**Dear Editor,**

**Many thanks for giving us the opportunity to revise our manuscript. We have now edited it taking editorial and reviewer comments into account. A point-by-point rebuttal follows. Please contact us if you have any further questions.**

**Yours faithfully,**

**Matthew Bowler** **and Stephanie Hutin  
  
Editorial Comments:**  
  
• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Done  
  
• **Protocol Language:** Please ensure that ALL text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.  
1) Examples NOT in imperative voice: parts of 1.1.1 , 1.1.7,

Section 1 has now been moved into supplementary data given reviewer comments and was changed accordingly.  
  
• **Protocol Detail:** 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) 1.1.1: It is unclear what exactly is done here because this appears to be a summary of several steps.

2) 1.1.4: Mention sonication frequency (Hz) and amplitude (W).  
3) 1.1.5: Mention column specifications.  
4) 1.1.6, 1.1.9: how is concentration performed? Mention filter cut off, centrifuge speeds (in g) and duration.  
5) 1.1.7: Mention dialysis membrane specifications (dimensions, MWCO,material)  
6) 1.1.8, 1.1.9: It is unclear what exactly is done here because this appears to be a summary of several steps.

Section 1 has now been moved into supplementary data given reviewer comments and was adjusted to the comments.

• **Protocol Numbering:** There must be a one-line space between each protocol step.

Done  
  
• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

1. The highlighting must include all relevant details that are required to perform the step. 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 included in the highlighting.  
   2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.  
   3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.  
   4) Notes cannot be filmed and should be excluded from highlighting.  
   5) Please bear in mind that software steps without a graphical user interface/calculations/ command line scripting (e.g. section 6) cannot be filmed.

Please see manuscript

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We think we cover this. Please see manuscript

• **Figures/Tables:**

1. Fig 1: Please add scale bars to all micrographs. Please make the panels uniform in dimensions. The right panel is much smaller than the left. Please increase the font size on the right panel to match the size of the font on the left panel.  
   2) Please upload each table as an individual Excel file.  
   3) Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

Please see the figures

• **Commercial Language:**JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are DECTRIS PILATUS3 2M, SPINE, etc.

1. Please use MS Word’s find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names.

Dectris Pilatus has been removed – SPINE is not commercial but describes a European standard mount so has been left.

• **Table of Materials:**Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as sonicator, columns, software,instruments, etc.  
  
• Please define all abbreviations at first use.

Done  
  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.  
  
• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**Comments from Peer-Reviewers:**   
  
  
**Reviewer #1:**  
Manuscript Summary:  
The manuscript describes how to prepare for executing an experiment in the MASSIF beam line at the ESRf, a highly successful resource for x-ray crystallography.  
  
Major Concerns:  
I find steps 1 and 6 in the protocol out of scope. The first is a purification protocol for a single protein, and defocuses from the real issues, what to do once you do have crystals. The last step is a tutorial in using ccp4i, which again does not add much in the context of this paper.

We have now moved these sections into the supplementary data

In contrast, there is a missed opportunity on seriously expanding on 5.1.2: at that step several results are available, and novice users dont know what to download! The top-scoring is (sometimes) not the best choice. Depending on the processing program used the "mtz" file that the user mostly needs has a different name! I would actually expand on this section seriously and comment:  
0. Why are there several choices for auto-processing?  
1. What are the criteria for setting something as top score?  
2. When should you think further than top-score (eg when a different space group is chosen by each processing program)  
3. When do you want the anomalous-processed dataset and when not?  
4. What are the content of each tarsal downloaded dependent on the processing program  
5. Are there general recommendations for which auto-processing result to use/

We changed the entire section

The list for materials needs to be modified accordingly.  
  
Minor Concerns:  
The details of the paper are excellent, I have no comments.  
  
  
**Reviewer #2:**  
Minor Concerns:  
A small point, but the special case of molecular replacement where the search model and the target crystal share the same unit cell and space group is often referred to a "Molecular Substitution" to prevent confusion with the more general method of Molecular Replacement that has no such limitations. I would not want to insist on this as a change, though, as referring to this case as Molecular Replacement is quite common. The description of how to carry out a molecular replacement manually are correct and helpful.  
  
  
**Reviewer #3:**  
Manuscript Summary:  
This article describes automatic data collection at a MX beamline, ID30A-1 in ESRF. It covers all the protocols from obtaining a beam time to determining a crystal structure.  This is quite informative to structural biologists and other researchers, and illustration  by movies are convenient for synchrotron beginners.  
  
Major Concerns:  
Therefore, most parts of it are reasonable, however, we felt redundant in the following protocols:  
1. Protocol 1. The purification and crystallization of GCSH.  This part might be required as "materials and methods" in a general articles (not JoVE).  However, it is a sample-dependent description. We felt it should be separately described as a supplemental.  
2. Protocol 6. The protocol for molecular replacement. This part is conducted manually. We felt it should be separately described.  
We felt both are not directly included in the pipeline. Instead of them, by adding a flowchart of the pipeline we can imagine its overview easily.

We have now moved these sections into the supplementary data

Minor Concerns:  
Moreover, please confirm the following a minor point:  
3. Line 277 search model must be in the same space group as the tested crystals.  
Why it should have same space group?

This speeds up the calculations and was required for older versions but thanks to the primitive cell reduction this is no longer a strict requirement.