**Responses to the comments**

We thank the comments from the editor and the reviewers. Those comments really helped us improve the manuscript. We made changes in the revised manuscript based on those comments. Since many changes have been made based on the comments, we did not highlight the changes in the revised manuscript. We provided responses to the comments one by one.

**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.

**Reply: We have thoroughly reviewed the manuscript for these issues.**

2. Please label/number the institutional affiliation of each author sequentially.

**Reply: Institutional affiliation labels have been added to the manuscript.**

3. Please expand the Short Abstract to briefly describe the applications of this protocol.

**Reply: we made the change based on the comment.**

4. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

**Reply: We made the change in the revised manuscript.**

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

**Reply: we made the changes.**

6. 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: Kimwipe, Eppendorf, Nalgene Rapid-Flow, Branson Sonifier, VWR Scientific, Agilent, etc.

**Reply: All language in the protocol text has been changed to avoid using commercial language.**

7. Please revise the protocol (e.g., 1.1.2, 1.2.3, 3.1.1-3.2.4, etc.) 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.

**Reply: Tense has been corrected in the protocol and certain phrases have been avoided when possible. Safety has been added to appropriate steps.**

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.

**Reply: Individual steps have been shortened to contain less than or equal to 4 sentences.**

9. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:

1.1.3: How is methanol (500 μL) injected, using a syringe pump?

2.2.4: What is used to cut?

4.3.2.1-4.3.2.3, 5.2.3: Please describe how these are done. Software must have a GUI (graphical user interface) and software steps must be more explicitly explained ('click', 'select', etc.).

**Reply: Changes have been made in the revised manuscript based on the comments. Details have been added where appropriate while trying to keep language as general as possible for other instrument users.**

10-12. 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. 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. 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.

**Reply: we highlighted the essential steps of the protocol in the revised manuscript based on the comment.**

13. Please revise to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. However for figures showing the experimental set-up, please reference them in the Protocol.

**Reply: The representative results have been revised and should have a better explanation of the technique for the readers.**

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

**Reply: we made the changes based on the comment.**

**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

This is a well-written, well-referenced and timely manuscript describing the use of CZE-MS for protoeoform analysis. Proteoform analysis is an emerging area of proteomics and the use of CZE for proteoform identification is under-developed. This article should have a strong impact on the field. The descriptions contain sufficient detail such that others should easily be able to easily reproduce the results.

Major Concerns:

None.

Minor Concerns:

-The introduction is especially well-written. The protocol contains several minor grammatical errors that should be corrected by the copy editors.

**Reply: Thank you for the nice comment. We went through the manuscript carefully and fixed some grammatical errors.**

-The quality of the figures in the review copy were low. These figures should be substituted with high-quality/high-resolution graphs prior to final publication.

**Reply: We provided high-resolution figures in the revision.**

-It would have been nice for the authors to show the improvement that injecting a larger amount of sample allows (e.g. by reporting the number of protoeoforms observed in the same set-up when injecting 1/10th the amount). However, I don't view this as a necessary experiment prior to acceptance. Simply would have better validated the claims made. This protocol will be highly valuable and is the major contribution.

**Reply: In our paper “Single-Shot Top-Down Proteomics with Capillary Zone Electrophoresis-Electrospray Ionization-Tandem Mass Spectrometry for Identification of Nearly 600 Escherichia coli Proteoforms. Analytical Chemistry. 89 (22), 12059-12067 (2017)”, we showed the difference in proteoform identifications from 100 nL injection volume to 1 µL injection volume.**

**Reviewer #2:**

The manuscript: Large-scale Top-down Proteomics Using Capillary Zone Electrophoresis Tandem Mass Spectrometry from Liangliang et al present an extended description of the approached used to perform top down analysis of intact proteins using CE-MS with neutral coated capillary, taken from previously published on Analytical Chemistry 89 (22), 12059-12067 (2017) and Analytical Chemistry. 90 (9), 5529-5533 (2018).

I found the text part of the material well written and organized and I look forward to see the video part of the contribution, being particularly interested on the fabrication of the sheath flow interface of the described setup.

One minor note:

As the article may interest also people approaching this field I suggest to include references on the top down proteomics initiative as from this resource are accessible settings and method that would extend the compatibility of the method to other MS platforms:

http://www.topdownproteomics.org/resources/methods/

And also include a repository concerning tools for TD data analysis

<http://www.topdownproteomics.org/resources/software/>

**Reply: Thank you for the nice comments. We included those two references in the revised manuscript.**

**Reviewer #3:**

Manuscript Summary:

The authors have submitted a JOVE article to describe their CE-MS setup for intact protein analysis. The topic is of interest to a general audience. The items below are provided to improve to the readability of the article. At times, the language gets very lax and is not technical.

Major Concerns:

-line 93: How is CE more sensitive than HPLC? Sensitivity refers to the ability to detect changes of a signal. Therefore, CE is not generally considered a detector.

**Reply: As we described in the manuscript, “CZE-MS has drastically higher sensitivity than widely used reversed-phase liquid chromatography (RPLC)-MS for analysis of intact proteins.” We did not say “CE is more sensitive than HPLC”. When a same amount of protein material is loaded into the CZE capillary and the RPLC column, CZE can deliver much narrower protein peaks to ESI-MS for detection with much lower sample loss than RPLC. The narrower peaks and lower sample loss from CZE are due to the lack of stationary phase with a large surface area. Therefore, CZE-MS is more sensitive than HPLC-MS for protein detection.**

-line 155: function should be functionalized?

**Reply: we made the change in the revised manuscript.**

-line 176: A more detailed safety description should be provided for using HF including the antedote

**Reply: Thank you for the great comment. We added the detailed safety description in the revised manuscript.**

-line 228: Settings are missing for this sonication. Is this a bath or a probe sonicator? Needs details.

**Reply: We made the change based on the comment.**

-line 252: what units? wt/v or v/v?

**Reply: We made the change.**

-line 255: ".... the goal is to approach the disulfide bonds..." I have no idea what this means

Reply:

**Reply: we made the change.**

-line 305: Fix is a generic term for repair. Do you mean to mount?

**Reply: Thank you for the comment. We made the change.**

-line 383: I would refrain from "obviously" it is negative voice. Such an article as this is a teaching tool.

**Reply: we made the change.**

-Figure 1: please refer as a diagram as opposed to a sketch

**Reply: We made the change.**

-Figure 3: Why is the cop of the electropherogram cut off? It should be scaled.

**Reply: There are very high abundant peaks in the electropherogram. If we scaled to 100%, the low abundant peaks cannot be seen clearly. Therefore, we used a zoomed-in electropherogram to show the low abundant peaks better.**