SPECTROSCOPY

Optical proteomics

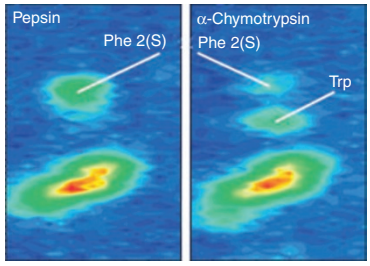
Researchers describe a method for protein identification and quantification based on electron-vibration-vibration two-dimensional infrared spectroscopy.

Before mass spectrometry-based protein identification became routine, researchers relied on the biochemical analysis of amino acid composition. David Klug of Imperial College London recalls looking into the various methods that people used in the early 1990s to obtain what we would now call 'proteomic' information. "I was surprised to discover that spectroscopy played no role whatsoever," he says.

Klug began to pursue an appropriate optical platform for protein detection and identification that would be convenient and complementary to other methods. Infrared (IR) spectroscopy, which works by detecting vibrational signatures of covalent bonds, is widely used in organic chemistry for molecular identification. However, unlike mass spectrometry and nuclear magnetic resonance spectroscopy, which had been also originally developed for small-molecules but have since been widely applied for protein analysis as well, there had been no efforts to extend IR spectroscopy in a similar manner.

One of the limitations was that a linear IR spectrum is quite congested. However, akin to how two-dimensional nuclear magnetic resonance spectroscopy methods made protein-structure analysis possible, Klug realized that two-dimensional methods could also facilitate IR-based analysis of proteins (2DIR). "The trouble with a linear IR spectrum is it falls underneath a whole bunch of other IR features. When you spread that out over multiple dimensions, the spectra are a whole lot more sparse, and so you can really start to see things which otherwise are simply hidden," he explains.

The result is a two-dimensional map of coupled molecular vibrations. "If you excite a molecular vibration with a short IR laser pulse, you can wait awhile and see if that vibrational energy arrives at another specific vibration nearby," explains Klug. "If it does, by definition, those two are coupled." Klug and his colleagues added another twist (first developed by John Wright at the University of Wisconsin): instead of reading out the information with an IR beam, they scatter a visible light beam, which allows detection of photons in the visible region of the spectrum. Visible photons are much easier to detect and thus improve the sensitivity. This variation on the technique is called electron-vibration-vibration (EVV) 2DIR.



EVV 2DIR spectra for pepsin and α-chymotrypsin. Phenylalanine and tryptophan amino acid cross-peaks are marked; the lower, more intense peak represents the methyl group. Copyright 2008, National Academy of Sciences, USA.

About a year ago, Klug and his colleagues showed that EVV 2DIR could be used to detect and quantify amino acid content in short peptides (Fournier *et al.*, 2008a). Now, they have extended the technique to distinguish proteins (Fournier *et al.*, 2008b).

Like the biochemical methods of old, protein identification by 2DIR is based on amino acid composition analysis. If you can determine the amino acid composition of a protein, you can compare it to a database of protein sequences and in almost all cases unambiguously identify the protein. Klug and his colleagues identified specific 2DIR features corresponding to three amino acids: tyrosine, phenylalanine and tryptophan. The methyl group peak serves as an internal reference for quantification. Though the work is still in a preliminary stage, the researchers showed that by monitoring the ratios of just three amino acids, they could distinguish 39 out of 45 protein pairs.

By monitoring the ratios of just five amino acids, the researchers report that they should be able to unambiguously identify 44% of the (unmodified) human proteome. With 6–9 amino acids, they project that they could identify as much as 90%. Though they have not tested this yet, Klug notes: "We've calculated the 2DIR spectrum of nearly all 20 amino acids now, so that gives us an idea of where to start looking, … but it may take us a bit of time to sift through and find good robust features that we can use reliably."

Though EVV 2DIR is very rapid and quantitative, Klug is not highly optimistic about its potential for protein identification in mixtures, for what mass spectrometry is used in modern proteomics. What it could be very useful for, however, is the quantification of post-translational modifications. The researchers have so far only done some preliminary work detecting phosphotyrosine, but Klug believes that the technique could be extended to a variety of post-translational modifications with distinct 2DIR spectra.

Klug stresses that these are still early days for this intriguing application of 2DIR, but he plans to see where he can push the limits of the technology. "We really have to work with our colleagues in more conventional proteomics to try and identify those areas where the method is most likely to be complementary to existing tools," he says. "We could develop the tool in all sorts of different directions now, and we just want to make sure we choose those directions sensibly."

Allison Doerr

RESEARCH PAPERS

Fournier, F. *et al.* Optical fingerprinting of peptides using two-dimensional infrared spectroscopy: proof of principle. *Anal. Biochem.* **374**, 358–365 (2008a).

Fournier, F. *et al.* Protein identification and quantification by two-dimensional infrared spectroscopy: implications for an all-optical proteomic platform. *Proc. Natl. Acad. Sci. USA* **105**, 15352–15357 (2008b).