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The Rise of Mass Spectrometry and the Fall of Edman Degradation

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Featured Article: Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal Chem 1996;68:850–8.[2]

Many of today's key proteins in biology and biomedicine were originally identified by protein chemical methods. Frederick Sanger obtained his first Nobel Prize for determining the sequence of insulin in the 1950s, and the Edman degradation, in which one amino acid after another is cleaved off the end of a protein or peptide and identified by HPLC, reigned supreme into the 1990s.

My background is in mass spectrometry (MS),[3] in particular electrospray ionization *(1)*, for which my advisor John Fenn won a Nobel Prize. On coming to the European Molecular Biology Laboratory (EMBL) in Heidelberg as a young group leader, I was determined to challenge what I saw as the old-fashioned chemical methods with high-tech and cool MS technology. Little did I know what I was up against, because this would require not only highly sensitive peptide sequencing by MS but also doing something with the short amino acid sequences we were able to obtain. We solved these problems by developing a highly sensitive nanoelectrospray method *(2)* and by using a peptide sequence tag algorithm *(3)*, with which we could locate the proteins in the newly available DNA sequence databases. This left the pesky issue of actually getting the proteins out of the polyacrylamide gels in which biologists were always isolating them. It was accepted that once proteins were in a gel, nothing could resurrect them for MS analysis, much less high-sensitivity MS analysis. This was thought to be doubly true when the proteins were not visualized by Coomassie blue but by 10- to 100-fold more sensitive silver staining, which presumably chemically oxidized or otherwise destroyed the proteins.

Andrej Shevchenko, one of my first postdocs, was a superbly trained and talented analytical chemist who came from St. Petersburg. He cracked the remaining problem by developing a robust "in-gel digestion protocol" that extremely efficiently extracted the trypsinized peptides from the polyacrylamide matrix—and in a form clean enough not to poison the electrospray or the mass spectrometer. In my first "big splash" paper as senior author, we showed that the combined procedure not only was a match for the classic methods but also could identify proteins at far superior sensitivities and in the presence of a vast excess of background proteins *(4)*. The article featured here adapted that protocol for silver staining and established that proteins were not drastically modified in that procedure. Apart from giving a detailed protocol, we established that MS identification could be "certain" rather than merely statistically probable. This assertion was helped by finding—to our great surprise—virus proteins and even mutated virus proteins in the gel bands of DNA-binding yeast proteins that we had used as an example. Only later did we learn that this was quite a common occurrence in yeast culture. We also sequenced open reading frames that had not yet been assigned as proteins. During the next year, we identified caspase 8, IκB kinase 1 and 2, the catalytic subunit of telomerase, the exosome subunits, U1 spliceosome components, and other proteins that are now famous.

With these results in hand, I approached the late Lennart Philipson, who was the director of the EMBL at the time, and asked him to communicate the paper to *Proceedings of the National Academy of Sciences*. Although Lennart was a great mentor to young scientists and thought highly of our work, he said that submitting this work to *Proceedings of the National Academy of Sciences* was a really crazy idea and that a general audience would never be interested in such an analytical paper! So *Analytical Chemistry* it was. Ten years later, we updated the protocol and brought it into the "proteomic age" by prefractionating proteins by 1-dimensional gels *(5)*. Of the 3 papers, the *Analytical Chemistry* article is now much more highly cited than the *Nature* paper, but they each have >1000 citations.

Nowadays, my own laboratory does little gel-based work anymore, since we typically work with unfractionated whole proteome samples that we analyze in rapid single shots *(6)*. However, biologists continue to use gels almost exclusively, and Shevchenko's in-gel method is

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[2] This article has been cited more than 6000 times since publication.
[3] Nonstandard abbreviations: MS, mass spectrometry; EMBL, European Molecular Biology Laboratory.

used every day by a vast number of researchers. It clearly has been a major contributor to establishing MS as a viable method in protein chemistry and proteomics.

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References

1. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science 1989;246:64–71.
2. Wilm M, Mann M. Analytical properties of the nanoelectrospray ion source. Anal Chem 1996;68:1–8.
3. Mann M, Wilm M. Error-tolerant identification of peptides in sequence databases by peptide sequence tags. Anal Chem 1994;66:4390–9.
4. Wilm M, Shevchenko A, Houthaeve T, Breit S, Schweigerer L, Fotsis T, Mann M. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. Nature 1996;379:466–9.
5. Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protocols 2006;1:2856–60.
6. Mann M, Kulak NA, Nagaraj N, Cox J. The coming age of complete, accurate, and ubiquitous proteomes. Mol Cell 2013;49:583–90.