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A Brief History of Mass Spectrometry

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A few of the great people and major discoveries that have shaped this century-old technique.

As the applications of MS rapidly expand, so does the number of mass spectrometrists. For example, in 2007, the American Society for Mass Spectrometry (ASMS) annual meeting drew >6000 participants to Indianapolis, Ind., for the 5-day event; the attendance figures for the 2008 and 2009 meetings are expected to be even higher. But the technique has not always had such a large following.

Development of MS was pushed at first by a few dedicated proponents. Originally, in the early 20th century, the technique was used to measure masses of atoms, and one of its first contributions to science was to demonstrate the existence of isotopes; this discovery fueled the contemporaneous ongoing debates about the structure of the atom. By the 1940s, chemists in the petroleum industry were using the mass spectrometer to measure the abundances of small hydrocarbons in process streams. It was not until the 1960s that natural-products scientists and other chemists really began to understand how complex molecules fragmented inside the instrument and to fathom the range of possible applications.

To really appreciate how the field of MS has expanded to its present-day size, it is helpful to look back and examine some of the great advances in the field and the people who made them happen. As in any other field of study, however, every advance in MS is built on all of the work that came before; every great innovator is standing on the shoulders of hundreds of people. This article is meant to highlight a few of the "greatest hits" and is not comprehensive by any means (Table 1 shows advances not covered in this article). More complete histories of MS are available both in print (e.g., Ref. 1) and on the web.

Table 1. Other important 20th-century advances in MS instrumentation.

What	Who	When
The first electron impact source (solids)	Dempster	1918
The first electron impact source (gases)	Bleakney	1929
TOF mass analyzer	Stephens	1946
Quadrupole mass filter and quadrupole ion trap	Paul	1953
Chemical ionization	Field	1966
Field desorption ionization	Beckey	1969
^{252}Cf plasma desorption	MacFarlane and Torgerson	1976
Triple-quadrupole mass analyzer	Yost and Enke	1978
FAB	Barber	1981

CATHODE RAYS AND THE RACE TO MEASURE *m/z*

Today, MS is mostly under the purview of analytical chemistry. Like most of its instrumental cousins, though, it was born in the field of physics. And like many great scientific discoveries, MS was "invented" while its discoverer, J. J. Thomson, was looking for something else.

Thomson was just 28 years old when he was offered the prestigious Cavendish Professorship at Cambridge University (U.K.). According to Per Dahl, now retired from Lawrence Berkeley National Laboratory and author of *Flash of the Cathode Rays: A History of J. J. Thomson's Electron*,[2] Thomson was in the right place at the right time. "He happened to be on the spot," Dahl says. "There was really nobody else quite suitable."

Thomson's training up to that point had been in theoretical physics, but the Cavendish Professorship was an experimental position. Thomson had to quickly choose a new program of experimental investigation, and so he chose a hot area of research: the transmission of electricity through gases.

Many scientists in the U.K. and in Europe were working on this topic at the time, and one major question they were trying to answer was: what is the nature of cathode rays? Some researchers thought they were made of particles and some thought they were waves, but despite 50 years of research, no one yet had a definitive answer. For those who believed in the particle theory, the race was on to measure the mass of these unknown particles.

Thomson participated in this race and ultimately prevailed, despite his somewhat poor laboratory skills. "He was said to be rather clumsy with his fingers and hands," says Dahl. "And yet he did this, so it's in a way one of the oddities in the history of science that he managed to come up with this experiment, after all." Luckily, Thomson's laboratory assistant, E. Everett, was a genius at building the apparatus needed for magnetic deflection of cathode rays.

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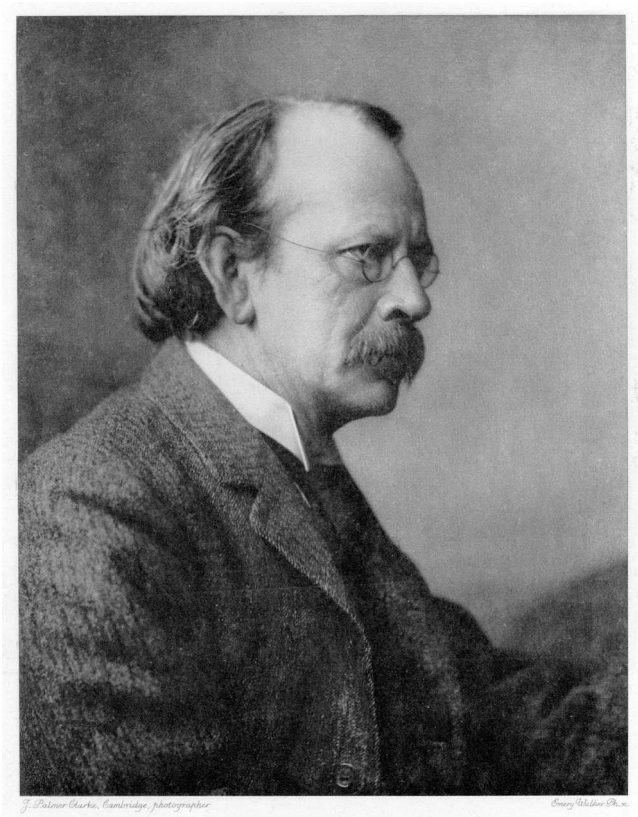


WIKIMEDIA COMMONS

Portrait of Thomson. (Courtesy of the Edgar Fahs Smith Collection/ Pennsylvania Library).

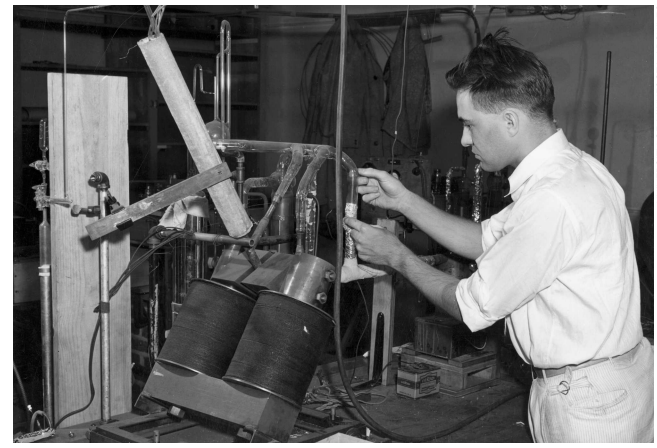
Thomson first used his apparatus to measure e/m (early physicists typically reported a charge-to-mass ratio, e/m, rather than the present MS standard of m/z) of these fundamental particles—electrons—in 1897. Two years later, again with the assistance of Everett, he built an instrument that could simultaneously measure e/m and e, thus indirectly measuring the mass of the electron. For this work in "discovering" the electron, he received the 1906 Nobel Prize in Physics.

Thomson's early work on cathode rays laid the foundation of the MS field. Ultimately, Thomson, with the help of his protégé Francis Aston (who would go on to win his own Nobel Prize in Chemistry in 1922), built what later would be recognized as the first mass spectrometer to measure the masses of charged atoms. This instrument used gas discharge tubes to generate ions, which were then passed through parallel electric and magnetic fields. The ions were deflected into parabolic trajectories and then detected on a photographic plate.

In the first three decades of the 20th century, Aston and other scientists redesigned the instruments to improve resolving power and began using them to separate and prove the existence of elemental isotopes. But it was the importance of isotopes to the Manhattan Project and World War II (WWII) that really pushed MS into prominence as a useful tool.

BRINGING MS TO THE MASSES

Until the 1940s, physicists still dominated the MS field, and they used the technique mainly to resolve questions about the fundamental nature of the atom. It took a modest man from Minnesota to show the world the practicality of MS.



UNIVERSITY OF MINNESOTA ARCHIVES

Nier with one of his early mass spectrometers.

By all accounts, Alfred Nier loved to build mass spectrometers. He began his academic career as an electrical engineer but, because of the paucity of engineering courses available at the time, eventually turned to physics for his graduate work at the Minnesota. Nevertheless, his early training served him well. "That gave him the skill set that he needed to be a very accomplished and knowledgeable person in electronics," says Michael Grayson, retired from Washington University and editor of *Measuring Mass: From Positive Rays to Proteins*.[1] "When he was forced to continue his education . . . in physics, then that combination was very unique because as an experimentalist, he had the physics background, and he also had the electrical design information that he needed."

Nier designed and built several revolutionary instruments, including the 60° sector field instrument, which greatly reduced the size and power consumption of the magnet. He later produced a design that still bears his name and that of his colleague E. G. Johnson: the Nier–Johnson mass spectrometer, which combines electrostatic and magnetic analyzers in a unique conformation. A variant of this design was commercially developed into one of the highest-resolving-power instruments of its day.

But Nier's real contribution to the MS field was his tireless promotion of the technique to people outside the tight community of physicists to which he belonged. "Al was involved in making mass spectrometry a discipline that could be used for other things," says Dennis Schlutter, who worked with Nier at the Minnesota for many years. "He sort of commercialized the instrument—not in the sense that he was trying to sell them, but [he] made them more useful and usable."

Nier's generosity with his time and resources is still legendary. "He was very selfless in giving his machines and ideas to others," says colleague Mark Kurz of Woods Hole Oceanographic Institution. "What was so great about Al Nier and why so many people were so devoted to him was that he was such a gentleman and helped so many people."

In one example, Nier helped biologists by preparing ^{13}C-enriched carbon. ("The material was of great interest to biologists, who could use it for tracer studies," Nier wrote years later.[3] "As a result, I gained many new friends.") He also assisted geochemists in determining the age of the earth by measuring $^{207}Pb/^{206}Pb$ in the planet's crust, among other achievements.[4]

One of his most notable accomplishments, however, was his contribution to U enrichment efforts during WWII. At the time,

scientists knew that one of the U isotopes underwent slow neutron fission, but they were not sure which one; nobody had yet been able to separate the two isotopes, ^{238}U and ^{235}U, to find out which was responsible.

Nier met Enrico Fermi, who was feverishly pursuing the question, at a conference in 1939. Nier agreed to attempt the separation by MS, but upon his return to Minnesota, the task was eclipsed by other duties. "Between lecturing 8 hours per week, perfecting the sector magnet mass spectrometer, [and] trying to separate ^{13}C by thermal diffusion . . . I was not looking for things to do, so the separation of ^{235}U was not high on my priority list," he wrote of this period in his life.[3] A not-so-subtle prod in a letter from Fermi eventually got things moving, and Nier was able to separate nanogram quantities of the U isotopes by MS. He mailed his samples to Columbia University's John Dunning, who was able to confirm that ^{235}U was the isotope responsible for the slow neutron fission, and the nuclear age was born.

MS GROWS ORGANICALLY

By the 1940s, mass spectrometers were commercially available, and MS was firmly established as a useful technique among physicists and industrial chemists. "When chemists in industry made use of MS, they used it quantitatively—so, to control production process, to find out how much of what is in a mixture," says Carsten Reinhardt, author of *Shifting and Rearranging: Physical Methods and the Transformation of Modern Chemistry*.[5] Industrial chemists knew the identities and structures of most of the molecules in their mixtures; they used MS only to measure concentration.

But nobody really understood what went on inside the instrument, and thus, its utility was limited to quantitative analysis—information that was not terribly useful to academic chemists. "I think the hard-core chemists saw it as kind of an unexplainable, voodoo, black magic kind of a tool," says Grayson. "So they held it at arm's length for quite some time." In addition, mass spectrometers were expensive, and many department heads balked at making such a huge investment in a technique that seemed esoteric.

But scientists were working on the problem. "The relationship of the mass spectrum to the molecular structure was a topic of research in industrial and government laboratories from the earliest Annual Conference on Mass Spectrometry and Allied Topics in 1954," says Grayson. "Significant work was reported, both in the literature and at these conferences."

Ultimately, the leadership of three chemists in the U.S., Fred McLafferty, Klaus Biemann, and Carl Djerassi, helped to change the prevalent negative attitude toward MS. Through methodical experiments, each scientist slowly teased out the fragmentation mechanisms of different classes of organic molecules, allowing chemists to determine the structures of unknown molecules by MS. These three scientists' body of work propelled MS into the consciousness of the chemistry community and laid the groundwork for modern biological MS research.[5]

McLafferty was ideally positioned to be one of the early proponents of MS in academia. Although his later career took him to both Purdue University and Cornell University, McLafferty began his work in industry, at the Dow Chemical Co. He describes his surprise introduction to MS at his initial job interview with Dow: "They told me when I arrived that I would spend a half day at the organics lab and the other half at the spectroscopy lab interviewing about mass spectrometry. I said, 'I've never seen a mass spectrometer. You don't need me'," he recalls. "Anyway, by the time they showed me the mass spectrometer, it looked like a pretty fun thing. That was 1950, and I've been in it ever since."

Of the three men, McLafferty focused the most on instrumentation and methodology. Reinhardt says that McLafferty was initially interested not in using MS to identify unknown compounds but rather in relating spectra to structure. "He established the rules and language of MS with compounds of known structure," Reinhardt says. The "McLafferty rearrangement" (a term coined by Djerassi) is an example of this mechanistic work.

Biemann, who spent his career at the Massachusetts Institute of Technology (MIT), had an equally fortuitous introduction to MS. As a graduate student in Austria, he received extensive training in organic synthesis, which he applied to natural products (specializing in alkaloids and peptides) as a postdoc at MIT. In 1956, at the behest of his funding benefactors, he attended a food-flavors conference in Chicago and heard a talk given by William H. Stahl. Stahl described using MS to identify fruit flavor components—mostly small organic compounds—by matching them to a database of known spectra.

At the time, Biemann had just been offered a position in the MIT chemistry department as an organic analytical chemist. "I had to think of what research to do, which couldn't be organic synthesis, obviously," he says. "It had to have some analytical flavor to it."

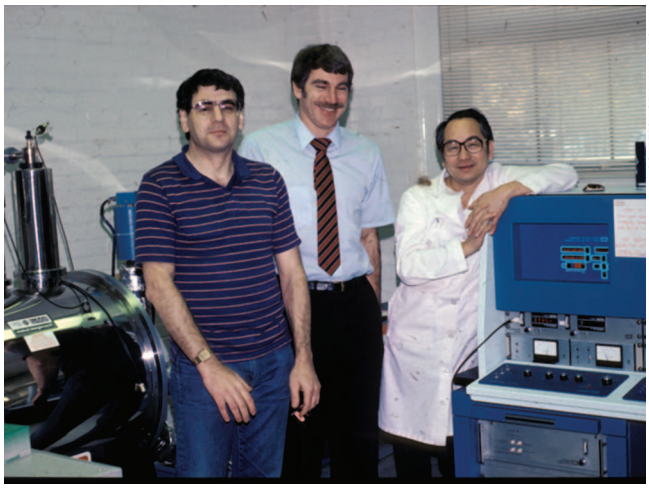
Stahl's talk inspired Biemann to research MS, and he concluded that the technique could be used on his molecules of interest for a completely different purpose—namely, to elucidate their structure. It was a novel and somewhat daring idea. "Nobody who knew anything about mass spectrometry would have dared to put those compounds into a mass spectrometer, because you were supposed to keep it clean," he recalls.

Biemann managed to persuade one company to let him try it out on just two compounds; it was enough to convince the MIT department head, Arthur Cope, that the idea was feasible. "He said, 'OK, I'll buy you a mass spectrometer, but you have to promise that it won't collect dust'," Biemann remembers.

With his new mass spectrometer, Biemann showed that the structures of complex molecules could be determined by MS. Biemann "was among the very first to apply [MS] to natural products of unknown structure," says Reinhardt. "He's clearly one of the pioneers in his field." Along the way, he also established rules for alkaloid and peptide fragmentation; he even devised an early method of sequencing peptides by MS, which ultimately laid the groundwork for modern proteomics.[6]

Djerassi, an equally prolific researcher who spent most of his academic career at Stanford University, came into the MS field somewhat later than McLafferty and Biemann. Djerassi had firmly established himself already as a natural-products chemist who focused on terpenoids and steroids when a conference talk about alkaloids given by Biemann in 1960 inspired Djerassi to apply MS to his work as well. "He recognized very early on . . . that you could find out about structures of steroids with mass spec," says Reinhardt.

Right away, Djerassi invited Biemann to Stanford to help set up an MS laboratory, and Biemann agreed. "I said yes, because



COURTESY OF ALAN MARSHALL

(Left to right) Comisarow, Marshall, and Tom Ricca (an electrical engineer who worked with Marshall at Ohio State University) in 1985 with the first commercial Nicolet FTICR MS.

I felt that if I develop a method, other people should use it too, and I should do anything to enable them to use it," Biemann explains.

Djerassi's work focused mainly on steroids, but, to a lesser extent, he also studied alkaloids. "Carl, during most of his life, was a very active researcher and very prolific and had a huge research group, so I created right there my most important competitor," Biemann says. Still, all three maintain enormous respect for one another and agree that the competition was healthy.

Though most of them have now retired from the field (McLafferty maintains a research "hobby" and has two postdocs in his laboratory at Cornell), their work certainly helped to broaden the field and to create the next generation of mass spectrometrists. "I remember once going to the International Mass Spectrometry conference in Berlin, and I think half the speakers were former postdocs or graduate students of mine," says Djerassi. Biemann has written about the MIT "Mass Spectrometry School", where many researchers in the field trained.[7]

McLafferty recounts a story from 1956 when he was contemplating a departure from MS research: "My friends said, 'What about mass spectrometry? Isn't it a pretty good field for you?' And I said, 'Well, I think it's developed about as far as it's going to go'." He turned out to be quite wrong, but in this case, he does not seem to mind.

THE ULTRAHIGH RESOLVING POWER REVOLUTION

Sometimes, two heads are better than one. For Alan Marshall and Melvin Comisarow, who were the first to apply FT to ion cyclotron resonance (ICR) MS, this was definitely the case.

Marshall and Comisarow first met at Stanford, where Marshall was a graduate student and Comisarow was a postdoc. Marshall obtained his first faculty appointment in 1969 at the University of British Columbia (UBC; Canada), and Comisarow followed 2 years later. At UBC, Marshall chose to work on NMR, whereas Comisarow focused entirely on ICR; it was this combination of expertise that led to the FTICR MS (sometimes abbreviated as FTMS) breakthrough.

ICR has been around since 1949, when J. A. Hipple first described the technique.[8] In an ICR instrument, charged particles rotate under the influence of a magnetic field. The ions are irradiated with an oscillating electric field, which drives the particles into a larger radius of rotation and into phase coherence (i.e., all ions of the same m/z are moving in sync). As the ions pass detector plates, their presence is recorded as an induced electric current. To obtain a full spectrum in ICR without FT, the irradiation frequency is kept constant while the magnetic field is swept through a range of values; this leads to long acquisition times.

Marshall and Comisarow's major breakthrough came when they realized that FT could be applied to ICR. FT is a mathematical manipulation that can deconvolute complex wave functions. A new method of performing FT that made the calculation 1000× faster had just been reported in 1965 and was being applied to several other analytical techniques, including NMR and IR, but MS had yet to follow suit.

Marshall recalls discussing the problem with Comisarow in the early 1970s: "I went to him and said, 'How come people in ICR aren't doing Fourier transform?' because I had learned about that from NMR," he says. FT was proving invaluable for increasing the speed of acquisition and sensitivity of NMR spectroscopy; Marshall wanted to see if they could improve on ICR as well.

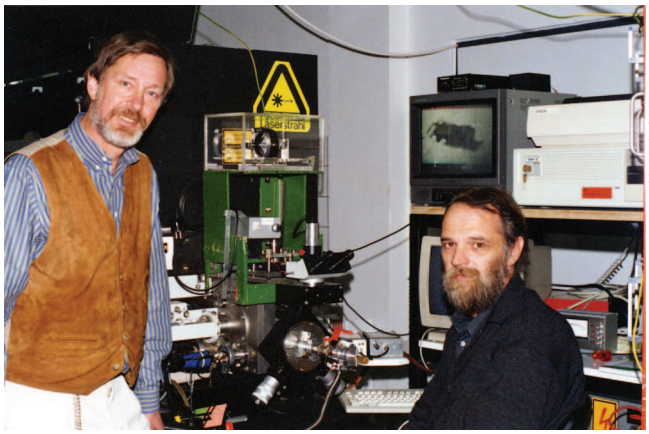
It worked. Now, instead of the magnetic field being varied to obtain the full spectrum, all the ions are measured at once. "Any time you can measure everything simultaneously and unravel it mathematically, you are going to get ultrahigh resolving power very fast without losing sensitivity," says David Muddiman of North Carolina State University. The advance was truly revolutionary. Because of its ultrahigh resolving power, FTICR MS is today one of the most valuable techniques for analyzing complex mixtures.

But Marshall and Comisarow did not stop with their initial experiments. Both have been tireless advocates of the technique for the past 35 years. "They not only said, 'Hey, this should work' and proved that it did work, but they pursued it and they believed in it," says Muddiman. "I think they are both people of perseverance. They're both true scholars; there's no doubt about that."

MAKING MOLECULAR ELEPHANTS FLY

By the 1980s, small organic molecules were routinely being analyzed by MS. However, proteins—especially large ones—and other macromolecules such as nucleic acids and complex carbohydrates provided more of a challenge. The problem was that, at the time, ionization relied on gas-phase collisions between the analyte and a charged particle; scientists had yet to figure out how to get large molecules into the gas phase without extensive fragmentation and decomposition. "Just about everything you can imagine was tried," says Dick Smith of Pacific Northwest National Laboratory. "It's been an area that's been worked heavily over the years."

A few techniques, including fast atom bombardment (FAB), plasma desorption, and thermospray ionization, were making narrow inroads into ionization of proteins, but none of them worked very well. They all required relatively high concentrations of small proteins—and they did not work at all for larger ones. Then, in 1988, ESI and MALDI appeared on the MS scene almost simultaneously. These ionization techniques revolutionized bio-



COURTESY OF FRANZ HILLENKAMP

Hillenkamp (left) and Karas with the LAMMA instrument used in early MALDI development experiments.

logical MS and are still the dominant forms of macromolecule ionization to this day.

John Fenn, who received the 2002 Nobel Prize in Chemistry for his development of ESI, gave a lot of credit to the early experiments conducted by Malcolm Dole of Northwestern University.[9] Dole showed that if he could dissolve nonvolatile solutes in volatile solvents and produce highly charged droplets of these solutions, evaporation of the solvent would leave intact gaseous ions of the solute.[10] "In principle, this would solve the problem of ionizing labile molecules, because there is no high energy involved, so they should survive," explains Matthias Mann of the Max Planck Institute of Biochemistry (Germany), who did his graduate studies with Fenn at Yale University. "[Fenn] thought that was a very nice idea . . . and he did try it [with proteins], but it didn't work."

It took another decade and a step back to simpler molecules to make ESI feasible. In the mid-1980s, Fenn and his postdoc Masamichi Yamashita tried again, this time focusing on small molecules. They started with vitamins, which are heat-labile and thus not amenable to most forms of MS ionization. "They dissolved a vitamin tablet and got a spectrum of it," says Mann. The m/z of each vitamin "was beautifully there in the mass spectrum, so this was very promising."

Fenn and his group moved on to amino acids, then high molecular weight polymers, and finally, for practical reasons, back to proteins. "We went to proteins because they had a nice, defined mass in contrast to the polymer," recalls Mann. When Fenn presented the work at the 1988 ASMS meeting, "everybody got really excited about it."

At about the same time, Franz Hillenkamp and Michael Karas, then of the University of Frankfurt, were developing a very different technique to address the same problem.[11] MALDI grew out of Hillenkamp's experience with the laser microprobe mass analyzer (LAMMA). Hillenkamp and his collaborators were attempting to map the spatial distribution of Ca^{2+} ions in heart muscle cells by using LAMMA, but background signals were making the spectra difficult to decipher. "This background appeared to have some sort of a general pattern, and I speculated one day when I was looking at it that it might be fragment ions from the organic matrix," Hillenkamp recalls. "So we changed the polymer, and indeed, we saw a change in the general pattern. So that triggered my idea that maybe one could even generate ions of organic molecules."

Who invented MALDI?

Koichi Tanaka of Shimadzu Corp. (Japan) shared the 2002 Nobel Prize in Chemistry with Fenn "for their development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules." Fenn's inclusion was for his work on ESI; Tanaka was recognized for his laser desorption method of protein ionization.

"Tanaka showed first that it could be done, that large proteins could be ionized, analyzed, and detected using any kind of laser desorption," says Catherine Fenselau of the University of Maryland College Park. "The success of Tanaka's engineering team also reminded the rest of us that ionizing proteins was not sufficient. It was also necessary to customize the rest of the instrument, especially the detector."

Although Tanaka's method uses another approach for soft desorption ionization, it is often erroneously referred to as MALDI. "More than once, I've been present in situations where the person introducing [Tanaka] says that he got the prize for developing MALDI," says Costello, who was president of ASMS when the award was announced. "He is faced then with starting his talk by saying he didn't."

The Tanaka method has some key differences. Although both methods use a laser for ionization, in MALDI, a chemical matrix absorbs the laser energy and transfers some of it to the analyte, whereas Tanaka's method uses a suspension of metal nanoparticles in glycerol for this purpose. In MALDI, the analyte is mixed into and surrounded by the matrix; in Tanaka's method, the analyte sits on the surface of the nanoparticles.

For many reasons, including its higher sensitivity compared with the Tanaka method, MALDI was the method that the MS community embraced. "The reality of it is, the techniques that [Karas and Hillenkamp] described and developed are the ones that everybody uses today," says Grayson. Mann agrees and says that it is important for the field to recognize the importance of the MALDI work, even if the Nobel committee did not.

Karas eventually came to work with Hillenkamp, and the researchers began carrying out a systematic study of the laser desorption of small organic molecules. "The key observation was that one day, for whatever reason, we looked at a mixture of two amino acids, and we saw that at the laser energy sufficient to generate ions of the tryptophan, there was also a signal for the alanine, which, when looked at alone, needed a much higher laser energy," Hillenkamp says. "So we saw that the aliphatic amino acid was riding piggyback on tryptophan." He says that looking at that data provided a flash of insight, and from there it just took lots of work to find a suitable matrix to make proteins fly. Karas and Hillenkamp ultimately published

their findings[12] and coined the name MALDI (sidebar, "Who invented MALDI?").

Catherine Costello of Boston University says that the development of ESI and MALDI really opened up the MS field to a whole new group of researchers. "It took MS out of the physical chemists' bailiwick and made it possible to put it into the laboratories where the biologists were," she says. "It really did make it much more user-friendly and much more possible to have people who wanted to use it not have to dedicate their careers to just working on the technique."

ESI and MALDI are still the methods of choice for ionizing proteins and peptides. "They are competing *and* complementary," says Hillenkamp. "Quite a number of analytical tasks you can solve with either of them." But each has its strengths and weaknesses. For example, it is easier to couple electrospray online to separation techniques such as HPLC. MALDI, on the other hand, is more tolerant of contaminants such as salts or detergents.

Development of the two technologies "just opened up all sorts of possibilities to the point that once in a while, you are running something and you catch your breath and think, 'Twenty years ago, I couldn't have imagined that we would ever be using these techniques to investigate problems like this one'," says Costello. "But now it's possible that each success whets your appetite to push them even farther. There really isn't a limit to where we can go." The future of MS looks to be as exciting as its past.

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