**Point-by-Point Rresponse to editorial and reviewers comments**

on manuscript entitled " The isolation of F1-ATPase from parasitic protist *Trypanosoma brucei*".

JoVE58334

We would like to take this opportunity to thank both reviewers for taking time out of their busy schedules to provide us with fair criticism that we believe has helped us to improve the manuscript.

**Editorial comments:**

Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

2. Table 1: Please provide the unit for protein concentration in the table.

*The unit (mg/mL) was stated in the originally submitted table.*

3. Figure 1: Please fix the typo “chlorform”, which should be “chloroform”.

*Corrected.*

4. Please provide an email address for each author.

*The missing email address was added to the Authors and Affiliations section.*

5. Please define all abbreviations before use.

*Several abbreviations were defined.*

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

*The abbreviations were changed. However, it is now in discrepancy with the requirements specified in the Instructions for Authors provided by JoVE (see the Technical Language section).*

7. 1.1.1-1.1.6: Please write the text in the imperative tense in complete sentences.

*Changed according to the requirements.*

8. 2.2: Please add more details to this step. This step does not have enough detail to replicate as currently written. Alternatively, add references to published material specifying how to perform the protocol action.

*Details and a relevant reference were added.*

9. 4.1/5.1: What volume of buffer is added and how long does it take to equilibrate?

*The volume of buffer and the recommended flow rate were added.*

10. 4.2: How is the flow rate controlled?

*The flow rate is one of the parameters that are set on the liquid chromatography instrument (e.g. AKTA Pure or similar).*

11. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.

*The reference list was generated using EndNote and the style file provided by JoVE and manually corrected (e.g. the scientific names were italicized). The abbreviated journal titles are dictated by the reference style and are also used in recent JoVE publications as well as in the example references in the Instruction for Authors (the manuscript template file). Shall we use the full journal titles in spite of these facts?*

*The volume and issue number are absent only from the reference #1 that is a book chapter, which does not have these specifications.*

**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
Gahura et al., describe a standard protocol for the purification of the F1 portion of the membrane-bound F1Fo ATPase which they have adapted for use with the human parasite Trypanosoma brucei. The authors clearly describe in the introduction the history behind the development of the purification protocol and what the final product can be used for. The protocol itself represents a standard protocol which is clear to follow but may not be in the correct style for a joVE article. The representative results are also clear apart from the sentence on line 202 and figure 2 (see below). The discussion is also adequate the pros and cons of the method. Thus in conclusion, the article represents a nice description of the protocol required to purify the F1 portion of F1Fo ATPases from T. brucei and illustrates how the protocol can be adapted for other organisms. When completed, this video will complement several other jove videos e.g. the purification of mitochondria and preparation of F1Fo ATPase vesicles for patchclamping.  
  
Major Concerns:  
None  
  
Minor Concerns:  
1) Line 202 states "the organic phase and precipitated interphase are discarded". It would be important to show an image or video segment indicating what is meant by these two terms.

*The Protocol describes this step in more detail (see 3.3), but we agree that it would be useful to visualize this step.*

2) In figure 2 where the dark green line in fig 2A indicates the F1 containing fraction but in figure 2B it is the light green colour. I think the color for the F1 containing fractions should be the same colour or all the shades of green should be different between the two images. Also a picture / diagram / or label should be included in the figure so that it is easy from the figure to determine which graph/gel image comes from which column.

*The misleading color-coding in the figure was changed and the graphs were labeled for easier orientation.*  
  
  
**Reviewer #2:**  
Manuscript Summary:  
Authors provided comprehensive and well-written protocol. The F1-ATPase purification from unicellular parasite Trypanosoma brucei is explained clearly, results are presented and discussed well. All steps in the protocol are detailed, clear and simple to follow. Included references are relevant and provide essential reading list for researchers investigating FoF1-ATP synthase complexes. Overall, the methodology is easily transferable and can be used for purification of complexes from other organisms. Publication of the protocol in JoVE would be very helpful to researchers in the field.  
  
Major Concerns:  
Long abstract - line numbers 40 and 41: Can authors elaborate on long-term storage of ammonium sulphate precipitate? Is precipitated F1-ATPase stable for weeks or months?

*We added details on the storage of ammonium sulfate precipitate in the last paragraph of Representative Results.*

Step 2.2. - Assays for protein concentration determination have limitations in sensitivity, compatibility with chemicals, etc. Can authors suggest an alternative to BCA protein assay or explain why they have used it in the protocol (steps 2.2. and 5.3.)?

*The solutions used in the protocol are devoid of any chemicals (e.g. reducing agents etc.).that interfere with any of the common protein assays. We routinely use the BCA assay, and therefore, we suggest it in the protocol, as each assay can give slightly different results. The data presented in the Table 1 were obtained with the BCA. However, principally, any assay can be used. We reflected this fact in the second paragraph of Representative Results.*

Step 2.3. - Disruption of particles using sonication is an arbitrary method completely dependent on a homogenizer brand and thickness of used probe. Can authors provide more details regarding the type or diameter of the probe in step 2.3. or in the Equipment section? Thin probe is not sufficient information for replicating this step.

*We agree that this information is essential. We added details on the probe in the step 2.3 and to the List of Material.*

Step 5.3. and Representative results line numbers 215-217- Concentration of F1-ATPase inhibitor azide used in the experiment is missing. Can authors provide it or insert a suitable reference?

*The concentration of azide was added.*  
  
Minor Concerns:  
Overall, I recommend thorough proofreading of the manuscript. In contains typos - for example the term "proton motif force" needs to be corrected.  
I recommend changing the title to - "The isolation of F1-ATPase from unicellular parasite Trypanosoma brucei " - it would increase the visibility of the protocol for researches working with different protists.

*We changed the title to "The isolation of F1-ATPase from parasitic protist Trypanosoma brucei”*

Steps and buffers 1.1.1, 1.1.2, and 3.3. - Can authors provide a recipe for the cocktail of protease inhibitors or clarify the final concentrations used in the protocol? There is a discrepancy in concentrations of amastatin and bestatin between 1.1.2 buffer B and step 3.3 - is there any reason for increase concentration of these two inhibitors from 10μM to 50μM?

*The discrepancy was corrected. In fact, in initial experiments we used 50 μM amastatin, but later we switched to sufficient 10 μM.*

Discussion - line numbers 278-281 -Clarification is needed for: …"The F1-ATPase tends to adhere to the membrane of the spin column." … Is this claim based solely on the protein concentration measurements (Table 1) or can authors provide a reference for this sentence?

*We slightly rephrased the statement. It is is based solely on our empirical experience with protein concentration measurement. The losses on the spin column were observed in each purification, but the extend was very variable. Reuse of the spin columns helps to decrease the losses, however, we do not want to recommend it in the protocol, as we are not sure how and how long the reused column can be safely stored.*

Fig. 2 - Bands on Coomassie gels are labelled either by nomenclature of subunits or molecular weight of a protein marker. Can authors unify this or add or molecular weight labelling to panel C?

*The MW labels were added to the panel C.*