

Time-of-Flight Mass Spectrometry

Tools for Proteomics Research, Bioagent Detection and Diagnostics

The worldwide effort to study the human proteome promises new drug targets, improved diagnostics and the ability to predict more reliably the beneficial and adverse responses to therapies. The analytical method leading this effort is mass spectrometry, with time-of-flight mass spectrometers and ion traps replacing the more traditional magnetic sector and double focusing instruments used for smaller molecules. Molecules analyzed by mass spectrometry must first be ionized and two new methods: electrospray ionization and matrix-assisted laser desorption/ionization

(MALDI), have made it possible to ionize the much larger molecules found in biological systems, such as peptides, proteins and DNA. Our laboratory at the Johns Hopkins University School of Medicine has been a major player in the development of time-of-flight mass spectrometers and their applications to biological and medical research. We have been fortunate in our collaboration with Shimadzu Corporation and their subsidiary Kratos Analytical to see the incorporation of several key technologies developed by our laboratory in their bio-analytical instruments.



Robert J. Cotter (PhD)

Prof. Pharmacology and Molecular Sciences

*Johns Hopkins University
Baltimore, MD 21205, USA*

Time-of-flight mass spectrometers

In a time-of-flight (TOF) mass spectrometer ions are formed in an ion source and accelerated by the potential difference (V) across the source into a drift region. [See Fig. 1.] Typically, a voltage of 20 kV is placed on the sample plate at the back of the source, and the ions are formed by a laser pulse whose duration may be from 1 nanosecond to as little as a few picoseconds. Once in the drift region, lighter ions travel faster than heavier ones. If the ion source is small (1-2 cm) and the drift length (D) large (1 meter or more), then the total flight time (t) of an ion is approximately the time in the drift region, or $t = (m/2eV)^{1/2}D$. Ions of any mass can be analyzed by the TOF so that it is attractive for investigating biological molecules. To improve mass resolution, a reflectron is generally

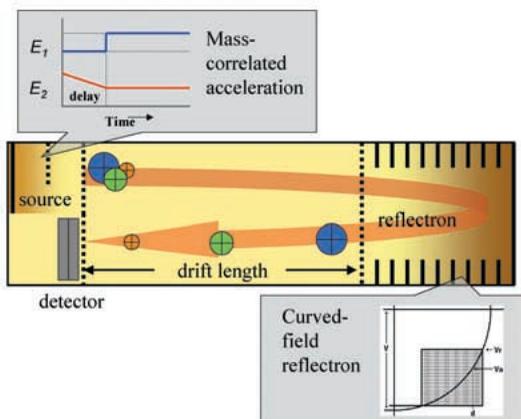


Fig. 1 TOF mass spectrometer and innovations described by author

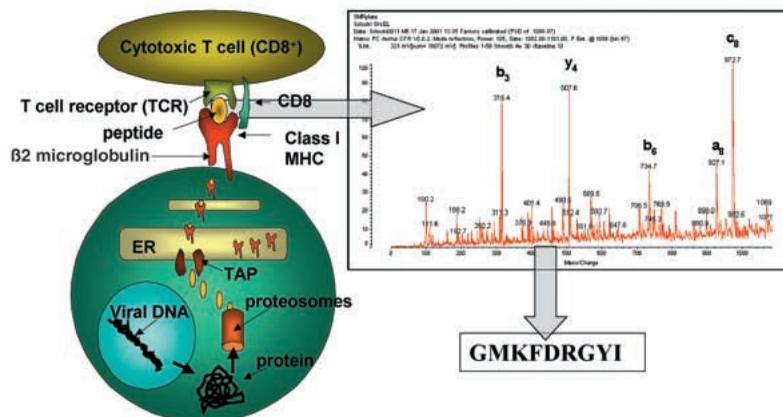


Fig. 2 Expression and mass spectrometric analysis of antigens

used to send the ions back along the same drift length, expanding the difference in arrival time at the detector between ions of different mass and compressing the differences in time between ions of the same mass.

Biology drives instrument development: the curved-field reflectron

Class I antigens are those derived from endogenous proteins that are displayed on cell surfaces by major histocompatibility (MHC) molecules and recognized by cytotoxic T-cells. [See Fig. 2.] In particular, the proteins are degraded by proteasomes, transferred to the endoplasmic reticulum (ER) by a transporter protein (TAP), combined with MHC and carried to the surface of the cell. Antigens are generally nine amino acids long, and the T-cell receptor/CD8 restricted recognition system must recognize both the antigen

and the MHC molecule as belonging to oneself. Failure to recognize the MHC molecule implies failure to recognize foreign cells and leads to rejection, as can occur following organ or bone marrow transplantation. Failure to recognize the antigen implies the presence of a tumor cell or a viral infection, and leads to cell cytotoxicity. The bound antigen/MHC complexes can be isolated by immunoprecipitation using an antibody to $\beta 2$ microglobulin, and the antigens released, separated by HPLC and analyzed by mass spectrometry. Obtaining the amino acid sequences of antigens can be difficult using TOF mass spectrometry, particularly if HPLC fractions reveal a number of peptides in their mass spectrum. The problem of sequencing mixtures provided one strong motivation for our development of the curved-field reflectron (CFR), a reflectron in which the voltage gradient follows the arc of a circle. This reflectron was used in

the MALDI 4 and AXIMA-CFR instruments and refocuses ions resulting from fragmentation in the flight tube. With the addition of an electronic gate for selecting a particular peptide mass, amino acid sequence ions can be obtained for each individual peptide in the mixture. In collaboration with Mark Soloski at Johns Hopkins we have used the AXIMA-CFR to characterize an endogenous peptide GMKFDRGYI derived from heat shock protein. This study, carried out by Suzy Ramirez in our laboratory, has also identified a molecular mimic GMQFDRGYL that is expressed when cells are infected with Salmonella, and may be responsible for an autoimmune response to self antigen following infection. Thus, the CFR developed by Timothy Cornish in our laboratory was intended to address a need in biological research.

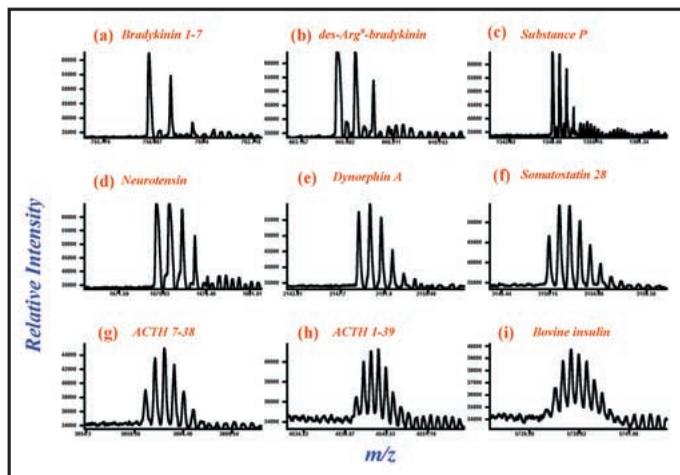


Fig. 3 Focusing ions over a broad mass range

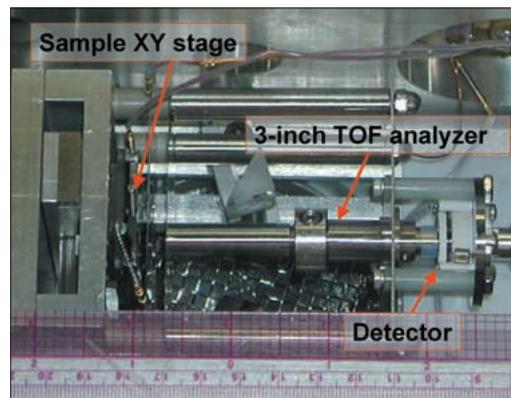


Fig. 4 Miniature mass spectrometer

Focusing all the ions: mass-correlated acceleration

As a means for improving the mass resolution of a TOF mass spectrometer, it is also common to pulse the first ion extraction region in the source (E_1) several hundred nanoseconds after the initial laser pulse. Known variously as time-lag focusing, delayed extraction or pulsed extraction, this method tends to focus only a narrow range of mass. Addressing the need for simultaneously focusing the wide mass ranges encompassed by partially-fractionated peptide mixtures from the enzymatic digestion of proteins, mass-correlated acceleration (MCA) was developed in our laboratory by Slava Kovtoun. This method adds an additional time-dependent field (E_2) in the second extraction region of the ion source to bring all masses into focus in a single spectrum. [See Fig. 3.] Mass-correlated acceleration is being used in our laboratory by Robert English to characterize biomarkers for biological agents, and will be incorporated in future AXIMA instruments.

The future: miniaturization

The AXIMA-CFR is a high performance mass spectrometer that is being used in a high-throughput mode by LumiCyte (Fremont, CA) to characterize the biomarkers for disease, in particular for prostate cancer. Once determined, these markers can be used diagnostically, and there would be a need for a miniaturized mass spectrometer that can be located at the point of care. An instrument for biological agent detection would be similar. As bioagent markers are determined by high performance instruments, an inexpensive and lower resolution instrument could be used to identify agents from simple molecular weight measurements. In work supported by the Defense Advanced Research Projects Agency (DARPA) we have developed a 3-inch TOF mass spectrometer that has a mass range in excess of 60 kDaltons and a mass resolution for peptides of up to one part in 1200. [See Fig. 4.] The biomarker signals expressed for viruses, bacteria and spores are generally peptides, so that identification by pattern recognition techniques is enhanced by comparison with genomic data for each

microorganism. This is a compelling approach because genomic data (and the proteins derived from the genome) are likely to be obtained far more rapidly for microorganisms than mass spectral libraries and are independent of ionization method. Thus, time-of-flight and other mass analyzers will continue to be useful as new ionization methods are developed. [See Fig. 5.]

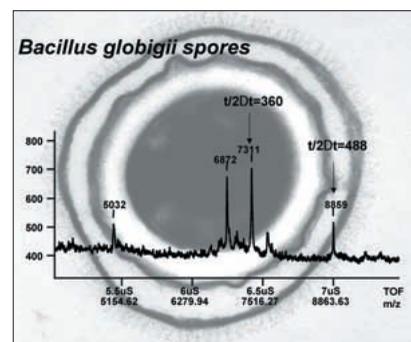


Fig. 5 Biomarkers for Bg Spores

From its early reputation as a low mass range, low resolution and low duty cycle instrument, the time-of-flight mass spectrometer has emerged as a major player in proteomics, having demonstrated subfemtomole detection limits for peptides, mass resolutions of more than one part in 15,000 and mass ranges into the hundreds of kilodaltons.

Robert J. Cotter is Professor of Pharmacology and Molecular Sciences at the Johns Hopkins University School of Medicine, and Director of the Middle Atlantic Mass Spectrometry Laboratory. He is currently Past President of the American Society for Mass Spectrometry and was President from 1998-2000.