

Matthias Mann: Mass Spectrometry Meets the Proteome



When molecular biologists recently raised their sights from genes to proteins, from decoding entire genomes to decoding entire proteomes, the tools of their trade had to change as well. Sequencing and identifying the myriad proteins in a single tissue sample requires entirely new technologies, and so the past decade has seen mass spectrometry emerge as the technology that powers the proteomic revolution.

The application of mass spectrometry to the biology of proteins, however, required a chemist's insight, and it was Matthias Mann, a student of the Nobel laureate John Fenn, who made it happen. This work has propelled Mann to the top five in Thomson Scientific's current update of *Essential Science Indicators*SM listing of the hottest scientists in Molecular Biology & Genetics, and to second overall among the **highest-cited** scientists in Europe, based on papers published in the last decade. Within that time, his blockbuster 1996 *Analytical Chemistry* article applying mass spectrometry to the sequencing of proteins (see [table](#) below) has garnered more than 2,400 citations, while its companion piece in *Cell* demonstrating the applicability of the technology has been cited



"Nowadays, we can read out the proteome in a quantitative way, in a large-scale fashion," says Matthias Mann of the Max Planck Institute for Biochemistry, Martinsried, Germany.

over 2,000 times. Mann has published another two papers with more than 1,000 citations each, and eight more with over 500, a remarkable record of high-impact productivity in the last ten years (and leaving aside pre-1996 collaborations with Fenn, notably two reviews that have now garnered more than 2,500 and 1,000 citations respectively). Mann also came in at #2 in this publication's 1998 ranking of highly cited chemists based on high-impact papers published between 1994 and 1996 (*Science Watch*, 9[4]: 1-2, [July-August 1998](#)).

Now 47, Mann began his career in physics and mathematics, receiving his bachelor's degree in 1982 from Georg August Universität Göttingen, Germany, and his master's in physics from the same institution two years later. He then moved to Yale, where he worked with Fenn and received his Ph.D. in chemical engineering in 1988. Mann then spent four years at the University of Southern Denmark, first as a post-doc then a senior scientist. In 1992, he went back to Germany to become leader of the Protein & Peptide Group at the European Molecular Biology Laboratory in Heidelberg. In 1998, Mann returned to the University of Southern Denmark as a professor of bioinformatics and director of the Center for Experimental Bioinformatics. In 2005, he became a director of the Max Planck Institute for Biochemistry in Martinsried, Germany, near Munich.

Mann spoke to *Science Watch* from his Martinsried office.



You did your doctoral work with John Fenn, who won the 2002 Nobel Prize in chemistry for his development of the electrospray technology. How did that come about?

I got into this field because of John Fenn. I was a master's student at the Max Planck Institute, and Fenn spent time there while visiting Germany. I worked on a project with him and he asked me if I wanted to do a Ph. D. at Yale, instead of continuing on at the Institute. That was in 1984, and I went to Yale and worked on the electrospray with him. That's still my most highly cited paper (J.B. Fenn, *et al.*, *Science*, 246[4926]: 64-71, 1989; roughly 2,600 cites to date). In fact, I've effectively been following that same technology since. Now we use it more as a tool. For many years, we were involved in the development of the tool.



You were pursuing physics at the time, weren't you?

I was getting my master's in physics when Fenn came to Göttingen. Originally, I wanted to do theoretical physics, but I could see two problems: one was that the golden age of theoretical physics had already been over for 60 years; the other was that you have to have a real talent for it. I could see that I wasn't cut out to be a very successful theoretical physicist, even though that was my main interest as a student.



How did Fenn see the applications for the electrospray technology at the time? Were proteins even in the picture?

He had the idea that he could use the electrospray simply to spray and ionize molecules and then put them in the mass spectrometer. The idea was that it would break through what was then the limitation of mass spectroscopy: that you cannot analyze substances that go to pieces when you try to ionize them. He wasn't thinking of proteins, but of small molecules like vitamin B12. This would be a method to get this molecule as an ion directly out of solution. He then had the idea that if this worked, doctors could take a urine sample and analyze the molecules in it with a mass spectrometer and then tell people what's wrong with them. Again, he was thinking of small molecules. People now have a similar idea with proteomics, but they want to do it with proteins.



What was the limiting factor in working with entire proteins?

By the late 1980s, electrospray was established as a very good method to get peptides or proteins into a form in which you could analyze them. I had always been interested, not so much in measuring the mass of proteins, but in sequencing peptides. At the time, the technology used to do that was known as Edman degradation, after Pehr Edman. This was a chemical technique; you cleave off one amino acid at a time from a protein or peptide and then determine what amino acid it was. That was the standard technique used to

identify new proteins until the 1990s.

SW It sounds excessively time consuming.

It is, and it's not very sensitive. You can't start doing fancy things with oligonucleotides, with RNA and DNA and proteins, if you're stuck in the dark ages. My idea was to use electrospray and peptide sequencing to give biochemistry and protein science a lift. That's been one of my ambitions throughout my research career. In order to do that, however, a number of things had to happen. If you had an isolated protein or peptide, you could analyze it by electrospray mass spectrometry. But you couldn't do that if you had the protein in a gel. And that's the form in which biologists always had their proteins. That was completely impossible for mass spectrometry. Our 1996 paper that's so highly cited is the one where we show you can indeed get the proteins out of the gel and analyze them with very high sensitivity by mass spectrometry.

SW How do you get them out of the gel?

The proteins are very difficult to get out. However, if we bring in an enzyme, trypsin, then the trypsin can migrate into the gel and cleave the protein into pieces, which can

Highly Cited Papers by Matthias Mann and Colleagues, Published Since 1996

(Ranked by total citations)

Rank	Paper	Citations
1	A. Shevchenko, <i>et al.</i> , " Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels ," <i>Analyt. Chem.</i> , 68(5): 850-8, 1996.	2,404
2	M. Muzio, <i>et al.</i> , " FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex ," <i>Cell</i> , 85(6): 817-27, 1996.	2,061
3	Y. Ho, <i>et al.</i> , " Systematic identification of protein complexes in <i>Saccharomyces cerevisiae</i> by mass spectrometry ," <i>Nature</i> , 415 (6868): 180-3, 2002.	1,176

come out. Although, actually, three things had to come together to make this technology viable: One was we had to figure out how to get peptides out of the gel. That's the paper 1996 paper, Shevchenko *et al.* Next, the electrospray had to be made more sensitive, and we developed a technique called nano-electrospray. This was a miniaturization; it would spray with an extremely low flow rate. That made the electrospray more sensitive by something like a factor of 100. That was the second thing. Then the third thing was our realization that if you just get a very short part of the sequence, what's called a peptide sequence tag, you can then put it in a computer algorithm and find the same peptide in a sequence database. Those three things together allowed us to really show that mass spectrometry could do the things that this Edman degradation had been doing before. Over the next few years, Edman degradation disappeared. And ever since, all protein analysis has been done by mass spec.

4	F. Mercurio, <i>et al.</i> , "IKK-1 and IKK-2: Cytokine-activated I [kappa]B kinases for NF-[kappa] B activation," <i>Science</i> , 278 (5339): 860-6, 1997.	1,095
5	M. Wilm, <i>et al.</i> , "Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry," <i>Nature</i> , 379 (6564): 466-9, 1996.	853

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SW When you started thinking about proteomics, how did your view as a physicist-turned-chemist differ from the mainstream thinking in the field?

Proteomic people started with the view of mapping all proteins on these 2D gels and then hoping to find something clinically or biologically useful. I never liked that technique. It's been around since the 1970s, and I thought the methodology was limited and of little potential. I didn't think you would get any great insights just by running normal tissue and cancerous tissue on these gels and trying to see what was different between them. So I started with the idea of using the

electrospray and these technologies I described for the very high-sensitivity identification of single proteins. For example, when we put all this together, we sequenced FLICE, a key protein in the apoptotic pathway. Our technique turned out to be very useful from day one. We purified this protein, sequenced it, and then cloned it and studied it. After we showed we could do that, we moved on to protein complexes—proteins that communicate in cells—and we characterized this machinery. So, this technology has been very useful all along. And only now are we coming to the point that we can do the experiment that the proteomics people envisioned ten years ago: to read out proteins all at once out of a cell. It's never been our goal and it's not what we did—we looked at a small number of proteins that had some functional context—but now we're at the point where we can do it.

SW **Can you give us an example of how you go from the electrospray and mass spectrometry to biologically or clinically useful information?**

Let's take a protein called caspase, which is involved in apoptosis. When we started this research we knew that apoptosis can be set in motion by a specific receptor on the cell surface. The receptor has various names. One of them is APO-1 receptor, and our collaborators had an antibody against it. They were able to pull out this receptor and use it to get the next proteins in the signaling cascade. The idea was that those proteins would associate with the receptor. If you had the antibody, you could pull out not just the receptor, but the other players in the signaling pathway. Our collaborators did this, and they fished out both the receptor and some of the other proteins. We then used our nanoelectrospray and peptide sequence tag technique to sequence those proteins. At that time, there wasn't a human genome sequence to go to for comparison. But the EST (for "expressed sequence tag") project had started—the first claim to fame of [Craig Venter](#) [see also]. We took our fragmentary data, the sequences of peptides from these proteins, and we couldn't find them in any database, which meant they were novel proteins. That was even more interesting. Then we found a hit in the EST database, a protein that

had a certain domain structure, suggesting it might be involved in [apoptosis](#). Our collaboration quickly cloned it and showed that, functionally, it was the next link in this apoptosis pathway. That was very nice, because we had just come out with all this technology, and we could use it on a biology project and show that it had important biological applications. People couldn't say, "Hey, that's a nice technology, but it could never be used in practice," because we had already used it on this very exciting apoptosis protein.



Where do you see mass spectrometry taking the field of proteomics in the next five years?

The future is now limitless. First of all, the field has become quantitative. Previously, one used mass spectrometry, as we did on the apoptosis protein, to find and sequence a single protein. That was good, but it wasn't quantitative. Now you can begin to do the kinds of things with proteins that people have only been doing so far with mRNA and microarrays. The advantage of doing them at a protein level is that proteins are the functional agents. When you look at mRNA on chips, you have a question as to whether the change you're seeing is at the mRNA level or down at a deeper level of regulation. Maybe it's at the protein level. Nowadays, we can read out the proteome in a quantitative way, in a large-scale fashion. Another area of work that's more specific to mass spectrometry is to look at modifications. We not only want to know what proteins are present in a sample, but also how they're modified. Are they in an active status? Are they phosphorylated, for example? This can now be done in a large-scale way by mass spectrometry. We can now look at the proteome quantitatively and examine how signaling pathways change by looking at how the proteins are modified, whether they're phosphorylated. By doing so, we have a very good handle on how cells process information. This will be a big theme in the field of systems biology. We can get the data and then people can build a mathematical formulation on top of that. One big problem with systems biology is that there isn't enough solid data to base the formalisms on. Now we can get that data.



Are there other applications we should keep our eyes out for?

There's another new field called "interaction proteomics." Here you use mass spec and proteomics to see which proteins talk to which other proteins. There are a lot of papers these days about large networks in the cell. These can be studied using mass spectrometry. And then there's the area of proteomics that researchers always had in mind from the beginning: they want to find biomarkers for disease. And that's also finally coming within reach. We hope that with further development we can look at the proteins in a urine sample, for instance, and then use them to classify patients. What disease course are they following? What drug will they respond to best? That's a little bit in the future, but it should be possible.



Here's a question I like to ask everyone these days: If you had unlimited funds and an ideal research environment, what one experiment would you pursue?

My team is now actually limited by the number of mass spectrometers we have for sequencing. At the moment, we have six of them. In an ideal world, I would have at least twice that number. I would start a number of large-scale projects, sequencing a lot of different proteomes, and I would use that data as input for bioinformatics. I would look at all the modifications of all the proteins. Nobody knows what they are on a proteomic scale, and nobody knows how they're regulated. I mentioned the example of phosphorylation, but there must be a lot more that we don't know. If I had to focus on just a few questions, I would look at how proteins are expressed and how they're regulated by this post-expression modification. Other proteins put methyl groups on them, phosphor groups, and that somehow regulates their activity. I think this will be a major theme in biology. It already is, but it could be bigger. And the mass spectrometers could read out all those changes and help us unravel this problem of regulation.■

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