

Development of Ion Mobility Spectrometry (IMS) and Advanced TOF-MS for Proteomics and Molecular Imaging

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Identification of individual proteins in complex biological mixtures by using matrix-assisted laser desorption ionization (MALDI) and high resolution, high-mass-accuracy TOF-MS has enormous potential for high-sensitivity and high-throughput applications. Such experimental capabilities can be further expanded, both in terms of sample throughput and information content, by incorporating ion mobility spectrometry (IMS) for post-ionization separation of the sample. In addition, advanced optical devices, *i.e.*, beam homogenizers and digital micromirror arrays (DMMA), and high speed multiplexed data acquisition electronics can be incorporated with IM-TOF-MS instruments for rapid, high-throughput molecular imaging of protein micro-arrays and tissue samples. This lecture will focus on the technological advances in IMS, TOF-MS and molecular imaging that have occurred in our laboratory over the past three years and the developments will be illustrated by recent applications in areas of chemical-biology that drive the technology development.

Since 1995 my laboratory has led the development of a variety of TOF-MS instruments. Our initial research was performed using a 7.7 meter reflectron TOF (PerSeptive Biosystems, Inc.) and more recent work has employed state-of-the-art instruments, *e.g.*, ABI Voyager STR (MALDI) and MDS Sciex Q-Star (ESI). Currently, we are developing a new ultra-high resolution MALDI TOF-MS instrument based on the 7.7 meter reflectron instrument, and our preliminary results suggests that we should be able to achieve mass resolution approaching 100,000 using this instrument. The combined capabilities of ultra-high resolution TOF-MS, rapid protein denaturation-enzyme digestion (thermal denaturation and the use of organic solvents), and high-throughput tandem mass spectrometry (TOF-TOF and ion mobility-TOF-MS) provide unparalleled approaches for proteomics scale research. To demonstrate the capabilities of high resolution TOF-MS for high-throughput applications, we initiated a collaborative research project to catalog the changes in protein expression of *E. coli* (specifically K-12). These studies are primarily aimed at identification of multi-subunit and multi-protein complexes in pathogenic and physiologic processes. The general method identifies expressed proteins using a combination of non-denaturing HPLC and MALDI-TOF-MS for peptide mass mapping. This approach allows us to identify the components of protein mixtures, esp. multi-subunit complexes and biochemical activities. The latter information is lost in more traditional proteomics studies of protein expression. We are currently continuing this type of work as well as developing new methods/techniques for studies of sumoylation and other types of post-translational modifications in a variety of sample types, ranging from purified protein samples, protein micro-arrays, as well as tissue sections.