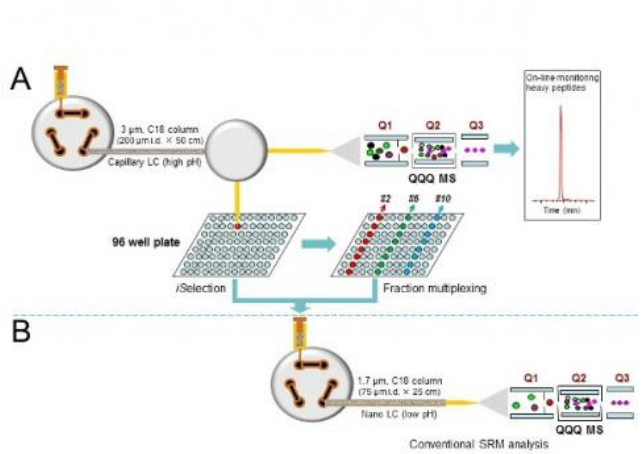
Mass spectrometry protein assays that match sensitivity of antibody-based clinical tests may speed drug discovery

3 September 2012



PNNL scientists developed a mass spectrometry-based technique called PRISM, illustrated here, to identify protein biomarkers associated with cancer and other diseases. The technique should be able to speed up development of protein-specific diagnostic tests and treatment.

(Phys.org)—Combining two well-established analytic techniques and adding a twist identifies proteins from blood with as much accuracy and sensitivity as the antibody-based tests used clinically, researchers report this week in *Proceedings of the National Academy of Sciences* Early Edition online. The technique should be able to speed up development of diagnostic tests and treatments based on proteins specific to certain diseases.

The team of scientists at the Department of Energy's Pacific Northwest National Laboratory found that their technique, called PRISM, performed as accurately as standard clinical tests known as ELISAs in a head-to-head comparison using blood samples from cancer patients. The tests measure biomarkers, proteins whose presence identifies a disease or condition.

"Clinical tests have almost always used antibodies to measure biomarkers, because antibodies can provide good sensitivity," said PNNL bioanalytical chemist Wei-Jun Qian, lead author on the study. "But it often takes a year and a half to develop antibodies as tools. Antibody development is one of the bottlenecks for new biomarker studies in disease and systems biology research."

Qian, Tujin Shi, Tom Fillmore and their PNNL colleagues worked out the highly sensitive PRISM using resources at DOE's EMSL, the Environmental Molecular Sciences Laboratory on PNNL's campus. The result is a simple and elegant integration of existing technologies that solves a long-standing problem.

The Competition

Researchers have long wanted to use mass spectrometry to identify proteins of interest within biological samples. Proteins are easy to detect with mass spec, but it lacks the sensitivity to detect rare proteins that exist in very low concentrations. Scientists use antibodies to detect those rare proteins, which work like a magnet pulling a nail out of a haystack.

Antibodies are immune system molecules that recognize proteins from foreign invaders and grab onto them, which allows researchers to pull their proteins of interest out of a larger volume, concentrating the proteins in the process. Because antibodies recognize only one or a couple of proteins, researchers have made treatments and tools out of them. Drugs whose generic names end in "-mab" are antibodies, for example.

For research purposes, the modern laboratory can produce antibodies for almost any protein. But that development process is expensive and time-consuming. If you have a new biomarker to explore, it can take longer than a year just to create an antibody tool to do so.

To get around the need for an antibody, Qian and the team concentrated the proteins in their samples another way. They used a common technique called high performance liquid chromatography, usually shortened to HPLC, to make the proteins about 100 times as concentrated as their initial sample. While an excellent step, they also had to find their protein of interest in their concentrated samples.

So they sent in a spy, a protein they could detect and whose presence would tell them if they found what they were looking for.

With a potential biomarker in mind, the team made a version that was atomically "heavier." They synthesized the protein using carbon and nitrogen atoms that contain extra neutrons. The unusual atoms added weight but didn't change any other characteristics. The heavier versions are twins of the lighter proteins found within the blood, cells, or samples. Although the twins behave similarly in the analytical instruments, the heavier twin is easily found among the sample's many proteins.

After adding the heavy version to the samples, the team sent the sample through the instrument to concentrate the proteins. The instrument spit out the sample, one concentrated fraction at a time. The fraction that contained the heavy biomarker was also the fraction that contained its twin, the lighter, natural protein. From this fraction, the team could quantify the protein.

Protein Spectrum

To prove they could use PRISM this way to find very rare proteins, the team spiked blood samples from women with a biomarker called prostate specific antigen, or PSA, that only men make. The team found they could measure PSA at concentrations about 50 picograms per milliliter. While typical of the sensitivity of ELISA tests, it represents about 100 times the sensitivity of conventional mass spectrometry methods.

"This is a breakthrough in sensitivity without using antibodies," said Qian.

Then they tested PSA in samples from male cancer patients and found PRISM performed as well as ELISA. Interestingly, PRISM measured three times the amount of PSA than the ELISA assay did. This result suggests that antibody-based ELISA tests fail to measure all of the forms of the biomarker. This is likely due to the fact that antibodies don't recognize all the different forms that proteins can take, Qian said, whereas PRISM measures the total amount of protein.

In addition to its sensitivity, PRISM requires only a very small sample of blood or serum from the patient. The team used only 2 microliters of the cancer patients' sample, a volume that would easily fit inside this small printed "o".

One drawback to the technique, however, is how many biological samples can be tested at once. Researchers want to test thousands, and antibody-based methods allow such high-throughput testing. But PRISM can only test several hundred samples per study. However, with the time researchers save not developing antibodies, the technique might still put them ahead in biomarker development.

For basic biology research, Qian said the method will be useful for studying biological pathways in cases where scientists need to accurately quantify multiple different proteins.

More information: Tujin Shi, Thomas L. Fillmore, Xuefei Sun, Rui Zhao, Athena A. Schepmoes, Mahmud Hossain, Fang Xie, Si Wu, Jong-Seo Kim, Nathan Jones, Ronald J. Moora, Ljiljana Paša-Toli?, Jacob Kagan, Karin D. Rodland, Tao Liu, Keqi Tang, David G. Camp II, Richard D. Smith, and Wei-Jun Qian, An antibody-free, targeted mass spectrometry approach for quantification of proteins at low pg/mL levels in human plasma/serum, *Proc Natl Acad Sci*, Early Edition online the week of September 3, 2012. DOI: 10.1073/pnas.1204366109

Provided by Pacific Northwest National Laboratory