

## New MS Tools Advance Biomolecular Studies

### Simpler, Cheaper, and Faster Instrumentation Facilitates Inclusion in Novel Applications

K. John Morrow Jr., Ph.D.

**M**ass spectrometry excels as a powerful technique to identify, quantify, and elucidate chemical substances. It is notable for its accuracy and sensitivity, and as a result, new applications and advances constantly pour forth. Recently launched innovative hardware and software for use in the life sciences were discussed at the "Pittcon" meeting held in Chicago.

"Accuracy of mass spectrometry can be divided into must-have versus nice-to-have situations," according to John Yates, Ph.D., principal investigator, Scripps Research Institute. Dr. Yates discussed examples of both categories of investigation. For example, in database searching, if the precursor ion mass is known with high accuracy, the speed of the search is increased, resulting in fewer candidate peptides that need to be tracked down. Peptide validation, in which the precursor ion mass accuracy can be used to filter and validate peptide identification is another situation, helpful but not absolutely necessary, for the success of the investigation.

Dr. Yates contrasted these investigations with ones that place a higher demand on the technology. For example, post-translational modifications represent "must-have high mass accuracy" situations in which validation of large mass and dealing with ambiguous and unanticipated post-translational modifications are more exacting. Other high accuracy tasks include quantitative proteomics and peptide mass fingerprinting.

As a case study, Dr. Yates and his coworkers considered arginylation analysis as an example of ambiguous post-translational modifications. Since many different residues and modifications can have similar molecular weights, it is necessary to have accurate measurements in order to avoid confounding one type of sequence modification with another.

According to Dr. Yates, highly accurate data is also of importance in identifying unanticipated and unknown modifications in a database search. These may include chemical modifications introduced during sample preparation, poor quality spectra, the presence of low-abundance peptides, and sequences not in the database. The team took advantage of BlindPTM, a database search engine designed to find unanticipated or unknown post-translational modifications. This search engine does not require users to list potential post-translational modifications, but it does require high mass accuracy precursor mass information.

Dr. Yates covered quantitative proteomics as a second example of an area requiring

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extremely accurate MS data, including a case study of the arginine-to-proline conversion in SILAC (stable isotope labeling with amino acids in cell culture). This method detects differences in protein abundance between samples, and has proven to be useful in quantitative proteomics.

Dr. Yates and his coworkers determined that, despite the power of the SILAC methodology, the ratio of light and heavy peptides can be incorrectly calculated due to the insertion of heavy isotope labels into proline through arginine catabolism. But high-resolution mass spectrometers can distinguish isotopes of converted heavy proline clusters from the heavy arginine clusters.

Finally, Dr. Yates discussed the applications of high-resolution mass spectrometry to peptide identification using a unique mass identifier, accurate mass tag, and peptide mass fingerprinting.

#### Coulometric Arrays

"Analytical techniques in small molecule drug discovery and development frequently



ESA Biosciences' CoulArray system relies upon the electroactive nature of molecules—a molecule's ability to be oxidized or reduced. The technology lends itself to the analysis of xenobiotic molecules and charged or uncharged pharmaceutical compounds.

involve liquid chromatography separation and detection of numerous compounds with diverse chemical structures," Ian Acworth, Ph.D., vp of HPLC products and services, life science tools at ESA Biosciences ([www.esainc.com](http://www.esainc.com)), explained. Common analytical approaches employ a suite of rapid generic chromatographic gradient methods, geared toward high-throughput analyses."

Dr. Acworth described an alternative approach to detection of complex molecu-

lar species. "Our objectives were to investigate the utility of coulometric array electrochemistry as a parallel detection technique for fast liquid chromatography methods," he explained.

Coulometric array detection is an advanced mode of electrochemical analysis; it employs an electrode that is 100% efficient and measures signals from all of the analyte passing through it by responding to substances that are either oxidizable

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## News Discovery & Development Briefs

### Sucampo Acquires Rights to RTU's Ophthalmic Drug

Sucampo Pharmaceuticals ([www.sucampo.com](http://www.sucampo.com)) will pay R-Tech Ueno (RTU; [www.rtechueno.com](http://www.rtechueno.com)) \$3 million for a license to its ocular disease drug, Rescula. The agreement gives Sucampo the right to market the product in the U.S. and Canada in open-angle glaucoma and ocular hypertension. The company will also develop the compound in other diseases of the eye.

Sucampo also will have the right of first refusal to commercialize in the U.S. and Canada any additional indications for which Rescula is developed by RTU. The company will pay RTU milestones related to its own development and commercialization goals. RTU will remain as the exclusive supplier of the finished product.

### Alethia Gets \$2.2M to Advance Preclinical mAbs

Alethia Biotherapeutics ([www.alethia.bio.com](http://www.alethia.bio.com)) received a \$2.2 million investment, which will be used to advance the development of the company's portfolio of thera-

peutic monoclonal antibodies. The firm's most advanced mAb targets a secreted factor called clusterin, which plays a role in tumor progression and invasion. In ovarian cancer, a mAb is being developed against an antigen that is overexpressed in more than 90% of ovarian tumors.

### Merck & Co. Funds Inflammatory Disease Program at Galapagos

Galapagos ([www.glp.com](http://www.glp.com)) entered into a multiyear global strategic alliance with Merck & Co. ([www.merck.com](http://www.merck.com)) to develop potential new therapies in inflammatory diseases. Galapagos will be responsible for the discovery and preclinical development of new small molecule candidate drugs based on its targets.

Merck has the option to acquire an exclusive license to each candidate drug, and upon exercise of such an option Merck will be responsible for the development and commercialization of the candidate drug. Galapagos may execute Phase I studies and will have the right to further develop and commercialize certain compounds

for which Merck does not exercise its exclusive option.

Under the terms of the agreement, Galapagos will receive an up-front fee of \$3.23 million from Merck. In addition, Galapagos is eligible to receive milestone payments that could potentially exceed \$251 million total for multiple products.

### Hadasit and Immuron Strike Codevelopment Deal

Hadasit, the technology transfer company of Hadassah Medical Organization, agreed to license its oral immune modulation technology to Immuron Limited in exchange for 19.99% equity in the company, and royalties on products Immuron develops using the technology. Hadasit will also provide clinical and laboratory services to Immuron.

Scientists from the Hadassah Medical Center claim to have demonstrated that the oral immune modulation approach, combined with dairy-derived antibodies and proteins used by Immuron can affect the activity level of regulatory T cells. n

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# Mass Spec Tools

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or reducible. The electrical output results from an electron flow caused by the chemical at the surface of the electrodes. If this chemical activity exhausts all the reactant driving the current to zero, the total charge that passes will be proportional to the mass of solute detected.

The technology lends itself especially to the analysis of food components, xenobiotic molecules, and charged or uncharged pharmaceutical compounds.

In Dr. Acworth's studies, fast gradient

chromatographic methods using short analytical columns and elevated flow rates and temperatures were used with post-column flow splitting for electrochemical array and serial diode array and MS detection. Metabolite analyses of urine obtained from rats demonstrated highly complementary detection.

Dr. Acworth and his coworkers found that 44 out of 45 compounds were detected by a combination of coulometric array with mass spec. Moreover, the electrochemical array augmented the metabolic-profiling capabilities of LC electrospray-MS through parallel detection of neutral and nonpolar redox active metabolites, from pg to µg levels.

"As many organic chemicals are thought to exert toxicity via redox processes, the acquired redox profiles may be particularly useful in drug discovery and development for tissue-specific modeling, diagnostic marker identification, and mechanistic insight to xenobiotic induced toxicity," Dr. Acworth concluded.

## DARTs Hit their Target

Yeping Zhao, Ph.D., principal research scientist at Roche Research Labs ([www.roche.com](http://www.roche.com)), discussed a recent development in mass spectrometry technology called direct analysis in real time (DART). This technology is an integral part of JEOL's ([www.jeolusa.com](http://www.jeolusa.com)) AccuTOF DART, which is aimed primarily at the quantification of small molecules in biological fluids. Combined with tandem mass spectrometry (MS/MS), it can be employed without sample preparation and liquid chromatography separation.

Ordinarily, biological materials must be subjected to elaborate preparative steps, including chromatographic separation, before they are analyzed in the mass spec-

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rometer. The DART approach bypasses these steps, however, by creating a dry, inert gas stream that contains long-lived, excited molecular species known as metastables. A grid at the exit of the DART apparatus acts as a source of electrons and reduces positive-ion/negative-ion recombination. The excited-state species can interact directly, desorbing and ionizing the sample.

The beauty of the system is that simply by placing the sample in the metastables stream, the analysis can be performed. Even substances bound to surfaces such as concrete, documents, food items, pills, and clothing are amenable to this approach. This means that the LC system and mobile phase are unnecessary and can be omitted from the train of equipment. When the system was used to detect experimental compounds in dog plasma, the accuracy and consistency was comparable to that obtained from a LC/MS/MS method with purified samples, according to Dr. Zhao, although it lacked the sensitivity garnered from the protocols using purified samples and a LC/MS/MS system.

Despite the noteworthy advantages of

the system, there are a variety of problems that remain to be addressed before the technology can be widely adopted. A pressing issue is that at least 20 to 30% of compounds tested cannot be ionized, and therefore, cannot be analyzed using the DART platform. Compared to highly purified material, the sensitivity is substantially lower, and special materials are needed for the sampling device to avoid the interaction between analytes and the surface of the tip.

With the resolution of these issues, however, the technology could be powerful, lending itself to such tasks as the search for early signs of degradation in documents, photographs, and physical structures, and in the analysis of forensic and archeological materials.

## Struggling with Detergents

"Detergents are widely used in protein extraction, solubilization, and denaturation," says Lisa Bradbury, Ph.D., proteomics R&D director at Pall ([www.pall.com](http://www.pall.com)), "yet their presence can interfere with the performance of mass spectrometry technology."

Given that detergents are a problem for so many downstream methods of protein analysis, a fast and efficient detergent-removal method could prove useful. Dr. Bradbury and her colleagues have evaluated the properties and behavior of SDR HyperD® resin, a mixed-mode chromatography medium. The mixed-mode properties of this resin result from a combination of a porous, spherical, silica bead with a hydrophobic polymer. The polymer is uniformly distributed throughout the silica pores allowing the specific interaction of small molecules in solution with silanol and hydrophobic groups.

SDR HyperD is effective for the removal of a family of detergents including NP-40, Triton X-100, SDS, Tween 20, and CHAPS from protein samples. Given its exquisite sensitivity to detergents, mass spectrometry can be used to detect extremely low levels of residual detergent, down to 100 ppm in protein samples.

SDR HyperD resin has a high capacity for all detergents tested, said Dr. Bradbury, and its application results in substantial improvement in MS signal intensity of proteins. Dr. Bradbury and her colleagues determined that SDR can be used in a prepacked column or with batch-mode

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## POINTS TO CONSIDER

- With improved instrumentation, mass spectrometry excels as a powerful technique to identify, quantify, and elucidate chemical substances.
- MS technology continues to move forward with advances that are simpler, cheaper, and faster, opening up new applications.
- In addition to long-standing applications including metabolic and biochemical analyses, a new generation of instruments have allowed MS technology to branch into the disciplines of forensics, anthropology, and history.
- The advanced technologies and their respective applications covered in this article include: coulometric array detection, high resolution mass spectrometry, DART (direct analysis in real time), comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS), and liquid chromatography electrospray ionization.

## Time of Flight (TOF) Mass Spectrometry Systems

Company	Product	Footprint (W x L x H)	Samples Processed/Hr	Sample-Detection Limits
Applied Biosystems/MDS <a href="http://www.appliedbiosystems.com">www.appliedbiosystems.com</a>	AB SCIEX TOF/TOF™ 5800	109 cm x 81 cm x 239 cm	>2,000	Neurotensin (1672.9175) @ 250 amol; 400 Laser Shots, S/N > 250:1; Regularly see S/N = 150 at 100 amol
Agilent Technologies <a href="http://www.agilent.com">www.agilent.com</a>	6230 Accurate Mass TOF LC/MS System	73 cm x 83 cm x 133 cm	1–60 <sup>a</sup>	2 pg of reserpine on column, with typical S/N > 40
Comstock <a href="http://www.comstockinc.com">www.comstockinc.com</a>	miniTOF II	23 cm x 56 cm	Varies	~10 ppm
Ionwerks <a href="http://www.ionwerks.com">www.ionwerks.com</a>	MALDI IMTOF	91 cm x 152 cm	10–100	Femtomole or less
Jeol USA <a href="http://www.jeolusa.com">www.jeolusa.com</a>	AccuTOF™-LC AccuTOF-DART™ AccuTOF-GCv	1 m <sup>2</sup> 1 m <sup>2</sup> 2 m <sup>2</sup>	1–10 10–500 1–10	pg pg fg to pg
Shimadzu Scientific Instruments <a href="http://www.ssi.shimadzu.com">www.ssi.shimadzu.com</a>	AXIMA Performance AXIMA Resonance	70 cm x 85 cm x 192 cm 72 cm x 95 cm x 192 cm	768 384	MS Mode: 250 amol Glu-Fibrinopeptide; MS/MS Mode: 2.5 fmol Glu-Fibrinopeptide MS Mode: 500 amol angiotensin-ii; MS/MS Mode: 500 amol angiotensin-ii
Thermo Fisher Scientific <a href="http://www.thermofisher.com">www.thermofisher.com</a>	Exactive	less than 1 m <sup>2</sup>	20–30 <sup>b</sup>	Typically sub ppb concentrations
Waters <a href="http://www.waters.com">www.waters.com</a>	LCT Premier XE XEVO QT of MS	83 cm x 80 cm x 65 cm 69 cm x 93 cm x 98 cm	20 spectra/sec 20 spectra/sec	Electrospray-positive ion signal; noise sensitivity of 10 pg reserpine of [M+H] <sup>+</sup> ion measured at m/z 609 (greater than 100:1) At 10,000 FWHM, mass at m/z 556 from 25 pg/µL leucine enkephalin (intensity of >2,040 counts per second).

<sup>a</sup> by LC/MS, depending on LC method conditions  
<sup>b</sup> depends on inlet mode, flow injection mode



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# Mass Spec Tools

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devices to readily accommodate a variety of experimental needs. Additionally, large proteins (> 60 kDa) cannot penetrate the pores of this resin, so protein losses are minimal. Dr. Bradbury cautioned that for moderately sized proteins in the 20 to 60 kDa range, the potential for protein loss is dependant on individual protein characteristics and sample composition, and thus should be determined on a case-by-case basis.

“We found that the SDR HyperD resin provides a means for efficient detergent removal,” Dr. Bradbury stated, “especially when used in combination with the Nanosep spin devices and AcroPrep 96-well filter plates, formats that are readily amenable for high-throughput applications.”

The analysis of metabolomic data using comprehensive 2-D gas chromatography with time-of-flight mass spectrometry

(GCxGC-TOF-MS) was discussed by John Heim, applications chemist at LECO (www.leco.com). His investigations illustrate how metabolomics can be strengthened through the addition of 2-D gas chromatography to the MS platform.

“Metabolomics presents challenges that, historically, have relied heavily upon standard quadrupole GC/MS, utilizing targeted methods of selected ion monitoring and tandem GC/MS/MS mass spectrometric techniques,” Heim stated. Two dimensional gas chromatography provides the resolution needed for the characterization of the small metabolite profiles.

The complex nature of metabolomic samples demands analytical solutions and instrumental methods that will identify the small molecule metabolomic profile completely as well as discover significant key components

of interest. In these studies, Heim and his colleagues investigated urine samples collected from diabetic and nondiabetic humans.

Comprehensive 2-D gas chromatography expands the peak capacity of the chromatographic separation, thereby, increasing resolution and analyte characterization necessary for complex biological samples. The high-data density and narrow-peak widths inherent to GCxGC analysis require a detection system able to characterize the peak shape and small molecule metabolite identification.

The group focused on the demonstration of the benefits of the system, in that TOF-MS provides the acquisition speed necessary for optimum characterization of the complex GCxGC separations. The studies were further aimed at evaluating the statistical powers of the Chroma TOF™ software for multivariate analysis of data sets represent-

ing diseased and nondiseased states. The statistical program allows the user to recognize previously unknown chemical differences between complex samples.

The use of this software could be significant in resolving problems in metabolome screening, in which confirmation of data may be problematic, even within the same laboratory. “This establishes a viable strategy identifying significant metabolic variation in complex biological samples from diseased and nondiseased states,” Heim concluded.

## Oleic Acid and Cardiovascular Disease

Huiling Liu, Ph.D., senior scientist at Agela Technologies (www.agela.com), discussed the use of liquid chromatography electrospray ionization mass spectrometry in the determination of oleic acid and its metabolites.

Oleic acid is an omega-nine, mono-unsaturated fatty acid found principally in plants. It has been shown to slow the development of heart disease, and promotes the production of antioxidants. There is a convincing body of experimental data indicating that oleic acid has a profound capacity to reduce blood pressure.

The major actor in this cascade may be the metabolite of oleic acid, 9,10-di-hydroxy stearic acid, which, acting as a double excitation reagent of endogenous peroxisome proliferation-activated receptors, could play a role in the prevention of atherosclerosis. In order to further understand the properties of these compounds, their pharmacokinetic performance has been monitored by determining their levels in plasma.

“We developed a high-recovery method for extracting the compounds from the plasma,” said Dr. Liu. A rapid, simple and sensitive solid-phase extraction, high-performance liquid chromatographic separation and tandem mass spectrometric detection system was designed for the quantitation of oleic acid and DHSA at sub-pg/mL levels.

Some recent studies suggest oleic acid incorporates into the cell membranes of blood vessels where it likely makes the cells more receptive to signals that reduce blood pressure. This is believed to occur through oleic acid’s cis configuration, which allows it to fit tightly into membranes. The mass spectrometry platforms designed by Dr. Liu and his collaborators will allow effective monitoring of oleic acid and its metabolites and aid in the understanding of how these substances offer protection from cardiovascular insult.

MS technology continues to move forward with advances in instrumentation that are simpler, cheaper, and faster, opening up new applications. It is noteworthy that the “Pittcon” presentations profiled, offer insights into questions of basic and applied science that were either difficult to engage or beyond the easy reach of most investigators before this technology came on the scene. In addition to long-standing applications including metabolic and biochemical analyses, a new generation of instruments have allowed mass spec technology to branch into the disciplines of forensics, anthropology, and history. **GEN**

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