

the Analytical Scientist

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D I G G I N G D E E P E R , B U I L D I N G B E T T E R



AN INTERVIEW WITH CATHERINE FENSELAU

Catherine Fenselau's impressive analytical science career has taken her from ancient ruins in Colorado to the analysis of lunar rock samples, from the early introduction of MS technology to the biomedical lab, to the heady days of fledgling mass spec journals. Here, Catherine reflects on the past, present and future of mass spec and explains why analytical science deserves more respect.





When I was a child, I wanted to be a “lady archaeologist”. We went to Mesa Verde National Park many times on family vacations, and I thought the archaeology there was just wonderful. One of the big mysteries of the Mesa Verde ruins is where the people went; they had a tough couple of decades with drought, inter-tribal warfare and even cannibalism... Then, after 1,300 years or so, they all left. Where did they go? I thought it would be great to find out – form a hypothesis and then dig out the proof. And I suppose that’s what really got me thinking about being a scientist.

FROM TITRATION TO SPACE STATION

As I got older I took the biology–chemistry–physics sequence that most American high schools provided around that time, but wasn’t sure which would be the best way to spend the rest of my life. When I got to Bryn Mawr College in Pennsylvania, I majored in chemistry – the curriculum captured my attention, but it was the engaging chemistry faculty who really hooked me in. Why not biology or physics? Perhaps I “titrated” myself into the right level of quantitation; at that time at least, chemistry was much more quantitative than biology, but physics was more quantitative than chemistry...

I was fortunate to work with Carl Djerassi at Stanford University for my PhD. And it just so happened to be one of the first labs to apply mass spectrometry to structure elucidation. You may know Djerassi’s name – he’s on the patent for the birth control pill – so you could say he changed western civilization. He was a natural products chemist, but also someone who really believed in the power of technology.

He had been impressed with Klaus Biemann’s success in applying mass spectrometry to undeciphered alkaloid structures. As someone interested in steroids, Djerassi wanted to use this fast, highly sensitive method to help elucidate steroid structures, so he got a mass spectrometer, hired two postdocs from European labs (where there were physical chemists using the technology) and, over the next two decades, took on 10–15 graduate students to develop the technology for his interests. I recognize a good opportunity when I see one.

In 1967, I moved to the NASA Space Sciences Laboratory for a postdoc with Melvin Calvin, where we practiced mass spectrometry techniques on rocks, in preparation for the analysis of lunar soil samples. NASA had a whole international consortium of labs to prepare for that project – and it seemed glamorous despite the analytical chemistry being pretty simple. Calvin and I published a paper in *Nature* (1), reporting on the behavior of olefins, and how they squeeze into the holes in model rocks. We

were working with high-end equipment, which was exciting – it was a very successful experience for me. However, I only stayed two years before moving on to my own position. My father always told me I should have waited till the moon rocks came back...

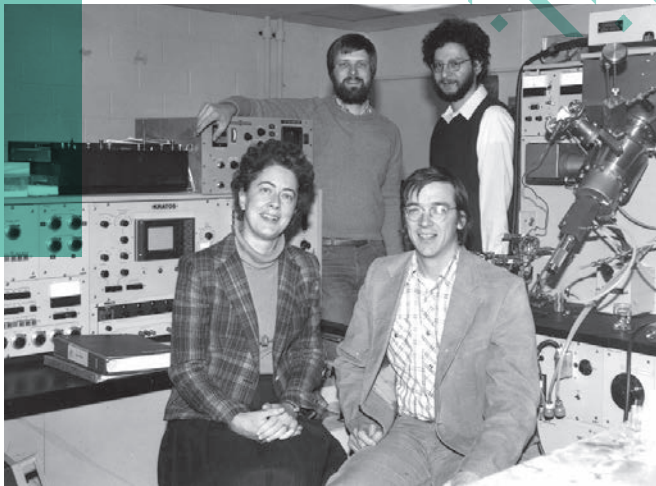
My husband and I both received job offers from Johns Hopkins School of Medicine, so I packed up my cats (and my husband), got in the car and drove across the country to Baltimore, where I have been ever since.

FORGING AHEAD

At Johns Hopkins, I was the first trained mass spectroscopist to join a US medical faculty. The problem? When I started, there was no mass spectrometer! I had to travel to the National Institutes of Health (NIH) labs to use their instruments until we were granted the funding to obtain our own. Though mass specs were a novelty for clinical applications, they had found real utility in monitoring the production of aviation fuel. Herbert Hoover’s grandson founded a company in southern California to manufacture instruments during the Second World War, so that all oil companies could use the same criteria. We bought an instrument made for this purpose, modified it and applied it to biomedical problems.

For me, one of the most delicious parts about being a scientist is the weekend when you know something that nobody else in the world knows; you made a finding in the lab on Thursday or Friday, but you have yet to communicate it. I was lucky enough to have a number of those weekends in this period of

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Clockwise from top left: Original team of the Middle-Atlantic Mass Spectrometry Lab in 1985 (Catherine Fenselau, husband Robert Cotter, James Yerger and David Heller). With sons Andy and Tom on Maui. Lecturing in Kuala Lumpur.



my career. One was our discovery that a common class of drug metabolites called carboxyl-linked glucuronides could alkylate proteins. When we eventually published our findings (2,3), pharma companies took notice, because it's a clear mechanism for toxicology. And so this new knowledge meant they could reformulate drugs to make them safer.

Some of the samples we analyzed back then were quite amenable and we got good answers. Others were not; for example, anything that had a phosphate group on it wasn't volatile enough. With our particular instrument, the sample had to be converted to the gas phase, but often it just burnt rather than vaporizing. I spent a lot of time at Johns Hopkins talking to other scientists to try and find a new way to bring these involatile molecules into the gas phase. A lot of folks recognized the limitation. And several of those scientists finally provided us with (Nobel-prize winning) solutions...

EXCITING TIMES

They were heady days. Mass spectrometry was so new that those of us who moved into medical schools at that time found that every analytical endeavor was novel, publishable and of considerable interest. At the University of Maryland, where I moved in 1987, we had a four-sector JEOL mass spectrometer (everyone who had one of these was totally thrilled with the engineering and capability, but the technology quickly became obsolete after the introduction of electrospray ionization empowered smaller instruments).

We used it, among other things, to measure the basicity of amino acids. Much of the fundamental work on peptide sequencing was dependent on understanding which parts of the



Clockwise from top left: With Melvin Calvin at UC Berkeley in 1966, examining a CEC 21-110 double focusing mass spectrometry system. Hiking in Colorado. Being presented with the 2012 ASMS Award for Distinguished Contribution in Mass Spectrometry by Scott McLuckey.



peptide were more basic – where the protons would be localized. The basicities of most amino acids had been measured; however, there was no value for arginine – nobody had found a standard compound to compare it with and thus to make an estimate of its basicity. I was at a conference in Europe, and saw a poster showing studies on some very basic compounds, one of which was strong enough to be used as a standard to quantitate arginine's basicity.

We went home, ordered it from a chemical supplier, popped out the measurement and shared it immediately with the community (4). Folks who needed that measurement were very pleased and referenced us, while some physical chemists complained – probably rightly – about the method we'd used; it was an estimate after all – but it was a pretty good estimate! It allowed analytical folks to develop their theories and their own methods. And it's one of my proudest and most pleasurable research memories.

SHAPING THE FIELD

In 1973, Carl Djerassi recommended me to be the co-editor of a new mass spectrometry journal called Biomedical Mass Spectrometry (now called Journal of Mass Spectrometry). Several journals started up around that time – mainly because JACS got tired of publishing the prolific output of the new mass spectrometry community! I worked on that journal for sixteen years. Then I became Associate Editor for the ACS journal, Analytical Chemistry. Importantly, that journal had decided that mass spectrometry was important and was expanding its coverage. I left ACS in 2015 – another 26 years was long enough!

It was an exciting time. I'd go to talks and conferences, find good papers, encourage their authors to write them up and publish them in peer-reviewed journals. I also spent a lot of time looking for reviewers, making sure the papers got corrected, resubmitted and published. There's a famous quote from Djerassi: "The research isn't finished until the paper's been peer reviewed and published." I have also always made an effort to encourage, include and honor women scientists in the field.

Editing journals gave me an invaluable opportunity to nourish the growth of the field, both with a new journal and then with a prestigious journal that was newly interested in mass spectrometry. It was a time when that area could be nurtured and shaped, and I was one of the lucky folks who got to do it. That was an exciting position to be in.

There's now a journal for everything and maybe too much gets published; for example, this last decade, the proliferation of electronic journals has made it possible to publish any observation – and they're not all interesting. But we work in a capitalist economy – the journals that don't get many papers submitted or that only

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publish boring papers are probably not going to make much money. The scientists themselves will select the winners and losers.

There has been a great deal of controversy around reproducibility or authenticity of work within peer-reviewed papers, and though I wouldn't say there's never been any false data in mass spectrometry, I'm not aware of it personally. Frankly, I don't think our rewards are high enough. There's probably more false reporting in fields where there's a very high payoff – where the secret you kept all weekend is going to get you a Nobel prize, not just delight the pharmaceutical industry. Analytical chemistry is a solid discipline that happens to be very useful across the whole spectrum of science – but it's not like discovering CRISPR. For me, it's satisfying when some other lab reproduces your work or takes the next step, because it demonstrates that your work was correctly reported.

SEPARATING PROTEINS, INTEGRATING COMMUNITIES

Currently, the research area I'm most excited about is the search for a mass spectrometry-based method for structural elucidation of polyubiquitins and ubiquitinated proteins. Ubiquitin is an 8,500-dalton protein that polymerizes and also attaches to other proteins in the cell, often determining the fate of both the proteins and the cell. In essence, it sends a message about where a protein should be moved and what it should do. The messaging seems to be dependent on the structures of mono- and polyubiquitin sidechains. So far, biochemists haven't had sensitive physical methods to characterize these branched proteins and read the ubiquitin code. So we've been working to develop a mass spectrometry approach. It

"EACH OF US CAN ONLY DO SO MUCH WITH ONE CAREER SPAN, BUT BY TRAINING OTHERS, YOU CAN MULTIPLY THAT IMPACT."

involves ionizing the intact branched protein, and then fragmenting it. The fun part is interpreting the spectrum! We've adopted some computer programs to help us locate how many branches there are and where they're attached to each other to unravel the structural puzzle (5). And I'm pleased to say that our approach is getting a lot of attention from the community.

Technologically, protein separation is still a major challenge in (biological) mass spectrometry. In the next decade or less, we need to see better separation techniques for proteins. That's not necessarily going to come out of the mass spec community, but whether it's a HPLC method, or a capillary electrophoresis method, we need something that will fractionate with higher resolution than what we have now.

In the mass spec field, I would say the most recent rapid advance was the Orbitrap – I don't think anyone saw that coming. I'm hoping we'll continue to see new ionization techniques and instrumental developments. But I don't ever want mass spectrometry to be a black box. Though there are some valuable applications where the instrument functions that way, ultimately if we're going to continue to evolve, we need to know what's inside and how it works. We need more sensitivity and we need more mass range. I can carry out a top-down analysis of a polyubiquitin that weighs 50,000 – but not 100,000... yet. I'm optimistic that as biology itself evolves, we'll be able to work on increasingly important problems, and thus make more and more impact – most likely in partnerships and collaborations.

Science always advances when we communicate with each other and I've always practiced collaborative science. Bioinformatics or computational science is helping to drive us forward and be more productive, so we now need programmers and bioinformaticists in our field. It's fair to say the field is data rich – and computing continues to have a massive impact. One person cannot know everything. I don't know in depth about the cell biology of a tumor-

bearing mouse, and I'm actually not very good at programming, so I work with collaborators. I also notice that the federal funding agencies appear to be thinking along similar lines – it seems easier to get money from the NIH if you have a team applying, rather than a proposal from a single laboratory.

WHERE SOCIOLOGY MEETS SCIENCE

I've had some exciting experiences in my career, but looking back, I think training 160 students and postdocs has been the biggest highlight. Each of us can only do so much with one career span, but by training others, you can multiply that impact. Several of my former PhD students have helped open new application areas for mass spectrometry. For example, Igor Kaltashov is one of the leaders in biophysical mass spectrometry, and Richard van Breeman has been a leader in the use of LC-MS to study metabolism of food additives and nutritional supplements.

An engaging professor needs to enjoy their subject, and they need to be interested. A sense of humor helps too. Djerassi was a terrific mentor, and I like to think that one of the major lessons I learnt from being in his lab was how to manage and run a research group. He engaged us intellectually in conversation and in how he wrote his papers. My own teaching style is probably very old fashioned; I still write on the chalkboard, I still stand up and lecture.

I think when students join the lab, there's an unspoken contract – they're supposed to work hard on my project, and I'm supposed to work hard to get them a job at the end. I think as educators it's our responsibility to teach the students to do lab work, but also to communicate their work. We need to teach them a little bit about sociology: if they go to a conference, everybody they meet is potentially a peer reviewer. Every introduction is an opportunity. I try to teach teamwork as well; some of the most difficult aspects of my research involve collaborations, as I mentioned. The grad students and postdocs involved in that work have to learn to communicate across scientific boundaries, which isn't always easy. You have to be patient. There are things that seem obvious to us, but not to our cell biology colleagues. And we also have a lot to learn from them.

I'm currently teaching a graduate course in biological mass spectrometry; there's widespread interest in the technology (I have students from three different colleges on campus, and five or six departments). A high percentage of the new students my department recruits actually want to be trained as bioanalytical chemists, because they see that we tackle important problems. They also appreciate that there's a lot of flexibility within a career in that area – you could end up being a government



Clockwise from top: With Bob Cotter at a rally in Washington DC. With grandchildren Nick and Rebecca in 2000. Sunny Wang receives her PhD.



regulator, go on to law school or pursue bioanalytical research...

The challenge for analytical chemistry as a whole is to be accepted as part of the frontline of chemical research. Often it is considered a supporting science, which suggests it's additional rather than essential. There's no official analytical chemistry position at Stanford, but there are two or three faculty members in chemistry and chemical engineering who are leaders in analytical chemistry. I trained in organic chemistry, came into mass spectrometry and essentially ended up in biochemistry. And I got my first job because somebody wanted to bring mass spectrometry into a medical school. In any case, I think my career shows just how helpful analytical chemistry can be – as well as how much fun an analytical chemist can have!

I often think about the fact that I didn't go into archaeology, but I don't regret it – because I plan to go into the field when I retire. Now I understand a little more how science works, I plan to check with archaeologists to see what advances they

have made in understanding the migration of the lost tribes of Mesa Verde. I want to build on what's already known.

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