THE UNIVERSITY of NORTH CAROLINA at CHARLOTTE
THE CENTER FOR BIOMEDICAL ENGINEERING AND SCIENCE

Center for Biomedical Engineering & Science
Department of Physics and Optical Science
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Charlotte, NC 28223

October 15, 2020

Dr. Nam Nguyen, Manager of Review
Journal of Visualized Experiments

Dear Dr. Nguyen:

We thank you and the reviewers for the critical reading and comments. With the revisions below, I hope the manuscript is ready for publication.

Response to Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

We checked for typos.

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

We used Summary as the heading, and followed your instructions.

3. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

We corrected the locations of reference numbers in text.

4. 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: NuPAGE, Azure Biosystems C400,

We removed the product names from the text, and modified the Materials Table accordingly.

5. 3.1: as the disulfide bonds are still intact, do you use any specific method to list expected fragments by taking into account 3D conformation?

We added a description: “Examine the amino acid sequence of BSA and…”

6. Lines 201-203: as smearing of the gel bands was observed even with your protocol, please comment (as you have done in lines 213-214) what might have happened and how to resolve this smearing.

We modified the description: “The gel band smearing was exacerbated with extended digestion times, in the presence of Au(III). The smearing was minimized when the protocol described above was used.”

7. Please obtain explicit copyright permission to reuse any figures 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].” [citation] could be just Dixon et al.#.

We attached the copyright permission from ACS. The figure legend was corrected.

8. Representative Results: lines 273-275: did you try an intermediate duration, e.g. 1 hour and lines 283-284: did you try adjusting to higher pH just before gel electrophoresis?

We believe the data presented are sufficient to describe the optimized conditions.

9. As we are a methods journal, please add limitations of the technique to the Discussion.

Thank you for this comment. We added the limitations of this technique: “For gel-based proteomics, a further improvement in the smearing may be necessary.”

10. 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. Please do not abbreviate journal names.

We corrected and used the specified reference style.

11. Figure 1 looks like a table. Please rename it as Table 1 and refer to it that way.

We renamed and referred to it as Table 1.

12. Please sort the Materials Table alphabetically by the name of the material.

We corrected the Materials Table.

Reviewer #1:

In this video protocol, Egusa et al. reported the method to identify the Au(III) binding domain in

serum albumins. Based on their recent papers (JPC Lett. 2020 etc.), they describe the detailed protocol on how to determine the red luminophore-forming domain in serum albumin-gold complexes using proteolysis. This red luminophore is the origin of metal clusters, although the mechanisms of the luminescence of the cluster are not fully understood. Therefore, this study is one of the important studies to clarify the origin of cluster formation as well as the basics of metal binding to protein. However, the current introduction is relatively narrow and specialized, and the protocol is of interest to the limited scientists. This reviewer recommends revising the introduction to strengthen the importance of the study, such as metal binding to proteins and cluster formation.

We thank the reviewer for the careful reading and positive evaluation. Following the reviewer's suggestion, we added in introduction to strengthen the rationale of this protocol: "Identifying the location of Au(III) binding and the luminophore formation in BSA is an important step."

Reviewer #2:

Manuscript Summary:

The manuscript describes an experimental protocol suitable for the controlled digestions of of proteins while preserving disulfide bonds as well as a protocol to minimize band smearing during gel electrophoresis in the presence of protein bound metal ions. These protocols facilitate the identification of the metal binding domains of the protein, which can be the interest in the case of many metal ion containing enzymes. The methods are demonstrated on the BSA/Au(III) system, which have a peculiar red fluorescence. The protocols are described clearly in details, which allows the readers to easily reproduce the presented methods. I recommend the publication of the manuscript as it.

Major Concerns:

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Minor Concerns:

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We thank the reviewer for the positive comments.

The annotated version is attached for your reference. We look forward to hearing from you soon.

Sincerely,

Shunji Egusa, Ph.D.
Assistant Professor