THE AUTHOR FILE

Ruedi Aebersold

Supercharged SRM: synthetic peptides bring high-throughput assays to targeted proteomics.

Teaching a mass spectrometer to measure proteins in a biological sample via selected reaction monitoring (SRM) is a well-established approach, but validating the assay for any given protein is slow and tedious. On page 43, Ruedi Aebersold, of the ETH Zurich and the

University of Zurich in Switzerland, and colleagues, describe an inexpensive approach to accelerate the development of SRM assays for targeted proteomics.

Like a bloodhound trained to a particular scent, SRM assays can find specific proteins within a sample by

telling the mass spectrometer which signals to look for. Even though many SRM assays can be run simultaneously on the same machine, such studies have typically examined only small numbers of proteins because assay development is slow, says Aebersold. This is because the spectra required for SRM assays are acquired in a slow-scanning instrument from noisy biological samples.

Aebersold decided to do away with the noise. Instead of using biological samples, he and his team developed assays using chemically synthesized 'proteotypic' peptides, unambiguously representing each protein of interest.

For their key set of experiments, the team selected 816 such peptides representing 156 yeast kinases and phosphatases, which are essential for many biological functions but can be hard to detect because of their low abundance. Synthesizing purified peptides costs about \$500 per peptide, but Aebersold's team reasoned that even a crude synthetic mixture, which costs about \$5–\$10 per peptide, would still be purer than a biological sample. They generated unpurified mixtures of peptides, grouping them into pools of about 100 peptides each. With these mixtures, they were able to generate SRM assays at a rate of over 100 per hour.

To conduct an SRM assay, researchers use a triple quadrupole (QQQ) mass spectrometer. In terms of speed and sensitivity, these machines are a far cry from 'sequencing Ferraris' such as the Orbitrap, explains Aebersold. Orbitraps (from Thermo Scientific) can quickly generate lists of peptides in a sample, but the more peptides in a sample, the lower the chance that any particular peptide will be identified. A QQQ

machine, though slower, can hunt for particular peptides. Still, Aebersold reasoned, with a sufficient number of SRM assays, QQQ machines could become very powerful at finding significant numbers of proteins. "We were faced with using a sequencing Toyota to generate the fragment-ion spectra and turning this instrument into a targeting Ferrari."

To test how well their SRM assays worked, the researchers decided to race a QQQ against an Orbitrap typically used for shotgun studies to compare how many kinases and phosphatases the machines would identify in yeast lysates. The high-performance tandem mass spectrometer found seven, or less than 5% of the targeted proteins. The QQQ machine found 110, or just over 70%.

"This is, for proteomics standards, a stunning result," says Aebersold. Other researchers have identified greater numbers of kinases using shotgun proteomics, he says, but those studies required hundreds of mass spectrometer runs, along with the corresponding demands for machine time, sample and human effort. "We were really excited to get basically a similar number of proteins in one hour," says Aebersold.

Aebersold believes the assays will readily transfer to other laboratories: "We think the assays are also usable in other brands of the same type of MS, but they may need to be slightly optimized."

What's more, the SRM assays generated in Aebersold's lab should work for a variety of conditions. For example, SRM assays developed with chemically synthesized peptides could be used with isotope labeling techniques, which allow samples representing different biological conditions to be examined quantitatively. And using synthetic peptides could be particularly powerful for measuring post-translational modifications, says Aebersold, as finding such modified peptides is difficult in biological samples.

His lab has made validated SRM assays available for about 1,500 proteins. Researchers simply need to download MS settings that uniquely identify the targeted peptide. He expects to offer assays for the full yeast proteome in the near future.

Would it be possible to generate SRM assays for all of the estimated 100,000 human protein isoforms? That depends on whether the proteins can be made to generate peptides that will fly through a mass spectrometer, Aebersold says. "The number," he says, "is not that frightening."

Monya Baker

"This is, for proteomics standards, a stunning result."

Picotti, P. *et al.* High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nat. Methods* 7, 43–46 (2010).