Weighing the Proteasome for Covalent Modifications

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Posttranslational modifications (PTMs) control protein function, but established peptide-based proteomic methods often fail to provide a comprehensive view of PTMs. In this issue of *Chemistry & Biology*, Gersch et al. describe an efficient combination of chromatographic separation and top-down mass spectrometry that together with an intuitive visualization tool allowed them to screen the proteasome for PTMs and covalently binding inhibitors.

Most proteins contain posttranslational modifications (PTMs) that are essential for their biological function. Given this importance, analytical methods developed to detect such modifications have become increasingly important. Among these methods, mass spectrometry (MS)-based proteomics has emerged as the most powerful approach due to its sensitivity, lack of bias toward certain proteins, and high-throughput capabilities (Mann and Jensen, 2003; Witze et al., 2007). However, well-established, routinely used proteomics methods based on tryptic digest of proteins followed by LC-MS/MS-analysis of the resulting peptides (known as bottom up proteomics) do not allow for comprehensive accounting of all PTMs due to several reasons. In reality, not all resulting peptide fragments will be found again, leading to incomplete sequence coverage. Another drawback is that peptides may not be specific for individual protein species or even proteins. Information about complex combinations of PTMs (e.g., on histones) and their relation to each other is also lost because they occur on disparate peptides. Moreover, some PTMs, especially phosphorylation, are cleaved more easily than the peptide backbone by commonly used fragmentation techniques (collision-induced dissociation), hindering identification of the site of modification. In order to overcome these limitations, intact protein mass spectrometry and top-down proteomics have been developed in which the mass of the intact protein and its fragments is determined with high-resolution mass spectrometry. For this purpose, FT-ICR-MS (Fourier-transform ion cyclotron resonance) instruments are commonly used, which, depending on their magnetic field, allow mass differences of < 1 ppm (part per million; e.g., < 0.02 Da for a 20 kDa protein) to be measured and, due to complete sequence coverage, individual protein species to be fully characterized.

The proteasome has been a natural testing ground for such MS-based methods in the search of PTMs (Lakshmanan et al., 2014, Loo et al., 2005, Sharon et al., 2007). On one hand, it is of special interest because of its high biological and clinical relevance as the most important player in protein breakdown in the cell; on the other hand, it represents an analytical challenge of considerable complexity featuring various catalytic and noncatalytic subunits, which are assembled differently depending on the disease state of the cell and numerous PTMs, such as Ser/Thr phosphorylation, N-terminal acetylation, and N-terminal truncation. This results in sample heterogeneity, which has imposed limitations on the analysis of samples and resulting data.

Sieber and coworkers (Gersch et al., 2015) present a straightforward analytical platform for PTM analysis of protein complexes, in which reversed-phase chromatography that allowed subunit separation, and concomitant FT-ICR-MS analysis were integrated to an intact protein LC-MS workflow. They devised a new data analysis tool called RoWinPro (rolling window spectral deconvolution of intact protein mass spectrometry datasets), which provides an automated analysis of the sets of scans over the complete run and very nicely visualizes shifts in retention time and mass resulting from modification of protein subunits in a 2D map. With this combination of techniques, Gersch et al., (2015) achieved several interesting findings.

The separation of protein subunits and subsequent HR-MS allowed for the investigation of the phosphorylation pattern of the proteasome subunits. The authors could confirm that the α7 phosphorylation is the only phosphorylation site in the yeast and human 20S proteasomes.

In a second line of research, Gersch et al., (2015) used their technique to study the interaction of the proteasome inhibitor carfilzomib, which is a clinically used anticancer drug, with the catalytic subunits of the proteasome and identify its covalent interaction points (Figure 1). While top-down MS has been used for studying PTMs before, it has been done less so for covalent modification by small molecule inhibitors. Considering the increasing interest in activity-based protein profiling (ABPP) and the renaissance in covalently binding drugs, this analytical tool will be of tremendous value for such research.

In summary, Gersch et al. (2015) have presented a very efficient analytical platform that allowed them to obtain a comprehensive view of covalent modifications of the subunits of the proteasome. Their tool should be of great help for research in posttranslational modification and covalent drug actions, especially in the context of protein complexes.

We expect that the recent developments will greatly increase the implementation of intact protein mass spectrometry and top-down proteomics in the scientific community. On the one hand, very sensitive, very high-resolution mass spectrometers have become available that allow the detection of the broad isotopic patterns of high-molecular-weight

molecules, such as intact proteins, next to small mass shifts introduced by PTMs (e.g., a disulfide bridge causes a mass difference of −2 Da). On the other hand, intact protein separation methods have been improved, which increases the sensitivity of the technique and allows top down profiling of complex proteomes (Catherman et al., 2014). Furthermore, suitable software for processing of top down proteomic data, such as the first software, ProSight PTM, developed and further improved by Kelleher and coworkers for top down LC-MS/MS data (Taylor et al., 2003, LeDuc et al., 2004, Zamdborg et al., 2007, Tran et al., 2011, Durbin et al., 2014), or the RoWinPro tool introduced by Gersch et al. (2015) for intact protein LC-MS data is now available. Together, both recent software and method development are pushing the limits of what can be analyzed and will likely lead to new functional insights.

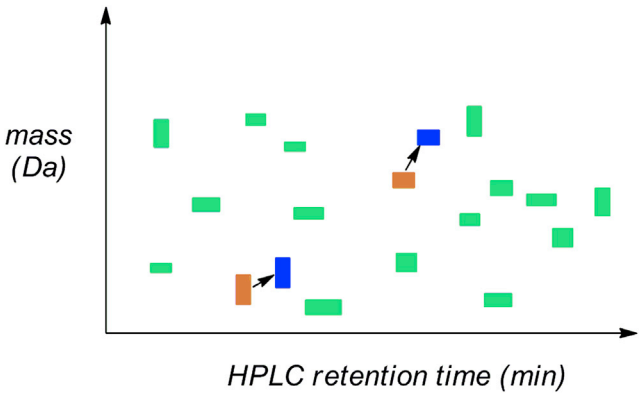

Figure 1. Visualization of Covalent Modification with Sample Maps
Green spots, protein subunits unaffected by covalent modification; orange spot, protein subunit in the absence of covalent inhibitor; blue spot, protein subunit after incubation with covalent inhibitor.

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