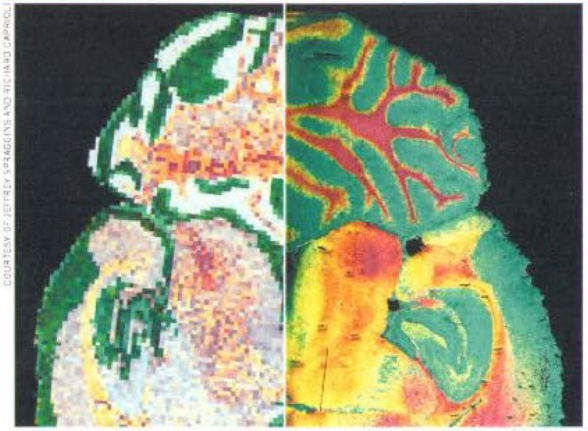
Chemical & Engineering News, June 2015



COURTESY OF JEFFREY SPRAGGINS AND RICHARD CAPRIOLI

DOUBLE EXPOSURE Images of a rat brain acquired with high mass (left) and high spatial resolution can be combined to predict an image with both qualities. Colors indicate three different mass-to-charge ratios of lipids.

A TASTE OF MASS SPECTROMETRY

Mass spectrometrists gather in St. Louis for **ASMS MEETING**

CELIA HENRY ARNAUD, C&EN WASHINGTON

EARLIER THIS MONTH, 6,100 mass spectrometrists met in St. Louis for the 63rd meeting of the American Society for Mass Spectrometry. Here is a sampling of the more than 3,000 oral and poster presentations that were given.

COMBINING TWO DATA SETS YIELDS HIGH-RES IMAGES

Mass spec imaging involves trade-offs among speed, spatial resolution, and mass resolution. Researchers at Vanderbilt University have found a way to obtain both high spatial and mass resolution in a reasonable amount of time by combining data from two different types of mass spectrometers.

A key advance for this technique is the ability to acquire matrix-assisted laser desorption ionization (MALDI) time-of-flight images at a rate of 50 pixels per second, said Jeffrey Spraggins, a researcher at Vanderbilt's Mass Spectrometry Research Center. At such speeds, the Vanderbilt team could acquire a megapixel molecular image of lung tissue from a cystic fibrosis patient in about six hours, he said. The spatial resolution allows them to see composition variations in the tissue.

But time of flight doesn't offer the same kind of mass resolution as Fourier transform ion cyclotron resonance, or FTICR. With FTICR, they can identify many more chemical species, especially lipids. The problem is that FTICR can take days instead of hours.

The answer is an image fusion method that combines data from MALDI time of flight and FTICR. First, the researchers take an image of an area of interest at high spatial resolution. Then they identify the smallest region that contains all of the molecules of interest and acquire an FTICR image of that small part of a similar sample.

To do the image fusion, they run a regression analysis to identify correlations and more advanced relationships between spectral features and spatial patterns observed in the different image types. They use those correlations to predict a high-spatial-resolution FTICR image based on the corresponding time-of-flight image. "Basically we're building a model based on a representative small area of the tissue," Spraggins explained. In this way, they achieve an image that has the spatial resolution of time of flight and the mass resolution of FTICR.

Spraggins acknowledges that such prediction-based imaging makes some people uneasy. But he points out that the process is mathematically similar to constructing a calibration curve, such as one used to predict the concentration of an unknown sample. In those experiments, a researcher measures a response for certain concentrations and then fits a line to those data to predict responses over a range of concentrations. "We're just doing this on much larger scales," Spraggins told C&EN.

Spraggins showed an example in which his team used image fusion to bring together a 280,000-pixel time-of-flight image of an entire rat brain tissue section and a 13,000-pixel FTICR image of just the right hemisphere. The predicted high-spatial-resolution FTICR image of the entire tissue section was then spot-checked by acquiring the actual high-spatial-resolution FTICR image of small areas.

"If we were to collect this same 280,000-pixel image on an FTICR, it would take five or six days instead of eight hours," Spraggins said.

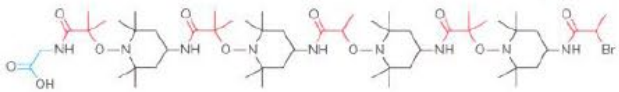
MASS SPEC READS CODES IN POLYMERS

Polymers could act as molecular bar codes that serve as anticounterfeiting features on high-value products, such as banknotes, pharmaceuticals, or specialty chemicals. But the polymer code would have to be carefully synthesized and easily read.

Laurence Charles of Aix-Marseille University, in France, described how mass spectrometry could read digitally encoded synthetic polymers (*Nat. Commun.* 2015, DOI: 10.1038/ncomms8237). The polymers were developed by her collaborator Jean-François Lutz of Charles Sadron Institute, in Strasbourg, France.

Lutz's team synthesizes the polymers using stepwise coupling reactions to add anhydride "data bits" connected by nitroxide spacers to a growing chain. They use two anhydrides that they define as the 0 and 1 bits.

First they react a symmetric brominated anhydride with a primary amine to form a substituted amide. Then they react a nitroxide with the brominated end of the amide through a radical reaction to yield an alkoxyamine. They alternate these two reactions until they've achieved the desired chain length. The C–O bonds of the alkoxyamine linkages, which are the most fragile bonds in the polymer backbone, preferentially break during tandem mass spectrometry. From the pattern of mass spec fragment ions, the researchers can easily read back the encoded sequence in either direction. The mass spectra are simple enough that high-resolution mass analyzers aren't needed, Charles said.



A binary code encrypted in a synthetic polymer can be read with mass spectrometry. The amide data bits (red) are separated by alkoxyamine spacers (black). This example contains the binary sequence 11010.

So far, she added, they've sequenced polymers encoding up to seven bits. Longer readable sequences will probably require the use of automated synthesizers.

LIPIDS ANALYZED IN BABIES' FIRST STOOL

Chemicals in the womb can have profound effects on fetal development. But measuring exposure can be difficult. Easily obtained samples, such as the mother's blood and urine, are more reflective of her condition than the baby's. But meconium—a baby's first stool—can be obtained shortly after birth and can reveal much about a baby's prenatal experience.

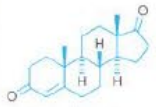
Nathaniel W. Snyder of Drexel University's A. J. Drexel Autism Institute described how he and his coworkers are analyzing meconium for steroids and other lipids as part of a study to identify prenatal risk factors for autism. Because meconium starts accumulating around a fetus's 12th week and isn't eliminated until after birth, it provides a time-averaged picture of a baby's metabolism during the last six months of gestation, a time window that's hard to access with other samples.

Males are significantly more likely than females to be diagnosed with autism. So some researchers wonder whether being male makes a child more susceptible to developing the disorder or being female protects a child. Such a bias makes sex hormones a good place to start looking for molecular markers of autism.

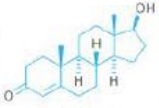
"Sex steroids are fairly difficult chemicals to study analytically because they all have very redundant chemical formulas," Snyder said. "You need good analytical tools, both liquid chromatography and tandem mass spectrometry."

Snyder analyzed sex steroids in 194 meconium samples. He targeted testosterone, androstenedione, and dehydroepiandrosterone. Because he used high-resolution mass spectrometry, he could do an untargeted analysis of other lipids in meconium. The untargeted analysis revealed thousands of other mass peaks that Snyder has yet to identify.

Because the study is ongoing, Snyder is still blind to the clinical outcomes of the children, which will be revealed after the entire cohort reaches 36 months. But the sex of the children is known. As a reassuring first step, the mass spec analysis of sex steroids in meconium has distinguished between males and females.



Androstenedione



Testosterone

If all goes well, Snyder will combine his data with others to identify ways to decrease the risk of autism. "If you can identify a contributing factor, then you can design a public health approach to prevent it," he said.

INTERLAB STUDY TESTS REPRODUCIBILITY OF SWATH ACQUISITION

For large-scale proteomic studies to be effective, researchers must be confident that different labs can analyze the same types of samples and get similar results using the same mass spec techniques.

One family of techniques used in these studies is data-independent acquisition methods, which allow researchers to capture tandem mass spectra of all detectable components in a complex sample.

For example, SWATH, developed by Ruedi Aebersold's lab at the Swiss Federal Institute of Technology (ETH), Zurich, and commercialized by instrument company Sciex, involves stepping through a series of mass windows. For each window, a full mass-range MS/MS scan is acquired at high resolution for the second MS stage. Data are processed in a targeted way using spectral libraries.

A group of 11 labs in six countries undertook a study to assess the reproducibility of analyses using SWATH. The study was modeled after one done for another mass spec technique several years ago by the National Institutes of Health's Clinical Proteomic Tumor Analysis Consortium. Christie Hunter, director of "omics" applications at Sciex, reported preliminary results from the new study.

The participating labs analyzed the same two samples of digested kidney cancer cell lysate. One was spiked with quality-control peptides to establish the methods and do an initial performance assessment. The second phase of the study involved a more complicated sample spiked with 30 synthetic peptides across a broad concentration range to assess reproducibility within and among labs. The researchers performed analyses on the same model mass spectrometer and processed the data centrally.

The teams also analyzed the data to see how many proteins from the background lysate they could quantify. The labs detected approximately 4,200 proteins over a 4.5-order-of-magnitude concentration range with reasonable reproducibility across labs.

The findings suggest, Hunter said, that large biomarker studies can be undertaken across instruments and sites.