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Title: Lipidico Injection Protocol for Serial Crystallography Measurements at the Australian Synchrotron

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Author Questionnaire

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

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

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 self-record interview statements outside of the filming date. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **47**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Connie Darmanin**: This protocol provides the opportunity for scientists to characterise protein crystals embedded in highly viscous materials, as well as other soft materials currently difficult to characterise using existing setups **[1]**.

1.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Peter Berntsen**: Using this method, the samples are prepared in glass syringes mounted directly onto the injector, making the injector system easy to use and minimizing sample waste **[1]**.

1.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. High Viscous Medium Crystal Preparation

2.1. To prepare crystals in a high viscous medium, first centrifuge the crystal solution at low speed **[1-TXT]** to form a soft crystal pellet **[2]**.

2.1.1. WIDE: Talent placing tube(s) into centrifuge **TEXT: 10 min, 1000 x g, 22 °C**

2.1.2. Shot of soft pellet

2.2. After removing the excess medium, fasten a coupler to the end of one, 100-microliter glass syringe **[1]** and load 28 microliters of the crystal solution into the syringe barrel **[2]**.

2.2.1. Talent attaching coupler to syringe

2.2.2. Solution being added to top of syringe

2.3. Slowly insert the plunger into the top of the syringe **[1]** and gently push the solution to the end of the coupler tip **[2]**.

2.3.1. Plunger being inserted

2.3.2. Solution being pushed to coupler tip

2.4. To remove bubbles, use one gloved finger to apply very gentle pressure to the top of the blunted coupler needle point to create a seal **[1-TXT]** and retract the plunger to draw the solution away from the needle tip to create a buildup of pressure within the syringe **[2]**.

2.4.1. Finger being placed onto needle tip *Videographer: Important step* **TEXT: Caution: Too much pressure may result in needle stick injury**

2.4.2. Plunger being retracted *Videographer: Important step*

2.5. Then remove the finger to quickly release the pressure and to burst any bubbles within the syringe **[1]**.

2.5.1. Finger being removed/bubbles being released

2.6. Alternatively, hold the syringe in one hand with the needle facing upwards and the plunger wedged between two fingers **[1]** and quickly rotate the arm holding the syringe in one direction 2-3 times **[2-TXT]**.

2.6.1. Syringe being grasped/secured

2.6.2. Talent rotating arm **TEXT: Caution: Slow rotation can result in sample loss**

2.7. The resulting centrifugal force will force any air bubbles out of the syringe **[1]**.

2.7.1. Shot of syringe with no bubbles

2.8. When all of the bubbles have been removed, use a fine spatula to add 42 microliters of high vacuum silicone grease directly to the top of a second 100-microliter syringe **[1]** and depress the plunger all the way to the end to remove any air bubbles and to ensure that there is no air gap in the end of the syringe **[2]**.

2.8.1. Grease being added to syringe

2.8.2. Plunger being depressed

2.9. When all of the bubbles have been removed from both syringes **[1]**, holding both syringes by the ends, attach the syringes with the coupler **[2]**.

2.9.1. Syringes being attached

2.10. To mix the samples together, gently sequentially depress both the crystal solution **[1]** and silicone grease syringe plungers 50-100 times **[2]**[2.1] until the materials are mixed thoroughly and appear to be homogenous **[3]**.

2.10.1. Crystal solution plunger being depressed

2.10.2. Silicone grease syringe plunger being depressed

2.10.2.1. Mixing sample together in syringes

2.10.3. Shot of homogenous solution

2.11. Then visualize the crystals under an optical microscope **[1-TXT]**.

2.11.1. Talent at microscope, viewing crystals in syringe **TEXT: Optional: View 1 microliter solution on glass slide with coverslip**

3. Crystal Concentration Determination

3.1. To determine the crystal concentration in the silicone grease **[1]**, count the number of crystals in a specific area using the optical microscope images **[2]**.

3.1.1. WIDE: Talent bringing crystals into focus, with monitor visible in frame

3.1.2. LAB MEDIA: 3_1_2: 00:03-00:10

3.2. To decrease the crystal concentration, repeat the crystal preparation using a higher volume of silicone grease **[1]** or dilute the crystal pellet in crystallization buffer prior to the syringe setup **[2]**.

3.2.1. Talent adding grease to syringe, with grease container visible in frame

3.2.2. Talent adding buffer to pellet, with buffer container visible in frame

3.3. To increase the crystal concentration, repeat the crystal preparation using a smaller volume of silicone grease, being mindful not to reduce the viscosity below 10 Pascal-seconds **[1]**.

3.3.1. Use 3.2.1.

3.4. When the appropriate crystal concentration has been obtained, move the entire sample into a single syringe **[1]** and replace the coupler with a 108-micron-inner diameter injection needle screwed very firmly into the base of the syringe **[2]**.

3.4.1. Sample being moved into syringe

3.4.2. Talent attaching injection needle

4. Injector Mounting and Control

4.1. To set up the injector, select **Tools** and **Homing Mode [1]** and **Negative Limit Switch** and **Start Homing** and let the software run **[2]** until the screw is retracted sufficiently for a 100-microliter syringe to fit under the screw **[3]**.

4.1.1. WIDE: Talent at computer, selecting Tools and Homing Mode

4.1.2. SCREEN: screen412: 00:17-00:28

4.1.3. Shot of retracted screw/screw retracting

4.2. Insert the needle of an empty syringe through the slit in the syringe holder to mount the syringe onto the injector **[1]** and line the syringe against the bracket **[2]**. NOTE: 4.2.1.-4.4.1. are merged

4.2.1. Needle being inserted *Videographer: Important step*

4.2.2. Syringe being lined up *Videographer: Important step*

4.3. Wrap one O-ring across the mid-section of the syringe **[1]**, attaching the ring to the hooks on either side of the syringe **[2]**, and loop a second O-ring around the hooks on the upper section of the syringe **[3]**. NOTE: 4.2.1.-4.4.1. are merged

4.3.1. O-ring being wrapped around syringe *Videographer: Important step*

4.3.2. Ring being attached to hook(s) *Videographer: Important step*

4.3.3. Second O-ring being looped around hook(s) *Videographer: Important step*

4.4. Then position one part of the second O-ring on the top of the glass syringe **[1]**. NOTE: 4.2.1.-4.4.1. are merged

4.4.1. O-ring being placed *Videographer: Important step*

5. Sample Syringe Mounting

5.1. To prepare the sample syringe, prime the syringe **[1]** and load the syringe onto the injector **[2]**. NOTE: 5.1.2. and 5.2.1. are merged

5.1.1. WIDE: Talent priming syringe *Videographer: Important step*

5.1.2. Talent loading sample syringe onto injector *Videographer: Important step*

5.2. Place the injector cap on the sample syringe plunger head **[1]** and move the drive screw to the top of the cap **[2]**. NOTE: 5.2.2. and 5.5.1. are merged

5.2.1. Talent placing injector cap onto plunger head

5.2.2. Talent placing drive screw at top of cap

5.3. In the injector control software, select **Velocity Mode** and **Activate Velocity Mode [1]**.

5.3.1. SCREEN: 531_and_541: 00:09-00:19

5.4. Set the **Setting Value** to 3000 revolutions per minute and click **Apply Setting Value [1]**.

5.4.1. SCREEN: 531_and_541: 00:21-00:31

5.5. As the screw approaches the cap on the syringe plunger head, change the **Setting Value** to 0 **[1]**. NOTE: 5.2.2. and 5.5.1. are merged

5.5.1. Screw approaching cap

5.5.1.1. Activate setting value on computer

5.6. Once contact is made, click **Apply Setting Value** to activate the preset value, stopping the screw instantly **[1]**~~**[2]**~~.

5.6.1. SCREEN: screen561: 00:14-00:17 ~~*Video Editor: please include 5.6.2. as inset*~~

5.6.2. ~~Shot of flow rate in calculator~~ ~~*Video Editor: please include as inset in 5.6.1*~~.

5.7. Then gently wiggle the cap to make sure that it is held firmly in place **[1]**.

5.7.1. Cap being wiggled

6. Running the Injector

6.1. To run the injector, change the **Setting Value** to 100 revolutions per minute **[1]** and visually inspect the tip to observe the sample as it begins to extrude **[2]**.

6.1.1. WIDE: Talent changing Setting Value, with monitor visible in frame

6.1.2. LAB MEDIA: To be provided by Authors: Shot of needle tip

6.2. After closing the hutch, decrease the **Setting Value** by decrements of 10 until a slow but stable stream of material is observed **[1]**[2].

6.2.1. ECU: Stream flow being slowed *Videographer: Important step*

6.2.2. SCREEN: shot562: 00:04-00:14 *Video Editor: please include as inset in 6.2.1.*

6.3. If the sample stream does not flow well, increase the **Setting Value** in 10-revolutions per minute-increments until the stream gets straighter **[1]**.

6.3.1. ECU: Stream flow being increased *Videographer: Difficult step*

6.4. If the stream does not stabilize, insert a polystyrene sample catcher under the sample stream to help guide the sample **[1-TXT]**.

6.4.1. Sample catcher being inserted **TEXT: This method works well for highly charged sample streams**

6.5. Alternatively, insert a venturi suction funnel into the sample catcher **[1]**, which can be connected to the air outlet tube located in the hutch at the Australian Synchrotron **[2]**.

6.5.1. Funnel being inserted

6.5.2. Shot of connecting funnel *Video Editor: please indicate air outlet tube when mentioned*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.4., 4.2.-4.4., 5.1., 6.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
Step 6.3. is the most difficult step as time needs to be taken to slow down the injector speed to gain stream stability. Two options are provided to help, steps 6.5 and 6.6, and if this doesn't work the sample viscosity needs to be increased by reconnecting the sample syringe to another syringe containing more silicone grease and mixing as in step 2.8. The amount of silicone grease to add is dependent on the consistency of the sample. Note: this testing should be done prior to beam time however if the sample volumes on the day are not accurate this can be easily fixed during beam time.

Results

7. Results: Representative Serial Crystallography Measurement

7.1. Determining the optimal sample running speed for the injector is crucial for the stream stability **[1]**.

7.1.1. LAB MEDIA: Figure 2

7.2. A slow flow rate results in an expansion of the silicone grease extruding from the injector **[1]**, while a faster flow rate produces a thinner stream **[2]**.

7.2.1. LAB MEDIA: Figure 2 *Video Editor: please emphasize Figure 2A*
7.2.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize Figure 2C*

7.3. Curling of the viscous media stream has regularly been observed **[1]**.

7.3.1. LAB MEDIA: Figure 3A

7.4. To overcome this issue in highly charged samples, a polystyrene catcher can be used to introduce a weak electrostatic force **[1]**.

7.4.1. LAB MEDIA: Figure 3B

7.5. In this analysis, a glass syringe containing 26 microliters of lysozyme crystals suspended in silicone grease was used **[1]**.

7.5.1. LAB MEDIA: Figure 4A

7.6. A sufficient amount of data was collected within 38 minutes **[1]**, enabling the generation of an electron density map surrounding one of the di-sulfide bonds in the lysozyme structure **[2]**.

7.6.1. LAB MEDIA: Table 1
7.6.2. LAB MEDIA: Figure 4B

Conclusion

8. Conclusion Interview Statements

8.1. **Peter Berntsen**: Remember to use a small volume of sample on a glass slide to assess the crystal concentration of the sample to determine whether the viscosity needs to be corrected **[1]**.

8.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera (Step 2.1.)

8.2. **Connie Darmanin**: The versatility of the injector allows the X-ray characterization of other highly viscous materials, for example, ionic liquid structures used in battery research or colloidal materials used in dairy products **[1]**.

8.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera