s8a.m8.o3 **Design of Inhibitors of the Hepatitis C Virus Serine Protease: Modelling and Experimental Results.** U.Koch, C. Gardelli, F. Narjes, V.G. Matassa, C. Steinkühler, *IRBM - P. Angeletti S.p.A., Via Pontina KM 30.60,* 00040 *Pomezia ,Italy*.
Keywords: drug design.

Inhibition of the proteolytic activity of the NS3 protease is a possible strategy for the development of anti-HCV pharmaceuticals. Unlike other serine proteases NS3 lacks well-defined binding pockets in its substrate binding region which renders the development of small molecule inhibitors of this enzyme a challenging task. To design potent inhibitors we tried to gain a more detailed insight into how the NS3-protease recognizes active site ligands. For this purpose the crystal structure of the enzyme was used as the basis for modelling inhibitor complexes which helped to understand the structure activity relationship. Results from these studies were employed to design site directed mutagenesis experiments which gave valuable informations about interactions between individial aminoacids and the ligand. The substrate binding region is characterized by a strong positive potential which is matched by the potential of its more potent ligands. The relevance of this remarkable electrostatic properties of the NS3-protease for catalysis, substrate recognition and inhibition will be discussed.

The results of the analysis were applied to design highly potent mechanism based inhibitors. Crystal structure analysis and NMR-spectroscopy confirmed the models employed. One class of inhibitors described is capable of forming a transient covalent bond with the hydroxyl group of the catalytic serine. The results obtained for different classes of «serine trap» inhibitors are interpreted in terms of the structural properties of the active site of the enzyme. Reactivity of the thiol group of the P1 cystein has been a major obstacle in this series. Chemically inert replacements of the P1-cystein have been designed and incorporated in various series of inhibitors. This provided a platform to design small and potent inhibitors whose peptidic character was increasingly reduced by peptidomimetic replacements.

s8a.m8.o4 **Towards understanding bacterial virulence: The first crystal structure of a *Legionella* protein.** R. Hilgenfeld[1], A. Vogel[1], J. Hacker[2], R. Köhler[2], G. Fischer[3], B. König[3] & A. Riboldi-Tunnicliffe[1]. *1 Institute of Molecular Biotechnology, Jena, Germany 2 Institute for the Molecular Biology of Infectious Diseases, Würzburg, Germany 3 Max Planck Unit „Enzymology of Protein Folding", Halle, Germany*
Keywords: Bacterial infections, virulence factors, *Legionella pneumophila*

The Macrophage Infectivity Potentiator (Mip) protein is a major virulence factor of *Legionella pneumophila*, the etiological agent of Legionnaires' disease. Mip is necessary for optimal intracellular infection by *Legionella* of human macrophages as well as for survival of the bacteria within the host cell. Furthermore, the Mip protein is also essential for survival and replication of *Legionella* in its natural hosts, i.e. amoebae and ciliated protozoa. Related Mip proteins have been identified as virulence factors in various *Chlamydiae* as well as in *Trypanosoma cruzi*. We present crystal structures for the Legionella Mip protein and for several designed mutants thereof, as well as for complexes between Mip and drugs such as FK506 and rapamycin. The three-dimensional structure of Mip turns out to be highly unusual, with two domains linked by the longest free-standing alpha-helix seen so far in biology. The N-terminal domain mediates dimerization of the protein through formation of an unusual four-helix bundle. The C-terminal domain harbours the peptidyl prolyl *cis/trans* isomerase (PPIase) activity of Mip and has the fold of the human FK506-binding proteins (FKBPs). Using a Mip mutant with deficient PPIase, we succeeded in visualizing, for the first time, an intermediate in the isomerization of a substrate oliogopeptide. Our structural results provide a basis for the design of novel drugs against intracellular parasites such as *Legionella* and *Chlamydia*.