Enhancing Ultraviolet Photodissociation Performance on a Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer for Small Molecule and Protein Analysis

Dustin D. Holden, Jae C. Schwartz, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA, USA, 95134

ABSTRACT

Purpose: Enhance the performance of 213 nm ultraviolet photodissociation for small molecules and proteins.

Methods: Full length trapping and product ion parking within the low pressure ion trap of a Thermo Scientific™ Orbitrap Fusion™ Lumos™ mass spectrometer platform.

Results: Increased photodissociation rate for high AGC targets, and reduced fragment ion over-dissociation.

INTRODUCTION

Ultraviolet photodissociation (UVPD) has been demonstrated to be a useful tool for the analysis of small molecules, lipids, peptides, and proteins. UVPD has been useful to extensively characterize protein sequences due to its unique ability to cleave the peptide backbone into all six main ion types (a, b, c, x, y, and z).[1] Along with last year's release of UVPD as a new hardware option for the Orbitrap Fusion Lumos Tribrid Mass Spectrometer, Mullen et al. presented the performance characteristics of UVPD and top-down utility using the 5th harmonic of a Nd:YAG laser.[2] In addition, Huguet et al. presented the utility of UVPD for small molecule structure determination.[3] This presentation represents some of the directions we are taking to enhance the current performance of UVPD on the Orbitrap Fusion Lumos Tribrid mass spectrometer for small molecule and top-down applications.

MATERIALS AND METHODS

Samples Utilized

Caffeine and MRFA from Thermo Scientific™ Pierce™ LTQ Velos ESI Positive Ion Calibration Solution. Angiotensin I from human, Sigma-Aldrich A9650. Insulin human recombinant, Sigma-Aldrich 91077C. Ubiquitin from bovine erythrocytes, Sigma-Aldrich U6253. Cytochrome c from equine heart, Sigma-Aldrich C2506. Apomyoglobin from equine heart, Sigma-Aldrich M1882. Carbonic Anhydrase II from bovine erythrocytes, Sigma-Aldrich C2522.

Test Method(s)

Samples, except for Caffeine and MRFA, were diluted to 1 pmol/µL in 50/50 MeOH/$H_2$0 w/ 0.1% Formic Acid and directly infused through ESI at 3 µL/min to a Orbitrap Fusion Lumos Tribrid MS equipped with UVPD (**Figure 1**). Scan parameters for calculating sequence coverages were 1e6 precursor AGC target, 120K OT resolution, 50 µScans averaged for insulin, ubiquitin, and cytochrome c, and 100 µScans averaged for carbonic anhydrase II. Analysis was performed in standard mode (IRM = 8.0 mTorr) for all experiments except for carbonic anhydrase II, which was performed in intact mode (IRM = 3.0 mTorr).

Data Analysis

MS/MS spectra were deconvoluted using the Xtract™ algorithm with a S/N threshold of 3, and searched against their respective sequence using ProSight Lite[4] with a 10 ppm mass tolerance.

Figure 1. Orbitrap Fusion Lumos Tribrid mass spectrometer equipped with UVPD. Shown is the assembly flange, laser head, steering optics, and vacuum interface window which are all behind the side cover.

Laser Description

The laser included with the UVPD hardware option is a Q-switched Nd:YAG laser (CryLaS GmbH) outputting the 5th harmonic at 213 nm. The laser pulse characteristics and relative size can be seen in **Table 1** and **Figure 2**, respectively. Photoactivation occurs in the low pressure trap (LPT) of the dual-pressure quadrupolar linear ion trap, while m/z analysis can occur in either the linear trap or the Orbitrap mass analyzer.

Table 1. Laser Characteristics

	Reference Value	Unit
Output Power (quasi cw)	3.75 ±0.5	mW
Pulse Energy	1.5 ±0.2	µJ
Peak Power	1.5	kW
Pulse Rep Rate	2.5	kHz
Pulse Width	< 1	nsec
Beam Dia	450 ±200	µm

Figure 2. CryLaS 213 nm Nd:YAG laser

RESULTS

Full Length Trapping

During a UVPD experiment, ions are initially transferred from the source to the LPT, where they are trapped in the center section and photoactivated by a laser beam as shown in **Figure 3A**, and then can be transferred to the Orbitrap analyzer for mass analysis. Since the LPT has an ion storage capacity allowing over a million charges to be trapped, it is possible to increase the signal to noise (S/N) of high resolution Orbitrap spectra by using the largest number of ions possible. This ability benefits experiments involving species that produce complex spectra or informative, low abundance fragments. Yet, at high AGC targets, coulombic repulsion of neighboring ions can cause a decrease in radial overlap of the ion cloud with the laser beam (**Figure 2A**). To avoid this issue, rather than sequester ions in the center section of the LPT during UVPD, a trapping volume gain of ~64% is possible by allowing ions to occupy the full length of the LPT as shown in **Figure 3B**.

Figure 3. LPT photoactivation scheme during A) conventional center section trapping, and B) full length trapping accomplished by leveling the DC offset of all trap sections while raising both end lens trapping potentials.

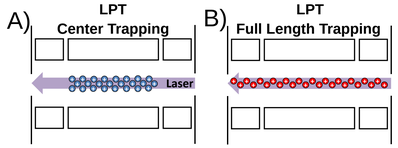
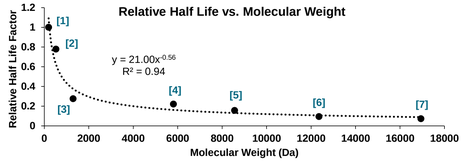
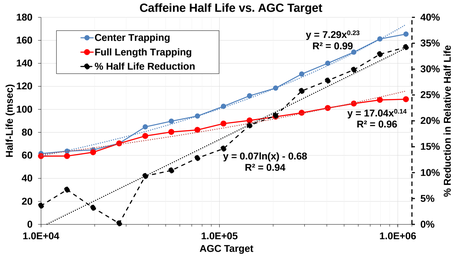


Figure 4. Plot of UVPD half life versus molecular weight relative to the half life of caffeine (195 Da) [1]. Relative half life factors are represented for MRFA peptide (524 Da) [2], angiotensin I (1297 Da) [3], insulin (5.8 kDa) [4], ubiquitin (8.6 kDa) [5], cytochrome c (12.4 kDa) [6], and apo-myoglobin (16.7 kDa) [7].



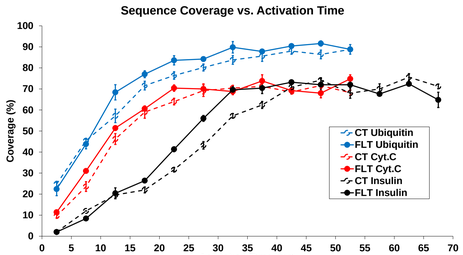
Half life, the amount of time required to reduce the precursor intensity by 50%, is a useful measurement for assessing laser alignment and photodissociation rate. Half lives were measured using both center and full length trapping schemes (**Figure 3**) for AGC targets ranging from 1e4 to 1e6 for the seven species shown in **Figure 4**, and normalized to caffeine half life values. There appeared to be an exponential decrease in relative half life with increasing molecular weight (**Figure 4**). Half life versus AGC target for caffeine is shown in **Figure 5** with nearly a 35% decrease in half life when using the full length trapping scheme at a 1e6 AGC target. Although not shown, similar % half life reduction values were observed for all of the other seven species represented in **Figure 4**.

Figure 5. UVPD half life values for caffeine using center vs full length trapping. The percent half life reduction utilizing full length trapping, relative to center trapping, for each AGC target is also shown. Trend lines for each data series are shown as dotted lines.



Since up to a ~35% decrease in half life at 1e6 charges is possible using full length trapping, a decreased activation time is expected to reach the optimal fragment signal to noise. Although not a direct measurement of fragment S/N, % protein sequence coverage calculations represent the bulk amount of identifiable fragments above a certain S/N threshold. In **Figure 6** sequence coverage values for the large peptide insulin (5.8 kDa), the small protein ubiquitin (8.6 kDa), and medium sized protein cytochrome c (12.4 Da) are shown for various activation times.

Figure 6. Sequence coverage values for ubiquitin (blue), cytochrome c (red), and insulin (black), comparing full length trapping (FLT), versus conventional center trapping (CT), during UVPD. Error bars represent standard deviation for five technical replicates.



In general, to reach maximum coverage, a ~20% decrease in activation time was observed for these three species using full length trapping versus conventional center trapping. Note, the percent decrease in activation time would be more dramatic for smaller species which generally require longer activation times. Despite this, ongoing research is underway to continue to increase the photodissociation rate even further.

RESULTS

Product Ion Parking

Early ion parking experiments were conducted by McLuckey et al. to essentially "park" a product species by reducing its reaction rate with the reactant through controlled resonance excitation using supplemental AC.[5] Weisbrod et al. applied the concept of ion parking to UVPD performed in a linear ion trap using a waveform to simultaneously excite multiple fragment ion species away from the direct path of a laser beam.[6] A comparison between conventional UVPD and UVPD with product ion parking is depicted in **Figure 7**. The following results represent ongoing development of UVPD product ion parking as a future tool for the Fusion Lumos Tribrid mass spectrometer.

Figure 7. UVPD performed in the LPT using A) conventional center trapping, and B) product ion parking with dipolar waveform resonance excitation applied. Parked fragment ions are represented as red ions. An example waveform frequency spectrum is shown with amplitudes in red.

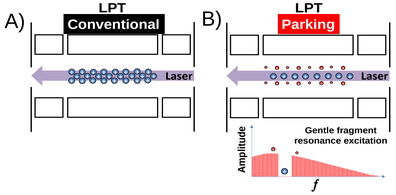
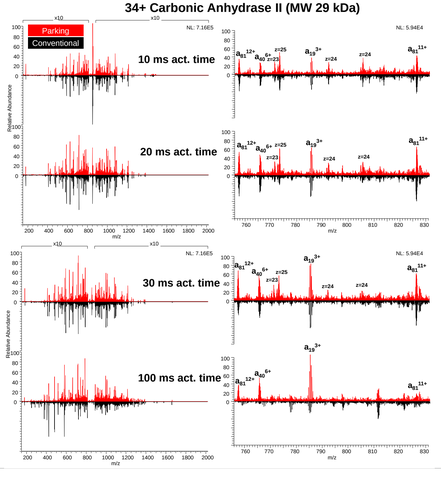
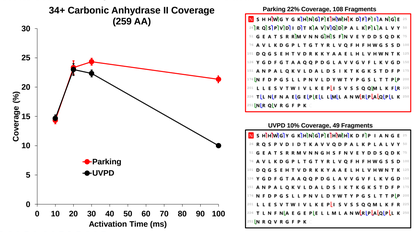


Figure 8. Butterfly spectra with similar scaling collected at various UVPD activation times of the isolated 34+ precursor ion of carbonic anhydrase II. Spectra collected with product ion parking is shown in red, with conventional UVPD spectra shown in black. On the right are zoomed sections (m/z 756-831) of each neighboring spectra.



Conventional and product ion parking UVPD spectra are shown in **Figure 8**. Product ion parking reduces the rate at which fragment ions undergo further dissociation that would otherwise occur, especially at extended UVPD activation times. Sequence coverages between conventional UVPD and UVPD with ion parking were comparable up to 100 ms activation times as shown in **Figure 9**. At an extended activation time of 100 ms, it is obvious that product ion parking preserves sequence coverage near the maximum value.

Figure 9. UVPD sequence coverage for 34+ carbonic anhydrase II while applying product ion parking (red) vs conventional UVPD (black). Sequence coverage maps are shown on the right for results obtained using either method at 100 ms activation time.



CONCLUSIONS

- UVPD half life decreases exponentially with sample molecular weight.
- Allowing ions to occupy the full length of the LPT (full length trapping) during UVPD provides a gain of ~64% in trapping volume over the conventional center trapping method.
- Full length trapping reduces precursor half life up to 34% at a 1e6 AGC target, allowing quicker photodissociation rates at high AGC targets.
- UVPD product ion parking reduces the rate of fragment ion over-dissociation, allowing a wider window of activation times to be used while retaining maximum spectral information.

REFERENCES

1. Brodbelt JS. "Ion Activation for Peptides and Proteins" Anal Chem 2016;88:30-51.
2. Mullen C, Weisbrod CR, Zhuk E, Huguet R, Schwartz JC. "Implementation of 213 nm Ultra Violet Photo Dissociation (UVPD) on a Modified Orbitrap Fusion Lumos" ASMS 2017; TP 397.
3. Huguet R, Sharma S, Mullen C, Canterbury J, Berhow M, Zabrouskov V, Stratton TJ. "UVPD as a Unique Fragmentation Tool for Complete Structure Determination and Substructure Identification of Small Molecules" ASMS 2017; TP 703.
4. Fellers RT, Greer JB, Early BP, Yu X, LeDuc, RD, Kelleher NL. "ProSight Lite: Graphical software to analyze top-down mass spectrometry data" Proteomics 2015: Feb 26;15(7):1235-1238.
5. McLuckey SA, Reid GE, Wells JM. "Ion Parking during Ion/Ion Reactions in Electrodynamic Ion Traps" Anal Chem 2002;74:336-346.
6. Weisbrod CR, Schwartz JC, Syka JEP. "Reducing Overfragmentation in Ultraviolet Photodissociation" U.S. Patent Application No. 2016/0358766 A1. 2016.

TRADEMARKS/LICENSING

© 2018 Thermo Fisher Scientific Inc. All rights reserved. ProSight is a trademark of Proteinaceous, Inc. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

Thermo Fisher SCIENTIFIC

PO65215-EN 0518D