Molecular functionalities of ADEP-insensitive ClpP proteins

Supervisors / institute:
Main supervisor: Prof. Dr. Heike Brötz-Oesterhelt
Co-supervisor: Prof. Dr. Karl-Erich Jaeger

Project background and description:
Acyldepsipeptide antibiotics of the ADEP-class act at the unprecedented bacterial target ClpP, the proteolytic core of caseinolytic the protease. ADEPs prevent protein-protein interaction between partners of the oligomeric protease complex and hence all of its natural functions. At the same time, ADEP binding induces conformational changes in the ClpP core that severely dysregulate its function. Over-activated ClpP degrades proteins in an uncontrolled manner thereby depleting bacteria of essential proteins. Although ADEPs showed excellent antibacterial activity against important human pathogens their pharmaceutical development was hampered by resistance development in target organisms. We have recently discovered that ADEP-treated bacteria acquire diverse mutations in ClpP, which seem to be linked to antibiotic resistance. For a variety of these, it is not possible to rationally develop molecular explanations for impaired antibiotic activity based on current knowledge. We therefore, aim at producing diverse ClpP mutants, analyse their oligomerisation behaviour, catalytic parameters, interactions with other partners of the protease complex, degradation of substrates and responsiveness to ADEPs. Here, ADEPs and the derived ClpP mutants serve as ideal tools to improve our understanding of the complex Clp machinery.
In addition, we aim at investigating, why the bacterial ADEP producing strain, a particular *Streptomyces* strain, is naturally resistant to its own product. For this project part we will establish within iGRASP growth conditions for ADEP production and a procedure for isolation of the natural product complex. Collaboration with the Jaeger group will be instrumental for ADEP production in the producer strain and for protein expression.

Aims of the project:
- (1) Molecular reason(s) for ADEP resistance of ClpP mutants derived from target pathogens and functionalities of mutated ClpP variants
  (2) Molecular mechanism of ADEP resistance of the bacterial producer strain
- Co-operations with the groups of Karl-Erich Jaeger (Biotechnology); Dieter Willbold (Struct. Biology); Successor of Prof. Büldt (Struct. Biology); Claus Seidel (Struct. Biology)

Requirements:
- Master degree in microbiology, biochemistry, molecular biology or a related discipline; alternatively 2nd state examination (Pharmacy)
- Experience in cloning techniques, protein expression, protein analyses, culturing of bacteria, sterile technique

Additional information:
http://www.pharmazie.uni-duesseldorf.de/Institute/pharm_bio/arbeitskreise/AK-Broetz-Oesterhelt