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Benjamin Werth
Sr. Science Editor - Chemistry | Biochemistry
JoVE

Dear Dr. Benjamin Werth,

Thank you very much for the possibility to improve the manuscript to publish in JoVE. We tried to address each of the questions and comments of the editorial board as well as the reviewers.

Please find our comments attached to this letter and the new version of the manuscript uploaded.

I am looking forward to hearing from you at your earliest convenience

Stephanie Hutin

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. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Thank you very much for the advice, we ask native speakers to control the text.

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 …”

We changed the summary accordingly.

3. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Please find the changes in the text.

4. 2.1.1: What medium is used? Please provide the composition. 27 or 37 C?

First at 37°C until OD600=1 and then at 27°C over night.

5. 2.1.2: How are cells harvested? What are the sonication parameters?

Please find the changes in the text.

6. 2.1.3: Please provide more details on the column use.

Please find the changes in the text.

7. 2.1.4: How are the fractions colored? Is a detector used for characterization?

The protein is intrinsically coloured bright yellow, hence the protein fractions are easily distinguishable.

8. 2.1.5: How large are the pores in the dialysis bag? How long was the dialysis process? How much is added to each bag?

We optimized the method using an S75.

9. 2.2.2: How are the seed beads used? How much is used?

It is actually easy to break them by tissue grinder.

10. Please specify all volumes and concentrations used throughout.

Please find the changes in the text.

11. Where are the hanging drop plates stored for crystal growth? What is the reservoir used and how much?

20 °C with a reservoir of 1 mL 100 mM HEPES at pH 6.75, 12% PEG8000 and 100 mM $MgCl_2$

12. 3.1; What is the cryosolution used?

It is the precipitation solution out of the well of the crystallization plate mixed with 20% glycerol.

13. 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We marked 2.5 pages of the steps which should be visualized.

14. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. 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.

We followed this advice.

15. Steps 7 and 8 are not appropriate for filming.

We are aware of it, but would like to leave it in the written protocol for completeness.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The manuscript submitted by Hutin et al. describes the use of MeshAndCollect at ESRF macromolecular crystallography beamlines for the structure determination of the model protein Cerulean. MeshAndCollect is a workflow to help the data collection of microcrystals in a serial fashion. It includes the raster scanning of a mesh containing a slurry of microcrystals, the identification and selection of crystals hits, and the collection and merging of many partial data sets. The authors briefly described the protocol used for the structure solution of high diffracting microcrystals of Cerulean, from the expression and purification of the protein, to the crystallographic data collection/processing using MeshAndCollect.

We thank the reviewer for his useful and thoughtful revision. Please find below our comments and corrections concerning each point.

The paper may be of interest for ESRF users who aim at determining structures from microcrystals.

Here we use an ESRF beamline, but the method can be used at any synchrotron.

The manuscript would need to address the following comments and corrections (see below) prior to publication.

Major Concerns:
- l100. The paragraph starting on l100 is of little relevance in the context of this paper and could be remove entirely.

We would prefer to keep this paragraph in the text. For this example we did not use a classical test protein, such as Lysozyme or Insulin, hence we do believe that have to introduce this protein most crystallographers are not familiar with.

- l111. The reasoning in selecting crystals that diffract to 1 Ang resolution is arguable since typical targets of SX experiments tend to diffract poorly.

Other examples of this protocol using less diffracting crystals are already published in literature (e.g. Zander 2015 or Santoni 2017). We wanted to present here a new example with a protein of biological interest that happens to diffract at 1A. The argument in the text has been edited accordingly.

- l182. Please explain the advantages of using a multi-axis goniometer like the mini-kappa when microcrystals on the mesh are expected to be randomly oriented.

The protocol being most suited for a sample holder which is flat and perpendicular to the beam, a multi-axis goniometer allows to better orient the whole support. Random orientation of the individual samples will be kept in any case

- l217. Why only 1.8 Ang resolution when crystals are supposed to diffract to 1 Ang (l111)?

Most protein crystals have an intrinsic variability in resolution ranges. The 1A value is obtained from bigger crystals with more intense beams. For the structural description please consult the **Lelimousin *et al.***[20] We took the sentence out to avoid confusion.

- l222. Please give the data collection parameters for the mesh scan, as well as an estimate for the duration of both the mesh scan and the DOZOR analysis (in a typical experiment with Cerulean).

The default parameters are included in the text.

- Table. Is the space group P222 correct? Isn't it supposed to be P212121 (like PDB 2WSO)?

Typing error, thanks for pointing it out. Has been edited to P212121.

Minor Concerns:

- l73. Citation Akey et al. is not relevant in that context as the crystals are not microcrystals. A review on SX may be a better choice here, for instance Diederichs et al. 2017 MiMB.

We changed it.

- l85. Please define "state-of-the-art X-ray source". When considering SX experiments, it usually implies microfocus undulator beamlines.

We specified it.

- l142. Please specify the loop size.

Specified in the text. We tend to prefer MiTeGen mesh 700x25, but all kind of supports is suitable, given that it can stay flat and perpendicular to the beam

- l56. Replace "use in structure determination protocols" with "use in structure determination"

We replaced it.

- l61. Remove "While"

We removed it.

- l65. Replace "classical" with "conventional"

We replaced it.

- l88. Replace "membrane protein crystals grown in lipidic and cubic phases" with "in meso grown microcrystals of membrane proteins"

We replaced it.

- l91. Remove "preliminary"

We removed it.

- l94. Replace "usually" with "typically". Explain why 10º.

We replaced it and added the explanation for the 10 degrees in the text.

- l117. Remove "2. Expression and purification" as it is already in l115.

We removed it.

- l318. Correct "small big crystals"

Thank you very much to bring that to our attention. We removed the word “big”. It slipped our proofreading.

Reviewer #2:

We are thankful for the useful revision of this reviewer and addressed his comments below:.

Manuscript Summary:
The manuscript describes the use of mesh and collect for structure determination of CFP cerulean. It largely repeats the work reported in Zander et al (2015).

Well, here we provide a detailed protocol which can be used step-by-step by the crystallography community experiments based on the publication of Zander et al (2015). We think that the method is so important that it should be available to the scientific community as a video and protocol.

Some of the referencing could be improved based on the first few I looked at. Are McPherson (reference 1) and Giege (2) really suitable references for the statement that a bottleneck in MX is the need for large, well diffracting crystals?

We took the references out given that it is common knowledge.

The manuscript seems to jump from talking about the need for large crystals to serial experiments. What about the well-established field of microfocus MX? This would seem too much more relevant than injector based serial experiments. MeshAndCollect is a development (and improvement / automation of) 'traditional' microfocus MX experiments rather than an answer to/improvement on jet/extruder SSX. It would be an improvement if the introduction reflected this.

We edited the text accordingly.

I understand that the text will be used as the basis of a video, but if the text is to accompany the video the protocols should be significantly reduced in length.

For the video we will just use 2.75 pages or less. Still the additional information will provide significant help to the experimentator to acquire good data.

Results/discussion

Line 283 - superimposition should be superposition.

We replaced it.

Line 318 - what are small big crystals?

Thank you very much to bring that to our attention. We removed the word “big”. It slipped our proofreading.