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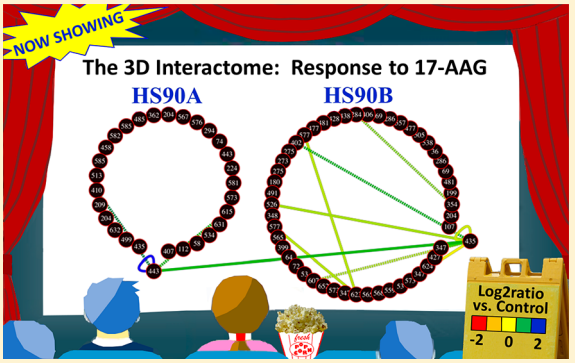
Tools for 3D Interactome Visualization

Andrew Keller, Juan D. Chavez, Jimmy K. Eng, Zorian Thornton, and James E. Bruce*

Department of Genome Sciences, University of Washington, Seattle, Washington 98195, United States

Supporting Information

ABSTRACT: In cells, intra- and intermolecular interactions of proteins confer function, and the dynamic modulation of this interactome is critical to meet the changing needs required to support life. Cross-linking and mass spectrometry (XL−MS) enable the detection of both intra- and intermolecular protein interactions in organelles, cells, tissues, and organs. Quantitative XL−MS enables the detection of interactome changes in cells due to environmental, phenotypic, pharmacological, or genetic perturbations. We have developed new informatics capabilities, the first to enable 3D visualization of multiple quantitative interactome data sets, acquired over time or with varied perturbation levels, to reveal relevant dynamic interactome changes. These new tools are integrated within release 3.0 of our online cross-linked peptide database and analysis tool suite XLinkDB. With the recent rapid expansion in XL−MS for protein structural studies and the extension to quantitative XL−MS measurements, 3D interactome visualization tools are of critical need.



KEYWORDS: *cross-linking, mass spectrometry, protein interaction networks, protein complexes, quantitative proteomics, dynamic interactome, cross-link database, heatmap, interactome movies, interaction surfaces*

INTRODUCTION

The use of quantitative cross-linking to compare protein interactions among samples is rapidly gaining popularity and importance.[1−3] Cleavable chemical cross-linkers, in particular, allow for the identification by mass spectrometry of cross-links in situ in cells, tissues, and whole organisms.[4−7] By providing distance constraints on protein residues, cross-links help elucidate the structures of proteins and protein complexes.[8−10] Quantitative cross-link data can be derived from samples isotopically labeled light or heavy, either at the level of the cross-linked peptides using technologies such as SILAC[3,11] or at the level of the cross-linker.[12] In either case, the light and heavy samples are combined together prior to XL−MS. Identified light and heavy cross-links are then quantified according to the peak areas of their parent ions, as acquired in MS,[1] to yield a ratio of abundance in the light sample (experimental condition) versus the heavy sample (reference condition). Quantitative cross-linked peptide information can be combined with protein abundance level measurements to delineate conformational and interaction changes due to posttranslational modifications, or protein interactor- or cofactor-induced allosteric changes, rather than changes in protein abundance.

A unique opportunity for new insight into biological function is presented by quantitative interactome measurements during perturbation. For example, visualization of interactome changes in samples treated with increasing concentrations of drugs or samples cross-linked longitudinally during an environmental perturbation can reveal functional conformational and protein interaction changes not evident in any other large-scale data. Combining the XL−MS data of multiple samples enables one to pool their cross-link identifications, which can then be quantified separately in each sample. Analyzing the data in this manner facilitates the researcher's ability to compare quantitative behavior among samples to find trends of interest. This is especially true in cases where samples have different experimental conditions but a common reference condition. Comparison of cross-link levels among the samples is then possible by comparing their abundance ratios with respect to that of the common reference condition. This gives the opportunity to visualize the interactions of related samples as a third dimension, in a 3D interactome, to help understand how the interactions change over time or with respect to varied perturbation levels.

We have previously reported XLinkDB,[13−15] a database that offers unique informatic tools for storing and visualizing protein interaction topology data. More recently, we have developed additional quantitative interactome analysis informatics tools to support the visualization of 3D interactome data sets. These tools are integrated into XLinkDB 3.0, which stores many cross-link characteristics, including the distance in the context of protein structures and the distance in known protein−protein interaction (PPI) databases. Support for quantitation now enables storage for one or more related samples of a cross-link log2ratio, standard error (among

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contributing replicates), and computed p value reflecting the likelihood that an observed log ratio significantly deviates from zero. Additionally, protein abundance level measurements of cross-linked proteins can also be integrated within XLinkDB 3.0. One can view and explore cross-link data in a browser, as either a table with a heatmap or as a PPI network in Cytoscape.[16]

MATERIALS AND METHODS

Cross-Link Data Upload to XLinkDB

Quantitative cross-link data in tab-delimited file format can be uploaded to XLinkDB at http://xlinkdb.gs.washington.edu/xlinkdb/. The file must contain a header line with the following column names:

1. PeptideA
2. ProteinA
3. Cross-linkPositionA
4. PeptideB
5. ProteinB
6. Cross-linkPositionB

Optional additional cross-link columns include:

7. Confidence
8. NumberIDs

Additional columns for quantitation can be added with names, for each quantified sample, as:

9. Exp_Ref_Type_log2ratio
10. Exp_Ref_Type_log2stdev
11. Exp_Ref_Type_pvalue
12. Exp_Ref_Type_chromatogram

where "Exp", "Ref", and "Type" are the experiment condition, reference condition, and quantitation type ("silac", "d0/d8"), respectively. They must in combination be unique for each included sample ratio. The Exp_Ref_Type_log2stdev, Exp_Ref_Type_pvalue, and Exp_Ref_Type_chromatogram columns are optional.

Values stored in columns of each nonheader entry of the file are:

1. First peptide sequence, without modifications.
2. Protein1, nsp1, wt1, Protein2, nsp2, wt2, ..., where Protein1, Protein2, ... is the ranked list of protein UniProt IDs corresponding to the first peptide, with the number of sibling peptides (nsp) and weight (wt) apportioned by ProteinProphet[17] when available. Only a single Protein is required. Other values are optional.
3. Position in sequence of first peptide, starting with 0 for the N-terminal residue.
4. Second peptide sequence, without modifications.
5. Protein1, nsp1, wt1, Protein2, nsp2, wt2, ..., where Protein1, Protein2, ... is the ranked list of protein UniProt IDs corresponding to the second peptide, with the number of sibling peptides (nsp) and weight (wt) apportioned by ProteinProphet when available. Only a single Protein is required. Other values are optional.
6. Position in sequence of second peptide, starting with 0 for the N-terminal residue.

Additional optional columns:

7. Probability that the nonredundant cross-link is correctly identified in the data set, a value between 0 and 1.
8. Number of identifications of the nonredundant cross-link in the data set.

Additional columns for quantitation:

9. Mean log2 ratio of experiment to reference condition, among contributing replicates.
10. Standard deviation of log2ratios among contributing replicates.
11. p-value assessing likelihood of observing mean log2 ratio by chance.
12. Chromatogram, enabling one to view the light and heavy parent raw data traces upon clicking a log2ratio value in the table or Cytoscape colored network edge. They must be extracted out of the raw data for the experiment and reference parent peaks ranging over the time of quantitation. As many as four chromatograms can be imported, separated by commas and written as an extended single line. The format is given below and requires square bracket delimiters as illustrated:

[[silac, ms1_exp, m/z, Rawfilename, NormalizationFactor],

[2580.8,0], [2581.4,0], [2582.0,0], [2588.8,281176], [2589.9,195922], ..., [2536.7,0]],

[[silac, ms1_ref, m/z, Rawfilename, NormalizationFactor],

[2580.8,0], [2581.4,0], [2582.0,0], [2588.8,190996], [2589.9,236953], ..., [2609.6,0]]

where ms1_exp and ms1_ref indicate the extracted ms1 data for the experimental and reference conditions, respectively. One must specify m/z values of the light and heavy parent peaks, Rawfilename, the name of the raw data file, and NormalizationFactor, the amount added to each log2ratio to center the distribution (0 when no normalization was applied). This is useful to display along with the chromatogram so researchers know how it resulted in the recorded log2ratio. "d0/d8" can be substituted for "silac" when appropriate. The following brackets each contain pairs of retention time in seconds and the integrated peak intensity within ppm tolerance.

Note that the first, third, fourth, and sixth columns must be unique in the uploaded file because they together define a unique cross-link. For the protein columns, when ProteinProphet results are known, one can include its apportionment of the number of sibling peptides and weight to the proteins, as indicated above, following the protein UniProt ID.

Quantitative Proteome Data Upload to XLinkDB

Quantitative proteome data associated with a network on XLinkDB can be uploaded in tab-delimited file format at http://xlinkdb.gs.washington.edu/xlinkdb/. The file must contain a header line with the following column names:

1. Protein
2. Exp_Ref_Type_log2ratio
3. Exp_Ref_Type_log2stdev
4. Exp_Ref_Type_pvalue

Values are as described above for cross-link data but with only a single protein in the first column, matching names of ProteinA or ProteinB in the cross-link data upload. The Type is "shotgun". Only the first two columns are required. Note that the first column value must be unique in the uploaded file.

Interaction Surfaces between Two Proteins

The interaction surfaces are computed by finding all amino acids in the structure or modeled docking PDB files with the two interacting proteins that are within a user specified Cα-Cα Euclidean distance from any residue of the other protein. The

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Peptide A	Protein A	Residue Number A	Peptide B	Protein B	Residue Number B	Network Distance	XL Distance (Å)	Number ReACT IDs	Confidence	Num Prot Pairs	log2ratio_1_Control	log2ratio_2_100nmAAG	log2ratio_3_250nmAAG	log2ratio_4_500nmAAG	log2ratio_5_1000nmAAG
FYEQFSKNIK	HS90A_HUMAN	443	FYEQFSKNIK	HS90A_HUMAN	443	1	36.47	35	1	1	-0.17	2.8	2.74	3.44	3.17
ILKVIR	HS90A_HUMAN	410	FYEQFSKNIK	HS90A_HUMAN	443	Intra	24.78	14	1	3	0.45	1.76	1.73	1.94	1.86
IMKAQALR	HS90A_HUMAN	615	FYEQFSKNIK	HS90A_HUMAN	443	Intra	20.83	104	1	2	0.06	0.55	0.94	1.26	1.1
FYEAFSKNLK	HS90B_HUMAN	435	FYEQFSKNIK	HS90A_HUMAN	443	1	####	74	1	1	-0.06	0.98	0.94	1.34	1.26
APFDLFENKK	HS90B_HUMAN	347	FYEAFSKNLK	HS90B_HUMAN	435	Intra	17.75	57	1	1	0.45	-0.03	0.37	0.01	0.74

Figure 1. Table view illustrating cross-link abundance ratios of various samples with respect to a common control as a sortable heatmap.

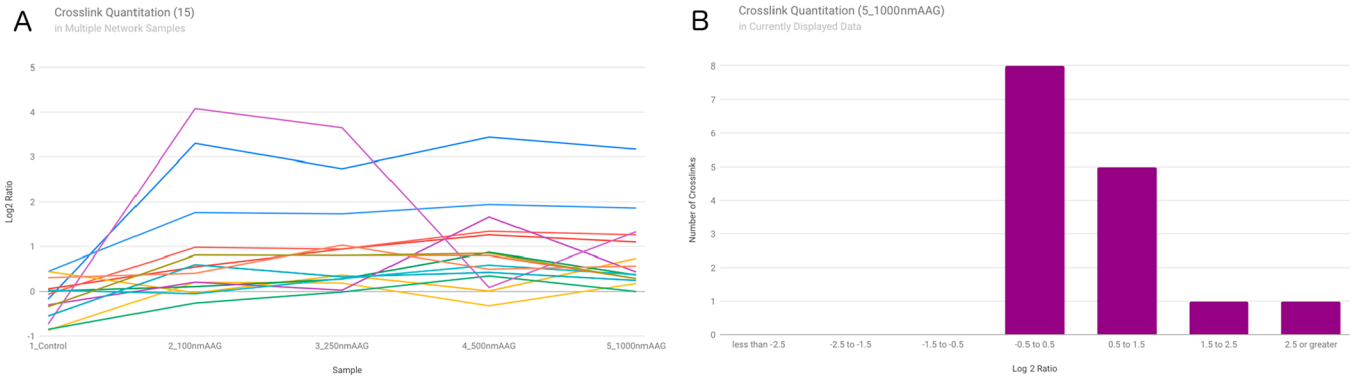

Figure 2. (A) Line chart showing cross-link quantitation log2ratio value trends in various samples. (B) Bar chart showing numbers of cross-links with various log2ratio values in a single sample.

surface residues of each protein are passed to the NGL Viewer with coloring instructions, along with cross-links.

Rotating View HTML Page

PNG files were downloaded from the XLinkDB Cytoscape PPI network colored, in succession, by multiple samples treated with increasing concentrations of 17-AAG and assembled into an HTML page using the Jquery code available at https://github.com/mcnitt/jquery-infinite-rotator. The HTML page was loaded into a browser and saved as a screenshot movie file with QuickTime.

RESULTS AND DISCUSSION

Table View

Cross-links uploaded to XLinkDB can be viewed in table format with one row per nonredundant cross-link. Information displayed for each cross-link, in columns, includes the released cross-linked peptide sequences and cross-link sites, proteins, distance in context of available protein structures and models, numbers of corresponding protein pairs, and numbers of identifications. With this information available for each identified cross-link, researchers can efficiently explore the data. One can sort by any column values or filter the table to view only cross-links with specific features, such as corresponding peptide or proteins, or numbers of identifications. Users have the option to display any or all sample quantitation information associated with the data set, including the log2ratio, standard error, and p value. Figure 1 shows selected rows of the table on XLinkDB displaying log2ratio values in several samples as a heatmap. By sorting columns, one can identify cross-links exhibiting similar abundance trends in the samples.

Charts summarizing some features of the displayed cross-link data, including cross-linked distance in the context of protein structures and models as well as quantitation log2ratios, are included at the bottom of the table. The distances are useful indicators of cross-links that are not consistent with known protein structures but possibly with alternative conformations. In cases where more than a single ratio is displayed in the table, plots of the multiple log2ratios for each cross-link are plotted as trend lines (Figure 2A), which, when clicked, go to a table with the single entry of that cross-link. When only a single ratio is displayed in the table, the number of cross-links having log2ratio values within various bounds are displayed as a clickable bar graph (Figure 2B), with links going to a table containing the contributing cross-links with ratios within its bounds. These features help one to explore the data set to identify and assess cross-links with quantitation values of interest.

A primary challenge involved in the analysis and interpretation of large-scale interactome data arises from the difficulties encountered in handling raw mass spectrometry and chromatographic data, protein structures, and models as well as offering seamless integration with other existing PPI database information. Within XLinkDB 3.0, users can make raw data available during the upload, and, if so, one can click on a log2ratio value in the table to immediately view the chromatographic profiles of contributing light and heavy

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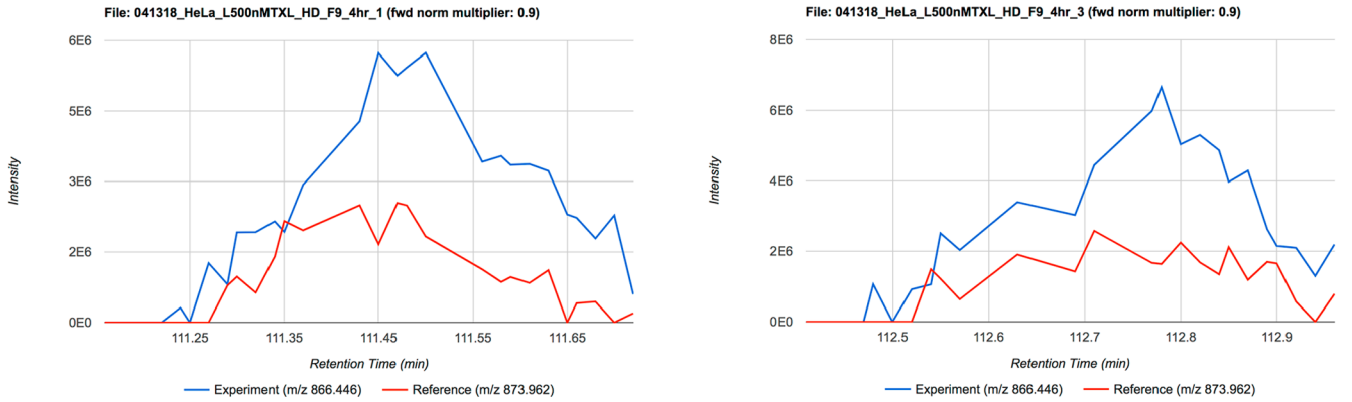

Figure 3. Example chromatographic traces of light and heavy parent masses in two replicate files, displayed upon clicking their cross-link log2ratio value in the table. The indicated normalization multiplier was applied to the peak area ratios to achieve a log2ratio distribution centered at zero.

parent peaks. This provides crucial assessment of the quality of the quantitative measurement (Figure 3).

Hyperlinks were also added in XLinkDB 3.0 to several other column values in the table, each leading to pertinent additional information. For example, clicking on the entry in the table column entitled "Network Distance" for cases other than "Intra" presents the paths through the network connecting the two cross-linked proteins found in other existing PPI databases. Clicking on the entry under the table column entitled "Num Prot Pairs" displays all proteins corresponding to each peptide, and, when available, their apportioned weights, as computed by ProteinProphet, indicating their relative likelihood as the origin of the cross-linked peptide. Clicking the "NGL Viewer" button displays the cross-link within the PDB structure in NGL (Rose and Hildebrand, 2015).[18] In the case of interprotein cross-links with an available multimeric structure or docked model, the user has the option of additionally coloring the interaction surface of both proteins at a specified distance (Figure 4). This can help illustrate and validate how the two interacting proteins may recognize and fit with one another. Surfaces defined in this manner can potentially be used to design drugs that bind to proteins to disrupt specific interactions.

Data displayed in the table can be downloaded as a tab-delimited file. Quantified data in this format can easily be subjected to clustering analysis to identify common trends among cross-links. Data can also be imported to Cytoscape to view the PPI network as a graphical display.

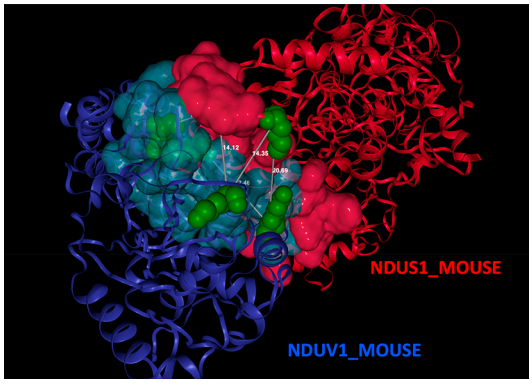

Figure 4. NGL Viewer showing identified interprotein cross-links spanning mouse mitochondrial proteins NADH-ubiquinone oxidoreductase (NDUS1) and NADH dehydrogenase flavoprotein 1 (NDUV1), with the interaction surfaces of each protein displayed at 10 Å in a model dimeric structure generated by iDock.[19]

Interaction Network View

It is often useful to view cross-link data as a PPI network with cross-links organized according to their corresponding proteins. In XLinkDB, one can view a network in a browser with Cytoscape, where nodes represent cross-link sites in proteins and edges represent nonredundant cross-links. Nodes corresponding to different sites in the same protein are normally grouped in circles according to UniProt ID, although any layout is supported. The interaction network clearly indicates which proteins interact with one another. One can explore the network, zooming in or out, and centering the view near any region. Users have the option to save the current view so that when they return to the network at a later time it will revert to that same position and zoom level.

Exploring the network is facilitated by options to color edges according to many cross-link attributes, such as the distance in the context of protein structures, the distance in known PPI networks, the confidence of identification, and numbers of corresponding protein pairs. When coloring by the various attributes, one can click on the edge to obtain the same pertinent information displayed upon clicking the value in the table view, as described above.

For networks with quantitative information, edges can be colored by any available sample log2ratio (Figure 5) as well as *p* value. When coloring by log2ratio, for which raw data is available, one can click on an edge to view the chromatograms of contributing light and heavy parent peaks. One can easily view and compare ratios corresponding to different contributing samples for cross-links of interest by saving a view near the cross-links and selecting edge coloring for each ratio, in succession. When edges are colored by log2ratio, the edge is solid if there is only a single corresponding protein pair possible but dashed if there are two or more. This can occur when two or more proteins share indistinguishable peptide sequences and is used to alert users in that case that the quantitation may not apply literally to the two displayed most likely corresponding proteins. When viewing the edges colored by the number of corresponding protein pairs, one can click on the edge to view all proteins corresponding to each peptide and, when available, their apportioned likelihoods, as computed by ProteinProphet. Proteome level quantitation information in data sets can be displayed as node colors of the

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J. Proteome Res. 2019, 18, 753−758

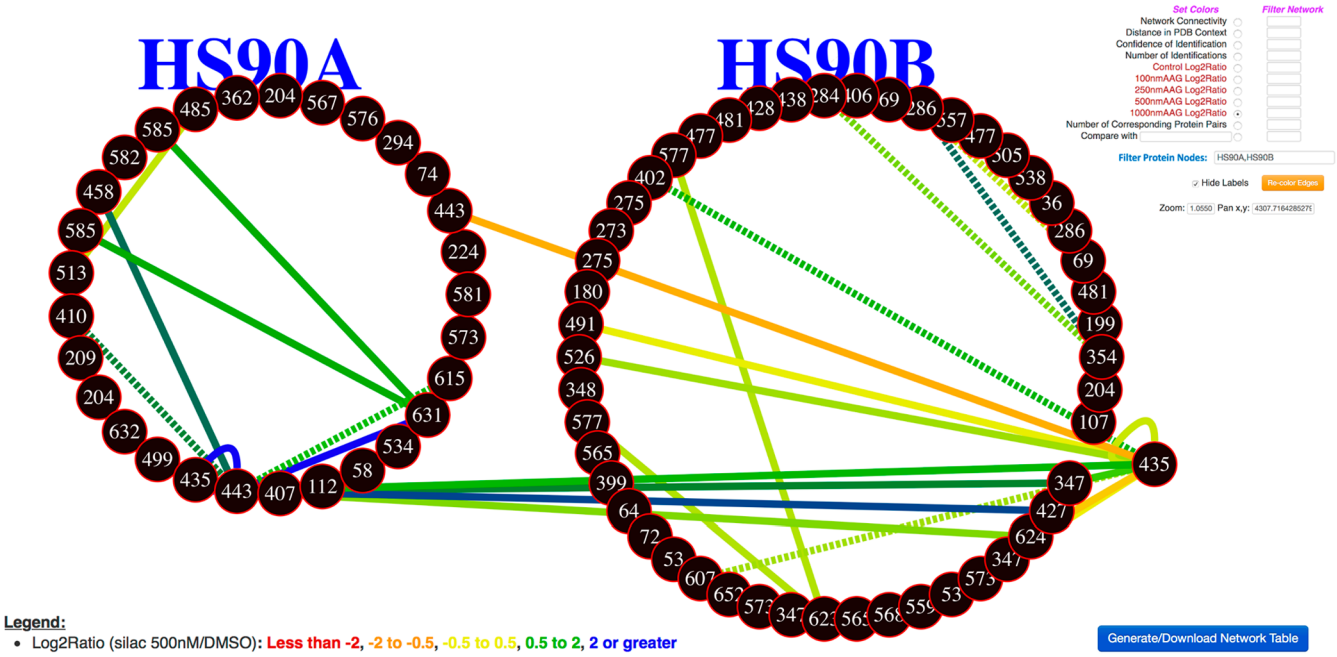

Figure 5. Cytoscape interaction network filtered for human heat shock proteins HS90A and HS90B, with edges colored by log2ratio in the selected sample, treatment of HeLa cells with 1000 nM 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG), an Hsp90 inhibitor, versus control.

same hue scale used to color network edges according to cross-link-level quantitation.

For each of the cross-link attributes by which edge coloring is possible, one can also apply filtering according to a specified range of values. For example, one can select to view only cross-links with low *p*-values with respect to some ratios and high *p*-values with respect to others. Or one can specify to view only cross-links with a single possible corresponding protein pair. One can also explicitly filter for a list of specified proteins, whereby only nodes of those proteins and edges connecting them are shown (Figure 5). One can obtain a table view of the network data currently displayed by clicking the "Generate/Download Network Table" button. This is especially useful when one has applied filtering criteria to view the network because those criteria are applied to the resulting table view.

Viewing the 3D Interactome

In cases in which a network has quantitation with respect to a number of related samples, one can use the XLinkDB Cytoscape network to construct a movie of images of the quantified PPI networks that comprise a 3D interactome. By coloring edges according to each sample log2ratio in succession and downloading for each a PNG file, one can easily assemble the network images into a rotating movie (see Materials and Methods). Viewing the movie as a 3D interactome, it is possible to experience the dynamic changes in quantified samples. For example, if the quantified samples are obtained over the time course of a perturbation, then observing the movie of time-successive images of the network with edges colored by quantitation gives a clear indication of the protein conformational changes occurring over time. Movies of the network images with quantitation corresponding to increased doses of a drug applied to a sample, in a similar manner, help to illustrate the changes in conformation induced by exposure to the drug. Changes observed among subsets of edges are strong indicators of alternative protein conformations.

As an example, a set of light SILAC-labeled human HeLa samples were treated with concentrations of the Hsp90 inhibitor, 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG), ranging from 0 (control) to 1000 nM.[20] Following treatment, cells were mixed with equal amounts of heavy labeled cells treated with no drug, cross-linked, subjected to ReACT XL–MS,[21] and quantified with MassChroQ.[14] The data were uploaded to XLinkDB, where the PPI network was viewed in Cytoscape, with cross-link edges colored in series by log2ratio of samples exposed to increasing doses of 17-AAG. Images of heat shock protein HS90A and HS90B components of this network were downloaded as PNG files, embedded in an HTML rotator, and captured as a movie with QuickTime (Figure S1). Viewing the movie as a 3D interactome, one can see drug-induced increased levels of HS90A homodimerization at residue 443 and HS90A–HS90B heterodimerization at residues 443 and 435, respectively. In contrast, no increase in analogous HS90B homodimerization at residue 435 was observed. The majority of changes occur with as little as 100 nM 17-AAG treatment, although some additional changes in observed levels of cross-links occur with higher doses of the drug. Exploring the 3D interactome, researchers can efficiently identify subsets of cross-links with similar quantitation changes that could specify alternative drug-induced conformations and that could be used to guide structure modeling.

CONCLUSIONS

Interactome data as acquired with XL–MS offer a new window through which to observe both protein conformational and PPI information in complex samples such as functional organelles, live cells, and tissues. We have previously developed a suite of informatics tools embodied within web-based XLinkDB that enable researchers to visualize large cross-linked data sets, resulting interaction networks, as wells as links on protein and protein interaction structures and models. Quantitative XL–MS studies are only beginning to reveal

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conformational and interactome dynamics but hold great potential for providing unique molecular-level insight into biological function. However, these data sets require further advanced informatics tools to enable multidimensional interactome visualization. To facilitate such exploration, we developed and implemented new tools to store and view quantitative cross-link data sets in XLinkDB in its 3.0 release, available online at http://xlinkdb.gs.washington.edu/xlinkdb/. These new capabilities allow researchers to visualize interactome dynamics in conjunction with proteome-level quantitation with respect to virtually any perturbation. Cross-links of interest can easily be assessed for the quality of their quantitation as well as other features such as consistency with known protein structures. In the future, we intend to enable upload of XL−MS data analyzed with XLinkProphet[22] and store the mass spectra of the released peptides, upon which the cross-link identifications are based.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.8b00703.

Figure S1. Movie of cross-links within and between human heat shock proteins HS90A and HS90B after treatment of HeLa cells with increasing concentrations of Hsp90 inhibitor, 17-AAG, versus control (PDF)

3D interactome of Figure S1 (MOV)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jimbruce@uw.edu. Tel: 206-543-0220.

ORCID

James E. Bruce: 0000-0001-6441-6089

Notes

The authors declare no competing financial interest.

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