Researchers 'hammer' proteins

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A team of chemists, led by an ASU professor, has come up with an elegant method for cutting proteins into more manageable pieces for analysis. The method, which uses industrial fillers commonly found in paint and light, could significantly aid the development of bioanalysis tools that identify human remains – and might aid ushering in the age of personalized medicine.

A prototype sample preparation method uses ultraviolet light and titanium dioxide to cut proteins. It could be ideal for field devices and new microfluidic lab-on-chip devices designed to rapidly analyze minute amounts of biological samples. The method was detailed in the article "Cleavage of Peptides and Proteins Using Light Generated Radicals from Titanium Dioxide," in a recent issue of *Analytical Chemistry*.

Proteins are relatively large and complex molecules made up of hundreds of thousands of amino acids. Cutting them into smaller sections allows researchers to work with more manageable pieces for analysis.

Currently, cutting proteins is achieved by using special enzymes called proteases that sever the chains of proteins at well-known locations. The protease trypsin, for example, cuts proteins at the locations of the amino acids lysine and arginine. Analyzing the residual fragments can identify the original protein.

But enzymes are finicky, requiring tight control of temperature and acidity, and the process of enzymatic digestion can be time-consuming,

lasting from a matter of hours to days.

The new work was led by Mark Hayes, an ASU associate professor of chemistry and biochemistry. Researchers working with Hayes include his former student, Barbara Jones, and Matthew Vergne, David Bunk and Laurie Locascio, all of the National Institutes of Standards and Technology. Titanium dioxide is commonly used in paint as a white pigment, but it also is a photocatalyst, so when it is exposed to ultraviolet light its surface becomes highly oxidizing, converting nearby water molecules into hydroxyl radicals – a short-lived, highly reactive chemical species.

“We are basically taking semiconductor phenomena and semiconductor materials and getting elegant specificity in biochemical applications,” Hayes says. “It shouldn't happen, but it did.”

In the experiments, titanium dioxide coatings were applied to a variety of typical microanalysis devices, including microfluidic channels and silica beads in a microflow reactor. By shining strong UV light on the area, the presence of a protein solution creates a small cleavage zone of hydroxyl radicals that cut nearby proteins at the locations of the amino acid proline.

The result surprised the team.

“The hydroxyl free radical is one of the most reactive species known,” Hayes says. “It wanders around desperately trying to steal electrons from anything. To find specificity and having things break at this proline residue was just a stunning result.

“From a chemist's or biochemist's point of view, we never would have predicted it. People who are trying to break up peptides and proteins work very hard at getting specificity, and that is why the enzymes they

use are so valuable – because they provide an exact cleavage location. We are basically hitting these poor things with a hammer."

The titanium oxide technique offers several advantages over enzyme cleavage of proteins. It's not too sensitive to temperature or acidity, like enzymes are, and it needs no additional reagents other than dissolved oxygen in the solution. It is a simple arrangement, Hayes adds, easy to incorporate into a wide range of instruments and devices, and titanium dioxide will last virtually forever in a wide range of conditions.

Enzymes, however, have to be treated carefully and stored in temperature-controlled conditions.

The target amino acid proline is relatively sparse in most proteins but it's found at key locations such as sharp turns in the molecule that aid analysis. It's also fast. In tests with the protein angiotensin I, the researchers detected cleavage patterns in as little as 10 seconds.

Hayes said the team now will focus on determining the exact mechanism that allows it to cut proteins with high specificity. He adds that the process could lead to a wider range of devices to incorporate protein analysis in their operation.

"This development will help lead to more robust analysis systems," he says. "The method could lead to field portable devices that can be used in military applications and for homeland defense purposes. In health care, it could lead to personalized medicine based on biological fingerprinting, where each person has a molecular 'fingerprint' associated with their health state."

Source: ASU