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Micro- and nanocrystal preparation for MicroED and XFEL serial crystallography by fragmentation of imperfect crystals

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Abstract

This protocol describes different techniques to fragment protein crystals for high-resolution structure determination by MicroED and serial crystallography at an X-ray free electron laser. These techniques involve agitation of the crystal of interest: 1) by vigorous pipetting, 2) by sonication, and 3) by vortexing. Aside from setup, the time needed to perform these steps ranges from seconds to minutes.

Subject terms: **Biochemistry** **Structural biology**

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Crystallography **Electron microscopy** **MicroED** **Proteins**
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Introduction

It must be noted that each protein crystal behaves differently to different forms of stress. Each method described here can be performed at varying degrees of intensity as needed.

Volume is a factor when dealing with a microcrystal sample whose final resting place is a 3 mm TEM grid. Therefore, it is up to the experimenter to optimize crystal concentration and vitrification conditions on the cryoTEM grid via cryoEM screening and adjust initial sample volume as necessary.

Once protein crystals in liquid media are ready to be harvested, fragmentation can proceed using

one or more of the three methods described below in the Procedure.

Equipment

Common equipment

- Microcentrifuge tubes, 0.6 mL/1.5 mL (Fisherbrand #02-681-257/05-408-129 or similar)
- Micropipettor with tips, 0.5-10 μ L/10-100 μ L (Eppendorf Research plus variable micropipettor, #3120 000.801/#3120 000.828 or similar; Eppendorf tips 0.1–10 μ L/2-200 μ L, #022491504/#022491539 or similar)
- Forceps or tweezers to manipulate crystal tray coverglass (if necessary)
- Razor blade (edge carefully cleaned with alcohol) or sterile scalpel
- Parafilm (to wrap microcentrifuge tube for protection if needed)

For sonication

- Sonicating bath (Elmasonic P30H Ultrasonic cleaner or similar)
- Water (for the sonicating bath)

For vortexing

- Vortex mixer with flat rotating rubberized head (Fisher Scientific Mini Vortexer with 3 inch head, #02-215-365 or similar)
- Glass beads (Hampton Research Seed Bead Glass Kit, #HR4-782 or similar)

Procedure

A.0. Vigorous pipetting The intensity of this simple procedure can be adjusted by varying the manual plunging force of the micropipettor during intake/discharge of the liquid. It may be necessary to cut the edge of the tip if crystals are too large to enter the tip; in this case carefully use a cleaned razor blade or sterile scalpel to slice off the thin edge to reveal a wider tip opening.

A.1. Place a new tip on a micropipettor.

A.2. Obtain a new microcentrifuge tube if separating crystals from source.

A.3. Use the micropipettor to intake the required volume of crystals in solution.

A.4. Discharge the solution either back to crystal source or to the microcentrifuge tube.

A.5. Repeat steps A.3–A.4 as needed, taking care not to disturb the sample with air bubbles.

A.6. Add well buffer/mother liquor if needed to dilute the sample.

B.0. Sonication Intensity using the suggested sonicating bath model can be adjusted by 1)

varying power, 2) varying frequency, 3) varying content of bath and crystal sample vessel, and 4) varying the time duration of sonication. We use the Elmasonic P30H because of its ability to reduce its ultrasonic frequency and power for gentle sonication. Therefore we generally keep the machine at its lowest power setting and only vary the duration of sonication (duration of crystal vessel/microcentrifuge tube contact with bath content, in this case, water); this is the method we present below. Other sonicating baths may be used, however the experimenter should test sonication intensity and adjust water bath contents and crystal sample as necessary to reduce or increase ultrasonic intensity.

B.1. Prepare an ultrasonic water bath by adding at least enough water to the container until the depth reaches 4–5 cm.

B.2. Place a new tip on a micropipettor.

B.3. Obtain a new microcentrifuge tube if separating crystals from source.

B.4. Use the micropipettor to intake the required volume of crystals in solution.

B.5. Discharge the solution to the microcentrifuge tube.

B.6. Add well buffer/mother liquor if needed to dilute the sample.

B.7. Close microcentrifuge tube. Wrap tightly with Parafilm if desired for further protection.

B.8. Set the sonicating bath to run continuously.

B.9. For 1–10 seconds, dip the bottom end of the microcentrifuge tube into the water, making sure the surface of the crystal solution inside the tube is below the surface of the water bath. Adjust sonication time duration depending on crystal fragility.

C.0. **Vortexing** Agitation intensity can be adjusted in this case by one or more of the following options: 1) changing bead size, 2) changing microcentrifuge tube size, 3) changing vortex speed, and 4) changing vortex duration (time). We typically use a glass bead of 0.5 mm diameter inside a 1.5 μ L microcentrifuge tube. Ensure that bead and tube temperatures are stabilized at sample temperature before proceeding.

C.1. Set the vortex mixer at the highest speed setting, and set power switch to auto mode (touch/pulse mode).

C.2. Place a new tip on a micropipettor.

- C.3. Obtain a new microcentrifuge tube if separating crystals from source.
- C.4. Use the micropipettor to intake the required volume of crystals in solution.
- C.5. Discharge the solution to the microcentrifuge tube.
- C.6. Add well buffer/mother liquor if needed to dilute the sample.
- C.7. Add glass bead to the microcentrifuge tube.
- C.8. Close microcentrifuge tube. Wrap tightly with Parafilm if desired for further protection.
- C.9. Use the flat rubberized head platform on the vortex mixer for stability.
- C.10. Place the bottom of the microcentrifuge tube in the center of the rotating platform until it actuates; vortex for 2 seconds.

Associated Publications

This protocol is related to the following articles:

- Atomic-resolution structures from fragmented protein crystals with the cryoEM method MicroED
M Jason de la Cruz, Johan Hattne, Dan Shi, Paul Seidler, Jose Rodriguez, Francis E Reyes, Michael R Sawaya, Duilio Cascio, Simon C Weiss, Sun Kyung Kim, Cynthia S Hinck, Andrew P Hinck, Guillermo Calero, David Eisenberg, and Tamir Gonen

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Competing financial interests

The authors declare no competing financial interests.

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Readers' Comments

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