

## Illuminating Proteins: Dual UV/IR Photodissociation in a custom FT-ICR system

Top-down mass spectrometry (TDMS) elucidates the molecular weight, characterizes proteoforms, and, in its native form, can even inform binding stoichiometry and higher-order structure of proteins and their complexes. The information gained is, however, largely dependent on the protein system in question and the dissociation technique used. Therefore, there is a great need for more efficient and informative fragmentation methods to complement continued improvements in mass analyzers. These are together essential for extending the top-down MS to larger and more diverse assemblies. Photodissociation approaches, such as infrared multiple photon dissociation (IRMPD) and ultraviolet photodissociation (UVPD), coupled with ultra-high-resolution MS, offer interesting avenues for multi-modal fragmentation schemes with high information content.

In this contribution, following up on the pioneering work by Halim et al. (JASMS 2016) we explore parallel 193 nm UVPD and 10.6  $\mu\text{m}$  IR laser activation applied to proteins using a custom laser-coupled 15-Tesla FT-ICR mass spectrometer. A rich and balanced fragmentation array of a/x, b/y, and z ions is produced when intact ubiquitin is exposed to UV and IR lasers simultaneously or sequentially in a single MS/MS experiment. Internal fragment inclusion in data processing further increases identified fragment numbers and improves average sequence coverage for denatured proteins. Our initial results demonstrate that the use of mixed photodissociation provides benefits for comprehensive protein characterization of denatured proteoforms and offers great promise for future native TDMS, especially when combined into multi-modal fragmentation schemes with other dissociation techniques available in an FT-ICR.

### User consent

yes

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