

# Mechanism of Action studies using systematic analysis platform

Jan-Martin Daniel, Kevin Ludwig, Melina Arts, Stefania DeBenedetti, Tanja Schneider



Institute for Pharmaceutical Microbiology, University of Bonn, Bonn, Germany  
German Centre of Infection Research (DZIF), partner site Bonn-Cologne, Bonn, Germany  
contact: janmartin.daniel@uni-bonn.de



## Abstract

Novel antibiotics with unprecedented mode of action (MoA) are urgently needed and so are new concepts for the discovery and analysis of antibacterial substances. Integral components of the drug development process are analysis of the MoA of antibiotics, as well as identification of the molecular target. Without this detailed knowledge, rational drug design is strongly hampered. We built up a comprehensive MoA platform, combining validated whole cell screenings and a biochemical assay platform that allows rapid identification of antibiotic mechanisms and targets on cellular levels. Initial screenings of substances and extract libraries in whole-cell based assays allow to identify bioactive compounds. Selected promoter-reporter fusions, provide first indications on the metabolic pathway affected, whereas the biochemical analysis platform, comprising more than 60 individual *in vitro* enzyme activity tests, further allows the identification of the specific target and characterization of the compound-target interaction on a molecular level.

### Cellular test systems

- Bioreporter strains
- Membrane integrity
- GFP fused fluorescent proteins, essential for cell wall biosynthesis
- antisense-RNA technology
- Accumulation of UDP-MurNAc pentapeptide
- Antagonization of antibacterial activity
- Cytotoxicity
- Lipid pool determination using HPLC

### Biochemical platform (>60 individual test systems)

- Cell wall biosynthesis: Peptidoglycan, wall teichoic acid, capsule and arabino-galactan biosynthesis
- Complex formation with HPLC purified cell wall precursors (e.g. Lipid II)
- Enzyme kinetics
- IC<sub>50</sub> determination of a vast variety from cloned and purified proteins, essential for cell wall biosynthesis