6. Summary

For the first time, this work presents systematic investigations of micro-arrayed sample preparation on MALDI-MS targets using a robotic multi micro-dispenser system. Generation, optimization and reliable application of piezo-jet micro-dispensers for sub-nano liter volume handling are explained. A patented electro-acoustic method for the precise and fully automatic positioning of micro scaled amounts of substances is described in detail. These means provide a great advantage towards the preparation of MALDI mass spectrometric samples for protein identification: Only femtomoles of proteolytic peptides have to be transferred into the mass spectrometer, yielding spectrometric data of acceptable quality and identical information content. This is ~ 1 % of the amount previously needed. Thus, high density MALDI-MS sample plates can be prepared and analyzed automatically.

Algorithms for the automatic interpretation of MALDI-MS spectra were invented and successfully applied. They comprise automatic extraction of mass spectrometric data like monoisotopic and average masses, quality factors, i.e., resolution and signal-to-noise ratio and export these data to existing databank search programs. A five fold gaussian curve fitting procedure, as well as the awareness of the isotopic distributions of natural occurring peptides are used to separate overlapping peak pattern.

The identity of functional proteins, involved in the process of bacterial conjugation, separated by one and two-dimensional gel electrophoresis and membrane blots was determined through mass spectrometric analysis of peptides derived from enzymatic digests. Analysis of primary structure and complex chemical behavior revealed the specific complexation of iron by TraH which is part of the relaxosome. Furthermore, the existence of an N-terminal lipid modification in the entry exclusion mediating TrbK was discovered. Additionally, cleavage of the predicted signal peptide of TrbM was confirmed. In the non-induced state, Lac-I, TraL, TrbG, TrbJ and TrbM were found to be the most prominent plasmid encoded proteins.

Consideration of predicted signal peptides drastically increases the sequence coverage of peptide mapping analysis when mass spectrometric data are used to search sequence databases. This characteristic feature is currently neglected by existing programs for MS-protein identification. MALDI-MS analysis of samples prepared with whole cells were used to determine the mayor component of extra-cellular pilus subunits. The matrix *trans*-3-indolyl acrylic acid, commonly used for the analysis of synthetic polymers, enabled the sensitive detection of the *trbC* gene product which was found to be mayor protein component of bacterial pili. “On-target” proteolysis of “protease resistant” pili was successfully used to examine the characteristic primary structure of the IncP-pilin. In *E. coli* the RP4 encoded, ribosomally synthesized and multiply processed TrbC is converted into a cyclic protein containing 78 amino acids. The head-to-tail connection of the former N- and C-terminus is realized in form of a peptide bond. At present, the resulting circular protein is the largest cyclo-peptide reported world wide. The gene products, *trbC* of the analogous plasmid R751 and *virB2* of the Ti plasmid from *Agrobacterium tumefaciens*, consist of a similar circular structure. A combination of mutagenesis experiments and MALDI-MS analyses peek into the cyclization-reaction and fundamentally yield a theoretical model of it’s chemical mechanism: In the periplasmatic space the serine-protease TraF cleaves a four amino acid residue peptide from the C-terminus of TrbC. It is proposed, that the resulting acyl-enzyme reacts via aminolysis involving the α-amine group of the TrbC N-terminus, generating cyclic TrbC* and the retained TraF.