Chemical Cross-linking Mass Spectrometry for Profiling Protein Structures and Protein-Protein Interactions

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Introduction

Proteins play a crucial role in cellular functions, and understanding their structures and interactions is essential for advancing the fields of biology, medicine, and biotechnology. In recent years, mass spectrometry has emerged as a powerful tool for investigating protein structures and interactions, complementing traditional methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

Chemical cross-linking is a technique that allows the identification of protein-protein interactions by forming covalent bonds between proteins. This method has become increasingly popular due to its ability to provide insights into the three-dimensional structures of proteins and protein complexes.

Strengths and Challenges of Chemical Cross-linking Mass Spectrometry

Chemical cross-linking mass spectrometry provides a number of advantages, including the ability to perform experiments in vitro and in vivo, as well as the ability to analyze complex protein mixtures. However, the technique also presents several challenges, such as the need for high sample purity and the requirement for computational tools to identify and validate cross-linked sites.

Cross-linking Reagents

Common chemical cross-linkers are non-cleavable homobifunctional and heterobifunctional cross-linkers. Homobifunctional cross-linking reagents contain identical reactive groups at both ends, while heterobifunctional reagents contain different reactive groups.

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can contain one photo-reactive group that is stable until it is exposed to high intensity UV light. Both homobifunctional cross-linkers and heterobifunctional cross-linkers can eliminate the spacer arm and mediate cross-linking between two proteins by creating a bond without an intervening linker. This type of cross-linker is termed as zero-length cross-linkers. The most widely used zero-length cross-linkers are carbodiimides, which mediate amide bond formation between a carboxylate and an amine group.

To facilitate the mass spectrometry detection and database search of cross-linked peptides, many novel cross-linking reagents have been developed, including cleavable cross-linkers, affinity-tagged cross-linkers, and isotopically tagged cross-linkers.

Placing a cleavable bond within a cross-linker structure can simplify the identification of cross-linked peptides. After cleavage, a cross-linked peptide behaves like a linear peptide with a modification, which enables the identification of cross-linked peptides using common protein database search engines. Cleavable cross-linkers can be cleaved by chemical reagents (e.g., DTT), by UV, or by tandem MS fragmentation (e.g. CID or ETD). The cleavable cross-linkers, such as Protein Interaction Reporter (PIR), have been applied for in vivo identification of protein-protein interactions [11]. Incorporating an affinity group into the structure of a cross-linker reagent enables the enrichment of cross-linked products. The typical affinity-tagged cross-linkers use biotin/avidin affinity purification strategies [12]. However, affinity-tagged cross-linkers are more bulky than conventional cross-linkers, which induce steric hindrance to prevent probing certain protein interactions. Alternatively, incorporation of an azide group provides a smaller cross-linker and enables click chemistry based enrichment [11]. An isotopically tagged linker substitutes one or more atoms in the cross-linker with heavy stable isotopes, usually deuterium or $^{13}$C. Peptides cross-linked with a mixture of heavy and light cross-linking reagents generate unique doublet peaks in the mass spectra, which not only facilitates MS detection of cross-linked peptides and but also enables targeted MS/MS of cross-linked peptides. Isotopically tagged cross-linkers have been used to investigate complex protein interaction networks at large scale [8,9].

Cross-linking Identification Algorithms

Cross-linked peptides pose challenges to their since conventional protein database search engines were designed for identification of linear peptides or linear peptides with modifications, but not for two peptides connected with a linker. Many MS and MS/MS search algorithms have been developed for different types of cross-linkers and their applications. xComb [13], MassMatrix [14], StarvX [15], X-Link identifier [16], and pLink [17,18] are commonly used for non-cleavable homobifunctional or heterobifunctional cross-linkers. By importing cross-linker information and protein FASTA database, xComb creates a linearized and concatenated cross-linked peptide database that can be used as a common search engine. MassMatrix is a comprehensive database search engine for MS/MS based proteomics, and it also provides algorithms for chemical cross-links identification. StarvX provides an easy-to-use graphical interface and shortens the processing time by only processing the MS/MS spectra of the precursor ions that match to the theoretical masses of cross-linked peptide candidates. MeroX [19], is designed for CID-MS/MS cleavable cross-links with a self-explanatory graphical user interface, similar to StarvX. BLinks [20] is designed for the PIR cross-linking applications. GPMAW [21] and xQuest/xProphet [8,22] can be used for identification of isotopically tagged cross-linking. GPMAW is suitable for small-scale analysis. xQuest can be used for large proteome-wide cross-linking studies [8,9].

Recently, xProphet was integrated into the xQuest to determine FDRs of large cross-linking data sets using a target-decoy strategy, which improve the scoring function and validation. xQ [23] is designed for quantitative cross-linking mass spectrometry using heavy and light isotopically tagged cross-linkers.

Conclusion and Perspective

The applications of chemical cross-linking mass spectrometry continue to grow. One direction for chemical cross-linking mass spectrometry is to capture real time proteome-wide protein-interaction networks in vivo. This can be achieved by the comparison of systematic interaction changes upon a specific stimuli or perturbation using cross-linking snapshots. Some pioneer studies have been applied in bacterial cells [8,10,24]. Another direction is quantitative cross-linking. Quantitative probing protein conformational changes or protein-interaction changes can be achieved by using heavy and light isotopically tagged cross-linkers [23,25]. The continuous advancement in MS instrumentation, cross-linking reagents, and informatics tools has great potentials to enable global scale, high-throughput identification and quantification of cross-linked products, making cross-linking mass spectrometry an important technique to profile protein structures as well as deciphering dynamic protein-interaction networks in vivo.

References


