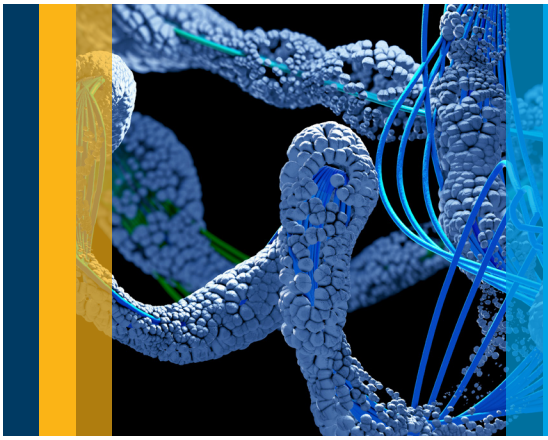

Agilent Case Study: University of Michigan

Mass Spectrometry, Large Biomolecules, and the Science of "More"

A conversation with Dr. Brandon Ruotolo, Professor and Associate Chair for Research, University of Michigan, Department of Chemistry

Mass spectrometry has a long and celebrated history as a foundational technique in biochemistry. Its ability to cleave molecules into charged fragments, then sort those fragments according to their mass-to-charge ratio, has been harnessed countless times to identify and quantitate a broad range of molecules of significant biological interest.

There's no denying that a tremendous wealth of knowledge has been – and continues to be – added to our understanding of biomolecular structure and function using these techniques. But occasionally the question arises: Are there ways we could improve, modify, or otherwise tweak this approach to tell us even more, even faster?

Count Dr. Brandon Ruotolo among those asking such questions. A professor of chemistry at the University of Michigan, Dr. Ruotolo and colleagues are looking for ways to make mass spectrometry analysis of macromolecules more quantitative, more nuanced, more information rich, and more reflective of the contextual reality of biomolecular structure, function, and interaction.

Native mass spectrometry

How do you take a technique that's really good at breaking molecules into pieces and modify it to keep large, often fragile, molecular entities intact throughout the measurement? Dr. Ruotolo explained. "It starts with advances in sample preparation and in the ion source, which is used to convert the biomolecule from its native solution phase into the gas phase for analysis. Big molecules have a keen ability to retain a 'memory' of their size and structure. In native mass spectrometry, we remove all the solvent away from the sample within a millisecond or so, without allowing enough time or imparting sufficient energy for the molecular structure to rearrange. In this way, you can kinetically 'trap' the protein or nucleic acid of interest in a form that is reflective of what it was before we ripped away all the solvent."

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This careful approach allows analysis of macromolecular structure that is closer to the native state – and therefore, more functionally relevant – than can be achieved with a more conventional mass spectrometry approach. "Using native mass spectrometry has allowed us to probe protein–ligand binding events and protein complexes, even to explore the organization of vast molecular machines like ATP synthases and ribosomes. It's a pretty far-reaching technology that's continually pushing the boundaries of how large and how complex of a biological apparatus we can put into the instrument and still rely on interpretable data coming out on the detector side. A lot of it just comes down to how carefully you perform the experiment."

Collision-Induced unfolding with the Agilent 6560 Ion Mobility Q-TOF

A lot of traditional mass spectrometry gains information by essentially heating molecules to the point where their covalent bonds break, allowing researchers to explore the nature of those pieces and learn about the whole. Dr. Ruotolo points out that valuable information can be obtained in this way, and a portion of his lab focuses on such experiments. But covalent bonds are only one of the forces responsible for macromolecular structure. A technique known as collision-induced unfolding aims to tease, rather than blast them apart, exploiting the fact that biomolecules use a host of noncovalent interactions to fold and unfold themselves, often in predictable and informative ways.

"As we gently heat these large molecules inside the instrument, through collisions with a background gas, we see that they get larger," Dr. Ruotolo explained. "We attribute that effect to unfolding, and although it may not correlate with how unfolding might progress in solution, we can use the data in a somewhat similar way. By analyzing the effect of that heating using the 6560 ion mobility Q-TOF – which allows sorting by size once the molecules enter the gas phase – we can quantitate which sizes are present at a given level of heating, and in what proportions. This can tell us a lot about the stability of the molecule or molecular complex, and by extension, its structure."

Scaling up with the Agilent RapidFire

Both techniques discussed so far are capable of providing vital information about macromolecular binding interactions – information that's of particular interest in drug discovery. However, in order to be practical in this context, the experiments have to be fast – biopharma labs need to rapidly screen large numbers of potential macromolecules in order to succeed. Throughput becomes an essential part of the conversation.

"When I was a student learning about native mass spectrometry, it was definitely not thought of as a high-throughput approach," Dr. Ruotolo said. "Work that proceeded at the pace of crystallography, spectroscopy, and electron microscopy could take months or even years to complete. In those days, we were very happy to run maybe 10 samples a day; that gave us plenty of data to add value and move a project along. Even then we were thinking, 'What if we could move faster?', but the engineering just wasn't there yet."

Still, the demands from the drug discovery sector kept the pressure on to discover ways to transform throughput. A number of innovations – better buffers, online separation technologies, many more – contributed to slimming the time needed to run a sample from maybe 15 minutes down to just a few.

Fast, yes, but not yet "screening" fast. Enter, RapidFire – which combines high-speed sampling, ultrafast automated solid phase extraction (SPE), and powerful mass spectrometry data acquisition into a platform that fully integrates with Agilent LC/MS systems.

"RapidFire has really enabled that next quantum leap; now we are in the realm of maybe 30 seconds to carry out a complete native mass spectrometry run, including sample preparation, injection, and analysis," Dr. Ruotolo said. "That's getting comfortably into screening-tool territory, and it's a very information-rich way to screen. I mean, a typical screening query might output a simple yes/no answer: Is it binding? Is it inhibiting the enzyme? Here, we get information about binding stoichiometry, the degree to which the target molecule is stabilizing or destabilizing the protein, maybe even as granular as which regions are involved, and can we consider pursuing an engineering approach to modulate the interaction. It's paradigm shifting to think about the ways this might span chemistry, biology, biomolecular engineering, and beyond."

Given the innovative nature of his research, Dr. Ruotolo admits it hasn't been completely straightforward to bring RapidFire online in his lab. "There's been a fair amount of replumbing and repurposing involved in allowing us to apply it to the sort of experiments we're interested in," he said. "We approached Agilent with these crazy ideas, sat down with their RapidFire and LC/MS teams, and together we worked out what was needed to enable this nonstandard pairing. There were some teething problems, as you might expect, but the support was very strong. They were interested in what we were trying to do, and that interest has continued as we've progressed toward our current setup."

Meta-analysis: Pursuing the next "more"

Despite his team's successes in incorporating RapidFire into an information- and context-rich approach for mass spectrometry, Dr. Ruotolo is already looking toward the next challenge.

"One of the things RapidFire is really allowing us to do is start to ask questions from a big data perspective," he explained. "When you start generating data on this scale, what can you learn about the robustness and reproducibility of what you're seeing? To what level can you quantify things? What sort of error bars can you really place on these values? We just completed a study where we looked at these questions across data generated in different labs. RapidFire is going to allow us to do a lot more meta-analysis, and it's the kind of data pharmaceutical companies really want to see. It's going to help us understand the technology better, yes, and also help us leverage it into whole new spaces with broad implications – from chemistry and protein engineering, across the life sciences, and into agriculture, personalized medicine, and much more."

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RA45475.1135300926

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© Agilent Technologies, Inc. 2024
Published in the USA, July 25, 2024
5994-7634EN

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