It is revealed that the phosphotyrosine residue of Cblin mediates the main contacts with positively charged pocket in Cbl-b TKB domain. Moreover, the detail binding mode between Cbl-b TKB domain and Cblin will be discussed.

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Keyword: complex, inhibitor, interaction

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Crystal structure of the leishmania major MIX protein

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Leishmania and *Trypanosoma* protozoan parasites of the order kinetoplastida are pathogenic to humans. These parasites shuttle between insect vectors and mammalian hosts where they cause disease. *Leishmania* species cause leishmaniasis, a spectrum of diseases that range in severity from skin lesions to serious disfigurement and fatal systemic infection. *Trypanosoma rhodesiense* and *Trypanosoma gambiense* are responsible for African sleeping sickness, while *Trypanosoma cruzi* causes Chagas disease in Central and South America. The WHO estimates that there are at least 2 million new cases of leishmaniasis each year. African sleeping sickness and Chagas disease, which are vastly underreported, each account for tens of thousands of cases per year.

There are currently no effective vaccines against these pathogens and existing drugs suffer from toxicity, variable efficacy and high costs. In addition, emerging drug resistance prompts the search for novel drugs, ideally directed against new targets. In our quest for such drug targets we have recently identified a mitochondrial membrane-anchored protein, designated MIX, which occurs exclusively in kinetoplastids. In *Leishmania,* MIX is expressed in all life-cycle stages including the amastigote stage present in the mammalian host. Deletion of one allele of *MIX* in *L. major* shows morphological and mitochondrial abnormalities, effects that are also seen in *T. brucei* epimastigotes in which MIX expression has been down-regulated by MIX gene-specific RNAi. Importantly, These parasites display reduced infectivity *in vitro* and reduced virulence *in vivo*.

We have determined the crystal structure of Leishmania major MIX (residues 45-195, referred to as MIX⊗45) using X-ray crystallography by the Multiple Isomorphous Replacement technique, to a resolution of 2.4 Å. MIX forms an all ⟨-helical fold comprising seven ⟨-helices that fold into a single domain. The distribution of helices is similar to a number of scaffold proteins, namely HEAT repeats, 14-3-3, and tetratricopeptide repeat (TPR) proteins, suggesting that MIX mediates protein–protein interactions. Accordingly, using co-purification and mass spectroscopy we were able to identify several proteins that may interact with MIX *in vivo*. Being parasite specific, MIX is a promising new drug target and, thus, the structure and potential interacting partners provide a basis for structure-guided drug discovery.

Keywords: parasite, protein, crystallography

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Identification of two imidazole binding sites in VAP-1

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Amine oxidases oxidatively deaminate amines to the corresponding aldehydes in a reaction where hydrogen peroxide and ammonia are produced. The mammalian amine oxidase vascular adhesion protein-1 (VAP-1) is classified as a copper containing amine oxidase (CAO). VAP-1 is a 180 kDa membrane-bound glycoprotein, which has a 2,4,5-trihydroxyphenylalanine quinone (TPQ) as its cofactor. In addition to the enzymatic function, VAP-1 is an adhesion protein, which is primarily involved in leukocyte trafficking to sites of inflammation. It has also been shown to be involved in glucose metabolism, differentiation of adipose cells, potentiation of hypertension and the vascular degradation that often occurs in Alzheimer's disease.

We have solved two new structures of a soluble, proteolytically cleaved form of VAP-1, which was extracted from human plasma. The structures were refined to 2.6 Å and 2.95 Å respectively. In the structures we found imidazole molecules, which were derived from the crystallization buffer. In the 2.6 Å structure, an imidazole molecule is hydrogen bonded to the TPQ cofactor, which is in an inactive on-copper conformation. In the 2.95 Å structure, an imidazole molecule is covalently bound to the active off-copper conformation of TPQ. A second imidazole binding site was also identified in both structures, as an imidazole is bound to Tyr394 close to Thr212 in the substrate channels. The imidazoles block the entrance to the active site and act as inhibitors of the enzymatic activity.

As VAP-1 is involved in leukocyte trafficking to sites of inflammation, inhibition of the enzyme could be of great importance in chronic diseases like rheumatoid arthritis, asthma and psoriasis. Small molecular inhibitors that block leukocyte trafficking to sites of inflammation could be used as new anti-inflammatory drugs against these chronic diseases. The new VAP-1 structures give new insights in the drug design based on secondary amine inhibition. Furthermore, inhibitors bridging the two imidazole binding sites might acquire specificity towards VAP-1, since the second binding site is unique in VAP-1.

Keywords: amine oxidase, vascular adhesion protein-1, imidazole

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Crystal Structure of a dimeric anti-HER2 human single domain antibody

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Human epidermal growth factor receptor-2 (HER2) is well described as a target for antibody in various tumor models [1]. Single-domain antibodies (sdAbs) derived from human VH are considered to be less soluble and prone to aggregate which makes it difficult to determine the crystal structures [2]. In this study, we isolated and characterized two anti-HER2 sdAbs, Gr3 and Gr6, from a synthetic human V_H phage display library. Size exclusion chromatography and surface plasmon resonance analyses demonstrated that Gr3 is a monomer, but that Gr6

is a dimer. To understand this different molecular behavior, we solved the crystal structure of Gr6 to 1.6 Å resolution. The crystal structure revealed that the homodimer assembly of Gr6 mimics the V_H-V_L heterodimer of immunoglobulin variable domains and the dimerization is, at least partially, attributed to amino acids in CDR3.

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Keywords: HER2, single-domain antibody

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Design, synthesis and X-ray crystallographic study of NAmPRTase inhibitors as anti-cancer agents

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NAmPRTase (PBEF/Visfatin) plays a pivotal role in the salvage pathway of NAD^+ biosynthesis. NAmPRTase has been an attractive target for anti-cancer agents that induce apoptosis of tumor cells via a declining plasma NAD^+ level. In this report, a series of structural analogs of FK866 (**1**), a known NAmPRTase inhibitor, was synthesized and tested for inhibitory activities against the proliferation of cancer cells and human NAmPRTase. Among them, compound **7** showed similar anti-cancer and enzyme inhibitory activities to compound **1**. Further investigation of compound **7** with X-ray analysis revealed a co-crystal structure in complex with human NAmPRTase, suggesting that Asp219 in the active site of the enzyme could contribute to an additional interaction with the pyrrole nitrogen of compound **7**. This work was supported by the "GIST Systems Biology infrastructure Establishment Grant (2011)".

Keywords: biomacromolecule, crystal_Structure, drug_ development

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Structural studies of DnaK in complex with proline rich antimicrobial peptides

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Bacterial infections are a major cause of death worldwide. Due to increasing resistance against the commercially available antibiotics over the past few decades, novel antimicrobial drug classes with new mode of actions are required for future treatments. Small proline rich antimicrobial peptides (PR-AMPs) from mammals and insects were identified to target the *E.coli* Hsp70 chaperone DnaK after cell penetration. Binding of the peptides to DnaK compromises the activity of the chaperone and thus the viability of the bacterial cells, in particular under conditions of stress. The non-lytic cell penetration of PR-AMPs to Gram-negative bacteria makes them a promising drug candidate against human infections. Therefore, structural informations about the interactions between peptide inhibitors and DnaK are necessary for a better understanding of the mode of action.

After recombinant expression of the substrate binding domain in *E.coli* and subsequent purification, we crystallized the domain with several PR-AMPs. Elucidation of the binding mode of the peptides and characterization of the substrate specificity of DnaK will allow a structure-guided development of peptide inhibitors as antimicrobial agents targeting DnaK.

Keywords: chaperone, antimicrobial, peptide

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X-ray crystal structures of Aminoglycoside-2''-phospho-transferase-IVa [APH(2'')-IVa]

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Bacterial resistance to antibiotics persists as a serious clinical problem and a threat to public health. Aminoglycosides represent a class of bactericidal antibiotics that interferes with bacterial ribosome function, causing mistranslation of mRNA to yield defective proteins and thereby causing a detrimental effect on the microorganism. Due to their extensive use, resistance isolates against almost all clinically relevant aminoglycosides have been discovered, including in life threatening species such as various enterococci strains. The major resistance mechanism for aminoglycoside antibiotics is enzymatically modifying the drug, which leads to poor ribosome-binding and decreased efficacy. In the clinic, high-level resistance to a number of important aminoglycosides used against enterococci infections is conferred by a members of the aminoglycoside-2''-phosphotransferase family. Among these enzymes, APH(2'')-IVa has been identified as a highly gentamicin-resistant resistance factor originally found in *Enterococcus casseliflavus* [1].

Our studies focus on the structural analysis of the APH(2'')-IVa protein with the goal of better understanding its active site architecture and reaction mechanism, which will enable the rational design of novel small molecules that inhibit this enzyme, and/or next-generation antibiotics with reduced susceptibility to resistance. We present here the first two binary crystal structures of this resistance factor in complex with a bound aminoglycoside. Comparison with the apo structure provides insight concerning the substrate selectivity of this enzyme. In particular, conformational changes upon substrate binding previously not observed for this family of proteins underline the active site diversity among the members of the APH(2'') subfamily, which are structurally closely related despite low sequence identity, and can serve to explain significant differences in their substrate preference (resistance profiles). Analysis of the interactions between enzyme and aminoglycoside reveals a distinct binding mode as compared to the intended ribosomal target. The differences in the pattern of interactions can be utilized as a structural basis for the development of improved aminoglycosides that are not susceptible to these resistance factors.

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