Identification of intact cross links in proteins and peptides using tandem mass spectrometry

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Chemical cross-links can provide spatial proximity between amino acid residues on proteins. LC-MS/MS is a powerful method to identify chemical cross-links in proteins. Two types of chemical cross-linking methods have been developed for this type of experiments: selective chemical cross-linking and nonselective photo-induced chemical cross-linking methods. The LC-MS/MS data for proteins with nonselective photo-induced cross-links can be extremely complicated and no software approaches have been reported to date for this type of analysis. We report a novel universal algorithm that can be used to identify peptides and proteins with selective and nonselective cross-links.

The algorithm was developed based on the MassMatrix database search engine by adding new algorithms for in silico digestion of proteins and fragmentation of theoretical peptides with consideration given to selective or nonselective cross-links. Cross-link identification is scored in XMapper.

The algorithm was tested using BS$_3$ (bis[sulfosuccinimidyl] suberate) cross-linked Cytochrome C. Five cross-links were identified and verified for spatial plausibility by comparison with its three-dimensional structure at optimized experimental conditions. It has also been demonstrated that the MassMatrix database search engine is capable of searching for intact cross-links in complex Escherichia coli proteome samples cross-linked by BS$_3$.

The algorithm for nonselective chemical cross-link search was validated using a tryptic digestion of bovine hemoglobin cross-linked by Sulfo-NHS-LC-Diazirine. The cross-link has one selective site on Lysine residues and the other nonselective site. Bovine hemoglobin forms heterodimer with two sets of identical chains (chains A and B). More than 200 photo-induced cross-links were identified at a FDR < 5%. These identified cross-links provide useful information about the 3D structure of the protein and are confirmed by the protein’s 3D structure.

Biography

Hua Xu is currently a research scientist at the Center for Proteomics and Bioinformatics at Case Western Reserve University, after working at the Proteomics and Informatics Services Center at the University of Illinois at Chicago for approximately three years. He received his Ph.D. from The Ohio State University.

Dr. Hua Xu has published 21 research papers on peer-reviewed scientific journals including Bioinformatics, BMC Bioinformatics, Journal of Proteome Research, Proteomics, Journal of the American Society for Mass Spectrometry, and Journal of Physical Chemistry B. He has also reviewed manuscripts for Journal of Proteome Research, Molecular & Cellular Proteomics, Bioinformatics, Analytical Chemistry, and Computational Biology and Chemistry.