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Seminar

In-vitro and *in-cell* cross-linking/mass spectrometry: from 3D-protein structure investigations to proteome-wide interactome studies

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In-Vitro and *In-Cell* Cross-Linking/Mass Spectrometry:

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Chemical cross-linking/mass spectrometry (XLMS) has emerged as a powerful tool for the 3D-structure analysis of proteins and protein complexes and is becoming increasingly popular in structural biology. The cross-linker has a defined length and imposes a distance constraint between the amino acids connected. The cross-linked proteins and protein complexes are enzymatically digested and analyzed by high-resolution mass spectrometry (MS). This permits the identification of the cross-linked amino acids yielding conclusions on their spatial proximity in the protein assembly. The resulting map of amino acid distances can be computationally reprocessed resulting 3D-structural models of the protein or protein assembly. The main advantage of XLMS over other protein structural techniques is that it creates comprehensive snapshots of the protein landscape with the minimum interference. XLMS experiments can be conducted within a few days making XLMS a highly attractive approach that complements existing high-resolution protein structural techniques, such as NMR spectroscopy, X-ray crystallography, and cryo-EM. On top of that, XLMS only requires minute protein amounts and is even applicable to intact cells. System-wide XLMS offers two key benefits: i) it allows capturing system-wide protein interactions for a comprehensive understanding of cellular signaling pathways and ii) it allows analyzing the conformation and the interaction of proteins in their native environment. Therefore, XLMS is currently one of the most promising MS-based approaches to derive 3D-structural information on very large and transient protein complexes as well as on intrinsically disordered proteins.

We developed and successfully applied sophisticated cross-linkers and integrated workflows to perform XLMS at all levels: from isolated protein and protein assemblies to highly complex protein mixtures, such as cell lysates and intact cells. Principles and recent applications of XLMS will be discussed.

References.

1 C. Iacobucci, *et al.*, *A Community-Wide, Comparative Study Towards Establishing Best Practice Guidelines*, **Nat. Commun.** (2018), under revision. (available at https://doi.org/10.1101/424697)

2. C. Iacobucci, M. Götze, *et al*., *A Cross-linking/Mass Spectrometry Workflow Based on MS-Cleavable Cross-Linkers and the MeroX Software for Studying Protein Structures and Protein-Protein Interactions*, **Nat. Protoc.** (2018), 13, 2864-2889.

3 M. Götze, C. Iacobucci, *et al*., *Cross-Linking/Mass Spectrometry for Proteome-Wide Interactome Studies: Protein Interaction Networks in Drosophila Embryos*, **Cell systems** (2018), under revision.