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International Journal of Mass Spectrometry 259 (2007) vii–xi
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Foreword

Donald F. Hunt

This issue of the *International Journal of Mass Spectrometry* is in recognition of Professor Donald Hunt's 65th birthday and his 40 years of research in mass spectrometry. It consists of a collection of papers contributed by former graduate students, postdoctoral fellows, colleagues, and long time friends celebrating this milestone in Don's life. The papers and reviews illustrate the impact Don's research and mentoring have had on the field of mass spectrometry, with papers ranging from instrumentation development to biological applications. These papers and the enthusiasm of the authors contributing them serve as a tribute to the impact Don's research and training has had in mass spectrometry and beyond.

Over the last 40 years Don's research interests have cut a wide path. As a graduate student, Don studied in the field of organometallic chemistry under Marvin Rausch and Peter Lillya at the University of Massachusetts. While in Dr. Rausch's laboratory Don also developed a strong interest in mass spectrometry, which he continued during post doctoral training in Professor Klaus Biemann's laboratory at the Massachusetts Institute of Technology. In the early 1960s, few laboratories were interested in pushing the frontiers of mass spectrometry, but the Biemann laboratory was busy extending mass spectrometry to the analysis of biomolecules. Don's postdoctoral research involved the development of a method to sequence small oligonucleotides using mass spectrometry, which also involved the development of suitable derivatization methods to make the nucleotides volatile enough for introduction into the mass spectrometer. In 1968, Don left MIT for a position as an Assistant Professor in the Department of Chemistry at the University of Virginia, with responsibilities that included supervision of a mass spectrometry service facility. At this time the research efforts of Biemann, Djerassi, McLafferty, Beynon and others were proving mass spectrometry to be an essential analytical method for natural product chemistry and organic synthesis as a means to deduce and confirm structure. With foresight characteristic of Don, he agreed to supervise the facility on the condition the Department acquire a chemical ionization source along with the high-resolution magnetic sector mass spectrometer they were about to purchase with NIH funds. Just a couple of years earlier, Field and Munson had pioneered the development of chemical ionization as a new soft ionization technique and Don quickly recognized the potential of this new method to solve a number of problems using mass spectrometry.

As a new Assistant Professor, Don returned to his roots in organometallic chemistry to initiate his independent research program while he began to develop his mass spectrometry interest. Don encouraged the use of mass spectrometry in his lab by urging his graduate students to monitor organometallic reaction products on a small scale using the mass spectrometer's inlet probe. If the reaction occurred as predicted, an ion should be observed at the expected m/z value once the probe was inserted into the mass spectrometer. Besides using the soft ionization attributes of CI to monitor the pseudo molecular weight of a molecule, its' structure and functionality could also be interrogated in the gas phase. Nitric oxide, oxygen, and argon/water were particularly useful reagents for studying the structures of organic compounds by either positive or negative ions. Don's research in chemical ionization mass spectrometry quickly outstripped his efforts in organometallic chemistry, and he discovered many new reagents to probe the structures of organic molecules with gas phase ion chemistry.

In the early 1970s, commercial versions of the quadrupole mass spectrometer began to appear. Based on the pioneering research of Wolfgang Paul, the quadrupole mass spectrometer was initially developed and commercialized by Finnigan Corp. (today known as Thermo Electron Corp.), a fledgling company in Silicon Valley. Don recognized the potential of this new technology, relative to magnetic sector mass spectrometers, as smaller and much faster scanning devices, which could be operated at much lower voltages, and would be ideally suited for gas chromatography and chemical ionization. Don approached Michael Story and Robert Finnigan (founders of Finnigan Corp.) to arrange a deal to purchase a quadrupole mass spectrometer with all that he had to spend at the time, $40,000, which was well below the retail price of the instrument. In exchange, Don offered to interface a GC to the instrument and develop CI methods and negative ion detection to improve the sensitivity of mass spectrometry. Mike Story and Bob Finnigan were not sure if Don's proposed research would be successful or even help Finnigan Corp. commercially but they were so impressed by what Don had proposed, they agreed to the collaboration. They were certain that Don would do his best to push quadrupole mass spectrometry technology ahead as fast as possible, exactly what this new analytical technique needed at this early stage of development. With this leap of faith, Mike and Bob had initiated what would be a long

1387-3806/$ – see front matter
doi:10.1016/S1387-3806(06)00507-0

Fig. 1. As early as 1957 in high school Don had a fascination for instruments.

and fruitful collaboration between Finnigan Corp. and Don (Fig. 1).

Don assigned the project to get the new quadrupole mass spectrometer operating as a GC/MS in the negative CI mode to second year graduate student George Stafford. At the time this was a fairly daunting task as the Finnigan 3200 instrument came equipped with a positive ion CI source, a mass range up to *m*/*z* of 800, and no data system or GC interface, but it was still the state of the art for quadrupole mass spectrometers at the time. George's objective was to interface a GC to the system and convert the mass spectrometer to detect negative ions and then develop a method to analyze amino acids with the system. A derivatization scheme needed to be developed for amino acids to volatize them into the GC. Don realized that a positive ion chemical ionization source creates thermal electrons and with the right derivative, electron capture might increase sensitivity up to a thousand-fold. The method was successfully demonstrated and shown to be much more sensitive than positive ion CI. However, George wanted to measure more accurately the differences between positive and negative ion CI, so he devised a strategy to measure simultaneously positive and negative ions (Pulsed Positive Ion Negative Ion CI, PPINICI). This was a provocative idea and Don was initially skeptical that it would work. After George demonstrated the concept, Don immediately saw the potential and developed many new ideas to apply the technique. This is one of Don's hallmarks as a mentor: he is always willing to allow students to try new ideas, and quick to realize their value (Fig. 2).

Don's collaboration with Finnigan Corp. benefited both parties in many ways. The Finnigan 3200 mass spectrometer used thermal sensitive paper to record a mass spectrum. Academic researchers were beginning to interface computers to mass spectrometers to make the process of recording and analyzing mass spectra faster and easier (*m*/*z* values had to be assigned to each peak by hand). Don became aware of INCOS, a small company in the San Francisco Bay area, that was selling a computer and software that could be interfaced to a mass spectrometer. Don alerted his collaborators at Finnigan Corp. that they should look at this new computer system as a way to improve their mass spectrometer. Finnigan Corp. forged a relationship with INCOS, eventually purchasing them, making Finnigan mass spectrometers one of the first to be commercially available with a fast, powerful data system years ahead of its time.

The methods developed for negative ion CI and PPINICI found application in the area of environmental chemistry, but Don quickly started looking for new applications for these methods. During a 1975 sabbatical in Professor Dudley William's laboratory at the University of Cambridge, Don was looking for new directions and started thinking about a mass spectrometry strategy to sequence oligonucleotides. Professor Williams suggested Don should go to the weekly lectures at the nearby Medical Research Council to hear about new developments in DNA techniques. After hearing a lecture by Frederick Sanger on his new method for sequencing DNA, Don abandoned the idea of sequencing oligonucleotides and began to think about protein sequencing. Consequently, the Hunt lab started developing peptide-sequencing strategies using the advantages of

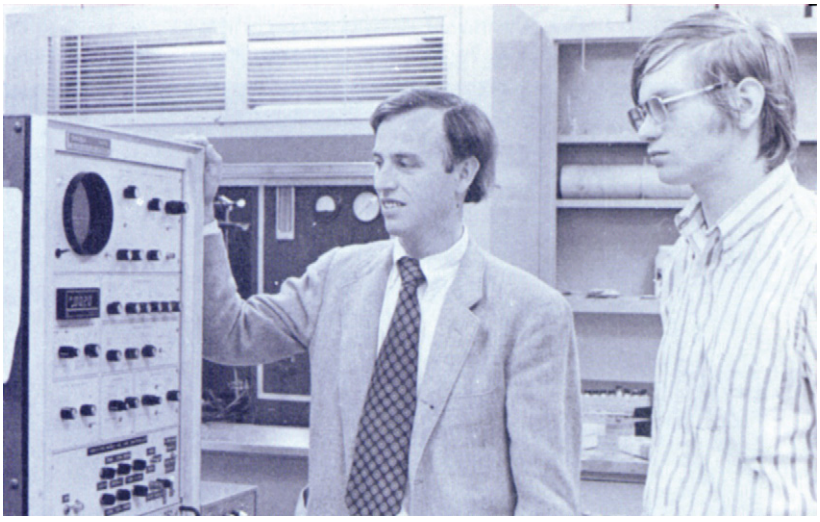

Fig. 2. 1976, Don pictured with George Stafford and Finnigan 3200 MS.

Fig. 3. 1976, Jeff Shabanowitz and Don pictured with rest of the group. (Left to right, graduate students: George Stafford, seated postdoc Bill Brumley, Alex Buko, undergrad Dave Lewis, Frank Crow, Satinder Sethi and postdoc Frank Botz.)

negative ion CI. The research progressed nicely, but was limited because CI is a soft ionization technique that produces very little fragmentation and, therefore, little in the way of sequence information. An important breakthrough for this research came in the form of the triple quadrupole mass spectrometer.

While attending the ASMS meeting in St. Louis in 1978, Don heard a talk by Rick Yost, a graduate student from Chris Enke's laboratory at Michigan State University, who reported on the feasibility of using low energy collision induced dissociation to fragment ions in experiments performed on a pentaquadrupole mass spectrometer in Jim Morrison's lab in Australia. Enke and Yost intended to develop their own computer controlled triple quadrupole mass spectrometer to do the same. Don was excited about the potential of this instrument and immediately approached Jeff Shabanowitz about constructing a triple quadrupole. After much debate and the realization that the components of three perfectly fine GC/MS single quadrupole instruments would be necessary, much to the dismay of the other graduate students, less than 2 months passed before they were shutdown and assembled into a TSQ producing its' first spectrum a week later. At the 1979 ASMS meeting, Don, Jeff and graduate student Alex Buko demonstrated the use of the triple quadrupole mass spectrometer to analyze derivatized peptides using isobutane CI. Within a few months, Don was able to convince Finnigan Corp. of the market potential of the TSQ by generating orders for five TSQs, and in 1980, Finnigan Corp. introduced the first commercial triple quadrupole mass spectrometer (Fig. 3).

While the TSQ helped with the peptide sequencing process by providing an efficient method to fragment peptide ions created by CI, the continued use of CI still necessitated derivatization of peptides for volatilization. Like most laboratories at the time, Don's group was also searching for methods to ionize intact peptides. Efforts to use lasers and rapid heating techniques to directly ionize peptides were being studied in the lab but not producing satisfactory results. These efforts put Don in the perfect position to pursue the new Fast Atom Bombardment method introduced by Michael Barber and colleagues in 1981. Barber's innovation was to dissolve peptides in a nonvolatile liquid, such as glycerol and then bombard the surface with high-energy particles. Don immediately combined FAB and tandem mass spectrometry to develop a new strategy to sequence peptides, which was published in *Analytical Chemistry* in 1981.

In September of 1983, the Hunt laboratory had no graduate students. Don had returned in 1982 from a Guggenheim and Fogarty supported fellowship in Howard Morris' laboratory at Imperial College in London with the intent of converting his laboratory fully to protein biochemistry. He graduated his students working on ion-molecule chemistry or environmental mass spectrometry and would not take any new students unless they had an interest in protein biochemistry. Don had big plans to start research in this new area and they included pursuing protein sequencing with an extended mass range (up to *m/z* 1800) on the triple quadrupole Jeff Shabanowitz had built. As Don slowly rebuilt his research group, he could often be seen in the laboratory working along side Jeff and his one new graduate student, John Yates. Don was still a very good and imaginative bench chemist and was quite a sight in the laboratory as he was always wearing his coat and tie while doing experiments.

With a promising new ionization method for peptides, Don began to think about the limitations of current instrumentation and became interested in FTICR. A major limitation to FTICR was sample introduction because the ICR required ultra-high vacuum. Once a sample was introduced through an inlet valve, the analyzer had to be pumped back down to 10^{-9} Torr. The high vacuum requirement was especially problematic for the FAB technique because by the time the analyzer reached proper vacuum the glycerol would be pumped away. Don's solution was to interface an ion source far away from the ICR cell and transfer ions into the magnetic field using rf-only quadrupoles. Differential vacuum pumping would maintain the vacuum in the ICR cell at the appropriate value. As Don often did with a new idea for an instrument, he approached Finnigan Corp. to discuss collaboration. However, Robert McIver of the University of California, Irvine had reached much the same conclusions about FAB and FTICR and just the week before had entered into an agreement with Finnigan Corp. to develop the idea. Don quickly agreed

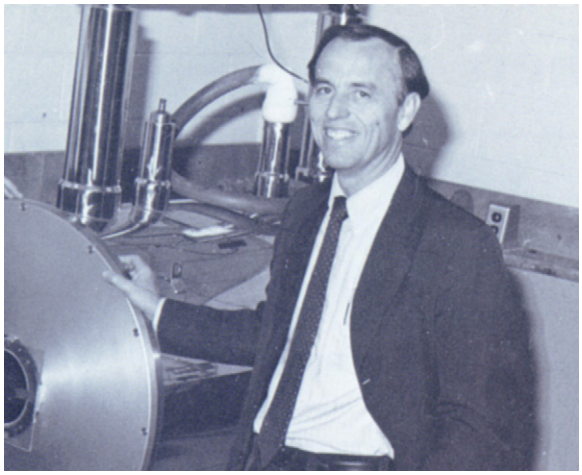

Fig. 4. 1987, Don pictured with 7 T magnet of QFTICR.

Fig. 5. 1994 Chicago ASMS. Left to right undergraduate Dalene Kottmeier, Don, graduate students Carthene Bazemore-Walker and Ron Hendrickson, 1987 graduate John Yates, Klaus Biemann and graduate student Pam Gulden.

to step out of the way to allow McIver to perform the initial and critical step of showing that ions could be injected through the fringing magnetic fields of the superconducting magnet and trapped in the ICR cell. McIver presented these experiments at the 1983 ASMS meeting in Boston. Don and Jeff with the help of Finnigan Corp. quickly built a similar instrument and published a series of seminal papers in FTICR mass spectrometry demonstrating peptide analysis with this instrument (Fig. 4).

The 1980s were a fruitful time in Don's laboratory. Techniques for protein sequence analysis were being refined and proteins were being sequenced. Methods were becoming more sensitive and tandem mass spectrometry was gaining ground on the standard protein sequencing method of the time: Gas Phase Edman Degradation. A highlight was the sequencing of the 1987 Protein Society Test Peptide using mass spectrometry. The success of Don's laboratory in this workshop helped legitimize mass spectrometry as a protein sequencing technique and demonstrated its potential to an audience of protein chemists. The power of mass spectrometry for protein sequence analysis was again dramatically improved with the introduction of electrospray ionization (ESI). Don quickly added this capability to his triple quadrupole mass spectrometers and through collaboration in 1991 with Arthur Moseley in Jim Jorgenson's laboratory, adapted microcolumn liquid chromatography to ESI. This combination of technology led to significant increases in sensitivity and capability for the analysis of complex peptide mixtures. Don spent the next decade training over 400 scientists from around the world how to use this new strategy to sequence proteins.

Armed with these new techniques, Don became interested in applying them to immunology in the early 1990s. Together with Professor Vic Engelhard, a colleague at the University of Virginia, new strategies to analyze both class I and II Major Histocompatibility antigens were developed. These methods led to the search for tumor specific antigens presented on the surface of cancer cells. The idea was to identify the cell surface antigens, nine residue peptides that trigger a patient's cytotoxic T-cells to destroy the tumor and then develop therapeutic methods to boost a patient's response to the antigens. Peptides that stimulate the immune system to recognize and destroy melanoma were the first to be found. Over the past 15 years, Don's laboratory studied many aspects of the cellular immunologic response and been responsible for the discovery of many antigens associated with a variety of diseases (Fig. 5).

As interest in FTICR continued to develop through the 1990s, Don and his group began focusing on the current limits of the technology and decided a tandem ion trap-FTICR instrument would overcome these limiting issues. Once again Don turned to his long time collaborator, Thermo Electron (formerly Finnigan Corp.), and tried to persuade them to support these efforts. At a dinner meeting at the Pittsburgh Conference in Orlando, Florida, Don worked hard to persuade the upper management of Thermo Electron to invest in this project. While the technology was interesting and had potential, was there a market for the instrument? In the end, the research project was successful and a linear ion trap-FTICR instrument (LTQ-FT) was commercialized meeting demand for an easy to use FTMS instrument.

At about the same time, Fred McLafferty's group at Cornell introduced Electron Capture Dissociation, which has the unique ability to fragment large polypeptides and proteins. The method uses thermal electrons to create unstable odd-electron cations of polypeptide and protein ions that readily fragment. The technique is well suited for use in the FTICR instrument because it does not compromise its' high vacuum requirements. Monthly publications on ECD indicated its potential as a new method to dissociate ions and Don began to think about alternative ways to achieve the same results. The parallels to the negative ion chemical ionization methods Don worked on years before were remarkable. If you could develop a method to create odd-electron cations of multiply charged peptides in an ion trap mass spectrometer, such a method might improve the fragmentation of peptides, particularly modified peptides such as phosphopeptides. Drawing on his expertise in gas phase ion chemistry the new method electron transfer dissociation (ETD) was developed. The method shows great promise to fragment highly charged peptides, polypeptides and even intact proteins in ion trap mass

spectrometers, and improves the fragmentation of phosphopeptides. Don's laboratory together with David Allis' have used these new capabilities to study the histone "code", or the collection of modifications to histones that regulate gene transcription. It is important to be able to place the many different modifications and patterns of modification found on histones into their functional context and methods such as ETD are essential to fragment histones to determine the locations of modifications.

Don's research career has spanned almost 40 years resulting in over 300 publications and many awards. He has witnessed and driven many changes in the field of mass spectrometry. Most importantly his involvement in the development and application of mass spectrometry to biological problems has made him an ambassador to the world outside of mass spectrometry where he has represented his community well. All who have worked with him know him as honest, sincere, and a pillar of integrity. He is encouraging and supportive of his students even long after they have left the laboratory and his 91 graduate students and postdoctoral fellows (and 11 current graduate students) fill the ranks of academics and industry following the passions that ignited in Don's laboratory. We would all like to wish Don a happy 65th birthday and many more years inspiring young minds.

John R. Yates III
Scripps Institute, United States

George C. Stafford
Thermo Electron Corporation, United States

Jeffrey Shabanowitz
University of Virginia, United States