Mass spectrometry for semi-experimental protein structure determination and modeling

Authors: Gerhard Mayer

1 Ruhr University Bochum, Faculty of Medicine, Medizinisches Proteom-Center, D-44801 Bochum, Germany

Gerhard Mayer
Ruhr University Bochum, Medical Faculty, Medizinisches Proteom-Center, D-44801 Bochum, Germany
Tel.: +49 234 32-21006; Fax: +49 234 32-14554; email address: gerhard.mayer@rub.de
**Abbreviations:**

| Abbreviation | Description |
|--------------|-------------|
| cryo-EM      | cryo-Electron Microscopy |
| CX-MS        | Cross-linking-Mass Spectrometry |
| EM           | Electron Microscopy |
| HDX-MS       | Hydrogen-Deuterium Exchange-Mass Spectrometry |
| IM-MS        | Ion Mobility-Mass Spectrometry |
| MS           | Mass Spectrometry |
| MS3D         | Mass Spectrometry to derive 3D structural information |
| NMR          | Nuclear Magnetic Resonance |
| PDB          | Protein Data Bank |
| PPI          | Protein-Protein Interaction |
| XL           | Cross-Linking |
| XRC          | X-Ray Crystallography |
**Summary/Abstract:**
The structure of proteins is essential for its function. The determination of protein structures is possible by experimental or predicted by computational methods, but also a combination of both approaches is possible. Here, first an overview about experimental structure determination methods with their pros and cons is given. Then we describe how mass spectrometry is useful for semi-experimental integrative protein structure determination. We review the methodology and describe software programs supporting such integrated protein structure prediction approaches, making use of distance constraints got from mass spectrometry cross-linking experiments.

**Keywords:** protein structure determination, integrative protein structure modeling, distance constraints, cross-linking
1. Introduction

Classically one either determines the protein structure with X-ray crystallography or NMR or one tries to model the protein structure from the sequence with either homology modelling, threading or ab initio (de novo) prediction [1]. These classical experimental methods are expensive and time-consuming and have some other shortcomings as shown by the disadvantages of these experimental methods listed in Table 1. Additionally the computational methods have their limits, e.g. homology modelling requires a known template structure from a protein sequence, which has at least 30% of sequence similarity to the target sequence and the sequence-structure profile matching of threading approaches, based on statistical similarities between sequential and structural properties. Cross-linking mass spectrometry is a high-throughput experimental method, which delivers data, useful in combination for restraining the search space of such computational methods.

2. Overview about experimental protein structure determination methods

Since the exact prediction of protein structures in general is still an unsolved problem, protein structures until now are determined by diverse experimental methods. These experimental methods can be divided into the well-known classical methods (X-ray crystallography, NMR, cryo-EM) and the modern seminal method of coherent diffraction, which give a more or less complete set of 3D coordinates for the constituent atoms and other (spectroscopic, MS-based [2] and other structurally important experimental data generating) methods. These methods give only partial structural information and hints, which somehow correlate with the protein structure. Many of them are spectroscopic methods, which either give global information and/or information about secondary structures, or information about dynamical structural changes, e.g. due to ligand binding. These experimental data are useful for deriving distance constraints for guiding computational structure prediction methods by considerably confining the configurational search space. Table 1 gives an overview of current experimental techniques, which are useful for getting protein structure related information or for protein structure determination.

Table 1: Different kinds of experimental methods for protein structure determination or for getting protein structure related information, useful as basis for semi-experimental protein structure prediction.

| Method | Advantages | Disadvantages | References |
|--------|------------|---------------|------------|
| A) Classical protein structure determination techniques | | | |
| Crystallization and X-ray crystallography (XRC) or neutron scattering (NS) | method with the highest resolution | requires crystallized proteins and therefore a larger amount of purified protein | [3,4] |
| | | not applicable for membrane proteins | |
| | | labor intensive, high cost | |
| | | slow | |
| | | access to a synchrotron is advantageous, since the resolution becomes better when using higher energetic radiation | |
| | | gives no information about protein dynamics due to the rigid crystal | |
- Loop regions are often found in unnatural conformational states (because in fixed crystals compared with the protein in solution).
- Images must be calculated from diffraction pattern by Fourier synthesis.
- Radiation damage of crystals possible.

### Nuclear magnetic resonance (NMR)
- Can give dynamic protein flexibility information since analysis occurs in solution.
- Yields proton-proton distance and chemical shift constraints.
- Only for smaller proteins (mainly < 30 kDa).
- Only for soluble proteins.
- Gives only ensemble information, requires lots of computational processing.
- Modest resolution.
- High cost method.
- High concentration of pure protein required.

### Cryo-Electron microscopy (cryo-EM)
- Gives directly images of larger structural parts like domains or α-helices.
- Higher resolution models possible by electron density map correlation, if the atomic structure is given.
- Medium to good resolution.
- Radiation damage possible.

### Holographic coherent diffraction mapping with X-ray free Electron Laser (XFEL), CDI (Coherent Diffraction Imaging)
- Requires only a single molecule.
- High resolution possible.
- Requires no protein crystal for diffraction.
- Still difficult to handle the experimental procedure.

### SAXS/SANS and WAXS/WANS (Small-, resp. Wide Angle X-ray / Neutron Scattering)
- Delivers information about flexible and weakly interacting proteins.
- Gives only low resolution information (shape reconstruction, radius of gyration).

### B) Spectroscopic methods

#### Short description

**CD (Circular Dichroism)**
- Fast and relatively easy measurement.
- Gives ratios for content of α-helices, β-sheets and random coils.
- Gives information about the secondary structure content by measuring the absorbance of polarized light (ellipticity in dependence of the wavelength $\lambda$).

**FTIR (Fourier Transform Infra-Red spectroscopy)**
- Gives mainly secondary structure information.

**FRET ( Förster Resonance Energy Transfer)**
- Mainly for determination of molecular interactions, conformational changes and quaternary structure.

**Tryptophan fluorescence**
- Gives information about the local electrostatic interactions (e.g. hydrations) of aromatic residues like tryptophan and tyrosine and allows to monitor conformational changes caused e.g. by ligand binding.

**2D-IR (Infra-Red) spectroscopy**
- The amide I backbone vibrations give specific signatures for different secondary structural motifs allowing to study protein dynamics on small time scales and to distinguish parallel from anti-parallel β-sheets.

**Deep UV Raman spectroscopy**
- Can be used for characterizing secondary structure composition.

**EPR (Electron Paramagnetic Resonance) and SDSL (Site-Directed Spin)**
| **Labeling** or **PELDOR / DEER (Pulsed Electron-Electron Double Resonance)** |  |
|---|---|
| allows to monitor conformational changes at the backbone level (SDS) |  |
| allows to measure distances in the nanometer range (1.5-8 nm) between paramagnetic centers like e.g. amino acid radicals (PELDOR / DEER) | [27-29] |

**C) Mass Spectrometric based approaches**

| **Protein Cross-linking (CX-MS)** | **HDX-MS (Hydrogen-Deuterium Exchange-Mass Spectrometry)** |
|---|---|
| requires only small amount of protein | targets hydrogens of amide groups from the protein backbone |
| can analyze protein mixtures | gives information about solvent accessibility and hydrogen bond status, which is dependent on secondary structure |
| is a High-Throughput method | continuous labeling can give information about the different conformations of a protein |
| structure determination of proteins containing flexible and disordered regions possible | pulse labeling allows to determine the influence of ligand binding on dynamic conformation changes |
| gets structure information also of weakly (transient) interacting complexes | requires handling with radioactive material |
| can uncover novel, so far unknown protein interactions with other proteins or other biomolecules (DNA/RNA, lipids, sugars) | back-exchange of deuterium limits precision of the measurements |
| is a relatively fast method | no structure information about protein core |
| derives distance constraints for structure refinement in homology modelling or for restraining the sample space in ab initio modeling |  |
| dead end modifications and zero-length cross-linkers can give information about solvent accessibility |  |
| allows the proteome-wide profiling to uncover PPI’s [30] |  |
| applicable also to membrane proteins [31] |  |
| semi-experimental method, i.e. typically it is combined with constraint-based modelling methods |  |
| requires either an enrichment step for the cross-linked peptides or the use of isotope-labelled cross-linkers |  |
| identification of the cross-links has in general quadratic complexity (exception: when using isotope-labelled cross-linkers) |  |
| CX-MS is most sensitive for highly abundant proteins. Therefore should be combined with pre-fractionation, e.g. size-exclusion chromatography or other enrichment methods, e.g. the use of affinity-tagged cross-linkers |  |

| **IM-MS (Ion Mobility-Mass Spectrometry)** | **Native MS** |
|---|---|
| multi-dimensional separation that allows to separate the components of protein complexes by size and shape in gas phase | gives quaternary structure information (stoichiometry and topology of protein complexes) |
| proteins are studied in a native-like structure state (since in gas phase) | study of membrane proteins not possible, since native MS work in aqueous solution |
| allows to determine the cross-section and radius of gyration of proteins complexes | gives only quaternary structure information (no structural information) |
|  | [42-44] |

**RAW TEXT END**
### Limited proteolysis combined with MS
- for isolation of protein domains
- allows to trace conformational transitions due to ligand binding
- requires only low time and effort
- gives only low resolution information

### Hydroxyl radical protein footprinting (covalent labeling) combined with MS
- gives information about solvent accessibility of the side chains of residues
- allows to monitor ligand-binding induced conformational changes
- no back exchange of labels like in HDX-MS
- relative reactivity of the residues must be taken into account

### Carbene footprinting
- allows the experimental determination of the solvent accessible surface area after laser-induced photolysis of diazirine into methylene carbine :CH₂, which is highly reactive and has a similar size as water
- can be used to determine the binding sites of protein-ligand and PPI interactions
- gives only information about the surface area

### Surface Induced Dissociation (SID)
- gives information about the connectivity, topology and the relative interface strengths of quaternary protein complexes
- can be combined with protein-protein docking approaches

### D) Experimental biochemical data
- Site-directed mutagenesis experiments
  - exchange of amino acid residues by site-directed nucleotide exchange in the coding gene
  - experimentally laborious and therefore costly
- Alanine scanning of protein surfaces
  - systematic exchange of amino acid residues by alanine
  - experimentally laborious and therefore costly

### E) Integrative methods either combining several experimental methods or combining computational methods with experimentally derived constraints
- combines information from several methods, especially computational and experimental methods, where the experimental data restrict the search space or guide the search
- semi-experimental method
- high efforts for integrating data from several sources into computational models required

### 3. Use of data produced by mass spectrometric methods for protein structure elucidation
As shown in Table 1, the different mass spectrometric based methods can be used to get information about distance restraints [66], solvent accessibilities of residues, stoichiometry of protein complexes, the global shape and radius of gyration and also about induced conformational changes in response to binding or interaction events.

The distance constraint information got by mass spectrometric cross-linking experiments (CX-MS) can be used theoretically to determine the 3D structure of proteins and protein complexes, if a great enough variety of cross-linkers regarding both their targeted amino acid residues and their spacer...
lengths is available. Until now, we are not aware that a protein structure is solvable solely by CX-MS alone. But the use of cross-linking either for supplementing the information got from other experimental structure determination techniques [59,62,67,68] or in combination with computational structure prediction methods [69] to predict the protein structures in a semi-empirical way - sometimes called hybrid methods - is already routinely done [1,70]. Often a small set of distance constraints got from CX-MS can help to distinguish between two or more possible protein structures. Mass spectrometry based approaches like CX-MS, HDX-MS and IM-MS can complement classical structural biology methods and provide beside identification and quantification information also information about protein and protein complex structure and the network of interaction between the proteins. By analysis of these interaction networks hints about the cellular phenotypic states in health and disease [71] and their system behavior [72] can be derived.

The spectroscopic and mass spectrometry methods are useful to complement the information got from classical methods or can be used in cases where the classical methods are not applicable. Examples are information about disordered parts of a protein, that cannot be got by X-ray crystallography, or the structure determination of large membrane complexes, which are neither amenable by X-ray crystallography (because lack of crystals), nor by NMR (because of their size) and where cryo-EM alone can give only very low-resolution information.

Integrated structure determination methods either combine several experimental methods, or they integrate one or more of the experimental spectroscopic, biochemical and/or mass spectrometry based methods with computational structure prediction methods to give semi-empirical solved protein structures. In the following we concentrate on such semi-experimental integrative protein structure modeling methods to illustrate how the information contained in the XLMOD ontology [7] can be used in CX-MS method and how it thereby can contribute to improved protein structure modeling resp. prediction.

In general the identification by a search engine is challenging, because the identification of the cross-links has in general quadratic computational complexity compared with the linear complexity of a database search in normal MS [73]. This is because one has to search for all possible binary combinations of peptides. This can either be done in an exhaustive search [74] or one explicitly encodes all the cross-linked peptides in the search database [75]. But also alternative strategies for the identification of cross-linked peptides, based on labelling of the used cross-linking reagents, were developed [76]. Beside of that also special cross-linking search engines [77,78], often used together with CID-cleavable cross-linkers [79] or isotope-labelled cross-linkers [80,81] are in use. Thereby the search can be made more efficient, because one now can easily detect the CID-fragment ion patterns [79] and / or neutral losses [34] respective the cross-linked masses by their characteristic doublets corresponding to the light resp. heavy forms of the used cross-linker [80,82]. Another method is to use cleavable cross-linkers and compare the mass shifts before and after the cleavage of the cross-linker [83]. Or one can use 18O labeling of the two oxygen atoms of the carboxyl group of the peptide C-terminus, leading to a mass shift of 8 Da for inter-peptide cross-links resp. of 4 Da for intra-peptide cross links and dead end modifications [84]. Instead of labeling the peptides one can also label the cross-linking reagents with 18O, leading to doublets separated by 4 Da for the cross-linked and the not
cross-linked peptides [85]. Other cleavable cross-linkers like BuUrBu generate characteristic doublet patterns by which the cross-linked peptides can be identified [86]. PIR (Protein Interaction Reporter) is another flexible technology for the design of cross-linking reagents for distinguishing dead end, intra- and inter-peptide cross-links [87]. These PIR cross-linkers are designed with two CID labile bonds, so that a specific reporter ion, possessing an affinity or click-chemistry reactive group for purification, is released after cleavage [87]. Beside the chemically and MS cleavable cross-linkers, reviewed by Sinz [88], there are also photo-cleavable cross-linkers available, e.g. pcPIR [73]. X-links is a special search engine developed for the analysis of data sets got from such PIR cross-linking experiments [89]. Another possibility is the usage of cross-linking reagents containing MS2 labile bonds, e.g. C-S bonds, so that after MS2 cleavage the two cross-linked peptides can be unambiguously identified in the following MS3 step [90].

Another category are photoactivatable cross-linkers having an (aryl) azide, benzophenone or diazirine (resp. the isomeric diazo) reactive group [91-93]. These photoactivatable cross-linkers are mostly not selective regarding the targeted amino acid. These photoactivatable cross-linkers are very useful, because using a UV light source one can control the cross-linking reaction and one gets highly reactive intermediates resp. excited states, which react non-selectively with a great variety of amino acid residue side chains [94]. This is advantageous, since by that one gets an increased number of distance constraints, in turn providing more information for determining the protein 3D structure [95,96]. Aldehydes such as glutardialdehyde and especially formaldehyde are broadly specific, i.e. they react with many amino acid residues and also with DNA, and the cross-link yield depends on the reaction conditions and reaction times, which therefore must be carefully controlled [97]. Therefore, they allow also the study of biomolecular protein interactions in living cells. Formaldehyde can also be used for the detection of protein-DNA interactions [98] and enable one to analyze also archived formalin-fixed samples [99].

The CX-MS method provides a set of experimentally determined distance constraints, which are used for the enumeration of all protein conformations, which are in agreement with theses constraints [100]. This constraint-based protein structure modeling method can not only used for predicting the tertiary structure of a single protein, but also for the quaternary structure of protein complexes. In the protein-protein docking method normally one gets in a first search step a very large list (often tens of thousands) of conformations and then a post-docking search process is performed in order to rank these conformations got from the first step [101]. But it was shown that even after this ranking the true complex structure often cannot be found at the top of the resulting ranked list. The integration of experimentally determined distance constraints from CX-MS or other experimental methods like SAXS can help to predict the real protein complex structure by sorting out all conformations, which are not in accordance with the experimental results [59]. According to [102] in general three distance restraints are enough to determine the PPI interface. By such experimentally derived distance constraints the conformational space to be searched by protein-protein docking programs, can be drastically reduced [103], so that in combination with efficient implementations like the fast 5D-manifold Fourier Transform (FMFT) [104] the high throughput determination of PPI interfaces becomes possible.
The constraint-based approach is based on distance geometry [105] and already used in homology modeling [106], where one uses a template protein with an as high as possible sequence similarity to the target protein to model the structure of the target protein. It can also be used for fitting structures into images got from cryo-EM [107], applied to structure determination either from distances between protons, yielded by NMR measurements [108] or from distances derived from SAXS profile data [109,110]. In distance restraints got from CX-MS data, one must take into account the distances between the Cα-atoms. For instance for the cross-linking reagent BS3 with a spacer length of 11.4 Å linking two lysine residues one must add twice the distance from the ε-amino group of Lys to the Cα-atom of Lys (6.4 Å), so that the total distance becomes 11.4 Å + 2 • 6.4 Å = 24.2 Å [111]. In addition, often a tolerance of ~3-6 Å is added to account for flexibility of a dynamic backbone, so that the recommended distance to use would be 27.2 – 30.2 Å. The optimal choice of a spacer length was systematically investigated by Hofmann et.al, which derived the following formula for it:

$$l_{opt} [\text{Å}] = k \sqrt{MW} + \sqrt{SCL1 + SCL2},$$

where MW is the molecular weight of the protein, SCL1 and SCL2 are the side chain lengths of the two cross-linked residues and k is a constant, which is 0.32 for Lys-Lys, 0.31 for Lys-Asp, 0.34 for Lys-Glu and Arg-Arg and 0.35 for Cys-Cys cross-links [112].

In general, with CX-MS experiments one can easily derive distance constraints for the maximum distance between two residues, whereas one must be careful in deriving minimal distance constraints, because for that one must take into account the cross-linker flexibility and the fact, that a cross-link cannot penetrate the surface of the protein [113,114].

Also one should be aware that sometimes the spacer arm cannot link two residues directly through the interior of the protein and that sometimes two residues are only not found to be linked to each other, since there are competitive suitable reactive residues at more optimal distances preferably linked [115]. Therefore, if one uses several cross-linkers with different spacer lengths, one should handle derived minimal distances with care. One possibility to handle that problem was implemented in Xwalk [113], an algorithm for validating cross-linking distances by calculating the solvent accessible surface distance (SASD) taking into account the cross-linker flexibility and that the cross-link will not penetrate the protein surface [113,114]. In addition, one can take the dynamic flexibility of the protein structure into account [116], as done by the DynamXL software. Another approach is to use specialized scoring functions, which assess beside the cross-link distances and their violations also the solvent accessibility in the scoring function [114,117].

Sometimes several distance constraints are in conflict with each other, what can e.g. be the case because of flexibility. Ferber et.al developed the XL-MOD software, which can handle that in an automatic way, by using self-organizing maps, which are a special class of neural networks, to test and score different combinations of restraints [102].

The de novo prediction of a protein from distance constraints alone is computationally very expensive, so that it is until now realistically only for peptides and small proteins, when one has unambiguous distance constraints [100]. For cases with additional uncertainty, e.g. due to unknown side chain conformations, and for larger proteins and protein complexes one has to use approximate methods, where the side chain representations are simplified by a super atom [112]. Therefore, CX-MS is useful for integrated protein structure prediction methods, where one uses the distance constraints to refine, i.e. to filter out incorrect models from a set of predicted models [115]. The predicted models stem
either from comparative (homology) modeling, to limit the sample space in *ab initio* modeling methods (which are based on molecular dynamics simulations using physical or knowledge-based, i.e. statistical force fields) [1], or are identifying the folds in threading (fold recognition) methods [118], which are based on sequence-structure profile matching.

Beside the support of tertiary structure predictions, CX-MS is an already established method for the elucidation of the quaternary structure of protein complexes. For that either restraints from CX-MS are integrated and used for improving the quality of either template-based [119] or FFT-based [103] protein-protein docking predictions or the distance restraints are used to construct a subunit interaction network with is then combined with homology modelling, as it is done by the SUMMIT algorithm [120]. CX-MS is combinable with other mass spectrometry methods for integrative protein modelling. Politis et.al. developed a weighted scoring function for integrating data about the overall shape (cross-section) obtained from IM-MS with stoichiometric information from native MS as well as intra-and inter-protein distance constraints from CX-MS for a restricted sampling of the conformational space [121].

4. Overview about tools integrating cross-linking information for protein structure prediction

Until now there are only few software programs available that support integrative structural modelling of proteins and protein complexes. Mass Spec Studio [78,122] allows the integration of data from HDX-MS [123], covalent labeling (protein footprinting) and cross-linking for the modeling of protein interactions with the protein-protein docking webserver HADDOCK (High Ambiguity driven protein-protein DOCKing) [124].

DockStar is an integrative software package for modeling of protein complexes that can integrate data from X-ray crystallography, NMR, comparative homology modeling and CX-MS [125]. It uses Integer Linear Programming (ILP) for the optimization of both knowledge-based interaction potentials as well as the satisfaction of constraints derived from CX-MS [125].

The Integrative Modeling Platform (IMP) [126] converts the information from five different experimental methods (SAXS profiles, EM2D images, EM3D density maps, the residue type content at the interface between the two proteins and distance restraints resulting from CX-MS) into a set of spatial constraints. These contraints must fulfilled simultaneously and are then optimized with a simulated annealing Monte Carlo approach followed by a refinement step [127]. This optimization based on the experimentally derived restraints drastically reduces the number of possible protein-protein docking models. IMP has several components ranging from a low level C++/Python library to user-friendly interfaces, which are integrated into the UCSF Chimera molecular visualization package [128] and allow a further integration with the homology modelling package MODELLER [129]. Another UCSF Chimera plugin useful for integrative modeling is XLinkAnalyzer [130], allowing one to integrate CX-MS data with the fitting of X-ray structures into electron density maps got from electron microscopy.

Another integrated modeling software, which can integrate distance restraints from cross-linking, is ROSETTA making use of the Xwalk algorithm [113]. It calculates the shortest path for a cross-link spacer, which leads only through solvent accessible space and not through the interior of the protein, and of XLdb, a database containing experimental cross-link data which are mapped to experimental structures from the Protein Data Bank (PDB) [131]. Kahraman et.al. describe 3 workflows based on
ROSETTA for data-driven comparative and for de novo modeling of a proteins tertiary structure, as well as for protein-protein docking.

I-TASSER (Iterative Threading ASSEmbly Refinement) [132] is a threading program for protein structure prediction, which allows to include experimentally gained distance restraints, either from cross-linking or from NMR experiments.

There are also software tools available for the visualization of cross-linking results, for instance xVis. It displays the cross links as circular, bar or network diagrams for the representation of the spatial restraints [133] (http://xvis.genzentrum.lmu.de/, accessed 02/2020), xiNET, showing interactive node-link diagrams [134] (http://crosslinkviewer.org/, accessed 02/2020) and the map viewer of XLPM [135] or VisInt-X (https://www.researchgate.net/publication/301766070_VisInt-X_Visualizing_Interactions_in_Cross-linked_proteins, accessed 02/2020). XLmap is a R package by which one can visualize contact maps with integrated of cross-link information [136].

Other interesting approaches are the specialized databases like XLinkDB 2.0 [137], integrating XL-MS data with PPI network analysis, PDB queries and homology models from the MODELLER [129] software or ProXL for visualizing, comparing, sharing, analyzing and quality control of CX-MS data [138]. MNXL [139] is a server which allows model validation based on cross-linking derived distance constraints.

Software packages supporting integrative protein structure modeling making use of cross-linking data are listed in Table 2 and a list summing up the available software tools and databases for cross-linking analysis, identification or visualization are given in Supplementary Table S1 and in the review of Yilmaz et.al. [140].

Table 2: Software packages for protein structure modeling able to integrate distance restraints derived from cross-linking data.

| Integrative modeling package | URL (accessed 02/2020) | References |
|------------------------------|------------------------|-------------|
| DockStar                     | http://bioinfo3d.cs.tau.ac.il/DockStar/ | [125]       |
| HADDOCK                      | http://www.bonvinlab.org/software/haddock2.2 | [124]       |
| Integrative Modeling Package (IMP) | https://integrativemodeling.org | [126-128]   |
| I-TASSER                     | http://zhanglab.ccmb.med.umich.edu/I-TASSER/ | [141]       |
| Mass Spec Studio             | https://www.msstudio.ca | [78,122,124] |
| MNXL                         | http://mnxl.ismb.lon.ac.uk/mnxl/ | [139]       |
| MODELLER                     | https://salilab.org/modeller/ | [129]       |
| ROSETTA                      | https://www.rosettacommons.org | [131]       |
| SSEThread                    | https://github.com/salilab/SSEThread | [142]       |
| UCSF Chimera                 | https://www.cgl.ucsf.edu/chimera/docs/morerefs.html | [128]       |
| XLink Analyzer - UCSF Chimera plugin | http://www.beck.embl.de/XlinkAnalyzer.html | [130]       |
5. Summary
The distance constraints got by the high-throughput CX-MS method can be used alone or in combination with constraints derived from other experimental methods to restrain, guide and accelerate the search of computational structure prediction methods. The resulting semi-experimental integrative structure prediction methodology are useful to model the structure of proteins, which cannot determined by one of the classical protein structure determination methods due to their limitations as listed in Table 1.

Acknowledgements:
Gerhard Mayer was funded by the BMBF grant de.NBI - German Network for Bioinformatics Infrastructure (FKZ 031 A 534A).

Teaser:
Cross-Linking is a high-throughput method that delivers distance constraints between two specific residues of a protein, useful as distance restraints to guide and improve computational structure modelling methods.
References:

1. Pi J, Sael L. Mass spectrometry coupled experiments and protein structure modeling methods. *International journal of molecular sciences*, 14(10), 20635-20657 (2013).

2. Serpa JJ, Parker CE, Petrotchenko EV, Han J, Pan J, Borchers CH. Mass spectrometry-based structural proteomics. *Eur J Mass Spectrom (Chichester, Eng)*, 18(2), 251-267 (2012).

3. Shi YG. A Glimpse of Structural Biology through X-Ray Crystallography. *Cell*, 159(5), 995-1014 (2014).

4. Jaskolski M. Personal remarks on the future of protein crystallography and structural biology. *Acta Biochim Pol*, 57(3), 261-264 (2010).

5. Guntert P. Automated structure determination from NMR spectra. *European biophysics journal : EBJ*, 38(2), 129-143 (2009).

6. Harris JR. Transmission electron microscopy in molecular structural biology: A historical survey. *Archives of biochemistry and biophysics*, 581, 3-18 (2015).

7. Bai XC, McMullan G, Scheres SHW. How cryo-EM is revolutionizing structural biology. *Trends Biochem Sci*, 40(1), 49-57 (2015).

8. Subramaniam S, Milne JLS. Three-dimensional electron microscopy at molecular resolution. *Annu Rev Bioph Biom*, 33, 141-155 (2004).

9. Longchamp JN, Latychevskaia T, Escher C, Fink HW. Non-destructive imaging of an individual protein. *Appl Phys Lett*, 101(9) (2012).

10. Latychevskaia T, Longchamp JN, Escher C, Fink HW. Holography and coherent diffraction with low-energy electrons: A route towards structural biology at the single molecule level. *Ultramicroscopy*, 159 Pt 2, 395-402 (2015).

11. Schlichting I, Miao J. Emerging opportunities in structural biology with X-ray free-electron lasers. *Current opinion in structural biology*, 22(5), 613-626 (2012).

12. Gallat FX, Matsugaki N, Coussens NP et al. In vivo crystallography at X-ray free-electron lasers: the next generation of structural biology? *Philos T R Soc B*, 369(1647) (2014).

13. Graewert MA, Svergun DI. Impact and progress in small and wide angle X-ray scattering (SAXS and WAXS). *Current opinion in structural biology*, 23(5), 748-754 (2013).

14. Schindler CEM, de Vries SJ, Sasse A, Zacharias M. SAXS Data Alone can Generate High-Quality Models of Protein-Protein Complexes. *Structure*, 24(8), 1387-1397 (2016).

15. Xu X, Yan C, Wohlhueter R, Ivanov I. Integrative Modeling of Macromolecular Assemblies from Low to Near-Atomic Resolution. *Computational and structural biotechnology journal*, 13, 492-503 (2015).

16. Greenfield NJ. Using circular dichroism spectra to estimate protein secondary structure. *Nature Protocols*, 1(6), 2876-2890 (2006).

17. Ranjbar B, Gill P. Circular dichroism techniques: biomolecular and nanostructural analyses-a review. *Chemical biology & drug design*, 74(2), 101-120 (2009).

18. Barth A. Infrared spectroscopy of proteins. *Biochimica et biophysica acta*, 1767(9), 1073-1101 (2007).

19. Kong J, Yu S. Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta biochimica et biophysica Sinica*, 39(8), 549-559 (2007).

20. Raicu V, Singh DR. FRET spectrometry: a new tool for the determination of protein quaternary structure in living cells. *Biophysical journal*, 105(9), 1937-1945 (2013).

21. Vivian JT, Callis PR. Mechanisms of tryptophan fluorescence shifts in proteins. *Biophysical journal*, 80(5), 2093-2109 (2001).

22. Ganim Z, Chung HS, Smith AW, Deflores LP, Jones KC, Tokmakoff A. Amide I two-dimensional infrared spectroscopy of proteins. *Accounts of chemical research*, 41(3), 432-441 (2008).

23. Demirdoven N, Cheatum CM, Chung HS, Khalil M, Knoester J, Tokmakoff A. Two-dimensional infrared spectroscopy of antiparallel beta-sheet secondary structure. *Journal of the American Chemical Society*, 126(25), 7981-7990 (2004).
24. Cheatum CM, Tokmakoff A, Knoester J. Signatures of beta-sheet secondary structures in linear and two-dimensional infrared spectroscopy. *The Journal of chemical physics*, 120(17), 8201-8215 (2004).
25. Shashilov VA, Sikirzhitski V, Popova LA, Lednev IK. Quantitative methods for structural characterization of proteins based on deep UV resonance Raman spectroscopy. *Methods*, 52(1), 23-37 (2010).
26. Balakrishnan G, Weeks CL, Ibrahim M, Soldatova AV, Spiro TG. Protein dynamics from time resolved UV Raman spectroscopy. *Current opinion in structural biology*, 18(5), 623-629 (2008).
27. Reginsson GW, Schiemann O. Pulsed electron-electron double resonance: beyond nanometre distance measurements on biomacromolecules. *Biochem J*, 434, 353-363 (2011).
28. Shi F, Zhang Q, Wang P et al. Protein imaging. Single-protein spin resonance spectroscopy under ambient conditions. *Science*, 347(6226), 1135-1138 (2015).
29. Czogalla A, Pieciul A, Jezierski A, Sikorski AF. Attaching a spin to a protein -- site-directed spin labeling in structural biology. *Acta Biochim Pol*, 54(2), 235-244 (2007).
30. Liu F, Rijkers DT, Post H, Heck AJ. Proteome-wide profiling of protein assemblies by cross-linking mass spectrometry. *Nature methods*, 12(12), 1179-1184 (2015).
31. Calabrese AN, Radford SE. Mass spectrometry-enabled structural biology of membrane proteins. *Methods*, 147, 187-205 (2018).
32. Rappsilber J. The beginning of a beautiful friendship: Cross-linking/mass spectrometry and modelling of proteins and multi-protein complexes. *Journal of structural biology*, 173(3), 530-540 (2011).
33. Sinz A. The advancement of chemical cross-linking and mass spectrometry for structural proteomics: from single proteins to protein interaction networks. *Expert review of proteomics*, 11(6), 733-743 (2014).
34. Sinz A, Arlt C, Chorev D, Sharon M. Chemical cross-linking and native mass spectrometry: A fruitful combination for structural biology. *Protein science : a publication of the Protein Society*, 24(8), 1193-1209 (2015).
35. Kukacka Z, Rosulek M, Strohalm M, Kavan D, Novak P. Mapping protein structural changes by quantitative cross-linking. *Methods*, 89, 112-120 (2015).
36. Xu X. Chemical Cross-linking Mass Spectrometry for Profiling Protein Structures and Protein-Protein Interactions. *Journal of Proteomics & Bioinformatics*, 8(12) (2015).
37. Benesch JL, Ruotolo BT. Mass spectrometry: come of age for structural and dynamical biology. *Current opinion in structural biology*, 21(5), 641-649 (2011).
38. Jaswal SS. Biological insights from hydrogen exchange mass spectrometry. *Biochimica et biophysica acta*, 1834(6), 1188-1201 (2013).
39. Rajabi K, Ashcroft AE, Radford SE. Mass spectrometric methods to analyze the structural organization of macromolecular complexes. *Methods*, 89, 13-21 (2015).
40. Mayne L. Hydrogen Exchange Mass Spectrometry. *Methods in enzymology*, 566, 335-356 (2016).
41. Leurs U, Mistarz UH, Rand KD. Getting to the core of protein pharmaceuticals--Comprehensive structure analysis by mass spectrometry. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 93, 95-109 (2015).
42. Marklund EG, Degiacomi MT, Robinson CV, Baldwin AJ, Benesch JL. Collision cross sections for structural proteomics. *Structure*, 23(4), 791-799 (2015).
43. Konijnenberg A, Butterer A, Sobott F. Native ion mobility-mass spectrometry and related methods in structural biology. *Biochimica et biophysica acta*, 1834(6), 1239-1256 (2013).
44. Zhong Y, Hyung SJ, Ruotolo BT. Ion mobility-mass spectrometry for structural proteomics. *Expert review of proteomics*, 9(1), 47-58 (2012).
45. Heck AJR. Native mass spectrometry: a bridge between interactomics and structural biology. *Nature methods*, 5(11), 927-933 (2008).
van Duijn E. Current limitations in native mass spectrometry based structural biology. *Journal of the American Society for Mass Spectrometry*, 21(6), 971-978 (2010).

Kondrat FD, Struwe WB, Benesch JL. Native mass spectrometry: towards high-throughput structural proteomics. *Methods in molecular biology*, 1261, 349-371 (2015).

Boeri Erba E, Petosa C. The emerging role of native mass spectrometry in characterizing the structure and dynamics of macromolecular complexes. *Protein science: a publication of the Protein Society*, 24(8), 1176-1192 (2015).

Fontana A, de Laureto PP, Spolaore B, Frare E, Picotti P, Zambonin M. Probing protein structure by limited proteolysis. *Acta Biochim Pol*, 51(2), 299-321 (2004).

Liu F, Fitzgerald MC. Large-Scale Analysis of Breast Cancer-Related Conformational Changes in Proteins using Limited Proteolysis. *Journal of proteome research*, (2016).

Kiselar JG, Chance MR. Future directions of structural mass spectrometry using hydroxyl radical footprinting. *Journal of Mass Spectrometry*, 45(12), 1373-1382 (2010).

Wang LW, Chance MR. Structural Mass Spectrometry of Proteins Using Hydroxyl Radical Based Protein Footprinting. *Anal Chem*, 83(19), 7234-7241 (2011).

Li KS, Chen G, Mo J et al. Orthogonal Mass Spectrometry-Based Footprinting for Epitope Mapping and Structural Characterization: The IL-6 Receptor upon Binding of Protein Therapeutics. *Anal Chem*, 89(14), 7742-7749 (2017).

Gomez GE, Mundo MR, Craig PO, Delfino JM. Probing protein surface with a solvent mimic carbene coupled to detection by mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 23(1), 30-42 (2012).

Manzi L, Barrow AS, Scott D et al. Carbene footprinting accurately maps binding sites in protein-ligand and protein-protein interactions. *Nature communications*, 7, 13288 (2016).

Seffernick JT, Harvey SR, Wysocki VH, Lindert S. Predicting Protein Complex Structure from Surface-Induced Dissociation Mass Spectrometry Data. *ACS Cent Sci*, 5(8), 1330-1341 (2019).

Kim KY, Frieden C. Turn scanning by site-directed mutagenesis: Application to the protein folding problem using the intestinal fatty acid binding protein. *Protein Science*, 7(8), 1821-1828 (1998).

Lefevre F, Remy MH, Masson JM. Alanine-stretch scanning mutagenesis: a simple and efficient method to probe protein structure and function. *Nucleic acids research*, 25(2), 447-448 (1997).

Alber F, Forster F, Korkin D, Topf M, Sali A. Integrating diverse data for structure determination of macromolecular assemblies. *Annual review of biochemistry*, 77, 443-477 (2008).

Hyung SJ, Ruotolo BT. Integrating mass spectrometry of intact protein complexes into structural proteomics. *Proteomics*, 12(10), 1547-1564 (2012).

Karaca E, Bonvin AM. Advances in integrative modeling of biomolecular complexes. *Methods*, 59(3), 372-381 (2013).

Vandermarliere E, Stes E, Gevaert K, Martens L. Resolution of protein structure by mass spectrometry. *Mass spectrometry reviews*, (2014).

Zhou M, Robinson CV. When proteomics meets structural biology. *Trends Biochem Sci*, 35(9), 522-529 (2010).

Leitner A. Cross-linking and other structural proteomics techniques: how chemistry is enabling mass spectrometry applications in structural biology. *Chem Sci*, 7(8), 4792-4803 (2016).

Merkley ED, Cort JR, Adkins JN. Cross-linking and mass spectrometry methodologies to facilitate structural biology: finding a path through the maze. *Journal of structural and functional genomics*, 14(3), 77-90 (2013).

Ryl PSJ, Bohlke-Schneider M, Lenz S et al. In Situ Structural Restraints from Cross-Linking Mass Spectrometry in Human Mitochondria. *J Proteome Res*, 19(1), 327-336 (2020).
67. Tran BQ, Goodlett DR, Goo YA. Advances in protein complex analysis by chemical cross-linking coupled with mass spectrometry (CXMS) and bioinformatics. *Biochim Biophys Acta*, 1864(1), 123-129 (2016).

68. Schmidt C, Robinson CV. Dynamic protein ligand interactions--insights from MS. *FEBS J*, 281(8), 1950-1964 (2014).

69. Politis A, Schmidt C. Structural characterisation of medically relevant protein assemblies by integrating mass spectrometry with computational modelling. *J Proteomics*, 175, 34-41 (2018).

70. Schneider M, Belsom A, Rappsilber J. Protein Tertiary Structure by Crosslinking/Mass Spectrometry. *Trends Biochem Sci*, 43(3), 157-169 (2018).

71. Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. *Nature*, 537(7620), 347-355 (2016).

72. Mayer G, Marcus K, Eisenacher M, Kohl M. Boolean modeling techniques for protein co-expression networks in systems medicine. *Expert review of proteomics*, 13(6), 555-569 (2016).

73. Yang L, Tang X, Weisbrod CR et al. A photocleavable and mass spectrometry identifiable cross-linker for protein interaction studies. *Anal Chem*, 82(9), 3556-3566 (2010).

74. Yu FC, Li N, Yu WC. ECL: an exhaustive search tool for the identification of cross-linked peptides using whole database. *BMC bioinformatics*, 17 (2016).

75. Yilmaz S, Drepper F, Hulstaert N et al. Xilmass: A New Approach toward the Identification of Cross-Linked Peptides. *Anal Chem*, 88(20), 9949-9957 (2016).

76. Petrotchenko EV, Borchers CH. Crosslinking combined with mass spectrometry for structural proteomics. *Mass spectrometry reviews*, 29(6), 862-876 (2010).

77. Petrotchenko EV, Borchers CH. Application of a fast sorting algorithm to the assignment of mass spectrometric cross-linking data. *Proteomics*, 14(17-18), 1987-1989 (2014).

78. Sarpe V, Rafiei A, Hepburn M, Ostan N, Schryvers AB, Schriemer DC. High Sensitivity Crosslink Detection Coupled With Integrative Structure Modeling in the Mass Spec Studio. *Molecular & cellular proteomics : MCP*, 15(9), 3071-3080 (2016).

79. Gotze M, Pettelkau J, Fritzscbe R, Ihling CH, Schafer M, Sinz A. Automated assignment of MS/MS cleavable cross-links in protein 3D-structure analysis. *Journal of the American Society for Mass Spectrometry*, 26(1), 83-97 (2015).

80. Petrotchenko EV, Borchers CH. ICC-CLASS: isotopically-coded cleavable crosslinking analysis software suite. *BMC bioinformatics*, 11, 64 (2010).

81. Rinner O, Seebacher J, Walzthoeni T et al. Identification of cross-linked peptides from large sequence databases. *Nature methods*, 5(4), 315-318 (2008).

82. Holding AN, Lamers MH, Stephens E, Skehel JM. Hekate: software suite for the mass spectrometric analysis and three-dimensional visualization of cross-linked protein samples. *Journal of proteome research*, 12(12), 5923-5933 (2013).

83. Bennett KL, Kussmann M, Bjork P et al. Chemical cross-linking with thiol-cleavable reagents combined with differential mass spectrometric peptide mapping--a novel approach to assess intermolecular protein contacts. *Protein science : a publication of the Protein Society*, 9(8), 1503-1518 (2000).

84. Back JW, Notenboom V, de Koning LJ et al. Identification of Cross-Linked Peptides for Protein Interaction Studies Using Mass Spectrometry and18O Labeling. *Anal Chem*, 74(17), 4417-4422 (2002).

85. Collins CJ, Schilling B, Young ML, Dollinger G, Guy RK. Isotopically labeled crosslinking reagents: Resolution of mass degeneracy in the identification of crosslinked peptides. *Bioorg Med Chem Lett*, 13(22), 4023-4026 (2003).

86. Arlt C, Gotze M, Ihling CH, Hage C, Schafer M, Sinz A. Integrated Workflow for Structural Proteomics Studies Based on Cross-Linking/Mass Spectrometry with an MS/MS Cleavable Cross-Linker. *Anal Chem*, 88(16), 7930-7937 (2016).

87. Tang X, Bruce JE. A new cross-linking strategy: protein interaction reporter (PIR) technology for protein-protein interaction studies. *Molecular bioSystems*, 6(6), 939-947 (2010).
88. Sinz A. Divide and conquer: cleavable cross-linkers to study protein conformation and protein-protein interactions. *Analytical and bioanalytical chemistry*, (2016).

89. Anderson GA, Tolic N, Tang X, Zheng C, Bruce JE. Informatics strategies for large-scale novel cross-linking analysis. *Journal of proteome research*, 6(9), 3412-3421 (2007).

90. Leitner A, Walzthoeni T, Kahraman A et al. Probing native protein structures by chemical cross-linking, mass spectrometry, and bioinformatics. *Molecular & cellular proteomics: MCP*, 9(8), 1634-1649 (2010).

91. Preston GW, Wilson AJ. Photo-induced covalent cross-linking for the analysis of biomolecular interactions. *Chemical Society reviews*, 42(8), 3289-3301 (2013).

92. Dubinsky L, Krom BP, Meijler MM. Diazirine based photoaffinity labeling. *Bioorganic & medicinal chemistry*, 20(2), 554-570 (2012).

93. Das J. Aliphatic Diazirines as Photoaffinity Probes for Proteins: Recent Developments. *Chemical reviews*, 111(8), 4405-4417 (2011).

94. Gomes AF, Gozzo FC. Chemical cross-linking with a diazirine photoactivatable cross-linker investigated by MALDI- and ESI-MS/MS. *Journal of mass spectrometry: JMS*, 45(8), 892-899 (2010).

95. Chen Y, Ding F, Dokholyan NV. Fidelity of the protein structure reconstruction from inter-residue proximity constraints. *The journal of physical chemistry. B*, 111(25), 7432-7438 (2007).

96. Lossl P, Sinz A. Combining Amine-Reactive Cross-Linkers and Photo-Reactive Amino Acids for 3D-Structure Analysis of Proteins and Protein Complexes. *Methods in molecular biology*, 1394, 109-127 (2016).

97. Toews J, Rogalski JC, Clark TJ, Kast J. Mass spectrometric identification of formaldehyde-induced peptide modifications under in vivo protein cross-linking conditions. *Analytica chimica acta*, 618(2), 168-183 (2008).

98. Lu K, Ye WJ, Zhou L et al. Structural Characterization of Formaldehyde-Induced Cross-Links Between Amino Acids and Deoxynucleosides and Their Oligomers. *Journal of the American Chemical Society*, 132(10), 3388-3399 (2010).

99. Srinivasa S, Ding X, Kast J. Formaldehyde cross-linking and structural proteomics: Bridging the gap. *Methods*, 89, 91-98 (2015).

100. Cassioli A, Bardiaux B, Bouvier G et al. An algorithm to enumerate all possible protein conformations verifying a set of distance constraints. *BMC bioinformatics*, 16 (2015).

101. Huang SY. Search strategies and evaluation in protein-protein docking: principles, advances and challenges. *Drug discovery today*, 19(8), 1081-1096 (2014).

102. Ferber M, Kosinski J, Ori A et al. Automated structure modeling of large protein assemblies using crosslinks as distance restraints. *Nature methods*, 13(6), 515-+ (2016).

103. Xia B, Vajda S, Kozakov D. Accounting for pairwise distance restraints in FFT-based protein-protein docking. *Bioinformatics*, (2016).

104. Padhoryn D, Kazennov A, Zerbe BS et al. Protein-protein docking by fast generalized Fourier transforms on 5D rotational manifolds. *Proceedings of the National Academy of Sciences of the United States of America*, 113(30), E4286-4293 (2016).

105. Crippen GM, Havel TF. *Distance geometry and molecular conformation* (Wiley, New York, 1988).

106. Sali A. Comparative protein modeling by satisfaction of spatial restraints. *Mol Med Today*, 1(6), 270-277 (1995).

107. Velazquez-Muriel J, Lasker K, Russel D et al. Assembly of macromolecular complexes by satisfaction of spatial restraints from electron microscopy images. *Proceedings of the National Academy of Sciences of the United States of America*, 109(46), 18821-18826 (2012).

108. Braun W, Go N. Calculation of protein conformations by proton-proton distance constraints. A new efficient algorithm. *Journal of molecular biology*, 186(3), 611-626 (1985).
109. Schneidman-Duhovny D, Hammel M, Sali A. Macromolecular docking restrained by a small angle X-ray scattering profile. *Journal of structural biology*, 173(3), 461-471 (2011).

110. Forster F, Webb B, Krukenberg KA, Tsuruta H, Agard DA, Sali A. Integration of small-angle X-ray scattering data into structural modeling of proteins and their assemblies. *Journal of molecular biology*, 382(4), 1089-1106 (2008).

111. Merkley ED, Rysavy S, Kahraman A, Hafen RP, Daggett V, Adkins JN. Distance restraints from crosslinking mass spectrometry: mining a molecular dynamics simulation database to evaluate lysine-lysine distances. *Protein science : a publication of the Protein Society*, 23(6), 747-759 (2014).

112. Hofmann T, Fischer AW, Meiler J, Kalkhof S. Protein structure prediction guided by crosslinking restraints - A systematic evaluation of the impact of the crosslinking spacer length. *Methods*, 89, 79-90 (2015).

113. Kahraman A, Malmstrom L, Aebersold R. Xwalk: computing and visualizing distances in cross-linking experiments. *Bioinformatics*, 27(15), 2163-2164 (2011).

114. Bullock JMA, Schwab J, Thalassinos K, Topf M. The Importance of Non-accessible Crosslinks and Solvent Accessible Surface Distance in Modeling Proteins with Restraints From Crosslinking Mass Spectrometry. *Molecular & Cellular Proteomics*, 15(7), 2491-2500 (2016).

115. Back JW, de Jong L, Muijsers AO, de Koster CG. Chemical cross-linking and mass spectrometry for protein structural modeling. *Journal of molecular biology*, 331(2), 303-313 (2003).

116. Degiacomi MT, Schmidt C, Baldwin AJ, Benesch JLP. Accommodating Protein Dynamics in the Modeling of Chemical Crosslinks. *Structure*, 25(11), 1751-1757.e1755 (2017).

117. Bullock JMA, Sen N, Thalassinos K, Topf M. Modeling Protein Complexes Using Restraints from Crosslinking Mass Spectrometry. *Structure*, 26(7), 1015-1024.e1012 (2018).

118. Young MM, Tang N, Hempel JC et al. High throughput protein fold identification by using experimental constraints derived from intramolecular cross-links and mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America*, 97(11), 5802-5806 (2000).

119. Xue LC, Rodrigues JP, Dobbs D, Honavar V, Bonvin AM. Template-based protein-protein docking exploiting pairwise interfacial residue restraints. *Briefings in bioinformatics*, (2016).

120. Taverner T, Hernandez H, Sharon M et al. Subunit architecture of intact protein complexes from mass spectrometry and homology modeling. *Accounts of chemical research*, 41(5), 617-627 (2008).

121. Politis A, Stengel F, Hall Z et al. A mass spectrometry-based hybrid method for structural modeling of protein complexes. *Nature methods*, 11(4), 403-406 (2014).

122. Rey M, Sarpe V, Burns KM et al. Mass spec studio for integrative structural biology. *Structure*, 22(10), 1538-1548 (2014).

123. Zhang MM, Beno BR, Huang RY et al. An Integrated Approach for Determining a Protein-Protein Binding Interface in Solution and an Evaluation of Hydrogen-Deuterium Exchange Kinetics for Adjudicating Candidate Docking Models. *Anal Chem*, 91(24), 15709-15717 (2019).

124. van Zundert GC, Rodrigues JP, Trellet M et al. The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. *Journal of molecular biology*, 428(4), 720-725 (2016).

125. Amir N, Cohen D, Wolfson HJ. DockStar: a novel ILP-based integrative method for structural modeling of multimolecular protein complexes. *Bioinformatics*, 31(17), 2801-2807 (2015).

126. Russel D, Lasker K, Webb B et al. Putting the pieces together: integrative modeling platform software for structure determination of macromolecular assemblies. *PLoS biology*, 10(1), e1001244 (2012).

127. Webb B, Lasker K, Velazquez-Muriel J et al. Modeling of proteins and their assemblies with the Integrative Modeling Platform. *Methods in molecular biology*, 1091, 277-295 (2014).
128. Yang Z, Lasker K, Schneidman-Duhovny D et al. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *Journal of structural biology*, 179(3), 269-278 (2012).
129. Webb B, Sali A. Comparative Protein Structure Modeling Using MODELLER. *Current protocols in bioinformatics*, 54, S 6 1-5 6 37 (2016).
130. Kosinski J, von Appen A, Ori A, Karius K, Muller CW, Beck M. Xlink Analyzer: software for analysis and visualization of cross-linking data in the context of three-dimensional structures. *Journal of structural biology*, 189(3), 177-183 (2015).
131. Kahraman A, Herzog F, Leitner A, Rosenberger G, Aebersold R, Malmstrom L. Cross-link guided molecular modeling with ROSETTA. *PloS one*, 8(9), e73411 (2013).
132. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nat Protoc*, 5(4), 725-738 (2010).
133. Grimm M, Zimniak T, Kahraman A, Herzog F. xVis: a web server for the schematic visualization and interpretation of crosslink-derived spatial restraints. *Nucleic acids research*, 43(W1), W362-369 (2015).
134. Combe CW, Fischer L, Rappsilber J. xiNET: cross-link network maps with residue resolution. *Molecular & cellular proteomics : MCP*, 14(4), 1137-1147 (2015).
135. Jaiswal M, Crabtree NM, Bauer MA, Hall R, Raney KD, Zybalov BL. XLPM: efficient algorithm for the analysis of protein-protein contacts using chemical cross-linking mass spectrometry. *BMC bioinformatics*, 15 (2014).
136. Schweppke DK, Chavez JD, Bruce JE. XLmap: an R package to visualize and score protein structure models based on sites of protein cross-linking. *Bioinformatics*, 32(2), 306-308 (2016).
137. Schweppke DK, Zheng C, Chavez JD et al. XLinkDB 2.0: integrated, large-scale structural analysis of protein crosslinking data. *Bioinformatics*, 32(17), 2716-2718 (2016).
138. Riffle M, Jaschob D, Zelter A, Davis TN. ProXL (Protein Cross-Linking Database): A Platform for Analysis, Visualization, and Sharing of Protein Cross-Linking Mass Spectrometry Data. *Journal of proteome research*, 15(8), 2863-2870 (2016).
139. Bullock JMA, Thalassinos K, Topf M. Jwalk and MNXL web server: model validation using restraints from crosslinking mass spectrometry. *Bioinformatics*, 34(20), 3584-3585 (2018).
140. Yilmaz Ş, Shiferaw GA, Rayo J, Economou A, Martens L, Vandermarliere E. Cross-linked peptide identification: A computational forest of algorithms. *Mass Spectrom Rev*, (2018).
141. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. *Nature methods*, 12(1), 7-8 (2015).
142. Saltzberg DJ, Hepburn M, Pilla KB et al. SSEThread: Integrative threading of the DNA-PKcs sequence based on data from chemical cross-linking and hydrogen deuterium exchange. *Prog Biophys Mol Biol*, 147, 92-102 (2019).
### Supplementary Table S1: Some software packages and databases for cross-linking analysis, identification or visualization

| Name of the software package                                      | URL (accessed 02/2020)                                                                 | References |
|-------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------|
| **Chemical drawing packages for spacer length determination**    |                                                                                        |            |
| BIOVIA Draw                                                      | http://accelrys.com/resource-center/downloads/freeware/                                |            |
| ChemBioDraw                                                      | http://www.cambridgesoft.com/Ensemble_for_Chemistry/ChemDraw/ChemDrawProfessional/    |            |
| CORINA                                                           | http://www2.chemie.uni-erlangen.de/services/telespec/corina/                           | 1          |
| **Databases for data-driven modeling**                           |                                                                                        |            |
| ProXL                                                            | http://yeastrc.org/proxl_demo/viewProject.do?project_id=1                             | 2          |
| XLinkDB                                                          | http://xlinkdb.gs.washington.edu                                                      | 3          |
| xComb                                                            | https://goodlett.umaryland.edu/xcomb.php                                              | 4          |
| XDB                                                              |                                                                                        | 5          |
| **Visualization tools**                                          |                                                                                        |            |
| UCSF Chimera                                                     | https://www.cgl.ucsf.edu/chimera/docs/morerefs.html                                   | 6          |
| VisInt-X                                                         | https://www.researchgate.net/publication/301766070_VisInt-X_Visualizing_Interactions_in_Cross-linked_proteins |            |
| xiNET                                                            | https://omictools.com/xinet-tool                                                     | 7          |
| XMap                                                             | https://omictools.com/xmap-tool                                                      | 8          |
| https://github.com/mammamals/XMap                                  |                                                                                        |            |
| xVis                                                             | https://omictools.com/xvis-tool                                                     | 9          |
| XWalk                                                            | http://www.xwalk.org                                                                | 10         |
| **XL analysis and identification (search) tools**                |                                                                                        |            |
| AnchorMS                                                         | https://omictools.com/anchorms-tool                                                  | 11         |
| ASAP                                                             |                                                                                        | 12         |
| BLinks                                                           | http://brucelab.gs.washington.edu/BLinks.php                                         | 13         |
| CLMSVault                                                        | https://gitlab.com/courcelm/clmsvault                                               | 14         |
| CLPM                                                             | http://www.cpan.org/modules/by-module/BioX/BioX-CLPM-0.01.readme                     | 15         |
| Software          | URL                                      | Notes |
|-------------------|------------------------------------------|-------|
| Crossfinder       | [http://doi.org/10.1093/bioinformatics/btv083](http://doi.org/10.1093/bioinformatics/btv083) | [16]  |
| CrossSearch       | ---                                      | [17]  |
| CrossWork         | ---                                      | [18]  |
| Crux              | [http://cruxtoolkit.sourceforge.net/crux-search-for-xlinks.html](http://cruxtoolkit.sourceforge.net/crux-search-for-xlinks.html) | [19]  |
| CTB (CoolToolBox), Virtual MSLab | [ldk@science.uva.nl](mailto:ldk@science.uva.nl) | [20]  |
| DynamXL           | [http://dynamxl.chem.ox.ac.uk](http://dynamxl.chem.ox.ac.uk) | [21]  |
| DXMSMS            | [http://www.creativemolecules.com/cm_software.htm](http://www.creativemolecules.com/cm_software.htm) | [22]  |
| ECL               | [http://bioinformatics.ust.hk/ecl.html](http://bioinformatics.ust.hk/ecl.html) | [23]  |
| FindLink          | ---                                      | ---   |
| FindX             | [http://findxlinks.blogspot.de](http://findxlinks.blogspot.de) | [24]  |
| ProteinXXX / GPMAW | [http://www.gpmaw.com/html/cross-linking.html](http://www.gpmaw.com/html/cross-linking.html) | [25]  |
| Hekate            | [http://andrewholding.com/research/hekate/](http://andrewholding.com/research/hekate/) | [26]  |
| Kojak             | [http://www.kojak-ms.org](http://www.kojak-ms.org) | [27]  |
| ICC-CLASS (Isotopically-Coded Cleavable Cross-Linking Analysis Software) | [http://www.creativemolecules.com/cm_software.htm](http://www.creativemolecules.com/cm_software.htm) | [28]  |
| JWalk             | [http://jwalk.ismb.lon.ac.uk/jwalk](http://jwalk.ismb.lon.ac.uk/jwalk) | [29, 30] |
| MasPy             | [https://pypi.python.org/pypi/maspy](https://pypi.python.org/pypi/maspy) | ---   |
| MassAI            | [http://www.massai.dk](http://www.massai.dk) | [31]  |
| MassMatrix        | [http://www.massmatrix.net](http://www.massmatrix.net) | [32]  |
| MaxQuant          | [http://www.coxdocs.org/doku.php?id=maxquant:start](http://www.coxdocs.org/doku.php?id=maxquant:start) | [33]  |
| MeroX             | [http://www.stavrox.com](http://www.stavrox.com) | [34]  |
| MS2Assign         | ---                                      | [35]  |
| MS2Links          | ---                                      | ---   |
| MS2Pro            | ---                                      | [36]  |
| MS3D              | ---                                      | ---   |
| Tool Name                  | Website                                                                 | Notes   |
|---------------------------|-------------------------------------------------------------------------|---------|
| MS-Bridge (ProteinProspector) | http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msbridgestandard | [37]    |
| MXDB                      | https://omictools.com/mxdb-tool                                          | [38]    |
| MSX-3D                    | ---                                                                      | [39]    |
| OpenPepXL                 | http://www.openms.de/comp/openpepxl/                                   | ---     |
| OpenProXL                 | ---                                                                      | ---     |
| PepSearch                 | https://omictools.com/pepssearch-tool                                    | ---     |
| PeptideMap (PROWL)        | http://prowl.rockefeller.edu/prowl/peptidemap.html                      | [40]    |
| pLink                     | https://omictools.com/plink-2-tool                                       | [41, 42]|
| pLink-SS (disulfide bonds) | http://pfind.ict.ac.cn/software/pLink/2014/pLink-SS.html                | [43]    |
| Popitam                   | ---                                                                      | [44, 45]|
| Pro-Crosslink             | https://els.comotion.uw.edu/licenses/3                                   | [46]    |
| pXtract                   | http://pfind.ict.ac.cn/software/pXtract/index.html                      | ---     |
| RNPxl                     | http://open-ms.sourceforge.net/publications/rnpxl/                      | [47]    |
| SearchXLinks              |                                                                          | [48]    |
| SIM-XL (Spectrum Identification Machine-XL) | http://patternlabforproteomics.org/sim-xl/                     | [49]    |
| SLinkS                    | https://xlinkx2beta.hecklab.com                                         | [50]    |
| StavroX                   | http://www.stavrox.com                                                  | [51]    |
| SQID-XLink                | http://chemistry.osu.edu/~wysocki.11/bioinformatics.htm                 | [52]    |
| XFDR                      | ftp://ftp.mi.fu-berlin.de/pub/OpenMS/develop-documentation/html/UTILS_XFDR.html | ---     |
| xiFDR                     | https://github.com/lutzfischer/xiFDR                                    | [53]    |
| XI                        | http://rappsilberlab.org/rappsilber-laboratory-home-page/tools/         | ---     |
| XiQ                       | http://rappsilberlab.org/tools/                                         | [54]    |
| xilmass                   | https://github.com/compomics/xilmass                                     | [55]    |
| XL-MOD                    | https://omictools.com/xl-mod-tool                                        | [56]    |
| Link | Description | URL |
|------|-------------|-----|
| X-Link | --- | [57] |
| X-Links | --- | [58] |
| XL!Link | yjlee@iastate.edu | [12, 59] |
| XLink | --- | [60] |
| XLink Analyzer | [http://www.beck.embl.de/XlinkAnalyzer.html](http://www.beck.embl.de/XlinkAnalyzer.html) | [61] |
| XLink-Identifier | [http://www.du-lab.org](http://www.du-lab.org) | [24] |
| XLinkProphet | --- | [62] |
| XlinkX | [https://xlinkx.hecklab.com](https://xlinkx.hecklab.com) | [63] |
| XL-MOD | [http://aria.pasteur.fr/supplementary-data/x-links](http://aria.pasteur.fr/supplementary-data/x-links) | [64] |
| XLPM (X-Linked Peptide Mapping Algorithm) | [http://binf-app.host.ualr.edu/~mihir/cgi-bin/xlpm.cgi](http://binf-app.host.ualr.edu/~mihir/cgi-bin/xlpm.cgi) | [65] |
| xQuest / xBobcat | [http://prottools.ethz.ch/orinner/public/htdocs/xquest/](http://prottools.ethz.ch/orinner/public/htdocs/xquest/) | [66] |
| xQuest / xProphet | [http://proteomics.ethz.ch/cgi-bin/xquest2_cgi/index.cgi](http://proteomics.ethz.ch/cgi-bin/xquest2_cgi/index.cgi) | [67] |
| xTract | [https://omictools.com/xtract-tool](https://omictools.com/xtract-tool) | [68] |
| XXXLink | --- | [31] |

**Structure model validation**

| Link | Description | URL |
|------|-------------|-----|
| DisVis | [http://milou.science.uu.nl/services/DISVIS](http://milou.science.uu.nl/services/DISVIS) | [69] |
| MNXL | [http://mnxl.ismb.lon.ac.uk](http://mnxl.ismb.lon.ac.uk) | [30] |
| PowerFit | [http://milou.science.uu.nl/services/POWERFIT](http://milou.science.uu.nl/services/POWERFIT) | [69] |

**References:**

1. Sadowski J, Gasteiger J, Klebe G. Comparison of Automatic Three-Dimensional Model Builders Using 639 X-ray Structures, Journal of Chemical Information and Modeling 1994;34:1000-1008.
2. Riffle M, Jaschob D, Zelter A et al. ProXL (Protein Cross-Linking Database): A Platform for Analysis, Visualization, and Sharing of Protein Cross-Linking Mass Spectrometry Data, J Proteome Res 2016;15:2863-2870.
3. Schweppe DK, Zheng C, Chavez JD et al. XLinkDB 2.0: integrated, large-scale structural analysis of protein crosslinking data, Bioinformatics 2016;32:2716-2718.
4. Panchaud A, Singh P, Shaffer SA et al. xComb: a cross-linked peptide database approach to protein-protein interaction analysis, J Proteome Res 2010;9:2508-2515.
5. Maiolica A, Cittaro D, Borsotti D et al. Structural analysis of multiprotein complexes by cross-linking, mass spectrometry, and database searching, Mol Cell Proteomics 2007;6:2200-2211.
6. Yang Z, Lasker K, Schneidman-Duhovny D et al. UCSF Chimera, MODELLER, and IMP: an integrated modeling system, J Struct Biol 2012;179:269-278.
7. Combe CW, Fischer L, Rappsilber J. xiNET: cross-link network maps with residue resolution, Mol Cell Proteomics 2015;14:1137-1147.
8. Schweppe DD, Chavez JD, Bruce JE. XLmap: an R package to visualize and score protein structure models based on sites of protein cross-linking, Bioinformatics 2016;32:306-308.
9. Grimm M, Zimniak T, Kahraman A et al. xVis: a web server for the schematic visualization and interpretation of crosslink-derived spatial restraints, Nucleic Acids Res 2015;43:W362-369.
10. Kahraman A, Malmstrom L, Aebersold R. Xwalk: computing and visualizing distances in cross-linking experiments, Bioinformatics 2011;27:2163-2164.
11. Mayne SL, Patterton HG. AnchorMS: a bioinformatics tool to derive structural information from the mass spectra of cross-linked protein complexes, Bioinformatics 2014;30:125-126.
12. Young MM, Tang N, Hempel JC et al. High throughput protein fold identification by using experimental constraints derived from intramolecular cross-links and mass spectrometry, Proc Natl Acad Sci U S A 2000;97:5802-5806.
13. Hoopmann MR, Weisbrod CR, Bruce JE. Improved Strategies for Rapid Identification of Chemically Cross-Linked Peptides Using Protein Interaction Reporter Technology, J Proteome Res 2010;9:6323-6333.
14. Courcelles M, Coulombe-Huntington J, Cossette É et al. CLMSVault: A Software Suite for Protein Cross-Linking Mass-Spectrometry Data Analysis and Visualization, J Proteome Res 2017.
15. Tang Y, Chen Y, Lichti CF et al. CLPM: a cross-linked peptide mapping algorithm for mass spectrometric analysis, BMC Bioinformatics 2005;6 Suppl 2:S9.
16. Mueller-Planitz F. Crossfinder-assisted mapping of protein crosslinks formed by site-specifically incorporated crosslinkers, Bioinformatics 2015;31:2043-2045.
17. Nadeau OW, Wyckoff GI, Paschall JE et al. CrossSearch, a user-friendly search engine for detecting chemically cross-linked peptides in conjugated proteins, Mol Cell Proteomics 2008;7:739-749.
18. Rasmussen MI, Refsgaard JC, Peng L et al. CrossWork: software-assisted identification of cross-linked peptides, J Proteomics 2011;74:1871-1883.
19. McIlwain S, Tamura K, Kertesz-Farkas A et al. Crux: rapid open source protein tandem mass spectrometry analysis, J Proteome Res 2014;13:4488-4491.
20. de Koning LJ, Kasper PT, Back JW et al. Computer-assisted mass spectrometric analysis of naturally occurring and artificially introduced cross-links in proteins and protein complexes, Febs Journal 2006;273:281-291.
21. Degiacomi MT, Schmidt C, Baldwin AJ et al. Accommodating Protein Dynamics in the Modeling of Chemical Crosslinks, Structure 2017;25:1751-1757.e1755.
22. Petrotchenko EV, Makepeace KA, Borchers CH. DXMSMS Match Program for Automated Analysis of LC-MS/MS Data Obtained Using Isotopically Coded CID-Cleavable Cross-Linking Reagents, Curr Protoc Bioinformatics 2014;48:8 18 11-19.

23. Yu FC, Li N, Yu WC. ECL: an exhaustive search tool for the identification of cross-linked peptides using whole database, BMC Bioinformatics 2016;17.

24. Du XX, Chowdhury SM, Manes NP et al. Xlink-Identifier: An Automated Data Analysis Platform for Confident Identifications of Chemically Cross-Linked Peptides Using Tandem Mass Spectrometry, J Proteome Res 2011;10:923-931.

25. Peri S, Steen H, Pandey A. GPMAW--a software tool for analyzing proteins and peptides, Trends in Biochemical Sciences 2001;26:687-689.

26. Holding AN, Lamers MH, Stephens E et al. Hekate: software suite for the mass spectrometric analysis and three-dimensional visualization of cross-linked protein samples, J Proteome Res 2013;12:5923-5933.

27. Hoopmann MR, Zelter A, Johnson RS et al. Kojak: efficient analysis of chemically cross-linked protein complexes, J Proteome Res 2015;14:2190-2198.

28. Petrotchenko EV, Borchers CH. ICC-CLASS: isotopically-coded cleavable cross-linking analysis software suite, BMC Bioinformatics 2010;11:64.

29. Matthew Allen Bullock J, Schwab J, Thalassinos K et al. The Importance of Non-accessible Crosslinks and Solvent Accessible Surface Distance in Modeling Proteins with Restraints From Crosslinking Mass Spectrometry, Mol Cell Proteomics 2016;15:2491-2500.

30. Bullock JMA, Thalassinos K, Topf M. Jwalk and MNXL web server: model validation using restraints from crosslinking mass spectrometry, Bioinformatics 2018;34:3584-3585.

31. Nielsen T, Thaysen-Andersen M, Larsen N et al. Determination of protein conformation by isotopically labelled cross-linking and dedicated software: Application to the chaperone, calreticulin, International Journal of Mass Spectrometry 2007;268:217-226.

32. Xu H, Hsu PH, Zhang LW et al. Database Search Algorithm for Identification of Intact Cross-Links in Proteins and Peptides Using Tandem Mass Spectrometry, J Proteome Res 2010;9:3384-3393.

33. Chen ZA, Fischer L, Cox J et al. Quantitative Cross-linking/Mass Spectrometry Using Isotope-labeled Cross-linkers and MaxQuant, Molecular & Cellular Proteomics 2016;15:2769-2778.

34. Gotze M, Pettelkau J, Fritzsche R et al. Automated assignment of MS/MS cleavable cross-links in protein 3D-structure analysis, J Am Soc Mass Spectrom 2015;26:83-97.

35. Schilling B, Row RH, Gibson BW et al. MS2Assign, automated assignment and nomenclature of tandem mass spectra of chemically crosslinked peptides, J Am Soc Mass Spectrom 2003;14:834-850.

36. Kruppa GH, Schoeniger J, Young MM. A top down approach to protein structural studies using chemical cross-linking and Fourier transform mass spectrometry, Rapid Commun Mass Spectrom 2003;17:155-162.

37. Chalkley RJ, Baker PR, Medzhiradszky KF et al. In-depth analysis of tandem mass spectrometry data from disparate instrument types, Mol Cell Proteomics 2008;7:2386-2398.

38. Wang J, Anania VG, Knott J et al. Combinatorial approach for large-scale identification of linked peptides from tandem mass spectrometry spectra, Mol Cell Proteomics 2014;13:1128-1136.

39. Heymann M, Paramelle D, Subra G et al. MSX-3D: a tool to validate 3D protein models using mass spectrometry, Bioinformatics 2008;24:2782-2783.

40. Fenyo D. A software tool for the analysis of mass spectrometric disulfide mapping experiments, Comput Appl Biosci 1997;13:617-618.

41. Yang B, Wu YJ, Zhu M et al. Identification of cross-linked peptides from complex samples, Nat Methods 2012;9:904-906.
42. Chen ZL, Meng JM, Cao Y et al. A high-speed search engine pLink 2 with systematic evaluation for proteome-scale identification of cross-linked peptides, Nat Commun 2019;10:3404.
43. Lu S, Fan SB, Yang B et al. Mapping native disulfide bonds at a proteome scale, Nat Methods 2015;12:329-U373.
44. Hernandez P, Gras R, Frey J et al. Popitam: towards new heuristic strategies to improve protein identification from tandem mass spectrometry data, Proteomics 2003;3:870-878.
45. Singh P, Shaffer SA, Scherl A et al. Characterization of protein cross-links via mass spectrometry and an open-modification search strategy, Analytical Chemistry 2008;80:8799-8806.
46. Gao Q, Xue S, Doneanu CE et al. Pro-CrossLink. Software tool for protein cross-linking and mass spectrometry, Analytical Chemistry 2006;78:2145-2149.
47. Kramer K, Sachsenberg T, Beckmann BM et al. Photo-cross-linking and high-resolution mass spectrometry for assignment of RNA-binding sites in RNA-binding proteins, Nat Methods 2014;11:1064-1070.
48. Wefing S, Schnaible V, Hoffmann D. SearchXLLinks. A program for the identification of disulfide bonds in proteins from mass spectra, Analytical Chemistry 2006;78:1235-1241.
49. Lima DB, de Lima TB, Balbuena TS et al. SIM-XL: A powerful and user-friendly tool for peptide cross-linking analysis, J Proteomics 2015;129:51-55.
50. Liu F, van Breukelen B, Heck AJ. Facilitating protein disulfide mapping by a combination of pepsin digestion, electron transfer higher energy dissociation (EThcD), and a dedicated search algorithm SlinkS, Mol Cell Proteomics 2014;13:2776-2786.
51. Gotze M, Pettelkau J, Schaks S et al. StavroX--a software for analyzing crosslinked products in protein interaction studies, J Am Soc Mass Spectrom 2012;23:76-87.
52. Li W, O'Neill HA, Wysocki VH. SQID-XLink: implementation of an intensity-incorporated algorithm for cross-linked peptide identification, Bioinformatics 2012;28:2548-2550.
53. Fischer L, Rappsilber J. Quirks of Error Estimation in Cross-Linking/Mass Spectrometry, Anal Chem 2017;89:3829-3833.
54. Fischer L, Chen ZA, Rappsilber J. Quantitative cross-linking/mass spectrometry using isotope-labelled cross-linkers, J Proteomics 2013;88:120-128.
55. Yilmaz S, Drepper F, Hulstaert N et al. Xilmass: A New Approach toward the Identification of Cross-Linked Peptides, Analytical Chemistry 2016;88:9949-9957.
56. Ferber M, Kosinski J, Ori A et al. Automated structure modeling of large protein assemblies using crosslinks as distance restraints, Nat Methods 2016;13:515-520.
57. Taverner T, Hall NE, O'Hair RA et al. Characterization of an antagonist interleukin-6 dimer by stable isotope labeling, cross-linking, and mass spectrometry, J Biol Chem 2002;277:46487-46492.
58. Anderson GA, Tolic N, Tang X et al. Informatics strategies for large-scale novel cross-linking analysis, J Proteome Res 2007;6:3412-3421.
59. Lee YJ. Probability-based shotgun cross-linking sites analysis, J Am Soc Mass Spectrom 2009;20:1896-1899.
60. Seebacher J, Mallick P, Zhang N et al. Protein cross-linking analysis using mass spectrometry, isotope-coded cross-linkers, and integrated computational data processing, J Proteome Res 2006;5:2270-2282.
62. Keller A, Chavez JD, Bruce JE. Increased sensitivity with automated validation of XL-MS cleavable peptide crosslinks, Bioinformatics 2019;35:895-897.
63. Liu F, Rijkers DT, Post H et al. Proteome-wide profiling of protein assemblies by cross-linking mass spectrometry, Nat Methods 2015;12:1179-1184.
64. Ferber M, Kosinski J, Ori A et al. Automated structure modeling of large protein assemblies using crosslinks as distance restraints, Nat Methods 2016;13:515-+.
65. Jaiswal M, Crabtree NM, Bauer MA et al. XLPM: efficient algorithm for the analysis of protein-protein contacts using chemical cross-linking mass spectrometry, BMC Bioinformatics 2014;15.
66. Rinner O, Seebacher J, Walzthoeni T et al. Identification of cross-linked peptides from large sequence databases, Nat Methods 2008;5:315-318.
67. Leitner A, Walzthoeni T, Aebersold R. Lysine-specific chemical cross-linking of protein complexes and identification of cross-linking sites using LC-MS/MS and the xQuest/xProphet software pipeline, Nat Protoc 2014;9:120-137.
68. Walzthoeni T, Joachimiak LA, Rosenberger G et al. xTract: software for characterizing conformational changes of protein complexes by quantitative cross-linking mass spectrometry, Nat Methods 2015;12:1185-1190.
69. van Zundert GC, Trellet M, Schaarschmidt J et al. The DisVis and PowerFit Web Servers: Explorative and Integrative Modeling of Biomolecular Complexes, J Mol Biol 2017;429:399-407.