Agilent case study: Asking Nuanced Questions

Agilent Collaborator Takes an Integrated, Multi-omics Approach to Metabolism

Adam Rosebrock was feeling unsettled. For years he had been using genomics to study what cells do when they do, well … what cells do. That is, which genes turn on or off as they go through cell division, for example, or various external insults.

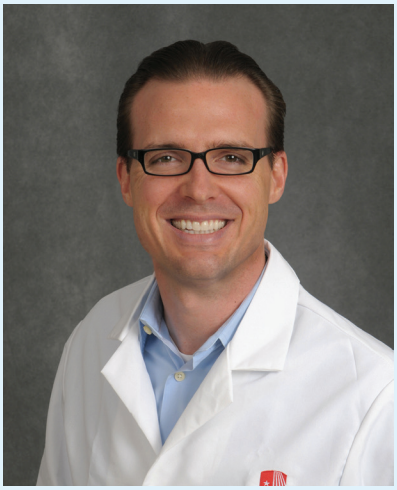
Simply put, he was not getting enough information. So Rosebrock, a classically trained microbiologist and biochemist, began to expand the scope of his investigations.

"My push into metabolomics and mass spectrometry was my unsettled feeling coming to a head," he says now. "I figured, if we wanted to measure what the cell was doing biochemically, we should just buckle down and measure metabolites directly."

He started to focus on measuring small molecules and the direct output of metabolism using what he calls "kick-butt mass spectrometers and fantastic liquid chromatographs" from Agilent.

One of the biggest shifts over the past few years, Rosebrock notes, is that scientists began to measure not just metabolite levels but reaction rates, or metabolic flux.

"What that has required is really a shift in instrumentation, from thinking of mass spectrometry for metabolites as being a triple quadrupole question to being an accurate mass question," he says. "My lab has been a proponent for accurate mass for a long time, and one of the reasons I'm really excited about Agilent's portfolio right now is the continued excellence and refinement of the mass spectrometry platform, especially the Agilent 6546 Q-TOF system."



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The platform is allowing Rosebrock to ask better, more nuanced questions.

"One of the most amazing ways that mass spectrometry has helped me as a biologist is getting insight into what's happening inside cells as they are healthy and as they are unhealthy, either by mutation or by environmental exposure, or by treatment with drugs or chemical agents," he says.

"For a long time, our phenotype, for geneticists in particular, was: Did my cell live or die? Or did my cell shrivel up and look really unhappy under the microscope? Those are broad descriptive terms. If the cell is dead, it can't really tell you anything else," Rosebrock notes. "Using mass spectrometry, though, we can start to ask about the biochemical state of the cell before the cell has really shut down and died. We can look at transitions between healthy cells and diseased cells. We can look at the changes inside the cell itself."

Rosebrock says that he was blown away by the ability of the 6546 to measure compounds across a wide dynamic range when the system was first introduced. Through continued refinement, he adds, the system is now especially adept at simultaneously measuring low abundance peaks and dealing with high-intensity signals.

"Using the 6546, we can measure, at a given retention time, not just one mass-to-charge ratio, but we get a really fantastic high-resolution view, a high-dynamic range view, of different co-eluting analytes," he says.

"We now have an easier time distinguishing crowded mass spectra and isolating individual isotopologues of our compounds in flux experiments using the 6546. Chromatography is still critical, but we have increased our biological feature counts and have better, more consistent, mass spectral peak shapes on the 6546. The system's increased resolution gives us confidence in individual mass spectral features as being isotopologues rather than some coeluting interference. This has enabled us to generate higher-quality flux data, higher quality isotope-labeling data, and be more confident in the results we generate."

Rosebrock's lab analyzes the data using homegrown software along with what he describes as the unsung hero of Agilent's metabolomics portfolio: MassHunter Profinder software.

"Cells maintain constant, homeostatic, levels of many metabolites across different environmental conditions and when faced with various intrinsic and extrinsic perturbations," he says. "The underlying biochemistry is anything but static, however. We are finding that cells drastically change the rates of metabolic reactions in order to keep metabolite concentrations stable."

To make matters even more complicated, scientists are also re-learning that metabolism is incredibly diversified and specialized.

"Even in our own bodies, there's very different metabolism that occurs across different tissues. Your muscles are metabolically different from your liver or your kidneys. Even you and I will have differences in our metabolic processes—and there is genetic variation that underlies why your liver might work differently than my liver does," Rosebrock says.

So he and his fellow research scientists are using everything they know and everything they can learn from microbiology, biochemistry, genetics, genomics, and metabolomics to understand the differences between individuals and populations. All in the hope of finding differences that can be exploited to selectively kill pathogens, or selectively kill tumors, while sparing the host.

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