A little pressure in proteomics analyses squeezes four hours into a minute

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Many coaches inspire better performance by pressuring their teams. Now, proteomics researchers are using pressure to improve the performance of their analyses. In a simple solution to a time-consuming problem, the researchers have found that adding pressure early in their protocol squeezes four hours of waiting into a minute.

"We were really happy to see how well it worked," said biochemist Daniel Lopez-Ferrer, a post-doctoral researcher at the Department of Energy's Pacific Northwest National Laboratory. "We're determining when and how to incorporate it into our analyses." Lopez-Ferrer and his colleagues reported their findings in July 8, 2008 *Journal of Proteome Research*.

Proteomics researchers learn about organisms by studying the proteins they make, and they want to be able to analyze large numbers of samples fast and sensitively. Cutting down the time-consuming steps would significantly increase the number of samples that could be analyzed. The first, slowest steps in most proteomics protocols requires at least four hours -- researchers often allow this to go overnight because of timing issues for the rest of the process. The team of chemists, biologists and physicists at PNNL investigated whether pressure would improve their process.

What takes so long is the breakdown of full-length proteins into smaller bits called peptides, which can be analyzed by measuring their individual masses. The most common way to break down proteins is to let an

enzyme eat through the bonds between the protein building blocks, or amino acids. Scientists have tried a variety of ways to help the enzyme digest these bonds faster, including using microwaves or ultrasound, with varying degrees of success.

Food processors have long been using high pressure to kill pathogens on food (think homemade preserves), and some evidence suggested that as pressure rises from low to high, some enzymes initially become more active before dying from the duress.

To determine if pressure would help in proteomics, the team used the protein albumin. The researchers incubated albumin with the enzyme trypsin at several different pressures for one minute each, then counted how many pieces into which trypsin cut albumin. At 10,000 pounds-per-square-inch up to 35,000 psi, trypsin appeared to maximally cleave albumin. For these tests, the team used facilities at the DOE's Environmental Molecular Sciences Laboratory on the PNNL campus.

The team then tested whether this process could be used for proteomics, in which large numbers of proteins are studied at once. So, they extracted the proteins from a bacterial culture and subjected half to the traditional overnight approach and half to 35 kpsi for one minute.

Overall, the resulting collection of peptides looked very similar between the two methods. The pressure method generated about 10 percent more unique peptides but cut slightly fewer total bonds than the traditional method. The researchers concluded that the benefit of time gained by the pressure method and the additional unique peptides outweighed the dip in total bonds.

Additionally, the team wondered how pressure sped up the enzyme digestion. To determine if pressure caused bacterial proteins to unfold and make their bonds more vulnerable to trypsin, the team turned to

myoglobin, a compact glob of a protein that packs a second, loose molecule inside. When they put myoglobin under 35 kpsi in the absence of trypsin, myoglobin lost its molecular parcel, suggesting that pressure opens up or denatures proteins, baring their bonds and giving trypsin more room to work.

Now, the PNNL researchers are integrating the pressurized digestion into their proteomics protocol where appropriate. In other work, Lopez-Ferrer and colleagues are testing whether ultrasound would also speed up this first step. They believe pressure and ultrasound might bring different advantages to the table of protein digestion, depending on the samples to be analyzed.

Source: Pacific Northwest National Laboratory

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