Implementation of 213 nm Ultra Violet Photodissociation (UVPD) on a Modified Orbitrap Fusion Lumos

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ABSTRACT

Details of the implementation of UVPD using a 213 nm laser into the Thermo Scientific™ Orbitrap Fusion™ Lumos™ platform are presented, along with results pertinent to top down proteomic experiments.

INTRODUCTION

UVPD has been demonstrated to be a useful tool for the analysis of peptides and proteins. Numerous studies have concluded that UVPD promotes all three types of backbone cleavages (resulting in a,b,c,x,y, and z type ions), and that UVPD preserves labile PTMs during activation such as phosphorylation, sulfation, and glycosylation.[1] While its application to the study of large polypeptides and proteins is relatively new, interest in the technique is rapidly increasing due to the sequence coverage provided, advances in proteome fractionation, and top-down informatics.[2,3] Last year Weisbrod et. al. presented the performance characteristics of UVPD using the 5th harmonic of a Nd:YAG laser.[4] This presentation is a continuation of that initial work and describes in detail the implementation of the 213 nm laser on a modified Orbitrap Fusion Lumos instrument including the performance related to top down protein sequencing.

MATERIALS AND METHODS

Sample Preparation

Apomyoglobin from equine skeletal muscle, lyophilized powder, Sigma-Aldrich A8673. 1 pmol/µL in 50/50 MeOH/H20 w/ 0.1% Formic Acid.

Carbonic Anhydrase II from bovine erythrocytes, lyophilized powder, Sigma-Aldrich C2522. 1 pmol/µL in 50/50 MeOH/H20 w/ 0.1% Formic Acid.

Test Method(s)

The sequence coverage reported for apomyoglobin and carbonic anhydrase was attained by infusing a 1 pmol/uL solution of the analyte at 3 uL/min into the instrument. The scan parameters were 5e5 precursor AGC target, 120K OT resolution, 100 uScans averaged, with the instrument in the low pressure mode (IRM = 3.0 mTorr). The UVPD irradiation time, and hence the number of laser pulses to which the analyte was exposed, was varied to find the maximum in sequence coverage.

Data Analysis

MS/MS spectra were deconvoluted using Hardklör[5], and searched against their respective sequences using ProSight Lite[6] with a 10 ppm mass tolerance.

Figure 1. Modified Orbitrap Fusion Lumos mass spectrometer with laser assembly interfaced to the rear of the ion trap manifold. Shown is the assembly flange, laser head, steering optics, and vacuum interface window which are all behind the side cover.

RESULTS

Laser Description

The laser employed is a Q3 series passively Q-switched Nd:YAG laser (CryLaS GmbH) outputting the 5th harmonic at 213 nm. The laser has an average power consumption of approximately 20 Watts, and is passively cooled on a heat sink that also functions as its mounting bracket. The laser pulse characteristics and relative size can be seen in **Table 1** and **Figure 2**, respectively.

Table 1. Laser Characteristics

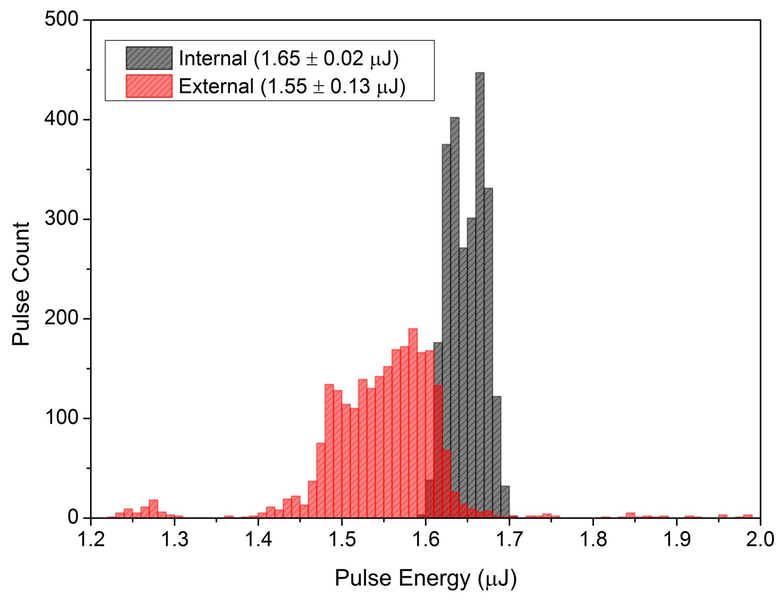
	Reference Value	Unit
Output Power (quasi cw)	3.75 ±0.5	mW
Pulse Energy	1.5 ±0.2	µJ
Peak Power	1.5	kW
Pulse Rep Rate	2.5	kHz
Pulse Width	< 1	nsec
Beam Dia	450 ±200	µm

Figure 2. CryLaS 213 nm Nd:YAG laser

The laser pulse energy is 1.5 ± 0.2 uJ/pulse at a 2.5 kHz repetition rate (0.4 msec/pulse). The beam diameter including divergence at the center of the ion trap is slightly larger than the simulated ion cloud diameter at normal AGC targets, and therefore no focusing optics are required. The laser trigger is provided by the instrument, which synchronizes the pulse to the scan function. Photoactivation occurs in the low pressure cell of the dual cell quadrupolar linear ion trap, while *m/z* analysis can occur in either the linear trap or the Orbitrap mass analyzer.

The laser's pulse to pulse stability is very good and has been optimized for variable delays between pulse trains to account for the overhead between scans associated with manipulating ions and m/z analysis. **Figure 3** shows the pulse variability for 2500 pulses measured using the CryLaS internal trigger and with the external trigger supplied by the instrument during the scan function.

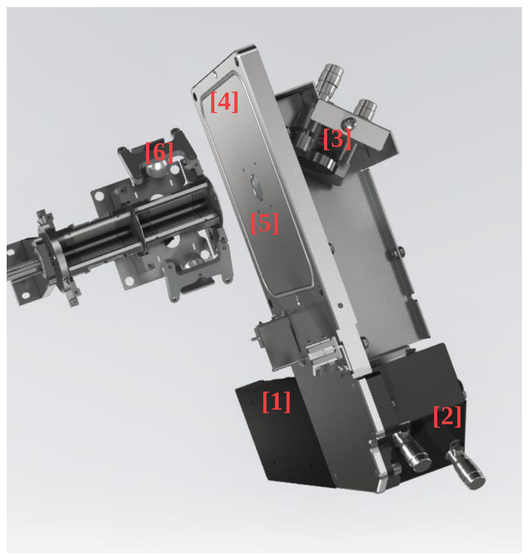
Figure 3. Histogram of the laser pulse energy measured using a Coherent J-10MT-10kHz-USB power meter. Black trace is from internal triggering of the laser at 2.5 kHz. Red trace is external triggering of the laser using the instrument scan function with 125 pulses/50 ms activation time and an approximately 50 msec pause between pulse trains.



UVPD Laser Integration to the Orbitrap Fusion Lumos mass spectrometer

As can be seen in Figure 4, the 213 nm laser is affixed to the system via a modified ion trap chamber back flange, which also houses the steering optics and vacuum interface window. All optics within the laser path are high quality UV grade fused silica, and are appropriately coated.

Figure 4. UVPD module and interface to the dual cell linear ion trap. [1] – Laser heat sink, [2] – Mirror1, [3] – Mirror2, [4] – Vacuum flange, [5] – fused silica vacuum interface window, [6] – dual cell linear ion trap assembly (sectioned).



The laser beam is manually aligned to the ion cloud in both ion traps using the actuators at each of the two mirrors. Alignment to the ion clouds in both ion traps was deemed advantageous as it ensures the maximum overlap of the laser beam with the ion cloud while minimizing the chance for photo-generated noise in the system. The Alignment Quality Score (AQS) characterizes the quality of the overlap and is described as:

$$AQS = \frac{\%PD_{HPT}}{\%PD_{HPT} + \%PD_{LPT}}$$

where %PD is the % photodissociation observed for a fixed amount of activation time, and HPT and LPT are the high pressure and low pressure cells, respectively. We therefore strive for an AQS = 0.5, while simultaneously maximizing the %PD in each cell, **Figure 5**. The UVPD module, once aligned, shows good stability with time, leading to a robust implementation requiring little additional user intervention, **Figure 6**.

Figure 5. Depiction of good laser alignment. The laser beam interacts with the total ion population in both ion traps.

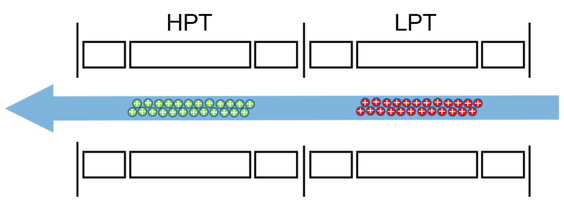
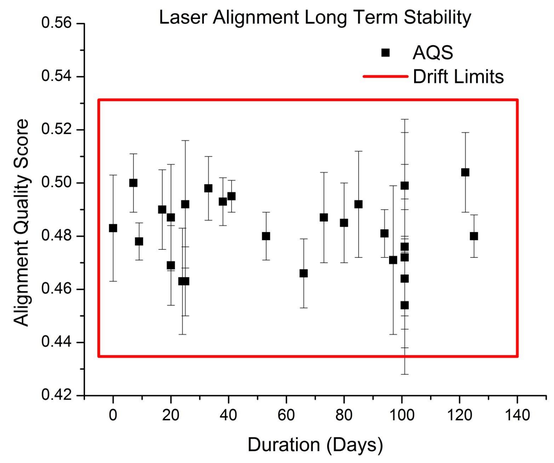


Figure 6. Laser beam – ion cloud overlap long term stability.



RESULTS – TOP DOWN PERFORMANCE

Sequence Coverage

The sequence coverage for UVPD of apomyoglobin and carbonic anhydrase as a function of activation time and charge states, is presented in **Figure 7**. The data in the figure was acquired according to the conditions described in the method section. In general, at the optimum activation times we achieve approximately 55% and 30% sequence coverage, respectively for 100 uScans. **Figure 8** compares the total bond coverage for the various dissociation methods, as well as the combined result of 95%, which indicates the unique sequence information provided by UVPD. The data in **Figure 8** represents a compilation of multiple activation times for each activation method. They are, 5,10, 17.7, 25, and 35 msec for UVPD; 3, 4, and 5 msec for ETD; 35 %NCE for CID; and 10 and 12 %NCE for HCD.

Figure 7. Sequence coverage vs. UVPD activation time for Apomyoglobin (16.8 kDa) and Carbonic anhydrase (29 kDa) obtained using the conditions described in the methods section for various charge states.

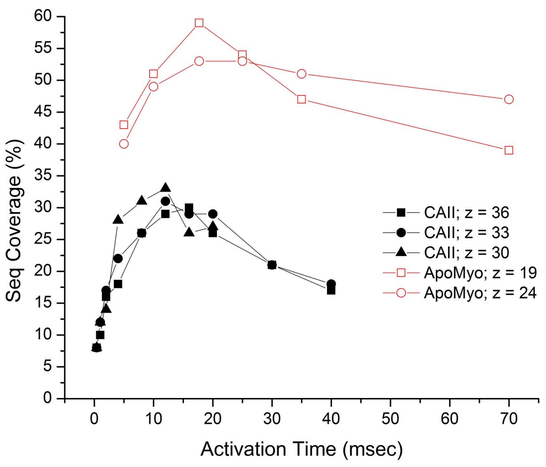
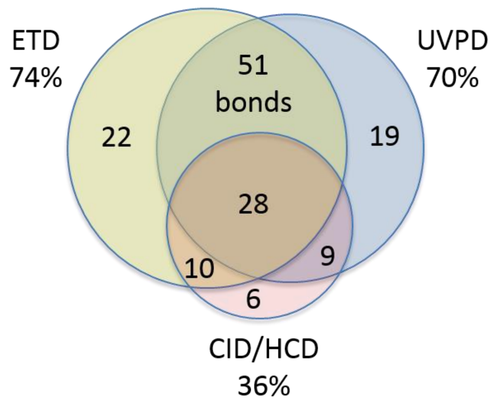


Figure 8. Bond coverage comparison for apomyoglobin $[M+24H]^{24+}$ charge state for UVPD, ETD, CID, and HCD, and for the combination.



CONCLUSIONS

- A compact, robust 213 nm solid state laser has been incorporated into the Orbitrap Fusion Lumos platform for the routine implementation of UVPD.
- Details of the implementation, including the laser characteristics, alignment strategy and stability, are described.
- The 213 nm UVPD performance for intact protein standards is shown to be very good, indicating its utility for top-down applications, including biotherapeutics.

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TRADEMARKS/LICENSING