Clathrates lead to MS without a matrix

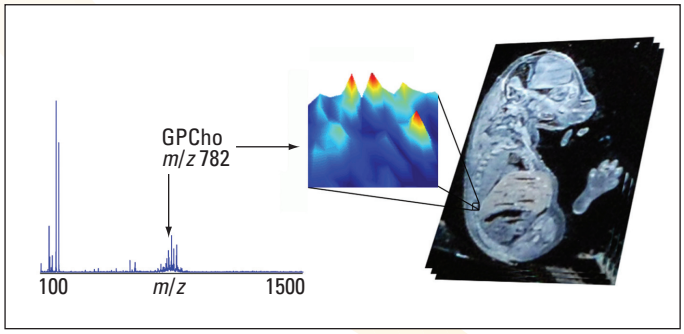
A new surface-based MS ionization technique enables the analysis of untreated biological samples.

If you were lying on a giant honeycomb that was zapped and exploded, vaporizing the honey underneath you, where would you go? Up. That's the principle behind the new MS ionization technique developed by Gary Siuzdak and colleagues at the Scripps Research Institute, the University of California Santa Barbara, and the University of Oregon (*Nature* **2007**, *449*, 1033–1063).

Nanostructure-initiator MS (NIMS), a laser desorption method, bypasses the need for matrix; instead, it uses clathrate nanostructures. Wikipedia defines a clathrate as "a chemical substance consisting of a lattice of one type of molecule trapping and containing a second type of molecule." In NIMS, liquid initiator molecules are trapped within a nanoporous silicon surface, and the analyte is deposited on top of this clathrate. When the surface is heated by a laser or ion beam, the initiator molecules are vaporized, and the analyte molecules go into the gas phase. Siuzdak says, "We've been trying to soften the surface up in order to make the molecules come off in an intact way."

Previously, the group had used porous silicon surfaces with their desorption/ionization on silicon (DIOS) technique. Then, postdoc Trent Northen had the idea of trying to remove the surface from underneath the sample while it was in a vacuum. "We kicked around ideas of how to do this, and eventually hit on this idea of making a surface very hot, which would vaporize a viscous, vacuum-compatible liquid," Northen says. In progressing from DIOS to NIMS, they switched from an n-type to a p-type silicon and found a simpler procedure for preparing the surface that yielded smaller pores.

The team tried a dozen different initiator molecules ranging from 200 to 14,000 Da, and the perfluorinated siloxanes turned out to be the best initiators for laser NIMS. Control experiments confirmed that the initiator was not acting as a matrix and that the phenomenon was a unique action of the clathrate, not the nanoporous surface or the initiator molecule individually. In addition to a laser beam, the team also tested the principle with an ion beam; they termed this method ion NIMS.



JON V. APON, TRENT NORTHEN, AND GARY SIUZDAK

MS imaging of glycerophosphatidylcholine (GPCho) localized around a developing vertebra from a mouse embryo section (right). The resulting 300-µm-wide false-color mass intensity image (expanded view) reveals a higher intensity surrounding the developing vertebra. Order of intensity: red > yellow > cyan > dark blue.

The group's second breakthrough occurred when postdoc Oscar Yanes came up with the concept of ablating the top layers of tissue samples with a higher laser power before performing NIMS analysis of the tissue at the lower laser power. "We think that for NIMS, we need a very, very thin layer of metabolites or peptides just in contact with or on top of the initiator," says Yanes. With this pre-ablation step, good spectra could then be obtained from tissue or blood without any sample pretreatment or the need for super-thin tissue slices.

Ole Jensen of the University of Southern Denmark highlighted the utility of being able to analyze biological fluids with minimal or no sample preparation. "In clinical diagnostics and prognostics, if this new technology can be used for very simple analysis of urine and blood and other biofluids, then it could be a way to move closer to the clinic and screen samples from many patients," says Jensen. "But again, before that can be achieved, the method has to prove its robustness and reproducibility." Like Jensen, Amina Woods at the National Institute on Drug Abuse is impressed with the lack of sample preparation in NIMS, but she would like to see more work done on the single cells and proteins, she says.

Siuzdak and colleagues find NIMS to be complementary in many ways to MALDI and ESI. For example, NIMS is better for analyzing metabolites than MALDI and is more tolerant of salt and yields different charge states than ESI. "But with metabolomics, we not only get good accuracy because we're in a relatively low mass range—which gives us some information in itself, like elemental composition—but on top of that, you also get MS/MS data . . . a *lot* of MS/MS data," Siuzdak says.

The researchers are handling the mountains of data they're producing with their own open-source bioinformatics software (XCMS) and the online metabolite database (METLIN) that they developed in collaboration with Ruben Abagyan's group and the Scripps Center for Mass Spectrometry.

Siuzdak is enthusiastic about the potential of NIMS for metabolomics. "I think this could open up another window into the metabolomics world." ■

—Christine Piggee