Crosslinking Mass Spectrometry

Optimized XL-MS workflows for protein-protein and protein-nucleic acid interactions

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Abstract

Purpose: To optimize XL-MS workflows for protein-protein and protein-nucleic acid interactions.

Methods: For protein-protein interaction, heterobifunctional photoactivatable crosslinker DizSEC (2,5-Dioxopyrrolidin-1-yl (2-(3-methyl-3H-diazirin-3-yl)ethyl)carbamate) were used to crosslink standard proteins. For protein- nucleic acid interaction, *E. coli* ribosomes were exposed to UV or nitrogen mustard and enriched using TiO_2. Separation was achieved using Thermo Scientific™ Vanquish™ Neo LC system with a 60 min gradient using EASY-Spray™ PepMap™ Neo column. Following separation, the peptides were detected on Thermo Scientific™ Orbitrap™ Ascend™ or Astral™ mass spectrometers, and data were analyzed using the XlinkX node or the NuXL node in Thermo Scientific™ Proteome Discoverer™.

Results: With optimized MS methods, DizSEC crosslinked peptides can be identified with high confidence. Moreover, we identified two to three times the number of unique RNA crosslinks on Orbitrap Astral MS.

Introduction

Crosslinking mass spectrometry (XL-MS) has grown dramatically as a key workflow for elucidating protein higher-order structure and mapping protein-protein or protein-nucleic acid interaction networks on a proteome-wide scale. UV light is one of the common ways to create crosslinks to investigate both types of interactions. However, challenges remain in sample preparation, instrument methods and data analysis. In this work, we optimized XL-MS workflows for photoactivatable crosslinkers SDA and DizSEC (cleavable) crosslinked samples and protein-RNA crosslinks. A new MS2-MS3 method was developed to improve DizSEC XL identifications for protein-protein interaction. Importantly, we further optimized protein– nucleic acid workflows on the Thermo Fisher Scientific™ Orbitrap™ Astral™ mass spectrometer and achieved two-to-three times identifications for XL-MS analysis.

Materials and methods

Sample preparation

Heterobifunctional photoactivatable DizSEC (2,5-Dioxopyrrolidin-1-yl (2-(3-methyl-3H-diazirin-3-yl)ethyl)carbamate) was used to crosslink standard proteins. E. coli ribosomes were exposed to UV or chemical crosslinkers. Crosslinked samples were digested, purified using TiO_2.

Liquid chromatography and mass spectrometry

Samples were separated by reverse phase-HPLC on a Vanquish Neo system using Thermo Scientific™ EASY-Spray™ PepMap™ RSLC C18 column. Peptides were eluted over a 60 min 4-50% gradient (A: water, 0.1% formic acid; B: 80% acetonitrile, 0.1% formic acid) at 300 nL/min flow rate. The crosslinked samples were analyzed on Thermo Scientific™ Orbitrap™ Ascend™ or Thermo Scientific™ Exploris™ 480 mass spectrometers in DDA modes. Specific LC and MS settings are shown in Figure 2,Tables 1 and 2.

Data analysis

Protein-protein crosslinking data were analyzed using Proteome Discoverer 3.2 software: the XlinkX node with the noncleavable, MS2 or MS2-MS3 search option for crosslinked peptides and SEQUEST™ HT search engine for unmodified, looplinks and monolinks peptides. Data were also analyzed using xiSEARCH/xiFDR. Carbamidomethylation (+57.021 Da) was used as a static modification for cysteine and methionine oxidation (+15.996 Da) as variable modification. Data were searched against a protein database containing 10 proteins including human serum albumin (P02768) or yeast enolase (P00924). The false discovery rate (FDR) was set to 1% at CSM and cross-link levels.

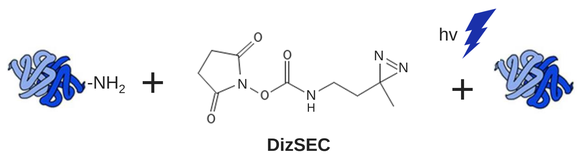
Protein-nucleic acid crosslinking data was analyzed using the NuXL node in PD 3.1. We calculate match-odds and subscores and employ semi-supervised score calibration using Percolator. Moreover, entrapment experiments have been employed using manually curated data ensuring proper false discovery rate (FDR) control.

Results

Protein-protein interactions

DisSEC is a novel MS-cleavable heterobifunctional crosslinker that connects Lys with any amino acid in proteins and forms Ureas. Ureas are doubly fissile under HCD/CID (across each N-carbonyl bond) and generate two distinct sets of fragment ions . The structure of the crosslinker is shown in Figure 1.

Figure 1. Structure and reactions of MS-cleavable heterobifunctional photo-cross-linkers DizSEC.



DizSEC

Results

Methods for DizSEC crosslinked samples

Collisional energy is crucial for cleavable crosslinkers. We compared three MS methods: MS2 SCE, MS2 SCE prioritizing charge states +4 to +6, and a new MS2-MS3 method for DizSEC. Optimized parameters are listed below.

Figure 2. Orbitrap Ascend MS acquisition methods for DizSEC crosslinked peptides prioritizing charge states +4 to +6.

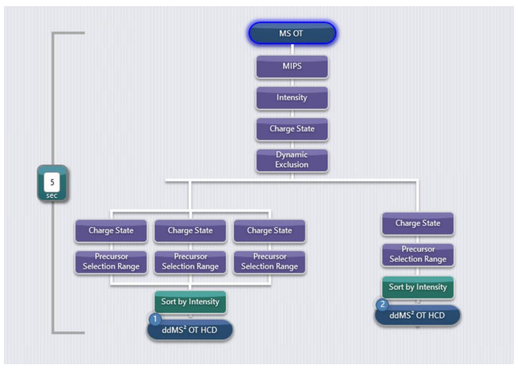


Table 3. Orbitrap Ascend MS acquisition parameter settings for DizSEC MS2-MS3 method.

Settings	MS2	MS3
Detector	Orbitrap	Ion trap
Resolution/Scan Rate	30K	Rapid
Normalized AGC Target (%)	100%	200%
Max. injection time (ms)	59	80
Charge states	3-8	2-6
Intensity threshold	$1e^4$	NA
Isolation width	1.6	2.5
NCE (%)	CID 25	HCD 30
Top speed/scans	5s	4 scans
Delta Mass (Da)	25.9942	

Figure 3. Examples of HSA DizSEC crosslinkers identified by (A) MS2 method and (B) MS2- MS3 method.

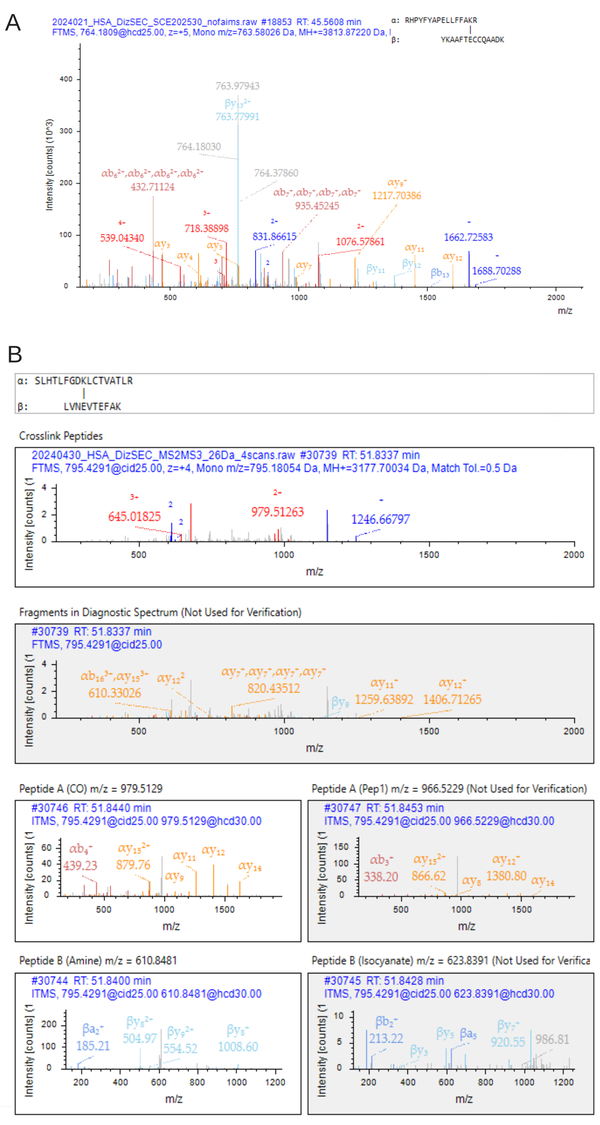
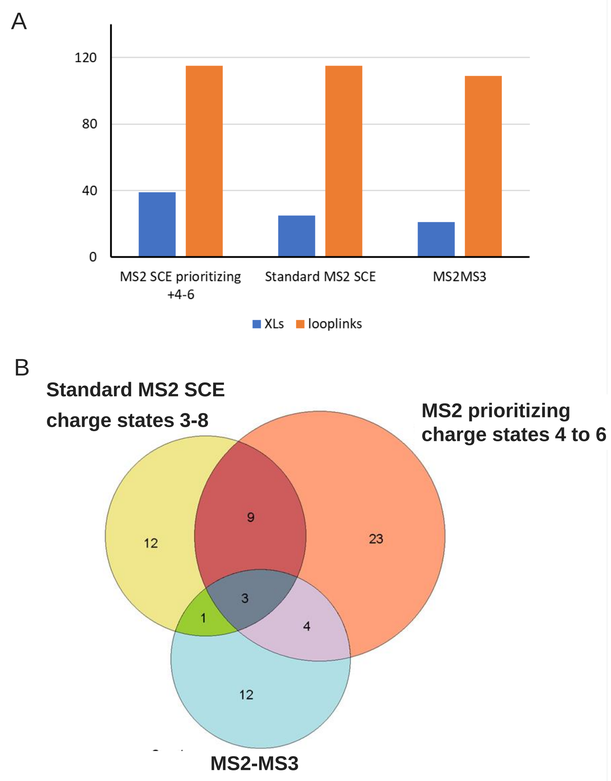


Figure 4. (A) HSA DizSEC crosslinks and looplinks identified using XlinkX MS2 search in different MS methods. (B) Venn diagram showing the overlap of identified HSA crosslinks on OT Ascend.



Protein-nucleic acid interactions

We developed DDA methods for NuXL identification and quantitation on OT Astral. *E. coli* ribosomes were crosslinked with UV light (215 nm) or nitrogen mustard .Optimized parameters are listed below.

Table 2. Orbitrap Astral MS crosslinker specific LC-MS acquisition parameter settings.

Settings	OT-OT	OT-Astral
LC gradient	4-50% B in 60 min	4-50% B in 60 min
MS1 scan	Orbitrap	Orbitrap
Resolution	120K	180K
Normalized AGC Target (%)	300%	500%
Max. injection time (ms)	20	5
Charge state	2-6	2-6
MS mass range, m/z	400-1400	400-1400
Intensity threshold	$1e^4$	$5e^3$
RF lens	50	50
MS2 scan	Orbitrap	Astral
Resolution	45K	Standard
Normalized AGC Target (%)	200%	100%
Max. injection time (ms)	105	20
Charge States	2-6	2-6
First mass (m/z)	150	150
Intensity threshold	$1e^4$	$5e^3$
Isolation width	1.6	1.6
NCE (%)	30	30
Top speed	2s	1s

Figure 5. Comparison of unique identified RNA crosslinks and spectrum matches from NM duplicate samples in OTOT and OT Astral DDA runs.

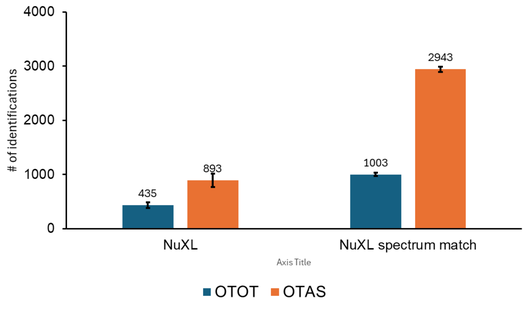


Figure 6. Venn diagram showing the overlap of unique identified RNA crosslinks in duplicates from (A) UV- or (B) NM samples in OT Astral DDA runs

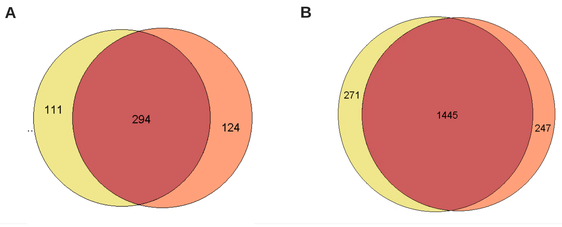
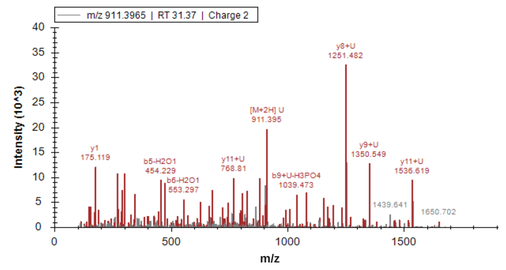


Figure 7. Example of Annotated MS2 spectrum of a UV induced RNA-peptide crosslink from OT-Astral.

The MS2 spectrum view in Proteome Discoverer of the peptide ATLGEVGNAEHmLR crosslinked with U



Conclusions

- Increased numbers of DizSEC crosslinked peptides were identified using the instrument method prioritizing high charge states 4-6.
- An optimized MS2-MS3 method for DizSEC crosslinker was developed to reduce false positives in XL identifications.
- NuXL workflow was benchmarked on the Orbitrap Astral™ mass spectrometer with UV- or chemically induced *E. coli* ribosome crosslinked samples.
- Using the Astral analyzer's high sensitivity and fast scan rate (up to 200 Hz), we identified two to three times the number of RNA crosslinks and spectrum matches for protein-nucleic acid interactions.

Availability

The NuXL node is released for Proteome Discoverer 3.0 and 3.1. The binary installer, documentation, and example data are available at https://openms.de/applications/nuxl/.

References

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