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# Quaternary structure modeling through chemical cross-linking mass spectrometry: Extending TX-MS Jupyter reports.

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Zurich, 30 October 2019

Dear Editor Wu,

We are pleased to submit our revised manuscript, "*Quaternary structure modeling through chemical cross-linking mass spectrometry: Extending TX-MS Jupyter reports*", for consideration by JoVE. The manuscript describes a method to enable biologists with minimal technical skills to analyze structural models and the supporting MS data in more depth compared to the standard report produce by the TX-MS method which we recently published in Nature Communications[1]. All the changes to the manuscript were tracked and each point raised by reviewers addressed in a separate rebuttal letter. We have uploaded high-resolution versions of the figures. Given the easily addressed reviewer comments, we are cautiously optimistic that the manuscript will be accepted, and we are looking forward to the next steps.

Sincerely,

Dr. Lars Malmström
University of Zurich

1. Hauri, S. *et al.* Rapid determination of quaternary protein structures in complex biological samples. *Nat Commun* **10,** 192 (2019).

**TITLE:**

# Quaternary Structure Modeling Through Chemical Cross-Linking Mass Spectrometry: Extending TX-MS Jupyter Reports

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**KEYWORDS:**

protein-protein interactions, host-pathogen interactions, chemical cross-linking mass spectrometry, protein docking, protein structure modeling, Jupyter Notebooks

**SUMMARY:**

Targeted cross-linking mass spectrometry creates quaternary protein structure models using mass spectrometry data acquired using up to three different acquisition protocols. When run as an iPortal workflow, the results are reported in a Jupyter Notebook. Here, we demonstrate how the Jupyter Notebook can be extended for a more in-depth analysis.

**ABSTRACT:**

Protein-protein interactions can be challenging to study yet provide insights into how biological systems function. Targeted cross-linking mass spectrometry (TX-MS), a method combining quaternary protein structure modeling and chemical cross-linking mass spectrometry, creates high-accuracy structure models using data obtained from complex, unfractionated samples. This removes one of the major obstacles to protein complex structure analysis, because the proteins of interest no longer need to be purified in large quantities. An automated computational workflow was developed to make the protocol more accessible to the community. The workflow creates a Jupyter Notebook, a graphical report summarizing the most important analysis results. Extending the Jupyter Notebook can yield more in-depth insights and a better understanding of the model and the mass spectrometry data supporting it. The protocol presented here demonstrates some of the most common extensions and explains what type of information can be obtained. It contains blocks to help analyze various MS acquisition data such as high-resolution

MS1 (hrMS1), data-dependent acquisition (DDA), and data-independent acquisition (DIA). The result of such analyses can be applied to structural models that are embedded in the notebook using NGLView.

**INTRODUCTION:**

Protein-protein interactions underpin the structure and function of biological systems. Having access to quaternary structures of proteins can provide insights into how two or more proteins interact to form high-order structures. Obtaining quaternary structures remains challenging: this is reflected in the comparatively small number of Protein DataBank (PDB) entries[1] containing more than one polypeptide. Protein-protein interactions can be studied with technologies such as X-ray crystallography, NMR, and cryo-EM, but obtaining a sufficient amount of purified protein under conditions where the methods can be applied can be time-consuming.

Chemical cross-linking mass spectrometry was developed to obtain experimental data on protein-protein interactions with fewer restrictions on sample preparation as mass spectrometry and can be used to acquired data on arbitrarily complex samples[2-9]. However, the combinatorial nature of the data analysis and the relatively small number of cross-linked peptides require that the samples be fractionated before analysis. To address this shortcoming, we developed TX-MS, a method that combines computational modeling with chemical cross-linking mass spectrometry[10]. TX-MS can be used on arbitrarily complex samples and is significantly more sensitive compared to previous methods[10]. It accomplishes this by scoring all data associated with a given protein-protein interaction as a set, instead of interpreting each MS spectrum independently. TX-MS also uses up to three different MS acquisition protocols: high-resolution MS1 (hrMS1), data-dependent acquisition (DDA) MS, and data-independent acquisition (DIA) MS), further providing opportunities to identify a cross-linked peptide by combining multiple observations. The TX-MS computational workflow is complex for several reasons. First, it relies on multiple MS analysis software programs[11-13] to create protein structure models[14,15]. Second, the amount of data can be large. Third, the modeling step can consume significant amounts of computer processing power. Consequently, TX-MS is best used as an automated computational iPortal workflow[16] that runs on large computational infrastructures such as computer clouds or clusters. To facilitate the interpretation of the results, we produced an interactive Jupyter Notebook[17]. Here, we demonstrate how the Jupyter Notebook report can be extended to yield a more in-depth analysis of a given result.

**PROTOCOL:**

**1. Register for an account at** https://txms.org.

1.1. Go to https://txms.org and click "**Sign Up**".

NOTE: We use the Rosetta modeling software. Because its usage is restricted to commercial users, we require registration to verify that the user belongs to an academic institution or a nonprofit organization.

1.2. Provide the requested information (i.e., name and email). Wait for the confirmation email with the user credentials.

NOTE: It can take up to 24 h to receive the user credentials because we need to manually verify that the user is from an academic institution.

**2. Run TX-MS.**

NOTE: Convert the vendor specific formats to mzML using the ProteoWizard MSConvert software[19].

2.1. Upload the MS data to https://txms.org. Click on "**Upload Data**" and pull the MS data, which must be in the mzML data format[18], to the upload box.

NOTE: Example data are available on https://txms.org. These data are also directly accessible through zenodo.org, DOI 10.5281/zenodo.3361621.

2.2. Upload two PDB files to https://txms.org. Click on "**Upload Data**" and pull the PDB files to the upload box.

NOTE: If no experimental structures exist, create models using, for example, Robetta[20,21].

2.3. Submit a new workflow and select the uploaded MS data and PDB files. Click on "**Submit Workflow**", and type in a title and a description. Click "**View Workflow**" and select the "**Cheetah**" workflow. Follow the wizard.

NOTE: Computing the result takes time, so please wait until the workflow finishes. The computation is carried out on a remote computational infrastructure. If you want to run TX-MS locally, please refer to Hauri et al.[10].

2.4. Inspect the Jupyter Notebook report using the online viewer. Click on "**View Workflow**" and scroll down to "**Report**".

**3. Install JupyterHub.**

3.1. Install docker as instructed at https://docs.docker.com/install/.

3.2. Download the JupyterHub docker container with the Jupyter openBIS extension. The general command is "**docker pull malmstroem/jove:latest**", but might differ on other platforms.

NOTE: For a general discussion on how to download containers, please refer to https://www.docker.com/get-started. It is also possible to download the container from zenodo.org, DOI 10.5281/zenodo.3361621.

NOTE: The Jupyter openBIS extension source code is available here: https://pypi.org/project/jupyter-openbis-extension/.

3.3. Start the docker container: docker run -p 8178:8000 malmstroem/jove:latest.

NOTE: The port that JupyterHub uses by default is 8000. This port is configurable, and the commands above need to be adjusted accordingly if changed. Port 8178 is an arbitrary choice and can be changed. The example URLs provided below need to be adjusted accordingly.

3.4. Go to the following address: http://127.0.0.1:8178. Log in using the username "**user**" and the password "**user**".

NOTE: The address http://127.0.0.1 implies that the docker container is running on the local computer. If the docker container is run on a server, use the server's IP address or URL (e.g., https://example.com). The docker container is based on Ubuntu Bionic 18.04, JupyterHub 0.9.6, and Jupyter openBIS extension 0.2. It is possible to install this in other operating systems, but this was not tested.

**4. Download the report.**

4.1. Create a new notebook by clicking **New**|**Python3** using the menu located near the top right part of the page. This will open a new tab with a notebook called **Untitled** (or something similar).

4.2. Click "**Configure openBIS Connections**" in the Jupyter tool menu.

4.3. Fill in the name: txms; url: https://txms.org; user: guest; password: guestpasswd.

4.4. Click "**Connect**".

4.5. Choose the new connection and click "**Choose Connection**".

4.6. Search for the report and click **Download**.

NOTE: There are many ways to search for the result. The easiest is to type in the workflow code (e.g., /CHEETAH/WF70).

4.7. Rerun the report by clicking **Cell**|**Run All**.

**5. Extend the report.**

5.1. Add a new cell at the bottom: **Cell**|**Insert Below**.

5.2. Type in the wanted code. For an example, please see the Representative Results section below.

5.3. Execute the cell by pressing "**Shift-Enter**".

**6. Upload the new report.**

6.1. Create a new dataset by clicking the **Upload** button.

6.2. Select the new report and upload it.

**REPRESENTATIVE RESULTS:**

TX-MS provides structural outputs supported by MS-derived experimental constraints. It works by combining different MS data acquisition types with computational modeling. It is useful to parse each MS data separately and provide visualization of the output structure. **Supplementary Data 1** contains an example notebook that can parse DDA and DIA data produced as the output of TX-MS. Users can select the XL of interest and, by running the notebook, the MS2 spectrum of that XL will be shown where different colors help to discriminate between fragments related to the first peptide, second peptide, and the combination. The XL can also be mapped to the structure using the NGLView widget embedded in a Jupyter Notebook.

Another cell in this notebook can help users to parse and visualize DIA data. Visualizing DIA data is more difficult, because the analyzed data need to be prepared in the correct format.

**Figure 1** shows an example structure of M1 and albumin with top XLs mapped on the structure. All XLs were obtained by TX-MS after parsing hrMS1, DDA, and DIA data, and the computational models were provided by the RosettaDock protocol.

As this report is a Jupyter Notebook, any valid Python code can be added to new notebook cells. For example, the code below will create a histogram over the MS2 counts, an indication for how well supported each cross-link is by the underlying data.

```
import seaborn as sns
sns.distplot(ms2['count']);
```

**FIGURE AND TABLE LEGENDS:**

**Figure 1: Structural model of *Streptococcus pyogenes* protein M1 and human albumin with XLs mapped on the structure.** The M protein is shown in gray and constitutes a homodimer. The six albumin molecules are presented as pairs in various shades of blue. Cross-links and distances are presented in red with black text.

**Supplementary File. Jupyter notebook data.**

**DISCUSSION:**

Modern computational workflows are often complex with multiple tools from many different vendors and complex interdependencies, high data volumes, and multifaceted results. Consequently, it is increasingly difficult to accurately document all the steps required to obtain a

result, thereby making it difficult to reproduce the given result. Here, we demonstrate a general strategy that combines the automation and ease of an automated workflow that produces a generic report, with the flexibility to customize the report in a reproducible fashion.

Three requirements need to be fulfilled for the protocol to work: First, the proteins selected for analysis need to interact in such a manner that the chemical cross-linking experiment can produce cross-linked species at a sufficiently high concentration to be detected by the mass spectrometer; different mass spectrometers have different levels of detection and are also dependent on the acquisition protocol as well as the choice of cross-linking reagent. The TX-MS protocol only allows for DSS, a lysine-lysine homobifunctional cross-linking reagent, but this limitation is largely due to the possibility that the machine learning step would need to be adjusted for other reagents. Second, the two proteins need either to have an experimentally determined structure, or need to be modeled using either comparative modeling techniques or de novo techniques. Not all proteins can be modeled, but a combination of improved software, as well as a constant deposition of experimental structures in the PDB expands the number of proteins that can be modeled. Third, the interacting proteins should remain sufficiently similar in their bound and unbound states so that the flexible backbone-docking algorithms in use by TX-MS can create quaternary structures of adequate quality to enable scoring. This requirement is relatively vague, as adequate quality is highly system-dependent, where smaller proteins of known structure are generally easier to compare than larger proteins of unknown structure.

In case of a negative result, first check that TX-MS found intra-links, that is, cross-links between residues that are part of the same polypeptide chain. If none are discovered, the most likely explanation is that something went wrong with the sample preparation or the data acquisition. If multiple distance constraints do not support the models, visually inspect the models to ensure that the conformation is supported by cross-linked residues in such a way that there is no obvious way to pivot one of the interactors without disrupting at least one cross-link. If there are cross-links longer than the permitted distance for the given cross-linking reagent, try to improve the modeling of the interactors by incorporating cross-linking data.

It is possible to use alternative software applications to accomplish equivalent results provided that the sensitivity of the chosen software is equivalent to the sensitivity of TX-MS. For example, there are online versions of RosettaDock, HADDOCK, and others. It is also possible to analyze chemical cross-linking data through xQuest/xProphet[5,6], plink[7], and SIM-XL[23].

We are continuously applying TX-MS to new projects[24] and thereby improving the reports produced by TX-MS to allow for a more detailed analysis of results without having to make the reports larger.

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**DISCLOSURES:**

The authors have nothing to disclose.

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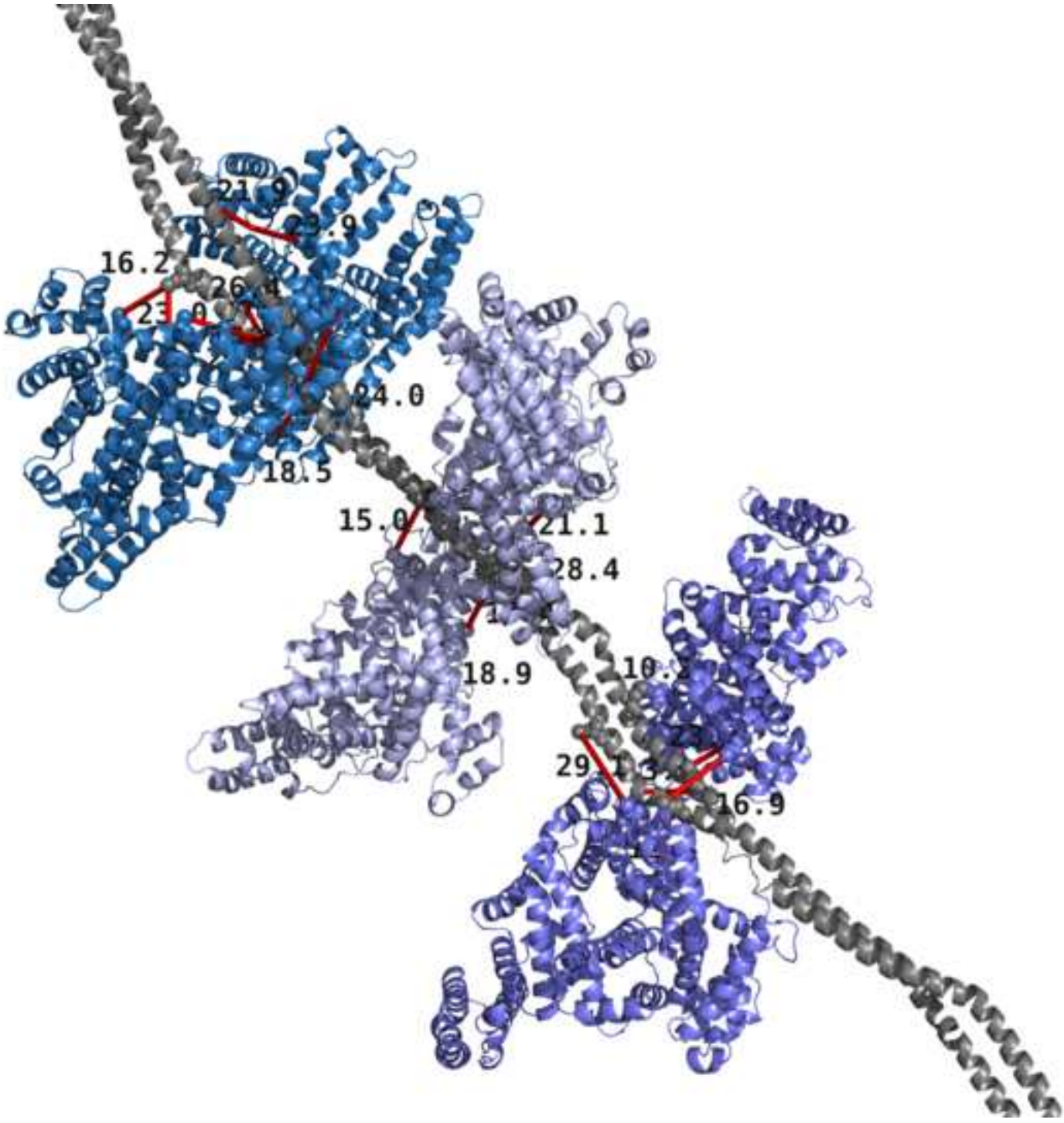
Figure

Click here to access/download;Figure;figure1.png



| Name of Material/Equipment | Company |
| --- | --- |
| Two Protein DataBank files of the proteins of interest. | N/A |
| An mzML data file acquired on a sample where the proteins of interest were crosslinked. | N/A |

| Catalog Number | Comments/Description |
|---|---|
| **N/A** | **Example files available on txms.org and zenodo.org, DOI 10.5281/zenodo.3361621** |
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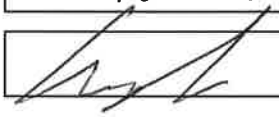
| Signature: |  |
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Rebuttal letter

We are pleased for the opportunity to submit a revised manuscript and appreciate the reviewer's and editorial staff's comments and insights. We were able to address all the concerns and hope that the reviewers and editors find the manuscript suitable for publication in the Journal of Visualized Experiments.

**Editorial comments:**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have gone over the text in detail and have removed spelling and grammatical issues.

2. Please obtain explicit copyright permission to reuse any figures (including TOC) from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

All the figures were newly created for this paper; data from previous papers were re-used, but the analysis was done specifically for this paper. We do not believe that we need to ask for permission to re-use the data as long as we cite the original paper. In addition, the previous papers were published in Nature Communication, a journal under a generous Creative Commons license stating:
"Under this license users are free to share (copy, distribute and transmit) and remix (adapt) the contribution including for commercial purposes, providing they attribute the contribution in the manner specified by the author or licensor"

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As such, we have fulfilled the license requirements by citing the paper.

3. Please do not use abbreviation in the title.

TX-MS is not an abbreviation but the name of a specific method. This name is an acronym, but the issue here is that we do specifically refer to the TX-MS method, not to "targeted cross-linking mass spectrometry" in general. We have, however, updated the title in accordance with a reviewer comment and we hope that the new title is permitted.

4. Please ensure that the references appear as the following:
Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, (YEAR).
For more than 6 authors, list only the first author then et al.

We are adhering to the requested citation format.

5. Step 4.2: Please specify the code.

The idea of the paper is that this input comes from the user, to provide the opportunity for the user to explore the data in greater detail compared to the original report.

6. Please remove the embedded figure(s) from the manuscript. All figures should be uploaded separately to your Editorial Manager account.

We have removed the embedded figures and uploaded the figures using the editorial manager account.

7. Each figure must be accompanied by a title and a description after the Representative Results of the manuscript text.

We have accompanied each figure with a title and description.

8. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

We have extended the discussion to cover the request explicitly.

9. Please revise the table of materials to include all essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

We have revised the materials.

**Reviewers' comments:**
**Reviewer #1:**
The manuscript as presented is minimal and it was not possible to fully review the work. What could be reviewed was not substantial enough to provide significant help to a non-expert and was poor for an expert. In general, the protocol only provides minimal detail and should be substantially expanded. Few of the steps are clear and the data linked from the manuscript is not easily found.

We have extended the manuscript to provide more help for users. We have also uploaded the needed input files and containers to zenodo.org and provide the DOI (10.5281/zenodo.3361621 ) in the manuscript.

Specific points are found below, these are not by any means exhaustive.
1) It feels highly disingenuous that they appear not to have cited any previous XLMS software?
E.g. xQuest (Rinner et al., Nat Methods 5(4):315-8, 2008; Walzthoeni et al., Nat Methods 9(9):901-3, 2012), pLink (Yang et al., Nat Methods 9(9):904-6, 2012), ProteinProspector (Chu et al., Mol Cell Proteomics 9:25-31, 2010; Trnka et al., 13(2):420-34, 2014), Hekate (Holding et al. J Proteome Res 12(12) 5923-593, 2013) and Kojak (Hoopmann et al., J Proteome Res 14(5):2190-198, 2015) etc.

We are now citing the papers pointed out by the reviewer.

2) On line 69 they mention that this method is "significantly more sensitive compared to previous methods" highlighting the lack of references to previous methods and without substantiating this claim.

We are referring to the TX-MS paper where we did indeed substantiate this claim to the satisfaction of the reviewers of that paper. The original submission of that paper contained a supplementary note that detailed our performance compared to established tools; however, that supplementary note was left out from the final paper as, through discussions with the Nature Communication editorial staff, we felt that such a comparison could not be carried out objectively as we know our tools better than the established tools. The supplementary note, in short, demonstrated that TX-MS was the only tool able to identify inter-crosslinks between proteins in the most complex samples, human plasma adsorbed onto live bacteria.

3) I cannot properly review step 1 - Run TX-MS as this requires me to sign up with name and organisation. As this is under peer review and to sign up would bypass the anonymous/blind peer nature of this review.

This is rather unfortunate, we sent login credentials for five reviewer accounts to JoVE to be sent to the reviewers, but something must have gone wrong. The login accounts are:

reviewer1 VaayMM1D
reviewer2 XM2CCipW
reviewer3 5rpu4TBi
reviewer4 8UHrPWzq
reviewer5 WG29f6yv

4) On line 94 it states "NOTE: There is example data available on txms.org", this should be included as a supplementary file or directly linked with a doi.

We uploaded data and containers to zenodo.org under DOI 10.5281/zenodo.3361621. The data from the TX-MS paper is also on zenodo.org and can be used as input, under DOI 10.5281/zenodo.3361621.

5) On line 96 they mention uploading two structures - this implies the method is limited to this number. The authors should clarify.

The current implementation is limited to two; we have clarified this in the text.

6) On line 101 it states "NOTE: There is example data available on txms.org" this should be included as a supplementary file or directly linked with a doi.

We uploaded data and containers to zenodo.org under DOI 10.5281/zenodo.3361621. The data from the TX-MS paper is also on zenodo.org and can be used as input, under DOI 10.5281/zenodo.3361621.

7) On line 115 it is not clear if "docker pull 116 malmstroem/jove:latest" a Windows, Mac or Linux terminal command. How is the average scientist meant to realise this? I had to run it in the "Docker Quickstart Terminal" not on the mac terminal.

We have made the instructions clearer by providing instructions for each operating system.

8) On line "Start the docker container: docker run -p 8178:8080 malmstroem/jove:latest" I have to correct 8080 to 8000 to make this command work.

We apologize for this error and have corrected it.

9) "Click on the log-in button on the openBIS Jupyter extension" Jupyter asked for a username/password to "Sign-in" that I have not been provided. (I found " guest/guestpasswd" and "user/user" on docker hub, but why I am searching for these?).

The fundamental issue is that we do require to log in due to the licensing for Rosetta. Rosetta is only free to use by academics, and hence, we need to verify that users are indeed academic. We have made changes to the manuscript clarifying this.

10) On line "Search for the report and click download" - is completely unclear how to do

this. Again I worked it out from the dockerhub page, but that wasn't particularly helpful either. At this point, I stopped as too much required information outside of the manuscript.

We have improved the manuscript to include better instructions.

11) On line 136 is states "We provide a few examples under the Representative results section" - these are not clearly signposted.

We have clarified this statement.

12) Table of Materials - "Two Protein DataBank files of the proteins of interest." - These should be either a supplementary file, linked to a copy with a DOI, or a direct link to PDB. As it currently stands it is an exercise for the reader to work out where the files are.

We uploaded data and containers to zenodo.org under DOI 10.5281/zenodo.3361621. One of these structures is a model and hence is not present in the PDB.

13) Table of Materials - "An mzML data file acquired on a sample where the proteins of interest were crosslinked/Example files available on txms.org." - should be placed with a link to the DOI file or attached directly to the manuscript, I could not find them on the website.

We uploaded data and containers to zenodo.org under DOI 10.5281/zenodo.3361621.

**Reviewer #2:**

Manuscript Summary:

Studying Protein- Protein Interactions (PPIs) has become very important for understanding biological phenomenon, esp. the origin of diseases. TX-MS, discussed in this manuscript/ video, is a new method for studying PPIs using Jupyter notebook. It combines Chemical Crossslinking (CXL)-Mass spectrometry (MS) with docking methods - bioinformatics tools (usually developed according to each laboratory's special requirements and writing the required Algorithms). Is this method dependant on Linux systems alone? Would that constitute a limitation? Adding docking data and analyzing these is an added step. We have also done such attempts. Will these necessarily add more quality to the results? Refining cryo EM results using CXL data is still time consuming even if one is using sophisticated software like HADDOCK (High Ambiguity Driven biomolecular DOCKing). TX-MS approach uses "Various MS acquisition data such as hrMS1, DDA, and DIA using the notebook using the NGL view".

We appreciate the reviewer's comments. This method is not dependent on Linux per se, but installation on other operating systems is challenging. Docker is solving this problem, providing a way to run the service anywhere. In this particular case, we carry out all the calculations as a service, allowing anyone with a browser to run TX-MS.

The example given describes only use of a Homobifunctionl Crosslinker, that too, only a di NHS crosslinker, which target mainly Lysine r residues and are less useful for studying intermolecular interactions. While it is true that Lysine residues are in abundance in proteins, yet PPI's are not restricted to studying Lysines alone. In the manuscript there is no mention of Heterobifunctional crosslinkers. Reference to A Sinz's work is completely missing-Pl do cross check this statement of mine. No reference is made to StavroX or MeroX (the latter being more suited for MS/MS cleavable crosslinkers), software developed in her laboratory, which help in identifying Intermolecular cross-linking better. For this StavroX/ MeroX software come in handy. Identifying the two crosslinked peptides/ proteins fragments is very difficult and usually only one of these is actually identified. In our recent paper in 2019, we have successfully identified the crosslinked fragments involving two proteins. Does TX-MS have the capability of doing so. For Chemical Crosslinking-Mass Spectrometry- Bioinformatics to emerge as a rapid, routine and reliable technique in hospital situations with large number of patient samples still remains a distant dream indeed.

The current implementation is only for a small subset of cross-linkers. The reason for this is that we have trained the machine learning step on this type of data; it is possible that the model also works for other cross-linkers, but we would like to verify this before making any claims. All software components other than the machine learning model are capable of handling other cross-links, as long as they are not cleavable.

Major Concerns:
Described is only the use of a homo bifunctional crosslinker

We have clarified this in the manuscript.

Minor Concerns:
Is it restricted to Linux systems?

It is not restricted to Linux, but as non-Linux installations are challenging, we solve this issue using docker.

**Reviewer #3:**
Manuscript Summary:
This manuscript introduces the protocol for TX-MS (targeted cross-linking mass spectrometry), a method capable of determining large complex structures, using a combination of high-resolution mass spectrometry, chemical cross-linking, and high-accuracy structure modeling. This method provides an alternative path to solving structures of protein-protein interactions, without requiring time-consuming structural biology experiments such as X-ray crystallography, NMR, and cryo-EM. The protocol introduced here demonstrates the procedures of running TX-MS and interpreting the results, using the

Jupyter Notebook platform.
This manuscript presents its potential importance; however, it lacks sufficient explanations and details for the users to repeat the procedures. In general, the current manuscript might not be qualified for publication in JoVE. After going through the manuscript, I have the following questions that preclude this work from publication in the current form.

We thank the reviewer for the comments and present an improved manuscript which we hope fulfills the requirements for publication in JoVE.

Major Concerns:
1. The title chosen by the authors is neither clear nor informative. The authors should use more general terms for the audiences to comprehend the key purpose of this work from the title. In the same vein, there are plenty of abbreviations running through the article without definition. It would be much more formal and apt to provide the full name of a term before using its abbreviation in the first instance; for example, TX-MS, XL, etc.

We have corrected this. TX-MS is the name of a method in this case.

2. Several sentences used in the abstract and introduction are vague. For instance, in the sentence "removing one of the major bottlenecks," the authors do not clearly explain what these bottlenecks are. In another sentence, "A graphical report summarizing the most important results," what do the authors mean by "the most important results?" More specific explanations would be appreciated.

We have clarified these vague statements.

3. In the section of PROTOCOL, the authors might over-simplify the steps for the first-time user to run TX-MS. First, there is no 'upload data' option on the page so I tried to click 'sign up' option followed by filling up the information to start with the tool. Unfortunately, the server directed to a strange page showing some texts (might be php codes), so I couldn't continue to review this work, even though I tried different web browsers (Chrome and Internet Explore).

We apologize for the inconvenience and have fixed the registration page. We also provided JoVE with login credentials to be used by the reviewers, but something must have gone wrong, and these were not communicated. The login credentials are:

reviewer1 VaayMM1D
reviewer2 XM2CCipW
reviewer3 5rpu4TBi
reviewer4 8UHrPWzq
reviewer5 WG29f6yv

4. If the authors can provide clear information about the version of the tools, or even the operating system used in this demonstration, that would be very useful. After all, there are many cases where the users cannot repeat the experiment due to conflicting versions of the tools or operating system. Also, is there any requirement for the Jupyter Notebook version to be installed before installing the JupyterHub? The authors should clarify all this.

We have clarified the versions of the tools and operating systems.

5. That would be helpful if the authors can note the way how users can run the Jupyter Notebooks on a public IP server, rather then a private computer using http://127.0.0.1 as the default address.

We have clarified this statement.

Minor Concerns:
Several grammatical errors need to be corrected.

We have corrected spelling and grammatical issues.

# Rebuttal letter

Editorial comments

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have gone over the manuscript in detail and removed spelling and grammatical issues.

2. Lines 191-231: Please write these as numbered steps.

We added numbered steps to the requested lines.

Reviewer 4

The protocol is already improved compare to the initial submission but in the line 302 the user could mention SIM-XL search engine (http://dx.doi.org/10.1016/j.jprot.2015.01.013), since this the only XL tool that produces a protein-protein interaction map as result, and can help in this protocol to validate the results.

We have added the requested reference.

Reviewer 5

As fairly pointed out by the authors, the major concern of the work is on the limitation to DSS (K-K) cross-linker only. This will restrict greatly the use of the tool presented here by the community as more and more new cross-links are developed and used. This work is another docking tool integrating cross-links data as several other exist and I am not convinced it will be largely used by the community because of its specificity.

We appreciate the reviewer's comments; adding support for other cross-linking reagents is technically possible but early work has indicated that we need to retrain the machine learning model used in the hrMS1 step. We will add support for all cross-linkers that we have sufficient amounts of data to train and validate the models.

There is quite a number of spelling error in the manuscript that need to be corrected. For example :

line 69 "and" should be removed

Removed.

line 294 "cross=linked" instead of "cross-linked",

Corrected.

line 300 "sessility" instead of "sensibility"

Corrected.

Jupyter notebook

Click here to access/download

**Supplemental Coding Files**

Supplementary Data 1.ipynb