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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.

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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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