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Scriptwriter Name: Domnic Colvin
Supervisor Name: Anastasia Gomez
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Title: Workflow and Tools for Crystallographic Fragment Screening at the Helmholtz-Zentrum Berlin

Authors and Affiliations:

Jan Wollenhaupt[1], Tatjana Barthel[1,2], Gustavo M. A. Lima[3], Alexander Metz[4], Dirk Wallacher[5], Elmir Jagudin[3], Franziska U. Huschmann[1,4], Thomas Hauß[1], Christian G. Feiler[1], Martin Gerlach[1], Michael Hellmig[1], Ronald Förster[1], Michael Steffien[1], Andreas Heine[4], Gerhard Klebe[4], Uwe Mueller[1], Manfred S. Weiss[1*]

[1]Helmholtz-Zentrum Berlin, Macromolecular Crystallography, Albert-Einstein-Str. 15, Berlin, Germany
[2]Freie Universität Berlin, Institute for Chemistry and Biochemistry, Structural Biochemistry Group, Takustr. 6, Berlin, Germany
[3]MAX IV Laboratory, BioMAX, Fotongatan 2, Lund, Sweden
[4]Philipps-Universität Marburg, Institute of Pharmaceutical Chemistry, Drug Design Group, Marbacher Weg 6, Marburg, Germany
[5]Helmholtz-Zentrum Berlin, Department Sample Environment, Hahn-Meitner-Platz 1, Berlin, Germany

Corresponding Authors:
Manfred S. Weiss (msweiss@helmholtz-berlin.de)

Email Addresses for All Authors:
jan.wollenhaupt@helmholtz-berlin.de
tatjana.barthel@helmholtz-berlin.de
gustavo.alvares.lima@gmail.com
alexander.metz@idorsia.com
dirk.wallacher@helmholtz-berlin.de
elmir.jagudin@maxiv.lu.se
franziska.huschmann@gmx.de
hauss@helmholtz-berlin.de
christian.feiler@helmholtz-berlin.de
gerlach-reise@gmx.de
michael.hellmig@helmholtz-berlin.de
foerster@helmholtz-berlin.de

michael.steffien@helmholtz-berlin.de
heinea@mailer.uni-marburg.de
klebe@staff.uni-marburg.de
uwe.mueller@helmholtz-berlin.de
msweiss@helmholtz-berlin.de

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**

   If **Yes**, can you record movies/images using your own microscope camera?

   No movies, only images, but there is an HDMI output (see next answer)

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Leica MZ7.5 microscope, there is a screw thread on top *(about 3.5cm diameter, a Leica-specific screw thread)*

   However, there is also a Leica IC80 HD module included, with gives out the video signal via mini-HDMI (HDMI-C) or analog video-out while the experimenter can still use stereo view. The HDMI resolution for the live view is: full-HD with 1920x1080 for 20 fps, or 1280 x 760 at 45 fps. The full HD option is probably the best way to record the steps to be performed under microscope. We hope the filming team can bring proper connectors/cables/recording devices.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 17
Number of Shots: 38

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Manfred Weiss:** The screening of small organic compounds, called fragments, using X-ray crystallography is an efficient method for identifying molecules that can be starting points for drug discovery or biochemical tool compound development.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Jan Wollenhaupt:** Crystallographic fragment screening reveals not only the identity, but also where and how the fragment hits bind on the surface of the protein under study.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Jan Wollenhaupt:** This technique can be used on every biochemically or medically relevant protein target for which suitable protein crystals can be grown.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Manfred Weiss:** The quality of the results is intimately connected to the quality of the sample handling. We believe that the following visual demonstration will help researchers to carry out high-quality experiments.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.5. **Manfred Weiss:** Demonstrating the procedure will be Tatjana Barthel, a doctoral researcher and PhD student from my laboratory.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Soaking Crystals

2.1. To begin, cut open the bag of the screening plate pre-warmed to room temperature **[1]**, then remove the lid and the foil from the screening plate **[2].**

2.1.1. Talent cutting open the pre-warmed screening plate.

2.1.2. Talent removing the lid and foil from the screening plate.

NOTE: 2.1.1 and 2.1.2 in one step

2.2. Decant the 5-milliliter soaking solution in the reagent reservoir **[1]**, then fill each of the 96 reservoirs of the plate with 40 microliters of soaking solution using a 12-channel pipette **[2].**

2.2.1. Talent decanting the soaking solution in the reagent reservoir.

2.2.2. Talent filling the plate with soaking solution.

2.3. Place the EasyAccess Frame on top of the screening plate **[1]** and secure it with the included clamps by sliding them onto the left and the right side of the device **[2].**

2.3.1. Talent placing the EasyAccess Frame on the screening plate.

2.3.2. Talent securing the frame with clamps.

2.4. Place the screening plate and EasyAccess frame under the microscope and slide open the first well by moving the respective acrylic glass tile of the frame **[1].**

2.4.1. Talent placing screening plate and EasyAccess Frame under the microscope

2.5. Add 0.4 microliters of soaking solution from the reservoir to the fragment-containing well using a fresh pipette tip **[1]**. Ensure that the drop covers the dried-on fragment **[2].**

2.5.1. CU: Talent adding soaking solution from the reservoir to the fragment containing well.

2.5.2. SCOPE: Talent observing the solution drop.

2.6. Place the crystallization plate that contains the target crystals under the second microscope and cut open the sealing foil at one of the wells **[1].** Using an

appropriately sized loop, transfer a crystal **[1.2]** to the well of the screening plate under the first microscope **[2].** *Videographer: This step is important!*

2.6.1. Talent cutting open the foil of the plate containing target crystals.

2.6.1.2 Added shot– Talent transferring crystal

2.6.2. SCOPE: Talent transferring target crystal to the first well of screening plate.

2.7. Wash the loop in the prepared glass spot plate **[1]**, then dry it by gently touching it to the tissue. Do this after every transfer to avoid cross-contamination **[2]**. Use the microscope to ensure that the crystal has been properly placed **[3].** Repeat the procedure for the second crystal **[4-TXT]**.

2.7.1. Talent washing the loop in glass spot plate.

2.7.2. Talent drying the loop using tissue paper.

2.7.3. SCOPE: Talent observing the crystal.

2.7.4. Talent repeating the procedure with the 2nd crystal. **TEXT: Duplicates are recommended**

2.8. Move on to the next well **[0]** and repeat the procedure for all remaining wells **[1].**

2.8.0 Added shot: Talent opening the next well and starting the procedure again

2.8.1. Talent transferring 2 crystals to the next well.

2.9. After crystals have been transferred to all 96 wells in the screening plate **[1]**, remove the screening plate along with the EasyAccess Frame from under the microscope and place it onto the bench or table **[2]**, then remove the EasyAccess Frame from the screening plate **[3].**

2.9.1. SCOPE: All crystals in drops present in one exemplifying row are shown.

2.9.2. Talent removing the screening plate from the microscope and placing on the table.

2.9.3. Talent removing the EasyAccess Frame from the plate.

2.10. Seal the screening plate with sealing foil **[1]** and place it in the crystallization incubator or cupboard to incubate for the previously optimized soaking time. Two hours of incubation is sufficient, but overnight may be more convenient **[2].**

2.10.1. Talent sealing the plate with sealing foil.

2.10.2. Talent placing the plate in incubator.

3. Harvesting Crystals

3.1. Prepare the Unipuck foam dewar with 3 unipuck lids and half fill it with liquid nitrogen, keeping it on the ground **[1].** Move the half-filled dewar to the bench **[2]** and fill it completely to the very edge. Keep it filled to the upper edge during the entire experiment and replace the liquid nitrogen frequently. **[3].** *Videographer: This step is important!*

3.1.1. Talent half filling unipuck foam dewar with liquid nitrogen.

3.1.2. Talent placing the half-filled dewar on the bench.

3.1.3. Talent filling the dewar completely to the very edge.

3.2. Retrieve the screening plate from the incubator **[1]** and remove its foil **[2]**. Place the EasyAccess Frame on top **[3].**

3.2.1. Talent retrieving screening plate from incubator.

3.2.2. Talent removing the foil from screening plate.

3.2.3. Talent placing the EasyAccess Frame on top of the plate.

3.3. Slide open the first well **[1].** Harvest one crystal from the drop **[2]** and flash-cool it in liquid nitrogen by plunging with a fast vertical movement **[3].** *Videographer: This step is difficult and important!*

3.3.1. Talent opening the first well with the pen tool.

3.3.2. SCOPE: Talent taking one crystal from the well. NOTE: Take after 00:13

3.3.3. Talent flash-cooling the crystal in liquid nitrogen. NOTE: Take second Take

3.4. Insert the sample in the proper puck position **[1]** and take relevant notes on the sample tracking sheet. Repeat the procedure for the second crystal **[2].**

3.4.1. Talent inserting the sample in proper position.

3.4.2. Talent taking notes on the tracking Excel sheet on the laptop or printed sheet.

3.5. Go to the next well and repeat the positioning of other crystals until all three pucks are full **[1].**

3.5.1. Talent sliding open next well and harvesting two crystals from this well.

3.6. Pre-cool the Unipuck bases in liquid nitrogen **[1]** and add them on top of the lids **[2].**

3.6.1. Talent precooling the unipuck bases in liquid nitrogen in a separate foam dewar.

3.6.2. Talent adding the unipuck bases on top of the lids.

3.7. Store the unipucks in storage racks in a transport dewar or storage dewar **[1].** Keep them in liquid nitrogen at cryogenic temperatures until measurement **[2]** Repeat the same procedure of harvesting and storing the crystals in unipucks for the remaining samples in the screening plate **[3]**.

3.7.1. Talent storing the 3 unipucks in a storage rack of a transport dewar.

3.7.2. Talent lowering the storage rack into the dewar and putting the lid on top.

NOTE: 3.7.1 + 3.7.2 in one step

3.7.3. Talent harvesting another crystal.

Videographer: Please film shot 4.2.1 from the results section.

Results

4. Photographic Snapshots of Some Crystalline Samples and Crystallographic Fragment Screening

4.1. The variability of morphologies of the crystals after performing the fragment soaking and crystal harvesting is shown here. Even crystals which look somewhat deteriorated were included as they still result in useful data **[1].**

4.1.1. LAB MEDIA: Figure 5.

4.2. Data was collected at the Beamlines 14.1 *('14-point-1')* and 14.2 *('14-point-2')* at the BESSY II *('bessie two')* synchrotron **[1]**. Diffraction data collection was performed for each sample **[2].**

4.2.1. Panoramic shot of Beamline control room. passing the door of the 14.2 beamline hutch and going into the 14.1 beamline hutch. *Videographer: Please film this*

4.2.2. LAB MEDIA: diffraction-data-collection-example-0p5degs.mpg.

4.3. The data was analyzed using FragMAXapp *(pronounce 'frag-max-app')* focusing on the combination of XDSAPP *('X-D-S-app')* for processing, fspipeline *('F-S-pipeline')* for structure refinement, and PanDDA *('panda' (like the animal))* for hit finding **[1].**

4.3.1. LAB MEDIA: Figure 6.

4.4. This resulted in 15 hits on the AR ('A-R') protein complex using a DMSO-free soaking condition. In a previous campaign of the F2X-Entry Screen *('F-two-X-Entry Screen')* against the same target, including DMSO in the soaking condition, 20 hits were found. This means that re-testing of those identified compounds succeeded in 75% percent of the cases without DMSO present **[1-TXT]**.

4.4.1. LAB MEDIA: Figure 7. **TEXT: Wollenhaupt, Metz et al., 2020, Structure**

Conclusion

5. Conclusion Interview Statements

5.1. **Tatjana Barthel:** For the success of the experiment, it is absolutely crucial that the crystals are handled properly and carefully during every step of the procedure.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6 and 3.3.*

5.2. **Manfred Weiss:** Crystallographic fragment screening has matured to a widely used technique and is often applied in academia and in the pharmaceutical industry.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.