Ile side-chain of the octapeptide fits into the hydrophobic concaved surface. The site 1, therefore, is attributed to the recognition site toward Hsp70. The octapeptide in the site 2 forms an anti-parallel β-sheet with β4 strand of domain I. This site is located just behind the site 1. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 213, Lys 217 and Lys 306 of Hdj1. The side-chain of Pro and Ile of the octapeptide are situated at the hydrophobic region which is flat and wider than the concave of the site 1. This region is suited for binding of the non-native polypeptides with hydrophobic side-chains bulkier than Ile. Hence, it is conceivable that the site 2 is the binding site toward non-native polypeptides. The point mutants at site 1 or site 2 are confirmed by determination of the structure that only one of the sites is be disrupted while the other site remains unchanged. These mutants reduced the cochaperone activity of