Two-dimensional mass spectrometry for top-down analysis and structural characterization of proteins

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Overview

- Narrowband 2D MS provides an easy visual method for the assignment, and location of modifications in top-down analysis of proteins.
- 2D MS can also be used for label-free quantification of modified proteins.
- We apply narrowband 2D MS for the covalent labelling of proteins for structural characterization.

Two-dimensional Mass Spectrometry

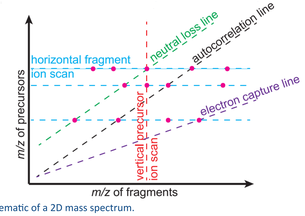

Figure 1: Schematic of a 2D mass spectrum.

2D mass spectrometry is a technique that has been developed for tandem mass spectrometry that correlates precursor and fragment ions without ion isolation. 2D mass spectrometry has been developed for Fourier transform ion cyclotron resonance mass spectrometry and has proven very useful for the analysis of complex sample. A 2D mass spectrum shows the following characteristic lines (see Figure 1):

- **The autocorrelation line** ($y = x$) shows the correlation of the prescursor ion signal with their own cyclotron radius.
- **Horizontal fragment ion spectra** ($y = m_{precursor}$) show the fragmentation patterns of each precursor ion.
- **Vertical precursor ion spectra** ($x = m_{fragment}$) show the precursor ions of each fragment ion.
- **Electron capture lines** (y = (n-p)*x/n) show the capture of p electrons by n-charged precursor ions.
- **Neutral loss lines** ($y = x + m_{neutral}$) show the loss of neutrals by precursor ions.

Narrowband 2D MS for top-down analysis of biomolecules

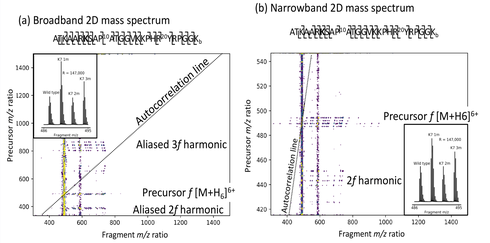

Figure 2: 2D (a) broadband and (b) narrowband ECD mass spectrum of the $[M+6H]^{6+}$ charge state of 26-residue histone peptides (non-modified, methylated, demethylated, trimethylated).

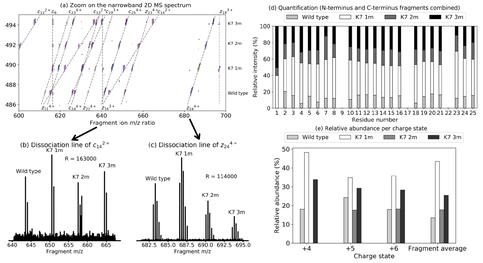

Figure 3: Narrowband ECD 2D mass spectrum of the $[M+6H]^{6+}$ charge state of 26-residue histone peptides (non-modified, methylated, demethylated, trimethylated): (a) zoom-in on fragment ion peaks, (b) dissociation line of c_{14}^{2+} fragment, (c) dissociation line of z_{24}^{4+} fragment, (d) relative quantification using fragment ion relative abundances, (e) relative quantification using abundances of different charge states.

- Maximum frequency reduced to fold over autocorrelation line.
- Same number of datapoints over smaller mass range.
- Increase in resolving power/precursor-fragment correlation.
- Dissociation lines: slopes 0.33, 0.5, 0.67: confirm ID and location of PTM.
- *m/z* difference: 14.0157 Da: methylations.
- c_6 and z_{18}^{3+}: vertical precursor ion scans: PTMs on 7th residue.
- PTM location achieved visually: relative intensities plotted without distinguishing fragment *m/z*.
- Comparable results from intensities of precursor ions, charge-reduced species, and fragment ions: 2D MS can be used for label-free relative quantification.

Fast Photochemical Oxidation of Proteins

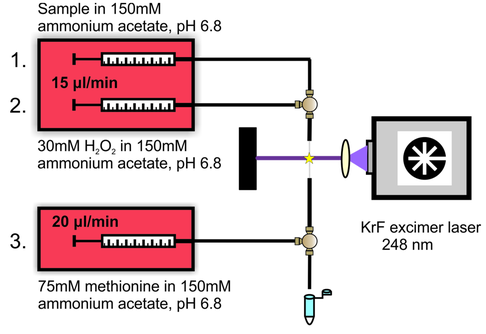

Figure 4: Schematic of FPOP setup.

- The protein in the presence of H_2O_2 can be oxidized with the help of the UV laser pulse.
- Fast covalent labelling of residues with modifications on various amino acids (+16 Da, +32 Da, +48 Da).
- Residues on the surface are easily oxidized but protected residues are not.

Top-down Analysis by 2D MS of Ubiquitin after FPOP Treatment

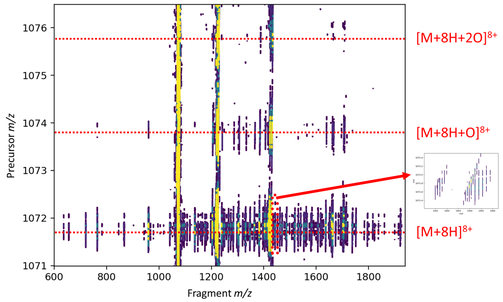

Figure 5: Narrowband ECD 2D mass spectrum of the $[M+8H]^{8+}$ charge state of ubiquitin after oxidation by FPOP on a 15T FT-ICR mass spectrometer with Paracell (zoom-in on two fragment isotopic distribution).

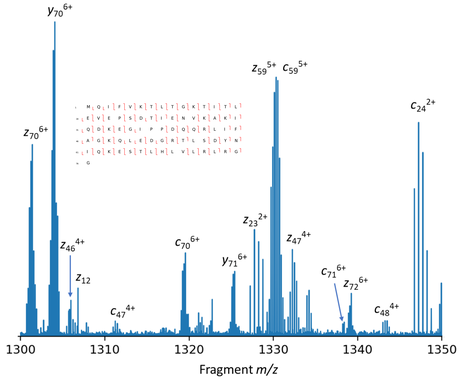

Figure 6: Added-up fragment ion scans for each isotope of $[M+8H]^{8+}$ of ubiquitin (non-oxidized) from the narrowband 2D mass spectrum in figure 5. The peaklist was analyzed through FAST-MS to assign the fragment peaks. Insert: 95% sequence coverage obtained from the added fragment ion scans for non-modified ubiquitin.

- Narrowband 2D MS (frequency range of 20kHz on a 15 T FT-ICR mass spectrometer) enables resolution of precursor ions on the isotope level (R = 8200 at *m/z* 1071).
- We added up the fragment ion scans of all the isotopes of M+8H]8+ of ubiquitin, which reconstitutes the isotopic distributions of the fragment ions comparable to one-dimensional tandem mass spectrum. An additional advantage of this is that it increases the S/N ratio.
- Using the FAST-MS top-down assignment software after internal mass calibration, we obtained 95% sequence coverage (3 missed cleavages outside of P residues) within 1 ppm mass accuracy.

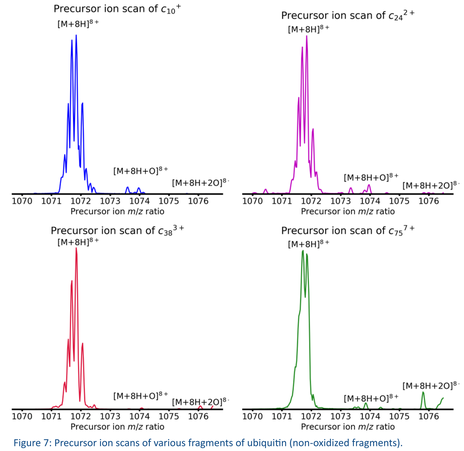

Figure 7: Precursor ion scans of various fragments of ubiquitin (non-oxidized fragments).

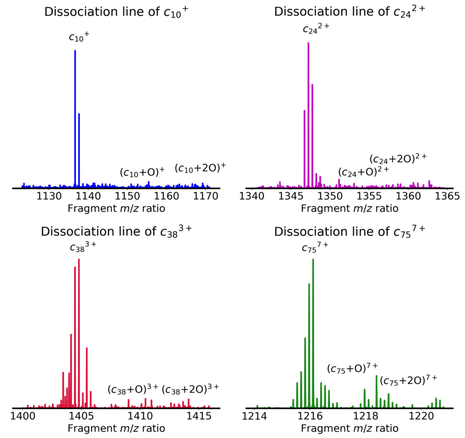

Figure 8: Dissociation linesof various fragments of ubiquitin (non-oxidized and oxidized fragments).

- Precursor ion scans show the fragments from all ubiquitin isoforms that do not contain any oxidations.
- Dissociation lines show the fragments from the ubiquitin isoforms that do contain oxidations.
- Both precursor ion scans and dissociation lines can be used to follow the rate of oxidation in ubiquitin for each residue.

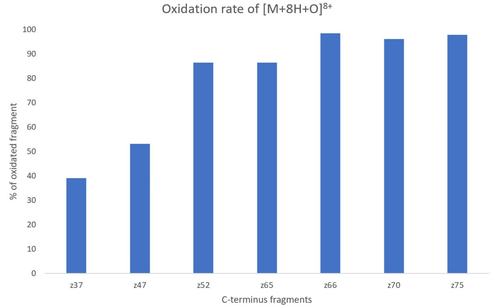

Figure 9: Rate of oxidation using the relative intensities of the C-terminus fragments of $[M+8H+O]^{8+}$ extracted from the narrowband 2D mass spectrum and calculated with the FAST-MS software.

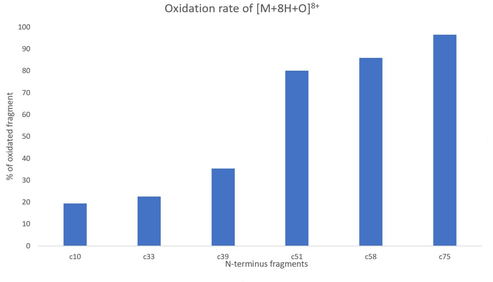

Figure 10: Rate of oxidation using the relative intensities of the N-terminus fragments of $[M+8H+O]^{8+}$ extracted from the narrowband 2D mass spectrum and calculated with the FAST-MS software.

Conclusion&Perspectives

- This study shows that narrowband 2D MS can be used for top-down structural characterization of proteins with FPOP oxidation.
- We can apply this method for the structural characterization of protein-ligand interactions (e.g. transcription factors binding with DNA).
- Narrowband 2DMS can also be applied to other covalent labelling techniques, such as acetylation.

Acknowledgments

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