Researchers can count on improved proteomics method

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Princeton University's Martin Wühr has improved upon his method to accurately count the proteins present in a cell under different circumstances. 'The TMTc+ method is in a kind of sweet spot compared to the other methods [of isobaric tagging],' Wühr says. 'It provides superb measurement accuracy and precision, it's at least as sensitive as any other method, and it's compatible with around ten times more mass spectrometers than TMT-MS3.' Credit: Martin Wühr, Princeton University Department of Molecular Biology

Every cell in the body contains thousands of different protein molecules and they can change this composition whenever they are induced to perform a particular task or convert into a different cell type. Understanding how cells function depends on proteomics, the ability to measure all of the changes in a cell's protein components.

In a recent paper published in the journal *Analytical Chemistry*, Martin Wühr and colleagues in Princeton University's Department of Molecular Biology described an improved method to accurately count the proteins present in a cell under different circumstances.

The basic tool for counting proteins is a machine called a mass spectrometer. Cell samples can be run through this type of instrument one at a time, but this is laborious and it can be difficult to detect any changes between different samples. An alternative approach is to label all of the proteins in a particular sample with a unique "isobaric" tag. Multiple samples—up to 11—can then be mixed together and run through the mass spectrometer at the same time, with the isobaric tag functioning as an identifying barcode that tells the researcher which sample the protein originally came from. This speeds things up and makes it easier to quantify any changes in the protein composition of different samples.

"However, with the simplest version of isobaric tagging, known as TMT-MS2, there are major difficulties in distinguishing real signals from background noise," Wühr explains. "That makes the readouts unreliable and only semi-quantitative."

A more complex version of isobaric tagging, called TMT-MS3, can improve this signal-to-noise problem, but it is slower and less sensitive. Moreover, it relies on a much more expensive type of mass spectrometer

beyond the reach of most researchers.

While he was a postdoc at Harvard University, Wühr developed a different approach to isobaric tagging that solved the signal-to-noise problem while remaining compatible with cheaper, widely available mass spectrometers. But the technique—known as TMTc—was not without its own problems, particularly a lack of precision that made it hard to obtain consistent results.

In their recent *Analytical Chemistry* paper, Wühr and two of his graduate students, Matthew Sonnett and Eyan Yeung, described an improved version of TMTc that they named TMTc+. By changing how the cell samples are prepared and altering the computer algorithm that extracts data from the mass spectrometer, Wühr and colleagues were able to address many of the limitations associated with the various methods of isobaric tagging.

"The TMTc+ method is in a kind of sweet spot compared to the other methods," Wühr says. "It provides superb measurement accuracy and precision, it's at least as sensitive as any other method, and it's compatible with around ten times more mass spectrometers than TMT-MS3."

Naturally, Wühr says, there is still room for improvement. TMTc+ only allows a maximum of 5 samples to be run at the same time, and the detection of proteins in these samples is relatively inefficient. Both of these problems can be solved by developing new types of isobaric tags. "We have to explore the chemical space of these tags and find ones that work really well," Wühr says. "To this end, we have started a collaboration with the Carell group, organic chemistry experts at the LMU Munich, and already published a proof of principle paper. Eventually, these efforts should lead to an approach that will allow researchers to count every protein in a cell as it changes its form and

function."

More information: Matthew Sonnett et al, Accurate, Sensitive, and Precise Multiplexed Proteomics Using the Complement Reporter Ion Cluster, *Analytical Chemistry* (2018). DOI: 10.1021/acs.analchem.7b04713

Michael Stadlmeier et al. A Sulfoxide-Based Isobaric Labelling Reagent for Accurate Quantitative Mass Spectrometry, *Angewandte Chemie International Edition* (2018). DOI: 10.1002/anie.201708867

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