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.

[1] M. Moasser *Oncogene* **2007**, *26*, 6577-6592. [2] F. Van Bockstaele, J. B. Holz, M. and H. Revets *Curr Opin Investig Drugs* **2009,** *10*, 1212-1224

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.

[1] S.F. Tsai, M.J. Zervos, D.B. Clewell, S.M. Donabedian, D.F. Sahm, J.F. Chow, *Antimicrobial Agents and Chemotherapy* **1998**, *42*, 1229-1232.

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