Fast Characterization Of The Proteoform Landscape In Human Tears Using Top Down Mass Spectrometry With Multiple MS/MS Strategies

Daniel Lopez-Ferrer[1], Romain Huguet[1], Ioanna Ntai[1], David Horn[1], Peter Raus[2], Claire Dauly[3], Vlad Zabrouskov[1], Andreas FR Huhmer[1] and Peter Verhaert[4]

[1] Thermo Fisher Scientific, San Jose, California, USA; [2] Miró Center for Oculoplastic Surgery, Geel, Belgium; [3] Thermo Fisher Scientific, France; [4] ProteoFormiX, Beerse, Belgium.

ABSTRACT

Purpose: Demonstrate the potential of top-down proteomics for mapping the proteoform diversity in the tear fluid. The proposed research workflow includes the use of Schirmer Tear Test strips (Clement Clarke International, Harlow UK) for sample collection, sample handling, and protein extraction in conjunction with various top-down fragmentation techniques on a chromatographic time scale.

Methods: Tear proteins extracted from the Schirmer strips in an aqueous solution are directly analyzed by LC-MS using an Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer modified with UVPD. MS/MS acquisition was performed using ETD, EThcD, HCD and UVPD fragmentations, detecting precursor and fragment ions with a resolution of 120,000 at m/z 200. Data analysis was performed using Thermo Scientific™ Deconvolution™ 4.0 software and ProsightPD nodes in the Thermo Scientific™ Proteome Discoverer 2.2 software.

Results: Our approach consists of a simplified two-step workflow. First, proteins are extracted from the Schirmer strips using a water-based buffer. Samples are then analyzed over a 30 or 60 min LC gradient by MS and MS/MS using various fragmentation mechanisms. One of the proteins represented in the dataset by a multitude of proteoforms is lacritin, which is a prosecretory mitogen capable of promoting basal tearing and low levels of which are associated with dry eye syndrome. Overall, this strategy offers a powerful option for discovery and characterization of potential tear biomarkers that could be used for the screening of diseases, both eye related or other.

INTRODUCTION

Tears maintain the health of the front of the eye and provide clear vision. Dry eye syndrome is defined as the low volume or low quality production of tears to sufficiently lubricate and nurture the eye, and affects up to 20% of the population above age 60. Currently, the presence of dry eye is determined either by subject self-reported history or by measuring tear flow using Schirmer strips. Here, we explore the use of top-down proteomics as a viable biomarker detection strategy for various eye related conditions / diseases.

MATERIALS AND METHODS

Sample Preparation

Tears were purchased from Lee Biosolutions, Schirmer Tear Test strips from Clement Clarke International. No further sample treatment was performed to the samples.

LC-MS

Samples were introduced via autosampler into the Thermo Scientific™ MAbPacTM RP 2.1X100mm column hyphenated to a Orbitrap Fusion™ Tribrid™ MS1 and MS2 scans were acquired at 120k resolving power.

Data Analysis

Raw files were analyzed using Proteome Discoverer 2.2 SW using the ProsightPD and Protein Center nodes.

PERFORMANCE EVALUATION OF UVPD FRAGMENTATION

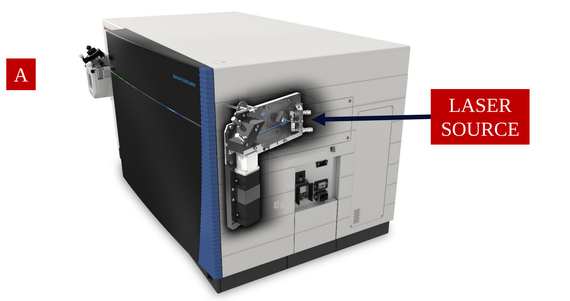

Figure 1. (A) Orbitrap Fusion laser source allows UVPD capabilities. (B) Schematic of different hardware parts including UVPD source (far right)

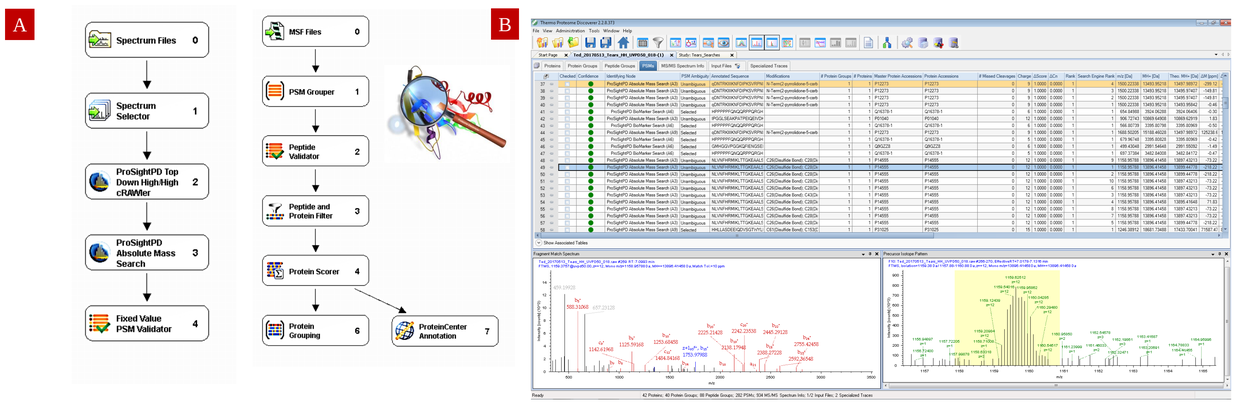

Figure 2. (A) Processing and Consensus workflows for top-down proteomics analysis in Proteome Discoverer 2.2 SW using ProsightPD node. (B) Screenshot of analysis output on Proteome Discoverer 2.2 SW, highlighting identified protein list, fragmentation spectrum for selected protein and full MS spectrum highlighting isolation window of precursor mass.

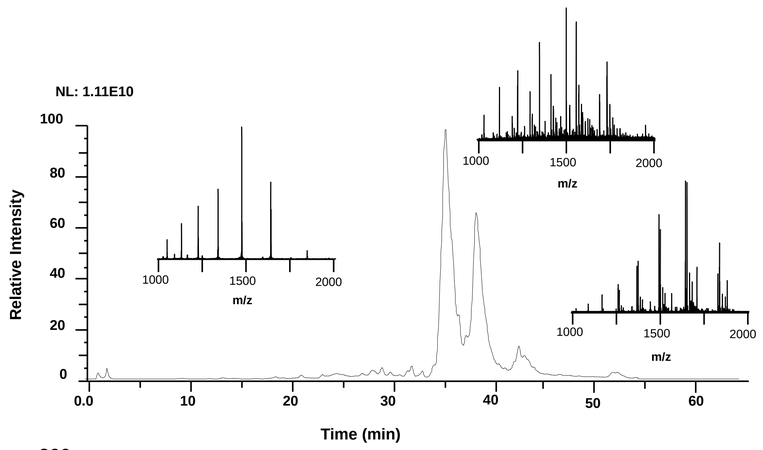

Figure 3. Representative chromatogram from a 60 min gradient of tears without any processing. Inserts show different full MS scans across the chromatogram.

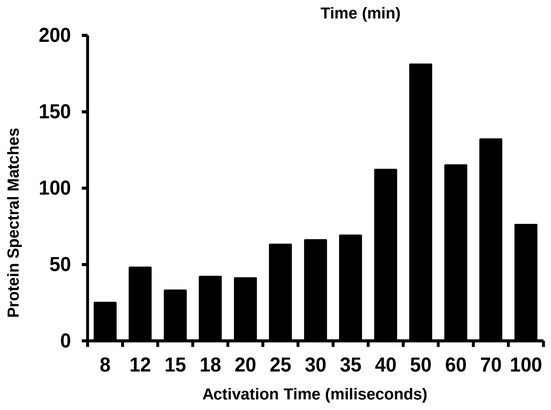

Figure 4. Histogram showing number of protein spectral matches in function of activation time during UVPD irradiation.

PROTEOFORM LANDSCAPE OF HUMAN TEARS

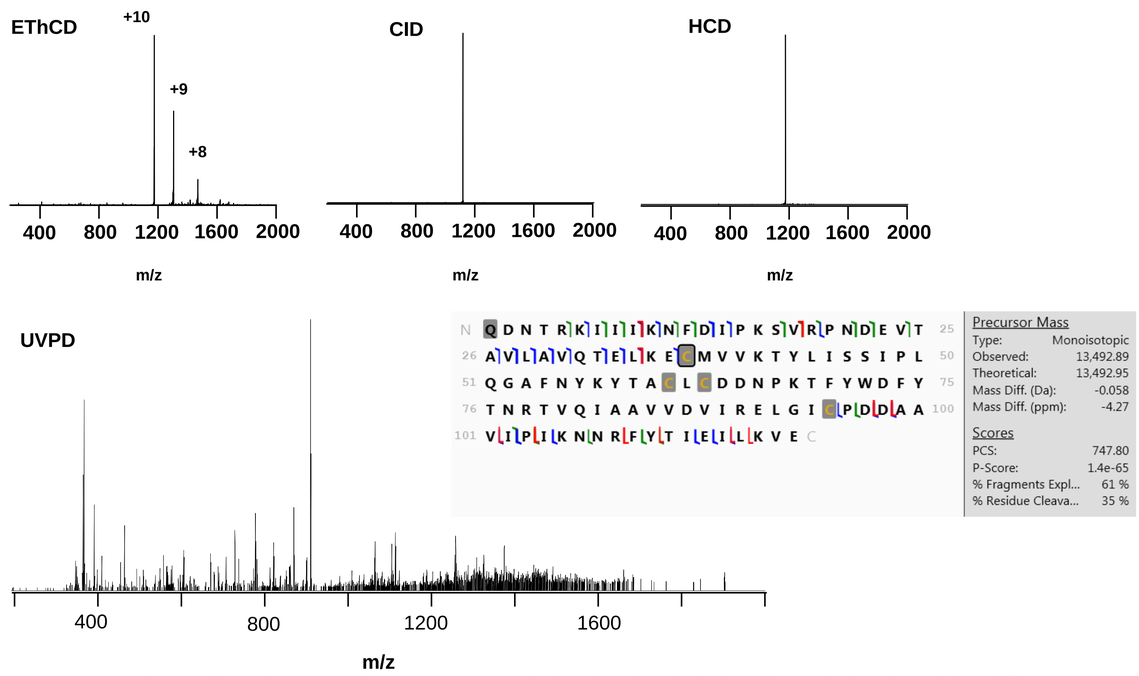

Figure 5. Comparison of four fragmentation spectra on a chain of Prolactin-induced protein (PIP) applying different dissociation techniques. Prolactin-induced protein in tears plays a role in host defense by binding to microorganisms. Due to its molecular function, PIP has a very stable structure characterized by two disulfide bridges making it very difficult to fragment in the gas phase. Interestingly, UVPD allows for a full characterization of the protein, allowing not only identification of the disulfide bridges that add to its stability, but also providing evidence for the conversion of the N-terminus glutamine residue to 2-pyrrolidone-5-carboxylic acid.

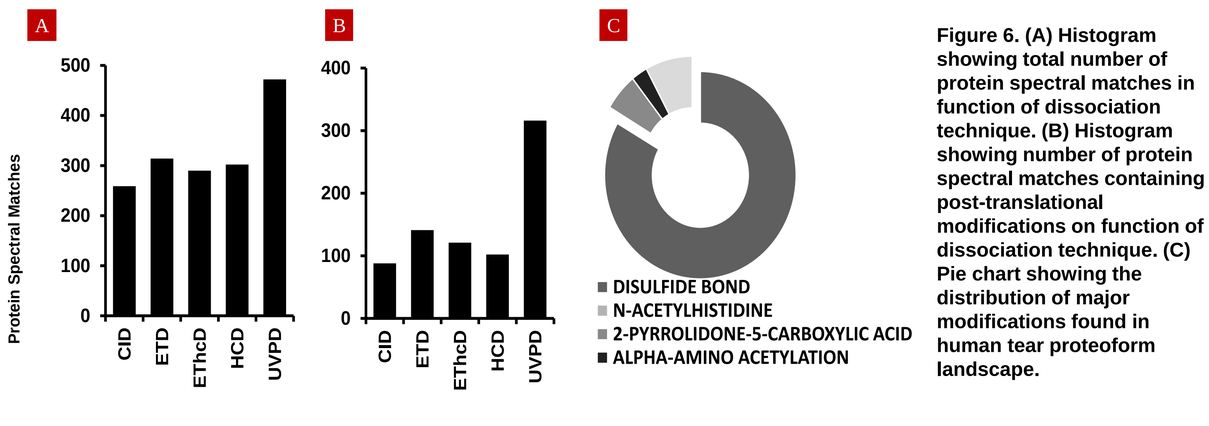

Figure 6. (A) Histogram showing total number of protein spectral matches in function of dissociation technique. (B) Histogram showing number of protein spectral matches containing post-translational modifications on function of dissociation technique. (C) Pie chart showing the distribution of major modifications found in human tear proteoform landscape.

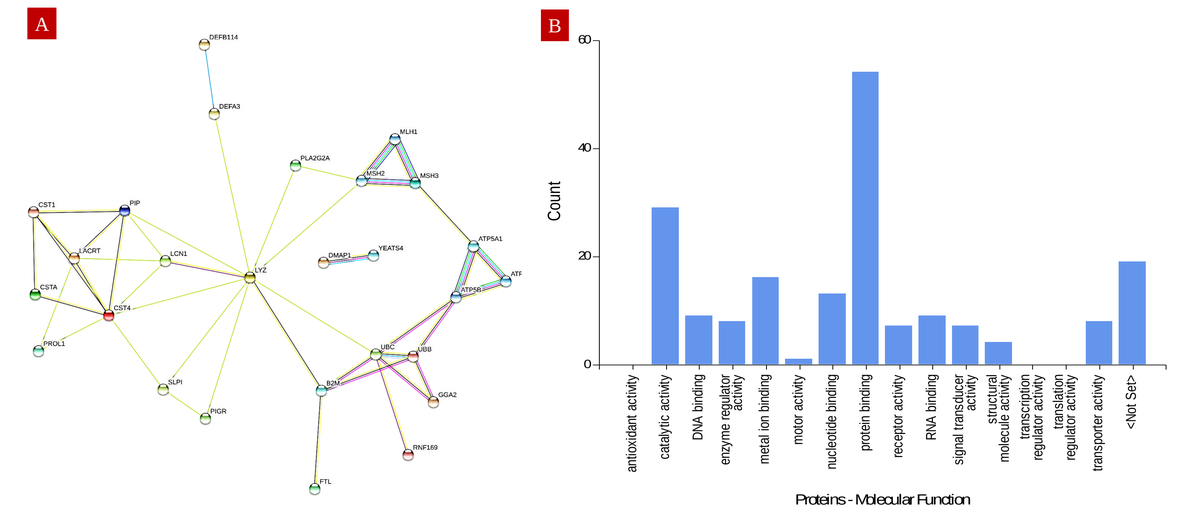

Figure 7. (A) Protein network built from identified proteoforms, highlighting four major clusters. Most significant are cystatin cluster (left side) containing proteins with endopeptidase inhibitor activity and Mut cluster (upper right) containing proteins with endopeptidase and DNA binding funtions. (B) Histogram of significant gene ontology terms classified by molecular function.

CONCLUSIONS

UVPD coupled to high resolution mass spectrometry and sophisticated bioinformatics tools represent a very easy and versatile combination for proteoform profiling of human body fluids, like tears.

- UVPD provides more protein identifications than any other dissociation technique.
- Combining UVPD dissociation, high resolution and accurate mass Orbitrap mass spectrometry and Proteome Discoverer allow for extremely sensitive analysis using minute amounts of sample.
- This work illustrates that robust profiling and monitoring of hundreds of proteoforms in tears in barely 60 min is possible, opening up this technology for single donor tear analysis in ophthalmological clinical research applications.

For Research Use Only. Not for use in diagnostic procedures.

REFERENCES

1. Cleland, T.P., DeHart, C.J., Fellers, R.T et al. (2017) J. Proteome Research 16: 2072-2079.

TRADEMARKS/LICENSING