INSTITUTE FOR RESEARCH IN BIOMEDICINE

"The Power of Cross-linking/Mass Spectrometry for Protein Structure Analysis"

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Abstract

During the last 15 years, chemical cross-linking combined with mass spectrometry (MS) and computational modeling has advanced from investigating 3D-structures of isolated proteins to deciphering protein interaction networks. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-linking data, three-dimensional structural models of proteins and protein complexes can be constructed. Most commonly, homobifunctional amine-reactive cross-linkers, such as *N*-hydroxysuccinimide esters, are used for studying protein conformations and for mapping protein-protein interactions.

One of our goals is to extend the arsenal of existing cross-linkers to obtain complementary structural information of proteins. To facilitate the identification of cross-linked products, we have designed MS cleavable cross-linkers creating characteristic marker ions in the fragment ion mass spectra. We have evaluated different fragmentation methods available on an Orbitrap Fusion mass spectrometer (CID, HCD, ETciD, and EThcD) in combination with a dedicated software tool, MeroX, for conducting fully automated analyses of cross-linked products. An alternative, more exploratory strategy relies on a novel cross-linking reagent, containing a TEMPO (2,2,6,6-tetramethylpiperidine-1-oxy) and a benzyl group. The aim for designing this cross-linker was to facilitate the assignment of cross-linked products by free radical initiated peptide sequencing (FRIPS).

A direct way to probe protein-protein interactions is by site-specific incorporation of genetically encoded photo-reactive amino acids or by non-directed incorporation of photo-reactive amino acids. These photo-reactive amino acids contain benzophenone or diazirine groups and are activated upon UV-A irradiation. As such, they possess the potential to study in vivo protein interactions. In my talk, I will give an overview of different cross-linking strategies and illustrate them for studying protein complexes of proteinkinase D, the tumor suppressor p53, and calmodulin-Munc13.