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Mass spectrometry supported determination of protein complex structure

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Virtually all the biological processes are controlled and catalyzed by proteins which are, in many cases, in complexes with other proteins. Therefore, understanding the architecture and structure of protein complexes is critical to understanding their biological role and function. Traditionally, high-resolution data for structural analysis of proteins or protein complexes have been generated by the powerful methods of X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. More recently, mass spectrometry (MS)-based methods have been developed that provide low-resolution structural information, which contributes to the determination of the native structure of protein complexes that have remained refractory to the high-resolution methods. Native MS and affinity purification coupled with MS (AP-MS) have been used to characterize the composition, stoichiometry and connectivity of protein complexes. Chemical cross-linking MS (CX-MS) provides protein–protein interaction data supplemented with distance information that indicates residues that are in close spatial proximity in the native protein structure. Hydrogen-deuterium exchange combined with MS has been used to map protein–protein binding sites. Here, we focus on recent developments in CX-MS and native MS and their application to challenging problems in structural biology.

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Introduction

The majority of proteins expressed in living organisms are part of protein complexes, which are further organized in dynamic protein interaction networks in the living cell [1]. To form functional protein complexes, proteins can stably or transiently associate with other proteins or different types of biomolecules such as nucleic acids or lipids. Such structures may consist of a few to hundreds of protein components. Macromolecular assemblies are known to play a crucial role in many of the cellular processes [2]. Therefore, their structural characterization is indispensible for a mechanistic understanding of the processes in living organisms and cells [3,4]. On the basis of large-scale AP-MS studies (reviewed in [5]), comprehensive protein–protein interaction data are available for the *Saccharomyces cerevisiae* proteome (∼6600 ORFs [6]) [7,8]. Gavin *et al.* [8] have estimated the total number of core complexes in yeast to be about 800, and in human to be about 3000.

Over the last decade, high-throughput structural biology initiatives were undertaken which aimed and largely succeeded in generating structural information for almost every protein family (see also the recent dedicated issue 'Structural Bioinformatics' [9] in Journal of Structural Biology). Additionally, the high-resolution structures of macromolecular assemblies of increasing size and complexity have been determined, as exemplified by the ribosome [10], the exosome [11,12], RNA polymerase II [13] and the 20S proteasome [14,15]. Despite these successes, large macromolecular assemblies are often refractory to high-resolution structure determination by X-ray crystallography or NMR spectroscopy, in part due to their high molecular mass and significant flexibility, indicating the need for alternative or complementary methods.

Over the last few years several mass spectrometry-based methods have been developed that generate low-resolution structural data of macromolecular assemblies. These data have been used, mostly via computational integration of complementary data types, to determine a range of increasingly complex structures [16–20,21••,22••,23••,24]. In general, MS based methods are applicable to soluble proteins or protein complexes, are for the most part not limited by the size of the protein complex analyzed, and the analysis is relatively fast and consumes only ng to μg amounts of sample. The mass spectrometric methods discussed in this article, the type of information and level of resolution they achieve and other characteristics are summarized in Box 1 and Figure 1.

In contrast to diffraction data from X-ray crystallography, structural data obtained by the mass spectrometric methods described in Box 1 does not provide 'complete' information about the three-dimensional (3D) structure

Box 1 Mass spectrometry based techniques for structural biology

Chemical cross-linking mass spectrometry (CX-MS): Amino acid residues in spatial proximity are physically connected by covalent bonds via a cross-linking reagent. The protein complex is cleaved into peptides, which are then analyzed by liquid chromatography-tandem mass spectrometry (LC–MS/MS). ***Data type:*** Direct binary contacts between subunits, low resolution distance restraints (20–30 Å).

Mass spectrometry of intact assemblies (native MS): Intact complexes of up to several MDa are directly analyzed by mass spectrometry. Solution and gas-phase dissociation into subcomplexes and hyphenation with ion mobility spectrometry is possible. ***Data type:*** Subunit composition, connectivity and stoichiometry of a complex and its subcomplexes; in combination with ion mobility separation information on shape via collisional cross section.

Hydrogen/deuterium exchange-mass spectrometry: Exchange of backbone hydrogen with deuterium atoms (or vice versa) is used as a proxy for solvent accessibility of regions of a protein complex. Deuterium incorporation is determined by mass spectrometry on the peptide level after rapid enzymatic digestion of the native protein. Practically restricted to smaller complexes. ***Data type:*** Changes in solvent exposure reveal conformational changes, ligand binding, etc.

Affinity purification-mass spectrometry (AP-MS): Interaction partners of affinity-tagged bait proteins are identified, and in some cases quantified from ‘pull-down’ experiments using conventional mass spectrometry-based proteomics workflows. ***Data type:*** Subunit composition and stoichiometry of a complex; indirect connectivity (networks, functional modules).

investigated. Therefore, such data are used most frequently in combination with complementary structural data obtained by different technologies to compute models of large protein complexes. The computational approach used to determine the architecture of an assembly can be summarized as an optimization problem in a 3D space (in analogy to solving a 3D puzzle). Structural data are represented in form of spatial restraints or volumes and the model (or an ensemble of models) that satisfies the input restraints and volumes is searched (for a detailed review see [25••]). The resolution and accuracy of the model strongly depends on the available structural data and ranges from coarse-grained topology models to near-atomic resolution models.

In this review we describe recent developments and applications of CX-MS and native MS, and discuss how these methods were used by integrative approaches to obtain molecular models of the 26S proteasome, the TRiC/CCT chaperonin and the mechanism of lipid binding to intact V-Type ATPases. Hydrogen-deuterium exchange-mass spectrometry has been reviewed recently by Konermann *et al.* [26] and hence is not discussed further in this review.

Chemical cross-linking mass spectrometry (CX-MS): recent developments and applications

The technology of chemical cross-linking of proteins, interfacing biology and chemistry, has a long-standing history in protein chemistry. About a decade ago, the combination of chemical cross-linking and subsequent identification of cross-linked residues by mass spectrometry was first reported in the literature [27,28].

For such studies, proteins or protein complexes are chemically cross-linked in solution under near-physiological conditions by using homobifunctional reagents, enzymatically digested to peptides, and the resulting peptides are subsequently analyzed by tandem mass spectrometry (reviewed in [29–32]). In recent years the technology has constantly evolved and a number of different implementations of the general concept have been described [17,28,33,34]. They share the major protocol steps ‘cross-linking’, ‘digestion’, ‘tandem mass spectrometry’, and ‘data analysis’, but differ significantly in the way these steps are being carried out. Broadly, two classes of methods have been developed, one around cleavable cross-linkers where the linked peptides are uncoupled before or during the mass spectrometric fragmentation process [35,36], and the other around non-cleavable cross linkers where ions of the cross-linked peptides are fragmented as single chemical entities [28,33]. The majority of recent reports on protein complexes analyzed by CX-MS have used non-cleavable cross linkers and are therefore further discussed below [18,21••,22••].

The commonly used homobifunctional cross-linker molecules and the mass spectrometric and data analysis tools have been recently reviewed in [37,31,38], respectively. In an enzymatic digest of a cross-linked complex, the number of cross-linked peptides is vastly lower as compared to the number of non cross-linked peptides. To enrich or detect cross-linked peptides in a complex sample, analytical enrichment strategies for cross-linked peptides based on physiochemical properties such as their higher relative charge or molecular weight have been developed [39,40]. Cross-linker molecules with additional functional groups for affinity-based enrichment (reviewed in [37]), and isotopically labeled cross-linkers that facilitate the selection of cross-linked peptides in a sample have been introduced [33,41]. Each one of these measures facilitates the selection of precursor ions of cross-linked peptides for fragmentation in the mass spectrometer. Subsequently, from the acquired fragment ion spectra the underlying sequences of the cross-linked peptides are identified by dedicated search engines. Recently, such software tools have been developed that facilitate the automated identification and statistical validation process of cross-linked peptides from tandem MS spectra [42,43].

By using these main steps, several groups were recently successful in applying the technology to various challenging large macromolecular assemblies.

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