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Title: Native Cell Membrane Nanoparticle System for Membrane Protein-Protein Interaction Analysis

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Author Questionnaire

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

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

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

Protocol Length
Number of Shots: **36**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Youzhong Guo**: This protocol will help researchers more accurately determine the native oligomeric state of membrane proteins by utilizing the native cell membrane nanoparticle system in conjunction with electron microscopy **[1]**.

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

REQUIRED:

1.2. **Youzhong Guo**: The advantage of this technique is that it provides accurate structural data on membrane proteins in a native cell membrane-like environment and can be utilized for future high-resolution structure determination **[1]**.

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

Introduction of Demonstrator on Camera

1.3. **Youzhong Guo**: Demonstrating the procedure will be Kyle Kroeck, a Postdoctoral Fellow from my laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Native Cell Membrane Nanoparticle (NCMN) Preparation

2.1. To prepare native cell membrane nanoparticles, resuspend 1 gram of the membrane pellet of interest in 10 milliliters of NCMN (N-C-M-N) buffer A **[1-TXT]**.

2.1.1. WIDE: Talent adding buffer to pellet, with buffer container visible in frame **TEXT: See text for all buffer and solution preparation details**

2.2. Use a glass Dounce homogenizer to homogenize the resuspended cell membrane sample at 20 degrees Celsius **[1]** and transfer the suspended sample to a 50-milliliter polypropylene tube **[2]**.

2.2.1. Sample being homogenized
2.2.2. Talent adding sample to tube

2.3. Add membrane active polymer stock solution and additional NCMN Buffer A to the sample to a final concentration of 2.5% membrane active polymer **[1-TXT]** and shake the solution for 2 hours at 20 degrees Celsius **[2]**.

2.3.1. Talent adding stock solution, with solution and buffer A containers visible in frame NOTE: This and next shot together *Videographer: Important step* **TEXT: *e.g.*, NCMNP1-1 or NCMNP5-2**
2.3.2. Sample on shaker *Videographer: Important step*

2.4. At the end of the incubation, ultracentrifuge the sample **[1-TXT]**.

2.4.1. Talent placing tube(s) into centrifuge **TEXT: 1 h, 150,000 x g, 20 °C**

2.5. During the centrifugation, equilibrate a 5-milliliter nickel-NTA (N-T-A) column with 25 milliliters of NCMN Buffer A **[1-TXT]**.

2.5.1. Talent adding buffer to column, with buffer container visible in frame **TEXT: NTA: nitrilotriacetic acid**

2.6. At the end of the centrifugation, transfer the supernatant onto the column **[1]** and use a syringe pump set to a 0.5 milliliter/minute a flow rate to load the supernatant onto the column at 20 degrees Celsius **[2]**.

2.6.1. Talent adding supernatant to column NOTE: This and next shot together

2.6.2. Supernatant being loaded onto column

2.7. Wash the fast protein liquid chromatography machine lines with enough NCMN Buffer B to completely flush the system **[1]** and connect the column to the chromatography machine **[2]**.

2.7.1. Talent flushing lines, with buffer B container visible in frame as possible *Videographer: Important step*
2.7.2. Talent connecting column to machine *Videographer: Important step*

2.8. Wash the column with 30 milliliters of NCMN Buffer B at 1 milliliter/minute flow rate **[1]** and collect the flow through **[2]**.

2.8.1. Talent adding buffer to column, with buffer B container visible in frame
2.8.2. Flow through being collected

2.9. Wash the column with 30 milliliters of NCMN Buffer C at a 1 milliliter/minute flow rate **[1]** and collect the flow through **[2]**.

2.9.1. Talent adding buffer to column, with buffer C container visible in frame
2.9.2. Flow through being collected

2.10. Elute the protein with 20 milliliters of NCMN Buffer D at a 0.5 milliliters/minute flow rate **[1]** and use a fraction collector to collect 1-milliliter fractions of the sample **[2]**.

2.10.1. Buffer D being added to column, with buffer D container visible in frame
2.10.2. Sample being collected in fraction collector

2.11. Then store the protein samples at 4 degrees Celsius **[1]**, using SDS-PAGE (S-D-S-page) to check the samples that correspond to the peaks observed on the fast protein liquid chromatography elution graph **[2]**.

2.11.1. Talent placing sample(s) at 4 °C
2.11.2. Talent adding sample to gel **TEXT: SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis**

3. Negative Stain Grid Preparation

3.1. To prepare negative stain grids, wrap a glass slide with filter paper with the carbon side facing up **[1]** and place the grids that are going to be used for the sample preparation onto the slide **[2]**.

3.1.1. WIDE: Talent wrapping slide with filter paper

3.1.2. Talent placing grids onto slide

3.2. Place the glass slide with the electron microscope grids into the chamber of a glow discharger between the two electrodes **[1]** and replace the glass lid, making sure the lid is centered and well-sealed **[2]**.

3.2.1. Talent placing slide into chamber between electrodes
3.2.2. Talent replacing/centering lid

3.3. Run the glow discharging machine **[1]** and make sure that the purple light generated by the plasma is visible **[2]**.

3.3.1. Talent running machine
3.3.2. Shot of purple light

3.4. When the machine is finished running, wait until the chamber has reached atmospheric pressure before removing the glass lid **[1]** and placing the slide onto the bench **[2]**.

3.4.1. Talent checking pressure and removing lid
3.4.2. Talent placing slide onto bench

3.5. Adjust the concentration of the purified protein samples to about 0.1 milligram/milliliter **[1]** and load 3.5 microliters of the protein sample onto the 10-nanometer thick carbon grid **[2]**.

3.5.1. Talent concentrating protein sample
3.5.2. Talent loading sample onto grid

3.6. After 1 minute, use a piece of filter paper to remove the liquid from the electron microscopy grid surface **[1]** and wash the grid surface three times with one 3-microliter droplet of water per wash **[2]**.

3.6.1. Liquid being removed NOTE: 3.6.1 – 3.8.1 in one shot in take 1 and 3.7.1 only in take 2 *Videographer: Important step*
3.6.2. Droplet being added to grid and/or being removed with filter paper NOTE: 3.6.2 take 2 should be 3.7.1 – 3.8.1 *Videographer: Important step*

3.7. After the last water wash, wash the grid surface two more times with 3-microliter droplets of fresh, filtered 2% uranium acetate per wash **[1]**.

3.7.1. Uranium acetate being added to grid and/or being removed from filter paper, with uranium acetate container visible in frame *Videographer: Important step*

3.8. After the last uranium acetate wash, stain the grid with a 3-microliter droplet of fresh,

filter 2% uranium acetate for 1 minute **[1]**.

3.8.1. Uranium acetate being added to grid *Videographer: Important step*

3.9. At the end of the incubation, use a new piece of filter paper to remove the droplet **[1]** and air dry the grid for at least 1 minute **[2]** before storing in the grid in a grid box for later use **[3]**.

3.9.1. Droplet being removed
3.9.2. Talent setting timer, with grid visible in frame
3.9.3. Talent placing grid into grid box

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.3., 2.7., 3.4.-3.7.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
n/a

Results

4. Results: Representative Negative Stain and Electrophoresis Gel images of Purified Acriflavine Resistance B (AcrB) Constructs

4.1. Here negative stain images obtained using transmission electron microscopy as demonstrated can be observed **[1]**.

4.1.1. LAB MEDIA: Figures 1A-1F

4.2. The negative stain image for the wild type acriflavine resistance channel protein B sample purified with detergent reveals a homogenous solution of monodispersed particles **[1]**, with the protein displaying a well-defined trimeric quaternary structure **[2]**.

4.2.1. LAB MEDIA: Figures 1A-1F *Video Editor: please emphasize Figure 1A*
4.2.2. LAB MEDIA: Figure 1A *Video Editor: please emphasize trimeric quaternary structure*

4.3. These trimeric structures correspond with the observed size exclusion chromatogram after purification **[1]**.

4.3.1. LAB MEDIA: Supplementary Figure 1 *Video Editor: please emphasize orange data line*

4.4. In comparison, in the negative stain image for the P223G (P-two-two-three-G) mutant, also purified with detergent **[1]**, a heterogeneous solution of polydispersed nanoparticles with a propensity towards aggregation but no observable native trimers can be observed **[2]**.

4.4.1. LAB MEDIA: Figure 1B
4.4.2. LAB MEDIA: Figure 1B *Video Editor: please emphasize nanoparticles*

4.5. These results are also supported by size exclusion chromatography **[1]**.

4.5.1. LAB MEDIA: Supplementary Figure 1 *Video Editor: please emphasize blue data line*

4.6. Similar results were observed for wild type **[1]** and mutant proteins after purification with the membrane active polymer NCMNP1-1 (N-C-M-N-P-one-one) **[2]**.

4.6.1. LAB MEDIA: Figures 1C and 1D *Video Editor: please emphasize Figure 1C*
4.6.2. LAB MEDIA: Figures 1C and 1D *Video Editor: please emphasize Figure 1D*

4.7. The use of the NCMNP5-2 polymer facilitates the generation of native cell membrane nanoparticles in much larger sizes **[1]**, allowing multiple acriflavine resistance channel protein B trimers to be imaged in a single native cell membrane particle **[2]**.

4.7.1. LAB MEDIA: Figure 1E
4.7.2. LAB MEDIA: Figure 1E *Video Editor: please add Figure 1I/zoom into red box in Figure 1E and show Figure 1I*

4.8. In the mutant sample, however, no trimer particles are observed **[1]**, even when looking at the large native cell membrane bilayer patches **[2]**.

4.8.1. LAB MEDIA: Figure 1F
4.8.2. LAB MEDIA: Figure 1F *Video Editor: please add Figure 1J/zoom into green box in Figure 1E and show Figure 1J*

4.9. SDS-PAGE analysis of the purified protein samples confirms the presence of acriflavine resistance B in all of the samples with a greater than 95% purity **[1]** at the predicted molecular weight of the protein **[2]**.

4.9.1. LAB MEDIA: Figure 1G
4.9.2. LAB MEDIA: Figure 1G *Video Editor: please emphasize bands in lanes 1 and 2*

Conclusion

5. Conclusion Interview Statements

5.1. **Kyle Kroeck**: When modifying this protocol for other proteins, it is important to experimentally determine the appropriate amount of membrane fraction and the length time and temperature for the solubilization process **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.1., 2.3.)

5.2. **Kyle Kroeck**: If the images of your sample reveal a homogenous solution of monodispersed particles with well-defined structural units, the sample can be used for high-resolution structure determination with cryo-electron microscopy **[1]**.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can skip for time*