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Title: Nano-Differential Scanning Fluorimetry for Screening in Fragment-based Lead Discovery

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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 something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

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?

   X 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, different rooms, same building**

Current Protocol Length

Number of Steps: 21
Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Anastassis Perrakis:** This protocol is useful for fragment-based lead discovery campaigns as an additional tool for selecting promising fragments as hit candidates.

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

1.2. **Misbha Ud Din Ahmad:** nanoDSF for fragment screening uses a limited amount of unlabeled protein sample and can be applied routinely for almost all protein targets.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

OPTIONAL:

1.3. **Alexander Fish**: This method can be used to screen any collection of ligands or for confirming hits from other methods.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1, 5.5.1 and 5.5.2*

Protocol

2. Plate Preparation

2.1. Take out a fragment plate from the -20 or -80-degree Celsius freezer **[1]** and let it thaw at room temperature with gentle shaking on a benchtop shaker **[2]**. Centrifuge the plate at 500 times *g* for 30 seconds to collect any drops sticking to the side of the wells **[3]**.

2.1.1. WIDE: Establish the shot of talent taking out the plate from freezer

2.1.2. Plate thawing on shaker

2.1.3. Talent putting the plate for centrifugation

2.2. Take an MRC 2-well crystallization plate and pipette 15 microliters of the protein stock solution into each sub-well using a multichannel pipette **[1]**.

2.2.1. Talent pipetting protein stock solution into each well using multichannel pipette

3. Fragment Nano-Dispensing by the Mosquito Robot

3.1. To check fragment and protein plate definitions on the Mosquito **[1]**, switch on the nanodispenser **[1]** and make sure that there are no obstacles around the moving components **[2]**.

3.1.1. Talent switching on nanodispensor

3.1.2. Moving components

3.2. Open the graphical user interface **[1]**, click on the **Setup** tab, and under **Deck Configuration**, check whether the type of plate in which the compounds are supplied and the one in which the protein is transferred are already present in the list of the **Available plates [2]**.

3.2.1. Talent at the computer

3.2.2. SCREEN: 3_2_2.mp4.mp4.00:00-00:07

3.3. If not, click **Options** and then **Plates** and create a new plate definition by filling in the correct values for the **Property** type **[1]**.

3.3.1. SCREEN: 3_3_1.mp4.mp4.00:00-00:25

3.4. Under the **Setup** tab, specify the plate positions under **Deck Configuration [1]**.

3.4.1. SCREEN: 3_4_1.mp4.mp4.00:00-00:04

3.5. Using the dropdown menu, choose **Greiner U bottom** plate for **Position 1** and **MRC 2**-well plate for **Position 2** and save the protocol **[1]**.

3.5.1. SCREEN: 3_5_1.mp4.mp4.00:00-00:17

4. Fragment Dispensing

4.1. Place the fragment plate at **Position 1** and the protein plate at **Position 2** of the deck **[1]**.

4.1.1. Talent placing fragment plate at position 1 and protein plate at position 2 of the deck

4.2. In the **Protocol** tab, click on **File** and choose the protocol saved in the previous step for dispensing the fragments. Define the volume of the fragments to be dispensed as 0.3 microliters **[1]**.

4.2.1. SCREEN: 4_2_1.mp4.mp4.00:00-00:021

4.3. For a typical plate, where columns 1 and 12 do not contain fragments, define the **Start** location to column 2 and the **End** location to column 11. In some plates, if columns 2 or 11 are empty, use columns 3 and 10 as start and end values **[1]**.

4.3.1. SCREEN: 4_3_1.mp4.mp4.00:00-00:20

4.4. Click on **Run** to start the program **[1]**. After dispensing, which takes about 2 minutes, is completed, remove the protein and the fragment plates from the robot **[2]** and seal them with an adhesive sealing film **[3]**.

4.4.1. SCREEN: 4_4_1.mp4.mp4.00:00-00:04

4.4.2. Talent removing the protein and fragment plates from the robot

4.4.3. Talent sealing the plates with adhesive film

4.5. Briefly centrifuge the protein plate at 500 times *g* for 30 seconds to collect any drops sticking to the sides of the wells before proceeding to the next step **[1]**.

4.5.1. Talent putting the plate for centrifugation

4.6. Visually inspect the wells of the protein plate for precipitation that might have occurred due to the addition of the fragments. If precipitation is observed in many wells, reduce the concentration of the fragments and repeat the experiment **[1]**.

4.6.1. Talent visually inspecting the wells

5. Preparing the Prometheus

5.1. Switch on the Prometheus instrument **[1]**. On the touchscreen, press **Open Drawer** to access the capillary loading module of the instrument **[2]**. Remove the magnetic strip

from the loading module, and clean the mirror with ethanol to remove any dust particles **[3]**.

5.1.1. Talent switching on the instrument

5.1.2. Talent pressing Open drawer on touch screen

5.1.3. Talent removing the magnetic strip and cleaning the mirror with ethanol

5.2. To transfer solution from the protein-fragments plate to the capillaries, place the protein plate and the capillaries next to the instrument for easy access to the capillary loading module **[1]**.

5.2.1. Protein plate and capillaries kept next to the instrument

5.3. Take one capillary, holding it at one end, and touch the solution in the protein plate with the other end of the capillary to transfer the sample. Always wear gloves, and make sure not to touch the capillary in the middle, as impurities from the gloves would affect the measurement **[1]**.

5.3.1. Talent holding the capillary with one end of the capillary in the sample

5.4. Place the capillary in the designated position of the holder, making sure it is properly aligned and centered. Repeat these steps to fill all positions in the loading module **[1]**.

5.4.1. Talent placing the capillary in the designated holder

5.5. At the end, place the magnetic strip on top of the capillaries to hold them in place **[1]**, and press **Close Drawer** to start the nano-DSF experiment **[2]**.

5.5.1. Talent placing the magnetic strip on top of the capillaries

5.5.2. Talent pressing close the drawer

6. Fluorescence Scan and Thermal Denaturation

6.1. Open the **Prometheus** application **PR.ThermControl**, and create a new project by clicking on **Start New Session**. First, do a **Discovery Scan** to detect the fluorescence of the samples **[1]**.

6.1.1. SCREEN: 6_1_1.mp4.mp4.00:00-00:41 *Speed it up!*

6.2. Alter the fluorescence signal of the samples by adjusting the **Excitation Power** of the laser **[1]**.

6.2.1. SCREEN: 6_2_1.mp4.mp4.0:12-00:50

6.3. For thermal denaturation, click on the **Melting Scan** tab. Set the **Start Temperature** to 20 degrees Celsius, the **End Temperature** to 95 degrees Celsius, and the **Temperature Slope** to 1 degree Celsius per minute, then click on **Start Measurement [1]**.

6.3.1. SCREEN: 6_3_1.mp4.mp4.00:05-0:34

Results

7. Results: Fragment Screening

7.1. Frequency distribution of the shift in melting temperature for the outer kinetochore Highly Expressed in Cancer 1 protein **[1]**, regulatory tetraricopeptide repeat domain of the monopolar spindle kinase 1 **[2]**, and SARS-CoV-2 3C-like protease are shown here **[3]**.

7.1.1. LAB MEDIA: Figure 5 *Video editor: Just show the Hec1 graph*

7.1.2. LAB MEDIA: Figure 5 *Video editor: Just show the Mps1 graph*

7.1.3. LAB MEDIA: Figure 5 *Video editor: Just show the Nsp5 graph*

7.2. Negative values indicate a reduction in the melting temperature in the presence of a fragment **[1]**, while a positive value indicates an increase in melting temperature **[2]**.

7.2.1. LAB MEDIA: Figure 5 *Video Editor: Emphasize the bars labeled with the negative vales (on the horizontal axis)*

7.2.2. LAB MEDIA: Figure 5 *Video Editor: Emphasize the bars labeled with the positive vales (on the horizontal axis)*

7.3. For Nsp5, all fragments have a destabilizing effect **[1]**, whereas for Hec1 and Mps1, both stabilizing and destabilizing hits are observed **[2]**.

7.3.1. LAB MEDIA: Figure 5 *Video editor: Please highlight Nsp5 graph*

7.3.2. LAB MEDIA: Figure 5 *Video editor: Please highlight Hec1 and Mps1 graph*

Conclusion

8. Conclusion Interview Statements

8.1. **Anastassis Perrakis:** This is cheap and quick method to screen fragment libraries. Other methods, such as NMR and X-ray crystallography, can show the exact binding of fragments, and are also available through iNEXT-Discovery.

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

8.2. **Misbha Ud Din Ahmad:** The results with the corona-virus protease helped to propose new compounds that can be developed into drugs against Covid-19.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 5*