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Commentary

Ultraviolet photodissociation enhances top-down mass spectrometry as demonstrated on green fluorescent protein variants

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Ultraviolet photodissociation (UVPD) is a compelling fragmentation technique with great potential to enhance proteomics generally and top-down MS specifically. In this issue, Cannon et al. (Proteomics 2014, *14*, 1165–1173) use UVPD to perform top-down MS on several sequence variants of green fluorescent protein and compare the results to CID, higher energy collision induced dissociation, and electron transfer dissociation. As compared to the other techniques UVPD produces a wider variety of fragment ion types that are relatively evenly distributed across the protein sequences. Overall, their results demonstrate enhanced sequence coverage and higher confidence in sequence assignment via UVPD MS. Based on these and other recent results UVPD is certain to become an increasingly widespread and valuable tool for top-down proteomics.

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Top-down MS analyzes intact proteins without digestion. There are multiple advantages to top-down MS, including the ability to access critical information that is lost by bottom-up methods such as: (1) detecting variations in protein sequence due alternative splicing (splice variants); (2) discriminating proteins arising from closely related genes (sequence variants); (3) identifying protein sequence variations within a population (mutations); (4) detecting postprocessing events (e.g. propeptide cleavage), and (5) measuring how combinations of posttranslational modifications coexist on single-protein molecules. Nonetheless, top-down proteomics remains far less common than bottom-up proteomics. This is due to multiple inherent challenges. One improvement that has gradually enhanced top down proteomics is increasingly fast and efficient fragmentation mechanisms that provide uniform and progressively more complete sequence coverage for intact proteins.

In this issue, Cannon et al. [1] use ultraviolet photodissociation (UVPD) [2–4] to perform top-down MS on sequence variants of green fluorescent protein (GFP) and compare the results to those from CID, higher energy collisional-induced dissociation (HCD), and electron transfer dissociation (ETD). UVPD has been used in various forms (wavelength, power, time, MS platform etc.) and for various purposes as early as 1984 [3–5]. In the past few years (starting c.a. 2010) the utility of UVPD for peptide fragmentation has become increasingly clear as the method has been refined for these purposes and implemented in linear ion trap and TOF instruments. UVPD, in the implementation used by Cannon et al., uses

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Abbreviations: **ECD**, electron capture dissociation; **ETD**, electron transfer dissociation; **GFP**, green fluorescent protein; **HCD**, higher energy collision induced dissociation; **UVPD**, ultraviolet photodissociation

a 193 nm ultraviolet laser to fragment the proteins with a single 5 ns pulse, an appealingly short time for duty cycle limited applications. The most attractive property of UVPD, as shown by their data, is its ability to produce x, y, z, a, b, and c ions simultaneously with similar yields for each ion type. This is compared to CID/HCD that mostly generates b ions and y ions and ECD/ETD that yields mostly c ions and z ions. Therefore, for one fragmentation site (bridging adjacent amino acid residues) there may be multiple fragment ions generated and identified by UVPD and this could greatly improve the confidence of assignment. Multiple fragment ions types may also yield higher sequence coverage if they are complementary. The Brodbelt group has previously applied UVPD to both bottom-up and top-down problems [2, 6]. In their previous bottom-up work, UVPD was shown to have similar efficiency to CID under comparable conditions for peptides from BSA and HT-1080 cell lysates. The UVPD data yielded higher peptide Xcorr scores and sometimes more identified peptides. In their previous top-down analysis of ubiquitin and myoglobin (common proteins used as analytical standards), UVPD showed significantly higher sequence coverage than CID, HCD, and ETD under similar conditions. Very recently the use of UVPD in an on-line top-down LC-MS method has been reported by some of the same authors, implying that the qualities shown in the current article scale well to fewer coadded spectra and shorter acquisition times [7].

The current work further extends the application of UVPD to the top-down analysis of larger and less standard proteins, five sequence variants (four mutants and wild type) of GFP, and makes a more complete comparison to other fragmentation methods. The samples were infused and 500 scans (for all fragmentation techniques) were averaged to achieve an impressive number of fragment ions and enable a thorough comparison of the fragmentation techniques. The results show, at least for this set of proteins that UVPD yields more confident assignments and often more complete sequence coverage than other techniques. With the extensive sequence coverage demonstrated by UVPD, combined with a very quick fragmentation time, the authors effectively demonstrate that UVPD is indubitably a powerful tool for top-down MS with a bright future.

Looking forward there remain some questions yet to be resolved for UVPD. First, UVPD also yields some less useful side-chain loss ions (v ions, w ions, etc.) [2]. These ions complicate the spectra and necessarily reduce the abundance of corresponding fragment ions without side-chain loss. More fragment types and more overall fragments complicate data analysis and have the potential to increase false positive rates in more complex unknown samples. Thus far, this issue appears to be outweighed by the utility of multiple fragment ion types. In our examination of their data, the presence of multiple inherently coupled fragment ions per amino acid residue appears likely to actually increase confidence in the correct sequence. Finally, this sort of quality result has garnered the interest of others to implement and utilize UVPD. As this technology becomes more widespread we are certain to learn more about this exciting capability.

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