Probing Antibody-Host Proteome Interactions via Intracellular Cross-linking

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ABSTRACT

Purpose: To investigate protein-protein interactions in intact CHO cells producing monoclonal antibodies (mAbs).

Methods: CHO cells expressing mAb were subjected to intra cellular cross-linking using tButyl-PhoX at various concentrations. The cells were lysed and the Endo plasmic reticular (ER) fraction was isolated. The eluate was reduced, alkylated and trypsinized followed by enrichment of phospho-xlinked peptides. Lastly, the peptides were subjected to LC-MS/MS using a Thermo Scientific™ Vanquish™ Neo LC coupled to a Thermo Scientific™ Orbitrap Eclipse™ mass spectrometer. Raw data were analyzed using Thermo Scientific™ Proteome Discoverer™ 3.0 software with XlinkX 3.0 node.

Results: Both intra- and inter- crosslinks were identified with high confidence provide important insight into proteome interaction in CHO cells.

INTRODUCTION

Development of monoclonal antibodies (mAbs) is often supported by comprehensive characterization of product quality (PQ) attributes of secreted proteins. However, elucidating how antibodies are formed in intact cellular vesicles via proteomics is not trivial. The cellular machinery regulating mAbs formation can be probed via the interaction landscape of the Chinese Hamster Ovary (CHO) proteome. We use structural mass spectrometry techniques such as chemical cross-linking with tButyl-PhoX to stabilize weak and transient interactors in intact CHO cells. Herein, we catalog cross-linked proteins in the Endoplasmic reticular (ER) transfected with different mAbs and mAb PQ profiles. The cross-linker stabilize the antibody-protein interactions and help to decipher specific protein complexes in their native configuration.

MATERIALS AND METHODS

Sample Preparation

CHO cells expressing monoclonal antibody (mAb), having significantly different product quality profiles were subjected to intra cellular cross-linking via tButyl-PhoX. The cells were treated with γ-phosphatase to remove endogenous phosphate. Endo plasmic reticular (ER) fraction was isolated and subjected to protein A purification. The eluate was reduced, alkylated and trypsinized followed by deprotection of tButyl group of xlinked peptides, and enrichment of phospho-xlinked peptides on FeNTA IMAC beads.

Liquid Chromatography and Mass Spectrometry

Samples were separated by reverse phase-HPLC using a Thermo Scientific™ Vanquish Neo system connected to an EASY-Spray™ PepMap™ RSLC C18 column (75 µm x 250 mm, 2 µm particle size) over a 60 min 3-65% 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 an Orbitrap Eclipse™ Tribrid™ mass spectrometer with Instrument Control Software version 4.0.

Data Analysis

The acquired spectra were analyzed using Proteome Discoverer™ 3.0 software using the XlinkX node 3.0 for crosslinked peptides and SEQUEST™ HT search engine for unmodified, looplinks and monolinks peptides. Carbamidomethylation (+57.021 Da) was used as a static modification for cysteine. Different crosslinked mass modifications for lysine were used as variable modifications for lysine or N-terminus in addition to methionine oxidation (+15.996 Da). Data were searched against the Cricetulus griseus database with 1% FDR criteria for crosslink spectra matches.

RESULTS

Figure 1. The workflow of intra cellular crosslinking sample preparation.

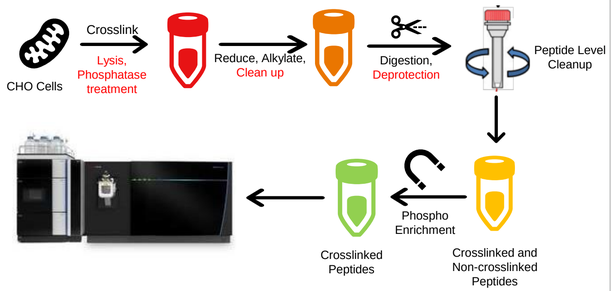
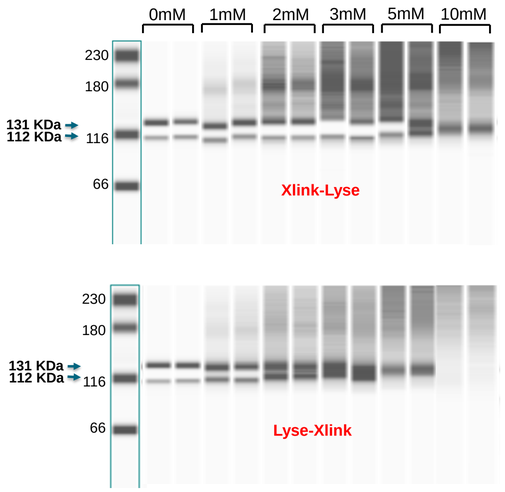


Figure2. The SDS-PAGGE immunobloted for ER protein Calnexin demonstrating levels of crosslinking under various conditions.



Cross-linking experiments were performed under two different condition (Xlink-Lyse vs Lyse-Xlink). In both cases, more crosslinked Calnexin was observed with increasing concentrations of tBu-PhoX. Importantly, the level and type of crosslinkers in calnexin depends on the protocol but in both cases significant amount inter and intra crosslinked species was produced.

Figure 3. Bar graph of total identified crosslinks (intra- and inter- XL) under different conditions.

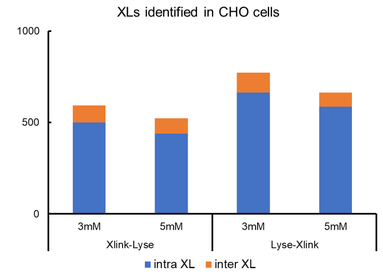


Figure 4. Venn diagrams of (A) the comparison of two replicates under Xlink-Lyse condition at 3 mM tBu-PhoX; (B) the comparison of two conditions (Xlink-Lyse vs Lys-Xlink) at 3 mM tBu-PhoX.

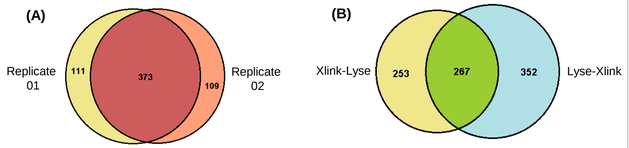


Figure 5. Identified crosslinks in ER protein Calreticulin and the MS/MS spectrum of the crosslink SDFG*K*FVLSSGK-WVES*K*HK from Calreticulin.

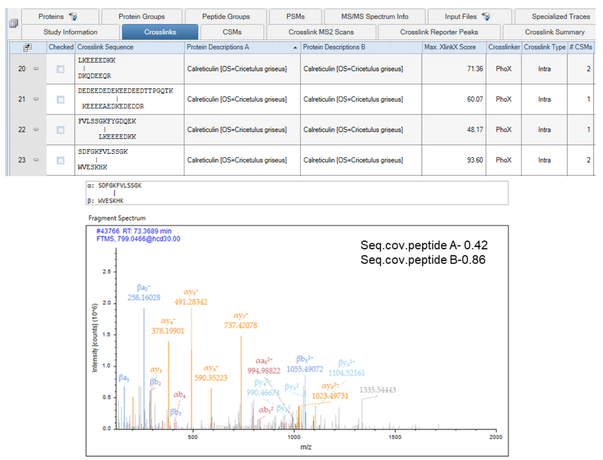
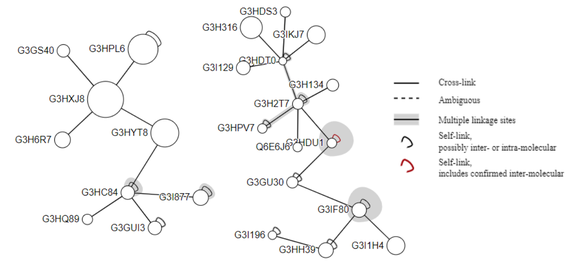


Figure 6. Identified proteome interactions in CHO cells.



CONCLUSIONS

- ER proteins in intact CHO cells were successfully crosslinked using the membrane permeable crosslink reagent tBu-PhoX.
- Good reproducibility of identified crosslinks were observed between replicates and more intra crosslinks were found under Lyse-Xlink condition compared to Xlink-Lyse.
- With the optimized crosslinking workflow, crosslinks from CHO cells were identified with high confidence.
- Identified crosslinked peptides provide important insight into protein-protein interactions during mAb expression.

REFERENCES

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2. Jiang, P., Wang C., Diehl, A., Viner, R., Etienne, C., Nandhikonda, P., Foster, L., Bomgarden, R. D., Liu, F. (2022). A Membrane-Permeable and Immobilized Metal Affinity Chromatography (IMAC) Enrichable Cross-Linking Reagent to Advance In Vivo Cross-Linking Mass Spectrometry. Angew Chem Int Ed Engl, 61(12):e202113937.

TRADEMARKS/LICENSING

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