xiVIEW: Visualisation of Crosslinking Mass Spectrometry Data

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Abstract

Crosslinking mass spectrometry (MS) has emerged as an important technique for elucidating the in-solution structures of protein complexes and the topology of protein-protein interaction networks. However, the expanding user community lacked an integrated visualisation tool that helped them make use of the crosslinking data for investigating biological mechanisms. We addressed this need by developing xiVIEW, a web-based application designed to streamline crosslinking MS data analysis, which we present here. xiVIEW provides a user-friendly interface for accessing coordinated views of mass spectrometric data, network visualisation, annotations extracted from trusted repositories like UniProtKB, and available 3D structures. In accordance with recent recommendations from the crosslinking MS community, xiVIEW (i) provides a standards compliant parser to improve data integration and (ii) offers accessible visualisation tools. By promoting the adoption of standard file formats and providing a comprehensive visualisation platform, xiVIEW empowers both experimentalists and modellers alike to pursue their respective research interests. We anticipate that xiVIEW will advance crosslinking MS-inspired research, and facilitate broader and more effective investigations into complex biological systems.

Keywords
Standards, protein networks, structures, software, data analysis

Abbreviations

CSV	Comma Separated Values
PPI	Protein-Protein Interaction
HUPO-PSI	Human Proteome Organisation - Proteomics Standards Initiative
mmCIF	The Macromolecular Crystallographic Information File (mmCIF)
MS	Mass Spectrometry
PDB	Protein Data Bank
RCSB	Research Collaboratory for Structural Bioinformatics
SVG	Scalable Vector Graphics
SASD	Solvent Accessible Surface Distance
XML	Extensible Markup Language

Introduction

Crosslinking mass spectrometry (crosslinking MS) is an experimental technique in which non-covalent interactions or proximities within or between biomolecules are covalently fixed with a crosslinking reagent and the crosslinked sites are identified by mass spectrometry [1].
It has emerged as an important technique for investigating proteins in their near native environment and is now a routinely used tool for structural biology. Numerous recent reviews of developments in the field are available [2–6]. Although primarily used to investigate protein-protein interactions and protein conformations, crosslinking MS may also include interactions with other classes of biomolecules, including other biopolymers and small molecules. The tool presented here, xiVIEW, is a visualisation and analysis tool primarily applicable to the use of crosslinking MS to investigate protein-protein interactions and protein conformations, though it has also been used to visualise protein-RNA interactions [7]. Table 1 summarises other visualisation tools designed specifically for crosslinking data, with the aim of locating xiVIEW within that space. Whilst some earlier crosslink identification software came with bundled visualisation tools, a more recent trend is to provide export to xiVIEW, for example, MS Annika [8].

Software	Spectra	Bars	Circle	Matrix	PPI	Grouped nodes	3D	Disto	Modelling / docking	Search software independent
XWalk [9]							PyMOL export			✔
Hekate [10]	✔						PyMOL export	✔		
XLinkDB [11–13]		~ (v3.0)	~ (v3.0)		✔		JMol		✔ (v2.0)	✔
Xlink Analyzer [14]							Chimera	✔	✔	✔
SIM-XL [15]	✔	✔	✔				Pymol export			
xiNET [16]		✔			✔					✔
xVis [17]		✔	✔							✔
XLmap [18]				✔						✔
ProXL [19]	Lorikeet	✔	✔ (v2.0)				pv			
Jwalk [20,21]							jsmol			✔
Mass Spec Studio [22]	✔						✔			

CLMSVault [23]	xiSPEC	xiNET			xiNET		jsmol	✔		✔
xiSPEC [24]	✔									✔
Cross-ID [25]	✔	✔	✔		✔	✔	DisVis export	✔		
PyXlinkViewer [26]							Pymol			✔
XlinkCyNET [27]		✔			Cyto -scape		pymol			✔
xiVIEW	xiSPEC	xiNET	✔	✔	xiNET	✔	NGL	✔		✔

Table 1. Overview of software in the same application space as xiVIEW. Rows are software packages, columns are features of that software, the first eight columns correspond to types of view. In order, the columns are: annotated spectra; residue level 2D network with proteins as bars; residue level 2D network with proteins as segments of circle; matrix view of residue level network (with contact map as background); PPI level network; PPI network with proteins grouped / collapsed to meta-nodes; visualisation of crosslinks on 3D structures; histogram of distances, modelling / docking capabilities; whether these feature are available independently of search software. In the case of Xlink-DB version 3.0 [13] the residue level network diagram omits the uncrosslinked amino acids and thus fails to show the crosslinks in the context of the protein sequence, this makes it less meaningful [16] (see Figure 1 therein).

The objective of xiVIEW is to provide the crosslinking mass spectrometry community with an easily accessible, standards compliant visualisation tool that integrates views of spectra, 2D networks and 3D structures. xiVIEW does not perform the identification of crosslinks or error control on these identifications. xiVIEW provides a comprehensive set of tools for visualising crosslinking MS data but does not address the problems of modelling or protein docking – instead exports are provided for other tools that specialise in this. Other crosslinking visualisation platforms that provide a similarly comprehensive set of visualisation tools are tied to specific search softwares. xiVIEW avoids this by reading the mzIdentML 1.2.0, the most recognised and supported standard for this data. Furthermore, xiVIEW integrates these views, providing synchronised selection and highlighting across them, this is not possible for the tools which export data to external viewers for 3D visualisation (see Table 1, ‘3D’ column).

The crosslinking MS community includes both academia and industry. It consists of research laboratories developing crosslinking tools (new crosslinker reagents, new workflows, new search software), research laboratories using crosslinking MS to investigate biological questions, and commercial enterprises, such as instrument vendors, who wish to make crosslinking MS easily accessible to their customers. Starting in 2015, this community initiated collaborative efforts to identify necessary improvements in the utility of crosslinking MS, leading

eventually to a list of 10 recommendations outlining areas that require work [1]. The work presented here addresses one of these - the accessibility of crosslinking MS data to experimentalists (recommendation 8). This recommendation calls for the development of parsers for data integration and the development of easily accessible visualisation tools.

xiVIEW is free to use at https://xiview.org. Being browser based, data can be easily shared via a unique URL. It is free open source software and can also be run locally. In addition, xiVIEW makes the crosslinking data itself more accessible by providing integrated views of different aspects of the data and allowing the user to make connections between them. Three different types of views of crosslinking data are essential to its interpretation and covered by xiVIEW: views of the annotated spectra, views of the crosslink network topology in two dimensions, and views of the crosslinks plotted on a chosen 3D structure.

The primary input file format to xiVIEW is mzIdentML 1.2.0. This is an XML file format endorsed by the Human Proteome Organization - Proteomics Standards Initiative (HUPO-PSI). HUPO-PSI develops open data standards in the proteomics field [28–30]. mzIdentML is the HUPO-PSI XML standard for peptide and protein identification results based on MS data. The first stable version of the standard (version 1.1) was formalised in 2012 [31]. At the HUPO-PSI meeting 2016 in Ghent (Belgium), an agreement was reached to support crosslinking data starting from v1.2 of mzIdentML [32]. mzIdentML allows FDR thresholds to be stated, and it can also encode the identifications that did not pass. A python library exists for writing mzIdentML [33]. Work is currently taking place to revise the mzIdentML standard, enhancing its support for crosslinking data in the upcoming version [34]. After these revisions have been accepted, xiVIEW will be updated to support the new features of the standard.

Results and Discussion

xiVIEW is a Multiple Coordinated View system [35]. This means it is a visualisation system that can simultaneously display several different representations of the data and in which user interactions, such as selection and highlighting, are synchronised in real time across these different views. Figure 1 gives an overview of the workflow, clarifying which data is input at which stage of the process, and which data is output.

The server side of xiVIEW consists of Python 3 code for reading mzIdentML files (https://github/rappsilber-laboratory/xi-mzidentml-converter), for serving the web pages containing the xiVIEW visualisation (https://github/rappsilber-laboratory/xiview-server), and for annotating spectra (this functionality is integrated into the web server). The mzIdentML reader is a revision of that implemented in xiSPEC [24]. It processes mzIdentML 1.2.0 files and writes the information into a relational database. This *converter* builds upon the underlying mzIdentML file *parser*, which is from the Pyteomics Python library (https://github.com/levitsky/pyteomics). The converter also serves as an integrity check of the mzIdentML file. If peak list files have been provided then this process ensures that the spectrum for each identification is correctly referenced and can be found by xiVIEW in the associated peak lists. The mzIdentML reader can be reused separately to provide easier access to data in mzIdentML files. In addition to

mzIdentML, xiVIEW supports two non-standard CSV file formats designed for crosslinked peptide identifications (https://xiview.org/csv-formats.php) although their use is discouraged. The supported formats for peaks lists are: mzML (https://www.psidev.info/mzml), mgf (https://www.matrixscience.com/help/data_file_help.html#GEN), and ms2 [36].

xiVIEW is browser based to enhance the accessibility of the crosslinking MS data, eliminating the need for software installation and facilitating easy sharing of datasets via URLs. The database populated by the xiVIEW mzIdentML converter enables faster loading of the web pages. Querying this database is faster than processing the mzIdentML files from scratch.

xiVIEW brings together three primary views - xiNET [16], for the 2D crosslink network (Figure 2), NGL [37,38] for the crosslinks on a 3D structure (Figure 2), and xiSPEC [24] for displaying annotated spectra (Figure 3d). Other views such as scatterplots, histograms or a circular 2D network plot are also available, see Figure 3. xiVIEW presents numerous options for filtering the data, again with the results being synchronised in real time across the views. By integrating these different views, xiVIEW allows both analysing data quality and looking for biological insights within the same working environment. It also allows separately uploaded datasets to be aggregated and/or compared.

The central 2D network view is a development of xiNET [16]. xiNET combines a protein-protein interaction level network and a residue-residue network in a single view. While the initial result of an crosslinking MS search are spectral matches, interpreting these requires aggregating the results to higher levels of consolidation - linked residue pairs or linked protein pairs. Displaying the residue-resolution information provided by crosslinking MS is essential to its interpretation [1]. Other tools also allow this [13,15,17,19,27]. xiNET has the advantage of allowing the level of abstraction (residue or protein) to be interactively chosen per protein. Other advantages of xiNET include being web-based, providing a meaningful representation of ambiguity in the position of peptides in the protein sequences (if this exists), and showing the linked peptides in the protein sequence [16]. It has been open source since its first release and was one of the first visualisation tools designed specifically for crosslinklinking MS data. xiVIEW comes with a new version of xiNET that provides the ability to assign proteins to protein groups, which can then be collapsed into a single meta node to further simplify the display. This can be particularly helpful when working with larger networks. Users have the flexibility to introduce these protein groups manually, generate them automatically based on GO terms, or upload an annotation file to define them.

xiVIEW incorporates xiSPEC version 2.0 [24] to provide the view of annotated spectra. xiSPEC provides an interactive view of how the peptide sequence matches to a fragmentation spectrum. As is the case with xiNET, it is browser based and open source. The annotation of the spectra depends on an annotation web service which is integrated into the xiVIEW server, see Materials and Methods. The input to this annotation service is: the spectrum peaks; the peptide sequences including modification masses and positions; charge; and configuration settings from the mzIdentML file. The output from it is the peaks annotated with the corresponding fragments. The aim of the spectrum view is to present a meaningful re-annotation of the spectra, it cannot

exactly match the annotation of the spectra as seen by the original search software. The limits on how closely the automatic re-annotation corresponds to the original search software annotation are determined by: the information that can be encoded in mzIdentML 1.2.0; which of that is present in the specific mzIdentML file being parsed; and which of that is actually parsed from it (the mzIdentML converter only extracts a subset of the information possibly present in an mzIdentML file). Additionally, xiSPEC enables users to re-annotate the spectra with user-controlled adjustments, facilitating easy hypothesis testing.

NGL [37,38] is used to display 3D structures, and also to read 3D structure files. 3D structures are supported in PDB and mmCIF file formats. Deposited PDB files can also be loaded by entering the four character PDB accession code into the xiVIEW interface. The selection of 3D structures is controlled from the visualisation web page (see Figure 1). Showing the crosslinks on a 3D structure requires knowing how to map the search sequences onto the sequences in the structure. Other tools exist that focus specifically on addressing this issue [26,39]. There are three parts to the problem of mapping crosslinks onto 3D structures. First, the search sequences must be assigned to chains in the 3D structure, bearing in mind there may be multiple chains with the same sequence in the structure (homo-oligomeric complexes). Second, the potentially differing search sequences and chain sequences must be aligned to place the link sites within the chain sequence(s), the XWalk [9] publication concludes by highlighting this problem. Third, in the case of homo-oligomeric complexes, crosslinks may arise from a residue pair within the same protein subunit or between its copies, there is a combinatorial increase in the number of possible crosslinked residue pairs determined by the number of chain copies, Xlink Analyzer [14] highlighted this problem. For structures held in the Protein Data Bank [40]first two parts of this problem could be addressed by using RCSB web services (1d-coordinate-server-api), however, this would limit the process to archived PDB structures rather than locally created structures and so xiVIEW uses its own internal alignment process. A Javascript implementation of the Smith-Waterman algorithm https://github.com/Rappsilber-Laboratory/bioseq-js, modified so that it does not penalise the first gap, is used for a pairwise comparison of search sequences and pdb chains. The algorithm is then used for the link site alignment between these pairs. To address the third part of the problem, xiVIEW provides options to show crosslinks between all matching chains in the 3D structure, or only between the two chains that result in the shortest crosslinked distance. It is possible to load multiple 3D structures, but support for this is limited; they cannot, for example, be moved in relation to each other as they can in XLink Analyzer [14].

xiVIEW can, however, export files containing the crosslinks mapped onto the 3d structure in various formats for more in-depth structural analysis or modelling (Figure 1). These 3D export formats are now described. Crosslinks can be written out as PDB files in which crosslinks are described using the “LINK” keyword, see Atomic Coordinate Entry Format Description Version 3.30. This is a generic format useful for loading crosslink data into tools that can only read a PDB file. xiVIEW also supports exporting crosslinks as a PyMOL (https://pymol.org) command file, in which crosslinks are described with the “distance” command. This allows users to open the crosslink data in PyMOL, a popular tool for analysing and visualising 3D structures which many users will already be familiar with. Crosslinks can be exported as ChimeraX [41]

pseudobonds; and the input format for the Chimera plugin XLinkAnalyzer [14]. These tools provide more advanced modelling capabilities. xiVIEW can export the text format used as input to JWalk [20], this allows the calculation of solvent accessible surface distance (SASD) although xiVIEW cannot display the SASD paths. Lastly, in the case where two structures have been loaded, xiVIEW can export a HADDOCK [42,43] restraint file [44] The restraint file allows HADDOCK to be used to perform docking - see https://www.bonvinlab.org/education/HADDOCK-Xlinks/ for guidance on using crosslink data in HADDOCK. The restraint file generated by xiVIEW may need to be manually edited to specify the crosslinker distance and chains/segment IDs involved in docking.

Integrating crosslink data and 3D structures within xiVIEW allows investigating the agreement between the observed crosslinks and the available 3D structure information from other technologies. Two of the views in xiVIEW that particularly indicate this are the distogram (histogram of distances) and the contact map, see Figure 3. Additionally, users have the option to colour the links based on distance in the loaded 3D structure, allowing the agreement to be visible in any of the network views, thereby providing information on crosslinking restraint satisfaction. Disagreement between the crosslink data and the 3D structure could result from one or many of several factors. These include: differences in structure resulting from different experimental techniques, with crosslinking examining the structure in solution, possibly even in the native context of the proteins; protein flexibility/disorder; the presence of multiple/alternative structured conformations; or additional binders. Disagreement could also be due to noise in the crosslinking data [45,46]. However, if the crosslinks that do not fit the structure cluster together then the differences are more likely to be due to differing experimental techniques or conformations [47].

xiVIEW automatically integrates protein sequence annotations from UniProtKB [48], when valid UniProtKB accession numbers are provided for the proteins in the uploaded search results. This integration uses the same alignment mechanism as the 3D mapping process, in this case computing an alignment between the search sequence and the canonical UniProtKB sequence. The calculated alignment ensures that the sequence annotations are shown in the correct positions on the search sequences, even if these sequences differ from the canonical sequences, for example because a tag was added. Thus, the dataset will be automatically annotated at the sequence level (post translational modification sites, domains, known disease variants) and at the whole protein level (gene ontology terms, annotation of protein complexes and grouping of proteins into them). All these annotations can be toggled on or off individually and overlaid with the structural and topological information, this allows exploring biological function and mechanisms while simultaneously probing the data underlying an hypothesis arising from analysing the crosslink network. Similarly, xiVIEW can automatically query the STRING [49] database of functional protein-protein interactions to integrate the data with previous knowledge. STRING score, or any other custom parameter can then be used to colour the edges of the network to give the user an idea of the weight of additional evidence underlying a particular protein-protein interaction. Custom sets of annotations for either proteins or links can also be uploaded via the visualisation web page (Figure 1).

xiVIEW can handle the ambiguity in link site position that arises from peptides occurring in multiple places in protein sequences (the protein inference problem). It does this by implementing the same approach that was present in xiNET across its network views – crosslinks with peptide position ambiguity are shown as dotted lines and all the alternatives are highlighted when one of these lines is moused over. Though this by no means solves the protein inference problem, it allows xiVIEW to deal with peptides potentially coming from multiple proteins and the rest of xiVIEW's functionality works as normal with these links. The new grouping mechanism in xiNET would allow homologous proteins (which will share a lot of peptides) to be assigned to a single group which could then be collapsed.

xiVIEW can load datasets covering entire proteomes and it has been used to create figures for large scale crosslinking studies [50,51].The protein grouping mechanism, whereby proteins can be assigned to groups and collapsed to a single meta-node, aims to make xiVIEW more usable with large (proteome scale) crosslink networks. It is inherent in node-link diagrams that they become harder to use as the network complexity increases, our approach to this problem is to simplify the network. This remains an ongoing area of development for xiVIEW.

As part of being workflow independent, xiVIEW is crosslinker independent. The information about linked residues comes from the input file and to visualise this xiVIEW can be agnostic of the linkage chemistry. The colour-coding for crosslink distances is defined through the interface, as is distance based filtering. Although the results of work flows using both cleavable and non-cleavable crosslinkers can be visualised in xiVIEW, it has limitations in representing the details of some cleavable crosslinker workflows - specifically, currently only one spectra can support a match in xiVIEW. This is closely related to limitations of mzIdentML 1.2.0 which will be addressed by mzIdentML 1.3.0. Addressing this issue in xiVIEW will be part of supporting mzIdentML 1.3.0 in xiVIEW. The new grouping mechanism in xiVIEW allows proteins which share peptides to be assigned to a single group which could then be collapsed.

All the views in xiVIEW export graphics. Where applicable, these are vector graphics (SVG) so that they can be used as a basis for publication figures. Hence, the whole workflow can be seen as: peptide identification and peak list data arriving in the visualisation from the database; 3D structure and annotation data being selected whilst the user works within the visualisation; and, graphics and files mapping the crosslinks onto 3D structures being output from the visualisation (Figure 1).

xiVIEW has been available online for use since 2018. There is widespread need for such a tool and xiVIEW is commonly used in the crosslinking MS field to visualise data – more than 1000 users have registered since 2018 and more than 6000 datasets were uploaded within the last year. Its recognised utility has encouraged the crosslinking field towards the use of data standards, mzIdentML 1.2.0 being the file format it reads. xiVIEW allows scientists to investigate and share their crosslink MS data in ways that otherwise would be very laborious and thereby advances the study of protein function.

Materials and Methods

There are online tutorial videos to demonstrate the features of xiVIEW at https://www.rappsilberlab.org/software/xiview/. Online documentation is available via the web interface.

Instructions to run a local installation of xiVIEW are at https://github.com/Rappsilber-Laboratory/xiview-server. Such an installation will have dependencies on Python 3.10, PostgreSQL v12 or higher, and upon the https://github.com/Rappsilber-Laboratory/xi-mzidentml-converter project which is used to populate the database with your data.

The actual JavaScript source code for the visualisation tool is at https://github.com/Rappsilber-Laboratory/build-xiview. This uses the backbone JavaScript library (http://backbonejs.org) to structure the code and manage the synchronisation between the different views. The three primary views of xiVIEW – 2D network, spectra and 3D structure – are each developed on the basis of existing software. The build-xiview project brings together the various submodules and dependencies of xiVIEW (xiNET, xiSPEC, NGL, backbone.js) and generates a single bundled JavaScript file. This project is not a dependency for running a local installation as the bundled JavaScript is already included in https://github.com/Rappsilber-Laboratory/xiview-server.

The development of xiVIEW followed a User Centred Design methodology – an iterative process in which the users are closely involved throughout, the focus being understanding the user's needs and the context in which they work.

The code for xiVIEW has been available on GitHub throughout its development with all collaboration on the code taking place in the open via GitHub, for example, when working with the developer of NGL on its integration. This process continues.

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