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Title: Measuring Transcellular Interactions through Protein Aggregation in a Heterologous Cell System

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **yes**

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

   yes

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

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

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Susana Restrepo:** This cell-based assay allows for the observation and quantification of *trans* adhesion interactions without the need for lengthy protein purifications or specialized equipment.

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

1.2. **Susana Restrepo:** Although the protein interactions tested here are relevant to neurobiology, this method may be applied to any area of research where protein adhesion is occurring intercellularly.

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

Protocol

2. Cell Harvesting

2.1. Forty-eight hours after transfecting the HEK293T cells, harvest the cells from the 6-well plate for aggregation **[1]**. First, wash each well twice with PBS **[2]**. Next, to gently disassociate the cells, add 1 milliliter of 10 millimolar EDTA to each well **[3-TXT]**. Then, incubate the plate at 37 degrees Celsius **[4]**.

2.1.1. Talent removes 6-well plate from incubator.
2.1.2. Talent washes wells with PBS.
2.1.3. Talent adds EDTA to each well. **TEXT: Protocol may not be paused until completion**
2.1.4. Talent places plate in incubator.

2.2. After 5 minutes of incubation, gently tap the plate to detach the cells, and harvest each well into a separate 15-milliliter conical tube **[1]**. Centrifuge the tubes at 500 x g and room temperature for 5 minutes **[2]**. *Videographer: This step is important!*

2.2.1. Talent gently taps plate, and then begins harvesting the wells into 15-milliliter conical tubes.
2.2.2. Talent places the tubes in the centrifuge.

2.3. While the cells are pelleting, prepare 6 incubation tubes by labeling the tops of microcentrifuge tubes with the experimental conditions **[1]**. Every permutation of GFP and mCherry should be used **[2]**. *Videographer: This step is important!*

2.3.1. Talent begins labeling tops of tubes.
2.3.2. Array of labeled tubes, showing all permutations.

2.4. Remove the supernatants from the 15-milliliter conical tubes, and resuspend the cells in 500 microliters of medium **[1-TXT]**. *Videographer: This step is important!*

2.4.1. Talent removes supernatant and resuspends cells. **TEXT: HEK media + 10 mM $CaCl_2$ + 10 mM $MgCl_2$ at 37 °C**

2.5. Using a hemocytometer, count the cells in each conical tube **[1]**. Then, aliquot 200,000 cells from each condition into the appropriate incubation tube, for a 1 to 1 mix in a total volume of 500 microliters **[2]**. After assessing baseline aggregation, described in the next section, place the incubation tubes in a slow tube rotator at room temperature **[3]**. *Videographer: This step is important!*

2.5.1. Talent counts cells using hemocytometer.
2.5.2. Talent adds cells from conical tubes to microcentrifuge tubes.
2.5.3. Talent places incubation tubes in a slow tube rotator.

3. Image Acquisition

3.1. To assess aggregation, at baseline and again after 60 minutes, pipette 40 microliters from the incubation tube onto a charged microscope slide. Baseline acquisition should be done as quickly as possible after addition of cells to microcentrifuge tube **[1]**. *Videographer: This step is important!*

3.1.1. Talent pipettes 40 microliters onto slide.

3.2. Image the slide under fluorescence in both the green **[1]** and red channels **[2]**. For each slide, capture images of three different fields of view in one focal plane **[3]**.

~~3.2.1. SCOPE: Slide in 488-nanometer channel (green). *Video editor, please show 3.2.1 and 3.2.2 together as a multipanel.*~~

3.2.1. Talent places slide on microscope, looks through the eyepiece, moves joystick to find the correct field of view and uses the focus nob on the microscope.

~~3.2.2. SCOPE: Slide in 561-nanometer channel (red).~~

3.2.2. Talent switches between both (red and green) channels of an already captured image.

~~3.2.3.~~

~~3.2.4. SCOPE: Image being captured, slide being moved, and another image being captured.~~

NOTE: Please keep VO as is and use the footage that the videographer did film. See note below for details.

Author NOTE: Due to technical limitations with the scope, the precise sequence of events presented here were not filmed. The capturing of each channel was not filmed. Instead, the videographer made some voice notes on the video he is sending in.

The videographer filmed me placing the slide on the microscope, looking into the eyepiece of the microscope and changing the focus. Then we have footage of the computer screen displaying images that were captured in both channels, and the talent toggling between the channels.

I do not know if the videogrpaher preserved the numbering in 3.2.

Results

4. Results: Point Mutation Enhances Binding

4.1. HEK293T cells were transfected with a protein of interest, a mutated protein of interest, or a ligand of interest, and co-transfected with a fluorescent protein **[1]**. Cell populations expressing the protein of interest were mixed with cell populations expressing the ligand of interest, and assessed for aggregation after 60 minutes **[2]**.

4.1.1. LAB MEDIA: Figure 1. *Video editor, emphasize parts A and B of Figure 1.*
4.1.2. LAB MEDIA: Figure 1. *Video editor, emphasize parts C and D of Figure 1.*

4.2. In overlays of images captured in the green and red channels **[1]**, aggregation appears as yellow puncta **[2]**.

4.2.1. LAB MEDIA: Figure 2. *Video editor, show only Figure 2A.*
4.2.2. LAB MEDIA: Figure 2. *Video editor, show only Figure 2A, and emphasize these images: bottom row of column 4, bottom row of column 5.*

4.3. Conditions in which cells were not expressing any synaptic ligands showed minimal aggregation **[1]**. Also, minimal aggregation was exhibited when only one of the two populations were expressing synaptic ligands **[2]**.

4.3.1. LAB MEDIA: Figure 2. *Video editor, show only Figure 2A, and emphasize the image in the top row of the first column.*
4.3.2. LAB MEDIA: Figure 2. *Video editor, show only Figure 2A, and emphasize the following images: bottom row of column 1, top row of columns 2, 3, 4, and 5.*

4.4. No aggregation was exhibited when the two populations of cells expressed incompatible adhesion molecules **[1]**.

4.4.1. LAB MEDIA: Figure 2. *Video editor, show only Figure 2B.*

4.5. Conditions with compatible adhesion molecules exhibited significant aggregation after a 60-minute incubation **[1]**. Surprisingly, the mutated protein of interest exhibited significantly more aggregation than its wildtype counterpart, suggesting that the point mutation enhances the protein's binding capabilities **[2]**.

4.5.1. LAB MEDIA: Figure 2. *Video editor, show only Figure 2C.*
4.5.2. LAB MEDIA: Figure 2. *Video editor, show only Figure 2C, and emphasize yellow and orange columns.*

Conclusion

5. Conclusion Interview Statements

5.1. **Susana Restrepo:** Mutations in many adhesion molecules are commonly linked to neurodevelopmental, neuropsychiatric, and addiction disorders. With this technique one can screen the effect of disease relevant point mutations on trans interactions.

5.1.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.