Protein Chemical Cross-linking/Mass Spectrometry: From raw data to fully immersive visualizations

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Abstract

Proteins are the base component that make all living organisms function. They are made up of amino acids and are responsible for the structure, function, and regulation of organisms' tissues and organs. They can exist alone or as part of a multi-unit structure. They interact with other proteins to form stable or transient complexes. Protein function, such as transporting signals or converting energy, is determined by its structure, therefore being critical that we understand protein complexes at the cellular level. Chemical cross-linking combined with mass spectrometry is an established method in protein chemistry to explain low-resolution 3D protein structures and interacting sequences in protein complexes. Chemical cross-linking of a protein enables scientists to understand how the protein folds, whereas intermolecular cross-linking between different proteins enables to determine which components interact and how and where they physically contact each other. Mass spectrometry is used to acquire distance information and distance constraints within a molecule. The challenge is to how to best take advantage of the information provided by these methods utilizing novel visualization analytics that can help explore much more detailed information on protein structure than traditional biomechanical methods, such as the Edman degradation which sequentially removes one residue at a time and the structures are observed through chromatographic procedures.

Additionally, data resulting from chemical cross-linking in most cases come in a raw format that makes it hard for the user to absorb the data and get a bigger picture of the existing interactions as well as getting a close insight on smaller reactions. This has motivated the department of Bioinformatics at the University of Arkansas at Little Rock to develop an algorithm named "X-Linked Peptide Mapping" that allows the analysis of the data and identify interacting peptides (short chains of amino acids) in an easier, more accurate way avoiding the limitations mentioned before. Since the field of chemical cross-linking mass spectrometry of protein-protein interactions hasn't been explored visually in a comprehensive way before, the results encouraged us at the Emerging Analytics Center at the University of Arkansas at Little Rock to develop an interactive web application that allows the user to visualize the results of the analysis including 2D information representations as well as 3D structural modeling representation of the interactions,. This paper presents preliminary research in this area, including the development of a prototype immersive environment to explore the molecular structures.

Introduction

Protein Complexes

Protein complexes usually appear as a group of two or more associated polypeptide (amino acid) chains, and scientists are interested in studying their structure and how they interact at the cellular level inside an organism because they control cell's growth and eventual fate.

The molecular structure of protein complexes can be determined by experimental techniques such as X-ray crystallography or nuclear magnetic resonance. [3] Since protein complexes are the cornerstone of most biological processes, they create diverse types of molecular structures that perform a wide range of biological functions. How far or how close these complexes are from each other regulate the selection and speed of interactions among them and therefore determine the cell's efficiency.

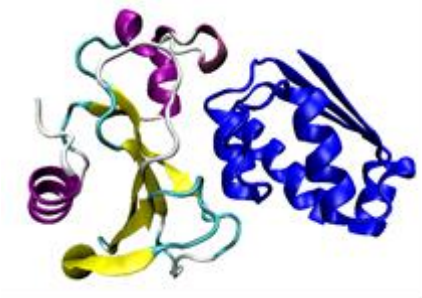

Figure 1. The Bacillus amyloliquefaciens ribonuclease barnase (colored) and its inhibitor barstar (blue) in a complex. From "Barnase-barstar-1brs" by Opabinia regalis - Self created from PDB entry 1BRS using the freely available visualization and analysis package VMD. Licensed under CC BY-SA 3.0 via Commons - https://commons.wikimedia.org/wiki/File:Barnase-barstar-1brs.png#/media/File:Barnase-barstar-1brs.png

Chemical Cross Linking (CX)

A cross-link is a bond that links one protein chain to another. They can be covalent bonds (sharing electrons pairs among atoms) or ionic bonds (electrostatic attraction between opposite charged ions). When crosslinking is used in biology, it refers to the use of a probe to link proteins together to check for protein–protein interactions, as well as other creative cross-linking methodologies. [5] As an example; when cross links are added to long rubber molecules, the flexibility decreases, the hardness increases and the melting point increases as well.

Protein Mass Spectrometry (MS)

Proteins' backbones are comprised of masses of amino acid residues, Mass spectrometry (MS) is an important emerging method used for the characterization and identification of proteins groups out of these masses of amino acid residues. Mass spectrometry works by breaking down the input protein into peptides and peptides into fragment ions then identify the mass of each piece by accelerating the fragmented ions and measuring mass/charge accordingly. Based on the identified mass it easy to match the identified ions to a database of previously known data or use De Novo's [28] method, a graph is drawn based on these findings and eventually identify the peptide then the whole protein. [27] [4]

Chemical Cross-Linking / Mass Spectrometry (CX/MS)

Incorporating cross-linking and mass spectrometry in bioinformatics has significantly improved modelling by mapping structural details of functional complexes in a solution [6]. Cross-linking is fast and economical, and mass spectrometers are widely available. Many efforts have been undertaken to tackle the current bottleneck of cross-linking/MS, namely the computational search tools for the identification of cross-linked peptides (short amino acids chains).

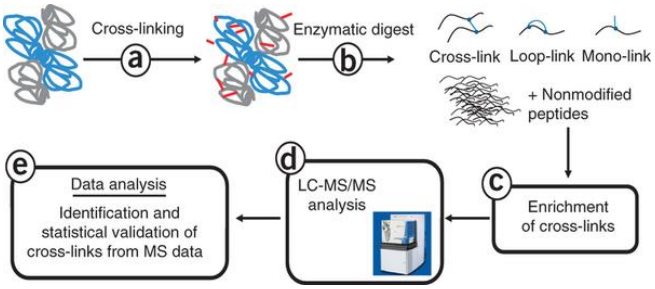

Figure 2. Typical workflow of an XL-MS experiment. From "Lysine-specific chemical cross-linking of protein complexes and identification of cross-linking sites using LC-MS/MS and the xQuest/xProphet software pipeline" Alexander Leitner,homas Walzthoeni&Ruedi Aebersold. Nature Protocols 9, 120–137 (2014)

X-Linked Peptide Mapping (XLPM)

Analyzing chemical cross-linking mass spectrometry (CXMS) data introduces several chemical difficulties in identifying the proteins incorporated. Current soft wares and algorithms like StavoX [29] are based on a cross-link data base search approach that is followed by matching the findings to the list of possible fragment matches. X-Linked peptide mapping [1] is the software tool developed by the Department of Bioinformatics at University of Arkansas at Little Rock (UALR) that is designed to help analyze and identify the data coming out of the process of chemical cross-linking mass spectrometry avoiding the drawbacks of other algorithms known, XLPM uses a probabilistic model to score the possible existing fragments to give a more accurate identification of the input protein. The XLPM probabilistic score gives the user the freedom of choosing the model which fits best to their data and still can use the score. In essence, XLPM probabilistic score provides the score for CXMS analysis that can be tailored for the CXMS analysis, in turn, providing better outcome for the CXMS analysis. This score is the start to long list of improvements in CXMS analysis. The basic data representation is a result of the XLPM algorithm which was designed for the analyses of CXMS data. XLPM identifies not only the peptide pair interacting with each other but also scores the pair of residues interacting with each other.

Visualization

We developed a web-based visualization tool that took the results from XLPM to provide the user with an interactive exploration of the data for analysis and insight. We coupled this 2D visualization with a 3D visualization of the molecular interactions based on user inputs from the 2D data analysis. The main components of the application are targeted for visualization in a conventional desktop environment through a web browser, although we also added a secondary module that enables immersive exploration of the molecular structures and interactions.

Implementation

The frameworks used in the online part is all based on JavaScript which have incredible benchmarks on the several browsers especially when computer graphics is involved with molecular structures. The virtual reality module of the project uses technologies based on C++ like OpenGL. The XLPM map viewer is the first attempt at visualization of chemical crosslinking mass spectrometry (CXMS) data. The three level visualization describes details of all the matched spectra for entire proteins. Heat map in the first level gives a profile of interaction between a pair of proteins on a single page. The second level gives in depth details of all spectra representing the interaction between a pair of peptides and the third level heat map for the residues interaction. Easy navigation, zoom and selection make the interpretation of CXMS results from XLPM analysis comprehensive.

Previous Work

There is a big body of work in the area of molecular visualization, with tools such as VMD [24], PyMol [30] and Protein Data Bank (PDB) [31] there have also been efforts to visualize protein docking and other multi protein interaction visualizations, such as Maya Molecular Toolkit [32] and Ligand Explorer [33]. Also there has been a trial at Boston University to visualize protein docking and design in an immersive environment (VRDD) [34]. However, the specific field of mass spectrometry has been hardly explored through visualization techniques Previous work that has been done was all based on graphical analysis of the resulatant data. “protViz” [25] which is R based check, analysis and visualizes mass-spectrometry data the visualizations are all based on 2d graphical visuals. “MZmine” and “Mass++” [26] are another tool that is used to analyses the mass spectrometry data in the same manner as “protViz” but they’ve added the ability to do batch processing analysis. These efforts focused mainly on 2D visualizations which did not present a direct visual of the molecular structure under study. Our focus is to provide bioinformatics scientists with a 2D/3D hybrid platform to explore, analyze and gain insight on protein complexes and their interactions.

Raw Data

XLPM [1] takes two protein sequences and potential cross-linked strands then searches in the available spectra to match the strand it has. XLPM resultant files shown in Figure 3a shows the file that comes out after running the algorithm in Figure 3b, XLPM receives information of protein sequences, a spectra file in mgf format, digestion enzyme, and cross-linker, static and variable modifications of amino acids, missed cleavage level, precursor ion tolerance in ppm and fragment ion tolerance in Daltons. XLPM generate temporary database of cross-linked pairs from given protein sequences. First, amino acids that may be cross-linked are marked in the protein sequence. A list of digested fragment sequences is generated. A missed cleavage is forced. In other words, digested fragments having only one amino acid at the C terminal, which can be cross-linked, are removed from the list. The digested fragments devoid of any amino acids that can be cross-linked are also filtered out. A database of cross-linked pairs is generated from the list of digested fragments including the change of the mass due to any amino acid modification.

A file in a mascot generic format is uploaded and read; data are extracted from it and parsed into precursor ions and fragment ions. The precursor ion masses are compared with the masses of cross-linked pairs in the database generated previously with the user defined error tolerance. Each matched cross-linked product is further analyzed using its MS/MS fragmentation pattern and scored.

```
TITLE   m/z Fragment1   Fragment2   Theoretical Mass    Actual Mass Char
CL_SITE_SEQ_1   CL_SITE_SEQ_2   SCORE
Zybaylov_103012_Rim1_03.9144.9144.5 821.415710449218750
kGALVYVEADAANYVFERDDGSkGTTLSLVQk    DDGSK   4102.0541
4102.03955078125    5.0000000000000 1.2625124730844
        1   1   1.23220944278137
        1   2   1.24736095793288
        1   3   1.2625124730844
        1   4   1.19686466046951
        23  1   1.03022425292789
        23  2   1.04537576807941
        23  3   1.06052728323092
        23  4   0.994879470616039
Zybaylov_103012_Rim1_03.9148.9148.5 821.417297363281250
kGALVYVEADAANYVFERDDGSkGTTLSLVQk    DDGSK   4102.0541
4102.04736328125    5.0000000000000 1.12617509584581
        1   1   0.954466677170317
        1   2   0.969618192321832
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Figure 3a. Resultant analysis from XLPM

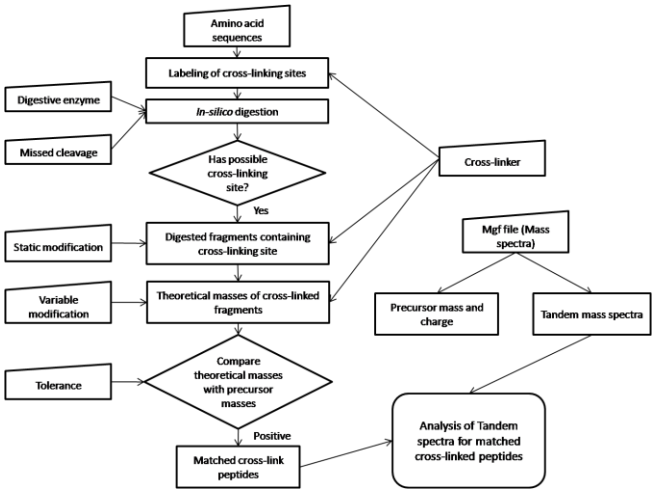

Figure 3b. XLPM Algorithm Flow Chart

2D Visualization

Sequence Files

Sequence files or FASTA format is part of the PDB specifications [31] for representing protein and peptide sequences after being sequenced. In our 2D visualization these files play an important role after being parsed in the program as a look up for all interacting peptides inside the given data file.

Visual Elements & Triggering Events

XLPM identifies not only the peptide pair interacting with each other but also scores the pair of residues interacting with each other. XLPM Map Viewer is a JavaScript based web tool that we have developed at the Emerging Analytics Center at the University of Arkansas at Little Rock to visually analyze the XLPM results dynamically. XLPM Map Viewer is based on co-occurrence matrix and tree map visualization techniques, it shows the protein-protein interactions between two proteins in three levels. The first level shows the highest ranked interactions between digested peptides as a co-occurrence matrix with color intensity reflecting the score. Selecting any of the interaction in the first level opens the second level of visualization. The second level of visualization shows all the precursor ions matching with the selected interaction. It also shows precursor ion mass, theoretical mass, charge, peptide sequences and the name of the spectra. Selecting one of the spectra shows the third level. Third level shows the details scores for interaction between each pair of residues. The visualization within XLPM map makes understanding of the interactions between a protein pair efficient by providing easy access to all levels of information. XLPM map viewer displays XLPM results in three levels. The first level is a heat map for showing the overall interactions between the parts of two sequences. Both the sequences are divided in to the fragments as per the digestion enzyme used assuming a complete digestion. The results from incomplete digestion is distributed among all the completely digested component peptides of the incompletely digested peptides. In the first level only the highest scored interaction is shown on the heat map for each pair of peptides. Clicking on any of the point on the heat map opens a level 2 visualization for that particular peptide pair. Level 2 is a tree map showing all the analyzed spectra that found the interaction between the peptide pair chosen in the first level. The tree map is arranged from top to bottom, left to right in deceasing order of the score for the spectrum. Mouse over each spectrum shows the details about the spectra including title, charge, theoretical and experimental mass. Level 2 visualization shows missed-cleaved sequence spectra also for the pair of sequence selected in the first level. Selecting any of the spectra in the tree map opens a third level of visualization. The third level of visualization is a heat map showing the cross-linking between pairs of residues. Figure 4 shows level 1 visualization heat map on the left. Figure 4 on the very right shows the level 2 visualization tree map. The number of components in the level 2 visualization depends on the number of spectra that led to identification of the interaction between this pair of digested peptides. Figure 4 shows all spectra that concluded the interaction between LEDAEGQENAASSELEHHHHHHH and GALVYVEADAANYVFER. The cross-linking between K and K is more likely than the cross-linking between K and E. The visualization allows zooming in and out to choose and select appropriate data point. Grey color at any stage of the visualization depicts zero score for that particular data point. XLPM map viewer provides the users a whole scale view of the interactions between a protein pair with its unique three level visualization. Mapping of these interactions on to protein three dimension structures is more informative in understanding the topology of the interaction. The knowledge acquired, thus, about the CXMS data can, in turn, be used to improve CXMS algorithms as well via the iterative process thus the additions of the interactions mapping onto protein three dimensional structures and mass spectra visualization in the XLPM map viewer will increase its applications immensely.

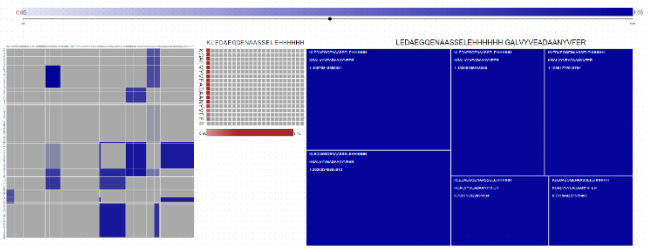

Figure 4. 2D Representation of XLPM data using matrices and tree maps

3D Visualization

The effort that has been done since the seventies in creating the PDB and other Biomedical data bases has led to the emergence and rapid development of the field of molecular modeling in bioinformatics, also the same efforts have led to an interdisciplinary endeavors to use the abundant data available to create a better experience for related users. Also the advancements in structural biology paved the way for the release of PDB files which describes proteins structures in 3D space accordingly the idea of regenerating these structures in computer graphics became obvious. We introduce an interactive viewer that maps the entire interaction based on the XLPM analysis based on the efforts that has been done before by 3DMol to view exactly the interacting peptides on the structures down to residue level. Analyzing PDB files and matching them to the results from XLPM files given the sequence files was the biggest challenge in the realm of parsing the PDB and creating the structures given the spaces between the molecules.

Breaching the gap between raw data, 2d graphical visualization, 3d protein modelling and virtual reality is our goal in this work. What really makes this work is the foundation that a lot people laid in the field of molecular graphics through the several ways they found to represent molecules based on PDB data, from Line representation to Electron density plot which exploits the field of quantum mechanics to give us an actual idea of how the molecule takes shape in space to Ball and stick models where bonds represented as sticks to Space fill models which suggests that the atom acquires a certain space based on a sphere to finally cartoon representation which is based on not representing atoms and bonds but rather a protein backbone with a smooth surface [18] based on a Bezier surface [19]. A Bezier surface is a surface of a degree (n, m) and is defined by a number of control points where a mesh of unit squares is mapped into a smooth-continuous surface embedded within a space of the same dimensionality therefore a Bezier surface can be defined as a parametric surface where the position of a point p is a function of the parametric coordinates (u, v) given by

$$\mathbf{p}(u,v) = \sum_{i=0}^{n} \sum_{j=0}^{m} B_i^n(u)\, B_j^m(v)\, \mathbf{k}_{i,j}$$

Then evaluated over the unit square where [19]

$$B_i^n(u) = \binom{n}{i} u^i (1-u)^{n-i}$$

Molecular modeling in cartoon representation is one of the most used ways to represent molecules in the field, it gives users a better way to understand interactions, in this work we are particularly using cartoon to model our work interactively. As a Java Script based project several libraries has been used to bring the modeling of the two proteins interacting together, the used library is called 3DMol [23], the library has been extended to be able to render more than one PDB model in the scene also to manipulate the camera and mouse interaction inside the application. Perhaps the challenge here was determining which pair of peptides are interacting based on the user input, which was solve by assigning each amino acid in the molecule and id and just send these ids to the 3D script to handle the coloring and the updating.

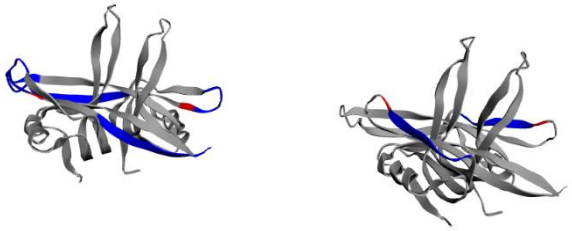

Figure 5. 3D Representation of the two protein molecules interaction facing each other in 3D space

Immersive Technology

The introduction of the CAVE system 1993 [20] has contributed to the marriage between scientific visualizations and virtual reality. VMD [24] can easily be integrated in the CAVE environment producing interactive high quality molecular models and simulations for scientific exploration..

Our pipeline starts with parsing the 2 intended PDB files for each protein and rendering them in the near field, The starts the navigates to the desired value

represented on the heat map matrix across the file, when navigating through the spectra of a chosen pair of peptides the user then is able to see the changes in the colors of the interacting peptides on the model represented in cartoon surface giving him a perspective through the 3D glass which gets tracked and translated through the VR-Juggler system [21]. The user has the freedom to walk around the model and see interactively which pair of peptides are interacting from any perspective. The system is designed to navigate to the amino acid residue level to show which pair of residues are interacting.

One challenge that we are facing right now is resolving the molecule rotation problem where each pair of interacting peptides has to face each other in a certain angle to be able to give the user a better understanding of the peptide level interaction. We are undergoing the development of this VR module and the plan consists of testing the results on several data files coming out of the XLPM algorithm with the corresponding protein PDB IDs.

Future Work

Molecular Dynamics Simulation

In Molecular Dynamics atoms are usually represented as single point masses inside Van Der Vaal potentials, according to the calculated parameters they come out as hard spheres also quantum methods are usually used to calculate the distribution of electronics charges across the molecules, bonds are usually represented as single harmonic oscillators with respect to the angles, several parameters control molecular dynamics simulations like start velocity and position, simulations are ran according to Newtonian physics with molecules transferring energy and momentum to one another via electrostatics and Van Der Vaal's interactions. VMD software packages introduce a clear implementations of molecular dynamic simulations. Through using open source packages like VMD we plan to incorporate molecular dynamic simulation into XLPM analysis as an experiment inside the CAVE environment to explore the potential of the proposed work.

Conclusion

This paper presents several ways to visualize the analysis and peptide identification done on CXMS results through XLPM on two platforms to enable bioinformatics scientists to gain better insight in their data and to address some of the mentioned details above. First off we are presenting a complete web application that captures all the details found in the resultant XLPM analysis files on several levels of data introducing visual elements reflecting interactively in real-time on a 3D model of the two interacting molecules in space with a calculated proximity and rotation, the second endeavor which is still under development and testing is working on an interactive immersive environment that would allow the user to be completely be immersed in an environment that will allow him to interact with the two molecules represented in the reaction with the 2D data representation on a tablet. Introducing the integration between 2D maps, Tree maps and 3D protein structures in an immersive VR environment might have a great impact on how we understand drugs and proteins, however several technologies will have to come together to make this work.

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