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## Mass spectrometry: playing catch up

Mass spectrometry is more than ever at the forefront of functional proteomics research. The technology has come a long way, but what does the future hold? Nathan Blow gets perspectives, predictions and wishes from key developers.

### WISHING & HOPING

- “I would like to see another 2–3 orders of magnitude increase in the sensitivity and scan speed.”  
John Yates, *The Scripps Research Institute*
- “As strange as it may appear for a mass spectrometrist to say, my wish for a next breakthrough is not in mass spectrometry. What we badly need now are better analytical techniques to observe the proteins in action in living cells and tissues.”  
Franz Hillenkamp, *University of Munster*
- “The ability to quantitatively measure and qualitatively identify extremely low level analytes that are in the presence of sample matrix components, which are many orders of magnitude more abundant in concentration.”  
Tim Riley, *Waters Corporation*
- “I think that if we can generate a robust method to generate high-fidelity, three-dimensional structures for protein assemblies over the next few years that would be great.” Carol V. Robinson, *University of Cambridge*
- “I would like to see continued acceptance of mass spectrometry by researchers in the biological sciences through continued integration of hardware and software tools.” Lester Taylor, *Thermo Fisher Scientific*
- “If and when the vendors get the software right, which directs the instrument, then the same instrument will be able to do much more. So I am hoping and waiting for that to happen.”  
Matthias Mann, *Max Planck Institute of Biochemistry in Martinsried*
- “I would like to see really good, sensitive, rapid and robust ‘top-down’ analysis of proteins.”  
Brian Chait, *The Rockefeller University*
- “I would like to see an instrument that could deliver accurate mass MS/MS data at the sensitivity and speed of an ion trap.” Neil Kelleher, *University of Illinois*

Is your wish on the list? Let us know either way by posting to *Methagora*, the *Nature Methods* comment forum. One way to make your wishes come true is to let the developers know what you really want. (Card design by Eszter Rabin.)

“What we have seen in the past decade was mostly improvements on existing technologies,” says Franz Hillenkamp of the University of Munster. John Yates of the Scripps Research Institute views mass spectrometry as basically evolving at a steady rate, with occasional leaps in the technology that spur researchers and developers forward. “Those usually occur

when there are new ionization techniques developed,” says Yates.

One thing is clear, however: consistent improvements in mass spectrometry instruments over the past decade have been enabling research that was unheard of before, driving the establishment of mass spectrometry as a critical technology for biologists.

#### Rise of the hybrids

When asked what the biggest innovation in mass spectrometry instrumentation has been in the last five years, Yates has a quick reply: “I would say that it has been the rise of the ‘hybrids.’” And this opinion is echoed by many mass spectrometry specialists.

Hybrid mass spectrometry instruments integrate systems with different



Matthias Mann thinks software improvements are crucial for the advancement of mass spectrometry.

operating principles into a single machine, combining the benefits of both. According to Yates, the quadrupole time-of-flight (qTOF) systems set the stage for researchers to demand increased resolution and mass accuracy from these hybrid instruments.

But it was development of another hybrid instrument that pushed the mass spectrometry field in terms of accuracy. “When the linear ion trap—[fourier transform mass spectrometer (FTMS)] systems came into being, that really filled a substantial unmet need in the field,” says Yates. Fourier transform ion-cyclotron resonance mass spectrometers (FT-ICR MS), or just FTMS, such as the apex-ultra platform from Bruker

Daltonics and the Varian 900-MS Series, are capable of very high resolution and very high accuracy, which was not previously attainable. One drawback to FTMS systems, says Yates, is that they tend not to be user-friendly, especially for the non-mass spectro-metrist.

One of the most recent and widely adopted of the hybrids arrived in 2006 when Thermo Electron introduced their hybrid LTQ Orbitrap system. “From a technical performance standpoint, this is an instrument that provides the high-resolution accurate mass capabilities that previously could only be achieved with superconducting magnet systems,” says Lester Taylor, Thermo Fisher Scientific’s director of Product Marketing in Life Sciences Mass Spectrometry.

The orbitrap mass spectrometer was first described in 2005 by Alexander Makarov and colleagues<sup>1</sup>. After ionization, ions are injected into the orbitrap where they circle around a central inner electrode ring flanked by an outer barrel-shaped electrode. Ion masses are mea-

sured from the frequencies within the image current generated by the orbiting ions. Matthias Mann of the Max Planck Institute of Biochemistry in Martinsried also pointed his finger directly at the LTQ Orbitrap as the hybrid instrument that he thinks has changed the face of the mass spectrometry. “That instrument has been a quantum jump in my opinion,” says Mann.

Yates agrees that the orbitrap technology is moving the mass spectrometry field forward. “The LTQ Orbitrap is a much more user-friendly instrument in terms of maintenance and care,” says Yates, when comparing it to the highly accurate FTMS systems. Although Yates feels the LTQ Orbitrap does not provide the same level of performance as FTMS systems, he is very happy with its combination of pretty good performance and ease of use.

#### ‘Punctuated’ ionization

Yates noted that most technological leaps during the evolution of mass spectrometry are the result of developments in ionization

methods for ionizing delicate, nonvolatile biological molecules. Today the most widely used ionization techniques for biological samples are matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI).

MALDI is a soft ionization technique, developed in the mid-1980s simultaneously by Franz Hillenkamp, Michael Karas and colleagues at the University of Munster, and Koichi Tanaka at the Shimadzu Corporation<sup>2,3</sup>. In this technique a laser beam, usually nitrogen, is focused onto a sample that is protected by a matrix (which is deposited onto a special plate). As the matrix heats up, the sample is ionized and transferred to the gas phase. In ESI, a technique developed by John Fenn and colleagues<sup>4</sup>, the substance to be studied is dissolved in a solvent, which is then highly charged and forced through a small capillary tube to form a fine mist of charged droplets. The solvent evaporates, leaving behind multiply charged gas-phase ions. "MALDI and ESI came along and presented two different workflows," says Yates. But he notes that it was ESI that filled a need in the community, providing the ability to perform online liquid chromatography with mass spectrometry, which allowed researchers to analyze more complex samples.

Carol Robinson's laboratory at the University of Cambridge is using mass spectrometry approaches to study native proteins complexes. One of her great wishes for the future of mass spectrometry is enhanced sensitivity. "Sensitivity is still a major obstacle, particularly for protein complexes, even though mass spectrometry is a highly sensitive technique. ESI has a lot of scope for improvement here."

The last ten years have seen the incorporation of these ionization techniques into mass spectrometry instrumentation. "In mass spec instrumentation, one of the biggest recent advances has been the adaptation of MALDI ion sources to many different mass analyzers," says Hillenkamp. He also cites the incorporation of MALDI into hybrid instruments such as FT-ICR combined with linear ion traps, orbitrap systems and even triple quadrupole systems.

Detlev Suckau, head of MALDI Applications Development and Proteomics at Bruker Daltonics, thinks that for MALDI, the development of TOF/TOF instrumentation was essential.

"Without the TOF/TOF, I am afraid that the MALDI-TOF as such would have had no future in the proteomics field," says Suckau. He also notes that this development has enabled MALDI to be used as a tandem mass spectrometry (MS/MS) discovery tool on par with ESI. And the multiple-step mass selection of MS/MS has opened new areas for mass spectrometry research, such as imaging of proteins and peptides. Bruker Daltonics developed its MALDI-based ultraflexIII TOF/TOF instrument for tissue biomarker discovery. The system relies on the smartbeam laser developed at Bruker Daltonics, which can be used with most MALDI matrixes and sample preparation methods. "I would not say that this is a new method, but the

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development of the smartbeam laser for MADLI-TOF/TOF shows exceptionally well that sometimes there are apparently minor technical innovations that provide access to new applications,” says Suckau.

Although both MALDI and ESI are the ionization techniques commonly used for analyzing complex biological samples, several new ionization techniques have come onto the scene in the past couple years for use with smaller molecular compounds. One of these is direct analysis in real time (DART) ionization developed by scientists at Jeol. DART ionization places samples in front of either helium or hydrogen gas streams containing electronically or vibrationally active neutral molecules. Other new ionization methods include desorption electrospray ionization (DESI) and surface-assisted laser desorption/ionization (SALDI).

Ionization of smaller molecules by these new techniques is very relevant now according to some scientists. Hillenkamp says that after much work on analysis of macromolecules, he now sees the analysis of smaller, pharmaceutically relevant molecules coming back again. But it is not clear what ionization methods will be mostly used for these molecules. “Whether any of the new ionization techniques will replace MALDI in this field remains to be seen.”

#### The user manual problem

“One of the most pressing issues regarding the application of mass spectrometry to the field of biology is training,” says Tim Riley, vice president and managing director of Pharmaceutical Business Operations at Waters Corporation. Riley is far from being in the minority on this issue—most developers see training and greater understanding of mass spectrometry principles as necessities for scientists wanting to use mass spectrometry in their research.

“This is a very complex technology, and researchers really have to be taught,” says Mann. But as more and more biologists seek out mass spectrometry solutions for their research, he predicts they will face problems. “This whole generation has been educated with a focus on genetics, and in some cases they cannot even perform proper immunoprecipitations or fractionations,” says Mann, “while the leading [mass spectrometry laboratories] have groups of 10–20 people that cover



The LTQ-Orbitrap is an example of the trend for hybrid mass spectrometers. (Courtesy of Thermo Electron.)

these areas. When biologists want to get into mass spec they get a grant for the instrument, hire one person and buy the software—and this makes it very difficult for them to do the kind of experiments they have read about in papers.”

Riley thinks that in some cases biologists might have bigger hopes for mass spectrometry than the technology is able to deliver right now. “Many researchers have overestimated the capability of the various types of mass spectrometers that they have employed, while underestimating the complexity of the biological samples that they need to analyze,” says Riley. He suggests that there has been a tendency to treat the mass spectrometer as a black box that always produces accurate and reliable answers, when in fact the complexity and dynamic range of many of the biological samples have proved too challenging for many mass spectrometry protocols.

To curb some of these training issues, Waters Corporation has worked to educate users on the performance of mass spectrometry instruments. “We provide training modules, quality control standards and test protocols to guide users through routine instrument performance checks to assure that high-quality, meaningful data [are] being produced,” adds

Riley. Even guides to mass spectrometry methods and applications directed toward biologists, such as the Focus published in *Nature Methods* two months ago, have been appearing with greater frequency in research journals.

#### The informatics challenge

Although training is on the mind of many experts, Mann and Riley feel very strongly that there is now a problem for both specialists and nonspecialists alike—software. From setting up the mass spectrometer for analysis to the management of the data, most developers will generally agree that improvements in each step would make a tremendous difference to users.

“The software that vendors make to control instruments has not kept pace with the advances in the instrumentation,” comments Mann, who is sure that when the software is made more robust, flexible and intelligent, the same instruments will be able to do much more. As an example of this he points to the headroom he sees available on orbitrap instruments: “They are very sensitive but limited in the total number of ions that can be analyzed.” To get high resolution on the orbitrap you need to wait for a full second for the computer analysis, but in fact it takes the instrument only a few milliseconds to perform the operation. So in principle



Carol Robinson's group analyzes protein complexes using mass spectrometry approaches.

the instrument has a headroom of ten- to a hundred-fold in sensitivity and dynamic range. "If that could be harnessed, it would make a huge difference [to have] a dynamic range that was higher by a factor of, say 10," says Mann.

Robinson also sees a need to optimize control of the presently available instruments. "We throw away 99% of the ion current we generate in a typical electrospray ionization experiment," says Robinson, "so a better and more efficient way of doing these experiments would have a great impact."

Although improving the software that controls the mass spectrometer would help both the advanced user and the beginner alike, "the most pressing issue right now in the proteomics field is data management," says Suckau. Even simple mass spectrometry experiments can produce incredible volumes of data. Lester Taylor notes that a complex liquid chromatography–mass spectrometry run can generate data files that are over 1 Gb in size—composed of many thousands of spectra, each containing many hundreds if not thousands of peaks. "It is just not feasible to manually interrogate that volume of data and get

meaningful answers without automated data processing methods." Taylor says that this is an area where Thermo Fisher Scientific is keenly aware of the need and is working on software solutions for these large datasets and workflows.

Neil Kelleher of the University of Illinois feels that a good way to address the software issues might be through a collaborative effort between all developers. "I would love to see an NIH-catalyzed collaborative where companies and principal investigators get together and work on these software issues," says Kelleher. He thinks without this form of collaborative interaction, advancement of this software might take longer than necessary.

Mass spectrometry has reached a unique point where small changes in either the software or hardware could open new doors for proteomics analysis by unleashing the full power of these instruments. And for the biologist moving toward mass spectrometry, take heart—with instrumentation all set, the goal of developers now is to make it all easier, faster and more reliable.

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3. Tanaka, K. *et al. Rapid Commun. Mass Spectrom.* **2**, 151–153 (1988).
4. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. & Whitehouse, C.M. *Science* **246**, 64–71 (1989).

**Nathan Blow is the Technology Editor for *Nature* and *Nature Methods* (n.blow@boston.nature.com).**

## SUPPLIERS GUIDE: COMPANIES OFFERING MASS SPECTROMETRY INSTRUMENTATION AND ACCESSORIES

Company	Web address
Adron Systems LLC	<a href="http://www.adronsystems.com">http://www.adronsystems.com</a>
Agilent	<a href="http://www.agilent.com">http://www.agilent.com</a>
Alcott Chromotography	<a href="http://www.alcottchromatography.com/">http://www.alcottchromatography.com/</a>
Analytica of Branford, Inc	<a href="http://www.aob.com">http://www.aob.com</a>
Applied Biosystems	<a href="http://www.appliedbiosystems.com">http://www.appliedbiosystems.com</a>
Astra Analytical	<a href="http://www.astranalytical.com">http://www.astranalytical.com</a>
Bergmann Messgerate Entwicklung KG	<a href="http://www.bme-bergmann.de/">http://www.bme-bergmann.de/</a>
BioTrove	<a href="http://www.biotrove.com">http://www.biotrove.com</a>
Bruker Daltonics	<a href="http://www.bdal.com">http://www.bdal.com</a>
Ciphergen Biosystems (Vermillion)	<a href="http://www.ciphergen.com">http://www.ciphergen.com</a>
Comstock Incorporated	<a href="http://www.comstockinc.com/">http://www.comstockinc.com/</a>
Evans Analytical Group	<a href="http://www.eaglabs.com/">http://www.eaglabs.com/</a>
Ferran Scientific	<a href="http://www.ferran.com">http://www.ferran.com</a>
Full Spectrum Analytics	<a href="http://www.fullspectrum-inc.com">http://www.fullspectrum-inc.com</a>
GB Scientific	<a href="http://www.gbscientific.com">http://www.gbscientific.com</a>
GE Healthcare	<a href="http://www.gehealthcare.com">http://www.gehealthcare.com</a>
Hitachi Instruments	<a href="http://www.hitachi.com">http://www.hitachi.com</a>
HT Laboratories	<a href="http://www.ht-labs.com">http://www.ht-labs.com</a>
Hybrigenics	<a href="http://www.hybrigenics.com">http://www.hybrigenics.com</a>
In Process Instruments	<a href="http://www.in-process.com">http://www.in-process.com</a>
Invitrogen	<a href="http://www.invitrogen.com">http://www.invitrogen.com</a>
Jeol	<a href="http://www.jeol.com">http://www.jeol.com</a>
Jordan TOF Products, Inc.	<a href="http://www.rmjordan.com">http://www.rmjordan.com</a>
Kore Technology	<a href="http://www.kore.co.uk">http://www.kore.co.uk</a>
Kratos Analytical	<a href="http://www.kratos.com">http://www.kratos.com</a>
Leco Corp.	<a href="http://www.leco.com">http://www.leco.com</a>
M-Scan	<a href="http://www.m-scan.com">http://www.m-scan.com</a>
Metabolic Solutions Inc.	<a href="http://www.metsol.com">http://www.metsol.com</a>
Monitor Instruments	<a href="http://www.monitorinstruments.com">http://www.monitorinstruments.com</a>
Mspec Group	<a href="http://www.mspecgroup.com">http://www.mspecgroup.com</a>
Perkin Elmer	<a href="http://www.perkinelmer.com">http://www.perkinelmer.com</a>
Photonis	<a href="http://www.photonis.com">http://www.photonis.com</a>
Prolab Resources	<a href="http://www.prolabresources.com">http://www.prolabresources.com</a>
Restek	<a href="http://www.restek.com">http://www.restek.com</a>
Scientific Instrument Services, Inc.	<a href="http://www.sisweb.com">http://www.sisweb.com</a>
Shimadzu Scientific Instruments	<a href="http://www.shimadzu.com">http://www.shimadzu.com</a>
Sigma Aldrich	<a href="http://www.sigmaaldrich.com">http://www.sigmaaldrich.com</a>
Thermo Electron Corp.	<a href="http://www.thermo.com">http://www.thermo.com</a>
Varian	<a href="http://www.varianinc.com">http://www.varianinc.com</a>
Waters	<a href="http://www.waters.com">http://www.waters.com</a>