PREFACE

Mass spectrometry has proven its applicability to the measurement of almost any molecule. However, different substances require special knowledge in the respective field and scientists tend to focus on their particular task in collaborations and meetings. Nevertheless, disciplines have become more and more interdisciplinary and scientists now have to acquire knowledge in many scientific fields other than our own. Especially mass spectrometrists who often find themselves learning about the history of their sample, be it from a zoologist, clinician, microbiologist, or biochemist. This was evident with the rise of proteomics, which reaches across species and research projects, uniting scientists through the methodology. This book covers research in the fields of biomacromolecules such as proteins, DNA, and other biopolymers studied with mass spectrometry.

Structural variability resulting from protein post-translational glycosylation vastly increases the range of biological functions produced from the genome. The chemistry of glycans differs from that of proteins and dictates the use of specialized mass spectrometric techniques for successful analysis. Moreover, the unique chemistry of each glycoconjugate class drives the need for tailored analytical techniques. Glycosaminoglycans (GAGs) are linear glycans that coat the surfaces of all animal cells, bound to syndecan and glypican proteoglycan core proteins. There, they play developmentally critical roles as co-receptors for many classes of growth factors and growth factor receptors. Animal cells grow in organized extracellular matrix environments consisting of proteins, glycoproteins and proteoglycans. GAGs serve to create and support immobilized growth factor gradients and play structural roles in these extracellular matrices. Although, due to their acidity, fragility and polydispersity, GAGs have traditionally proven difficult to analyze using mass spectrometry, effective new approaches have emerged over the past few years. Chapter 1 summarizes structural biochemistry of GAGs and the uses of mass spectrometry in their analysis, with an emphasis on recent developments that have proven effective.

FTICR (Fourier transform ion cyclotron resonance) mass spectrometry offers several advantages for the analysis of biological samples, including excellent mass resolution, ultra-high mass accuracy, and a wide mass range. Chapter 2 gives a short overview of recent applications and technological developments of FTICRMS, which are relevant to the field of protein analysis. Tandem mass spectrometry, "bottom up" and "top down" approaches for protein analysis as well as methods for studying protein conformations are described and illustrated with examples.

In chapter 3, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and isoelectric focusing (IEF) of pure recombinant human MIA (melanoma inhibitory activity) protein, as expressed in CHO cells, indicated that part of the protein was post-translationally modified. Whereas standard positive ion mode MALDI analysis of intact MIA revealed an additional peak 80 Da higher than the theoretical mass, IEF showed three major protein bands which could all unequivocally be identified as MIA isoforms by peptide mass fingerprinting. Negative ionization MALDI mass spectrometry on a quadrupole TOF instrument indicated that the differences between these isoforms were due to one or two 80 Da modifications confined to tryptic fragment 59-76. The identity of the 80 Da post-translational modification (PTM) was determined to be a Tyr-O-sulfation through enzymatic removal of the 80 Da moiety from MIA by arylsulfatase treatment, and through the absence of the 80 Da modification(s) in MIA harvested from cells cultured in the presence of chlorate, which is known to inhibit Tyr-O-sulfation. The PTM being sulfation was further corroborated by its metastability upon MALDI-TOF MS analysis. It is concluded that the CHO produced MIA is partially post-translationally modified by Tyr-O-sulfation at positions 69 and/or 70 (^{59}LFWGGSVQGD**YY**GDLAAR^{76}). Protein Tyr-O-sulfation, has been reported to serve various functions, including modulation of protein-protein interactions. Combination of this notion with the observation that the eukaryotic MIA protein shows only a weak binding towards laminin and fibronectin leads to the speculation that the putative *in vivo* metastasis promoting activity of MIA may be based on the interaction of MIA with an as yet unknown factor via Tyr-(O)-sulfation enhanced binding kinetics.

Many prokaryotes use polyhydroxyalkanoates (PHA) as storage compounds for carbon and/or energy in the form of water-insoluble inclusion PHA granules in the cytoplasm. The surface of such granules is covered by proteins which also affect their form and structure. In chapter 4, phasin PhaP1 protein was identified in high abundance on the grana surface of *R. eutropha* employing gel electrophoretic separation and mass spectrometry. Homologous phasins PhaP3 and Pha4 were visualized using 2D-PAGE in the PhaP1 knock-out mutant, but no evidence for the presence of PhaP2 could be found. All detected phasins exhibit isoforms whose structural differences are still a matter of investigation, although C-terminally truncated isoforms of PhaP1 were detected with FTICR-MS.

The Münster Conference on Single Cell Analysis was held in Münster on November 16, 2004. Chapter 5 is an overview of topics discussed at the conference. Chapter 6 contains the speaker abstracts from the conference.