Dear Editor,

We thank you and the reviewers for your suggestions to improve our manuscript. We have revised our manuscript accordingly and have summarised our changes below.

**Editorial comments:**

Changes to be made by the Author(s) regarding the written manuscript:

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

*O We have read the article carefully.*

2. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

*O We have revised this section. It currently has 50 words.*

3. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

*O We have used SI abbreviations.*

4. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

*O We have ensured that there is a space between all numbers and their corresponding units.*

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Rigaku, S-MAX3000, MicroMax, Mac OS, YouTubeTM, etc.

*O We have ensured that no commercial trademark symbols are present in the manuscript.*

6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. Please move the discussion about the protocol to the Discussion.

*O We have revised this section and Discussion accordingly.*

7. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

*O We have revised this section to include the changes recommended.*

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

*O We have revised this section to include the changes recommended.*

9. Please include single-line spaces between all paragraphs, headings, steps, etc.

*O We have implemented this suggestion throughout the manuscript.*

10. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

*O The Protocol section is highlighted by the bold-font title –* ***PROTOCOL*** *in grey background*.

11. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

*O We have implemented this suggestion In Protocol section.*

12. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

13. Please number the figures in the sequence in which you refer to them in the manuscript text.

*O Figures are numbered according to their order of description in the text.*

14. As we are a methods journal, 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  
15. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

*O We have implemented these suggestions in a revised Discussion section.*

**=====================================================================**

**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
In this manuscript the authors provide a protocol for using SAXS (small angle X-ray scattering) to determine hybrid structures of macromolecular assemblies. The authors provide a recent example of the complex of full length nidogen-1 and laminin which assembles into an extended curved shape. This manuscript is well written and provides a very nice introduction to SAXS including an overview of its benefits and limitations. This manuscript is acceptable for publication, however I have concerns about the current format of the protocol because it contains numerous lengthy paragraphs that should be broken down into smaller segments.  
  
Major Concerns:  
The protocol is written in paragraph format, which differs from the typical JoVE protocol format. The authors provide very detailed descriptions of each step in the SAXS data processing pathway but they combine everything into one paragraph and don't break things down into discrete steps. For example Section 3.4, should be broken down into individual steps as follows:  
3.4.1 Load the GNOM module ….  
3.4.2 Generate the p(R)plot …  
3.4.3 Quality control check of the data …  
3.4.4 Adjust the Dmax …

*O We have addressed reviewers’ suggestions in a revised Protocol section.*

Minor Concerns:  
1. SAXS Sample Prep: This section is not really part of the protocol but a comment since no specific details are included. The authors should either include specific details or make this a simple note about sample prep and not part of the protocol.

*O We have included the importance of sample preparation in* ***SAXS Sample Preparation and Data Acquisition*** *section.*

2. Data Acquisition: This section is also more of a comment and not part of the protocol. The authors should either include the details for specific data collection of the Lamin complex or format this as a comment/note and not the protocol.

*O We have revised* ***Protocol*** *section to implement reviewers’ suggestion.*  
  
**Reviewer #2:**  
Manuscript Summary:  
The JOVE manuscript from Mrozowich et al. "Structural Studies of Macromolecular Interactions in Solution using Small Angle X-Ray Scattering" seeks to provide a detailed protocol for scientists using SAXS as a tool in their research. The manuscript is well timed and with some additional work should achieve this aim.  
  
However, a major concern is that the detailed description of the ATSAS software is very much out of date. The authors describe in detail how to use the original windows version of PRIMUS, which is no longer in common use (at the very least this is what the package maintainers say during their many workshops and recent publications). I feel that if the authors were to describe their protocol using the latest cross-platform version (primusQt for example) it would be a more useful and appropriate contribution. An alternative is to provide the analysis steps using another modern program that has been published and available such as bioXtas RAW (Hopkins, J.B., Gillilan, R.E. and Skou, S., 2017. BioXTAS RAW: improvements to a free open‐source program for small‐angle X‐ray scattering data reduction and analysis. Journal of applied crystallography, 50(5), pp.1545-1553.; Nielsen, S.S., Toft, K.N., Snakenborg, D., Jeppesen, M.G., Jacobsen, J.K., Vestergaard, B., Kutter, J.P. and Arleth, L., 2009. BioXTAS RAW, a software program for high-throughput automated small-angle X-ray scattering data reduction and preliminary analysis. Journal of applied crystallography, 42(5), pp.959-964). The authors indicate that another program called Scatter can be used, however, there appears to be no publication in a peer reviewd journal of this software, so perhaps it is better to focus on ATSAS and RAW.

*O We have revised the* ***Protocol*** *section for PrimusQt version. Due to the constraints with space (~2.5 pages for* ***Protocol*** *section), we were unable to include the detailed description of other data analysis packages suggested by the reviewers. However, we recommend that subsequent protocol/methods papers should be published in the future describing the packages suggested by the reviewer.*

In addition, several

suggestions to improve the reader experience are:  
  
1. Make it clear that the scattering phenomenon under discussion is elastic.

*O We have made this clear in the Discussion section.*

*2. Provide a better explanation of the determination of concentration dependence (not just suggest "the Rg of each data set should be compared to avoid interparticle interaction").*

*O We have briefly described this in the* ***Principles, Benefits, and Limitations of SAXS*** *section.*

3. In the SAXS Sample Preparation section: explain why particle size is an important parameter in the range of concentrations one should consider.

*O We have included this in* ***Principles, Benefits, and Limitations of SAXS*** *section.*

4. Ensure that a figure is made available of the Guinier analysis and also the Kratky plot, showing graphically what interparticle interaction and "foldedness" look like.  
  
*O We have revised Figure 2 to include Guinier and Kratky plots.*

And finally, make sure that the aims of the manuscript as defined in the abstract are clearly met:  
  
1. protocol is provided for elucidating the "specific domains" that mediate interactions. I don't think this is clear, a protocol for basic analysis is provided but I do not it currently addresses this specific aim sufficiently.

*O We have revised sections of the manuscript to address this change.*

2. method is provided for calculating hybrid solution structures... I do not think it is currently clear to the reader that what is described provides a method for doing this. A little work is required to address this.

*O We have revised sections of the manuscript to highlight that SAXS can be used to study macromolecules and their complexes.*

3. "allows large numbers of samples to be analysezed..." This I don't think is addressed at all.

*O We have revised this sentence in Abstract.*  
  
**Reviewer #3:**  
Manuscript Summary:  
This manuscript reviews the method of SAXS to study macromolecules in solution. When combined with other high resolution structural methods, SAXS analysis provides important insights into the mechanisms of macromolecular interactions. The manuscript has reviewed the basic and essential methods, such as Guinier, P(r) analyses, as well as the ab initio methods to restore molecular shapes using DAMMIN and Monsa. The manuscript is certainly worthy to be published.  
  
Major Concerns:  
The authors might also discuss some of the recent progress in ensemble analysis of SAXS data, which is quite useful when combining with NMR methods.

*O We have included a paragraph in the Discussion section for this. We believe that the combination of SAXS and NMR for ensemble analysis merit a separate publication to provide an in-depth description of benefits of these two powerful tools.*

Minor Concerns:  
Some of the sentences in the text are long, and awkward to read.

*O We have revised sentences to ensure that they are not too long and awkward to read.*