Gary Siuzdak on Shooting Viruses through the Mass Spectrometer

July 04, 2003

At A Glance:

Name: Gary Siuzdak

Age: 41

Position: Director, Center for Mass Spectrometry, The Scripps Research Institute, since 1990

Associate professor (1996-2000 assistant professor), Department of Molecular Biology, The Scripps Research Institute, since 2000

Prior Experience: PhD in Physical Chemistry, Dartmouth College, 1990

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How did you become interested in mass spectrometry?

I obtained my PhD at Dartmouth College, in physical chemistry, [and] I got involved in mass spec by building a time-of-flight mass spectrometer for work on small molecules in my third year of graduate school. It seemed like such a great tool for such a wide variety of things. After I finished graduate school in 1990, I came right here to Scripps Research Institute as director of mass spectrometry, and I have been here ever since.

What is the focus of your research?

One [area we work in] is metabolomics; we have a strong effort in small-molecule analysis from biofluids. In conjunction with that, we are also developing new technologies for [studying] small molecules and for protein identification. One of those technologies is desorption/ionization on silicon, DIOS, which we originally published back in 1999 in Nature.

What is DIOS?

DIOS is essentially the same [as MALDI], except it has no matrix. You directly desorb and ionize molecules right off a porous silicon surface. Since there is no matrix, there also isn't any matrix background. There are a couple of special things about the porous silicon surface. One is, it's very strongly UV-absorbing. It also is a thermal insulator, so when the laser hits the surface, facilitating desorption, the energy doesn't get dispersed throughout, it stays localized to where the laser is striking, and there is a very rapid heating on the surface. Also, it's very porous, so the surface area is quite high: For every cubic centimeter of porous silicon, there is on the order of 200 square meters of surface area. [Now] we can [also] chemically modify the surface; it took us about three years to figure out how to do this effectively. The different types of functional groups we put on the surface actually change the way the desorption/ionization process behaves for certain types of molecules. We are in the process of making chips that are specific for certain types of molecules, such as carbohydrates or peptide digests, or oligonucleotides.

What is DIOS most useful for?

[For] high-sensitivity measurements of protein digests, but it's also used for looking for small molecules. Recently we have been able to go down as low as 100 attomoles on a protein digest. [Also] we have recently submitted a paper on using the surfaces for quantitative analysis.

You also study viruses by mass spectrometry?

We started doing work on viruses in the early 90s, and one of our first experiments was

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to send intact viruses through a mass spectrometer. It was an electrospray ionization experiment on a triple quad instrument, but we set up the quadrupoles in RF-only mode, which allowed high mass ions to pass with some crude mass selection. We electrosprayed the viruses into the mass spectrometer, with a countergas to reject neutrals, and collected them on a plate inside the instrument. Electron micrographs showed they stayed intact. In fact, we tested their infectivity after they passed through the instrument, and they remained infectious. That was a fun set of experiments.

What we have been able to do subsequently, largely through interactions with Henry Benner at Lawrence Livermore National Laboratory, is to analyze the intact viruses and get mass measurements on them. We recently did electrospray ion mobility experiments on intact viruses and were able to measure the radius of [each of] the viruses in the gas phase.

In a separate set of experiments, we exposed the virus to proteases, and we looked at the initial cleavage sites. What we saw is completely different from what we should have observed based on the X-ray crystal structure: The [first sites] that we saw getting cleaved were internal to the virus. It turns out that the virus capsid is really dynamic. It's so dynamic that it's fairly porous. [In subsequent studies, we were actually able to] pass small molecules through the viral capsid, which can react with the genetic material to inactivate the virus.

Recently, we have been trying to capture novel virus receptors from cells using affinity capture. We have been able to identify a novel receptor for adenovirus using very basic LC-MS/MS approaches. We are just getting ready to submit this [for publication].

You are also involved in a plasma biomarker project?

Mass Consortium, a company that I started back in 1994, has been doing this project with another company. We were looking at a set of individuals that had a disease and another set of individuals that were very sick but didn't quite have the disease yet. The idea here was to identify any sort of biomarkers to see if we could predict which individuals were going to get the disease. We were actually applying a multi-dimensional LC-MS/MS approach developed by Zhouxin Shen at Mass Consortium. When we tried this initially, we were completely unsuccessful with it. That was because we were getting totally swamped with all of the proteins that are prevalent in the plasma samples, like albumin, transferrin, IgGs. That's when Agilent contacted us and told us about this immunodepletion technology that they had [licensed from Large Scale Biology], and they let us use it, and it actually worked extremely well. It got rid of a lot of the proteins that were causing problems for us in terms of masking out all the other, less prevalent proteins. But it still wasn't very useful for us, because even though we could see a lot of the proteins that we wanted to see, we didn't have a very good, reliable way of quantitating them, or even getting semi-quantitative information on them. At the same time, Agilent told us about this Spectrum Mill software that they were licensing from Millennium. The program is really nice because the algorithms are very fast for identifying the proteins, easily on the order of four to ten times faster than anything I have seen previously. On top of that, it also gives you an idea of how much of one protein is in one sample vs. another [using] a color coding scheme. That, in addition to the immunodepletion technology, allowed us to identify three to four different proteins that we believe could act as biomarkers for the onset of the disease.

How did Mass Consortium come about?

Originally a lot of companies became interested in what we are doing here at Scripps, and I just thought it would be a good idea to start something separate. Mass Consortium started off as a service company back in 1994. Over the years, it [became] more involved in developing mass spec technology — some DIOS technology is being developed there. Waters has licensed the technology [and] we are hoping they will be coming out with it this year sometime.

But [Mass Consortium] is also doing work in proteomics and in metabolomics. They are collaborating with other companies to identify novel biomarkers. Also, they offer hands-on proteomics short courses.

You wrote a book called *Mass Spectrometry for Biotechnology*, and you are about to publish a new one. What has changed over the years?

The first book I finished in 1995, and in the last eight years, the changes in the field have just been incredible. People are now using mass spectrometry to look at the structure of proteins, [and] they are using it now much more routinely than they were

back then to do protein ID work. The reliability of doing protein ID work, both with MALDI and LC-MS/MS approaches, was for the most part unheard of at that time.

What are the technical challenges that proteomics researchers are still facing?

One is coverage, because if you don't have complete coverage on a protein, you can't say whether you have identified all of the sites [that have] been posttranslationally modified. Eric Peters at the Genomics Institute of the Novartis Research Foundation has been able to do LC-MALDI experiments. These experiments, by their design, allow you to get better coverage. With electrospray, when you spray the sample out, you often get significant signal suppression of a certain percentage of the peptides. MALDI is valuable because it can look at a simultaneous mixture much more effectively than electrospray can. For that reason, an LC-MALDI type approach could become quite effective at identifying posttranslational modifications. For the same reason, that's why we are developing LC-DIOS.

The second issue is quantitation. Obviously Ruedi [Aebersold] and Steven Gygi have made great strides in that area, but your quantitation can never be too good. What's interesting is that mass spectrometry has already proven that it can be a very quantitative tool in the pharmacokinetics area, and I think that is proof that it's going to be a quantitative tool in the proteomics area. It's just a matter of time and using the right technology. In many respects, the technology is already here.

You could start talking about the top-down [approach], and how that could be useful. The big issue with the top-down technology is separating out the proteins. Top-down, if you have a pure protein or a mixture that isn't too complex, can be very powerful, especially if you combine that with things like electron capture dissociation. But the reality is that the best way to separate [complex protein mixtures] is to first digest them and separate out the individual peptides.

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A trio of researchers involved in the Cancer Genome Project at the Wellcome Trust Sanger Institute published a review article outlining what's known about the cancer genome, remaining questions, and the future of the field.	Prometheus Laboratories licensed US rights to Rosetta Genomics' recently introduced microRNA-based tests and has invested $8 million in the molecular diagnostics firm.	The NIH Challenge Grants program will include support for studies that explore bioethical questions and for developing new information technology for use in personalized medicine.	In bioinformatics grants, San Diego Supercomputer Center's Michael Baitaluk was funded to extend an analysis platform called Biological-Networks, while George Casella at the University of Florida got an award to develop methods to delve into SNP association and clustering data.