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Title: Mapping Dysfunctional Protein-Protein Interactions in Disease

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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? **no**

3. **Filming location:** Will the filming need to take place in multiple locations? **yes**

   One street block apart

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **no**

Current Protocol Length
Number of Steps: 26
Number of Shots: 49

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Anna Rodina:** Our work maps dysfunctional protein–protein interactions directly from native cells and tissues, revealing how disease rewires cellular networks.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 1*

~~What technologies are currently used to advance research in your field?~~

1.2. **Chander Digwal:** Unlike most interactomic methods, dfPPI requires no genetic engineering and scales to patient cohorts, using one multiplexed capture per sample.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.12.1.*

CONCLUSION:

~~What significant findings have you established in your field?~~

1.3. **Souparna Chakrabarty:** dfPPI allowed us to uncover mechanistic and therapeutic insights that other approaches simply can't reach.

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

~~What research gap are you addressing with your protocol?~~

1.4. **Shujuan Wang:** dfPPI brings interactomics to the level of real-world disease cohorts—under native conditions—enabling precise mechanistic and therapeutic hypotheses.

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

~~What questions will future research focus on?~~

1.5. **Anna Rodina:** We're now expanding dfPPI to map network-level changes in neurodegenerative diseases such as Alzheimer's and Parkinson's.

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

Videographer's Note: 1.5 is mislabeld as 1.4. Clip for 1.5 is B111_B109_1017XF_001

Videographer: Obtain headshots for all authors available at the filming location.

2. Sample Preparation for Affinity Capture

Demonstrator: Anna Rodina

2.1. To begin, prepare a protein extraction buffer containing 20 millimolar Tris, 20 millimolar potassium chloride, 5 millimolar magnesium chloride, and 0.01 percent NP-40 *(N-P-Forty)* **[1]**. Add protease and phosphatase inhibitors immediately before use **[2]** and keep the buffer on ice **[3]**.

2.1.1. WIDE: Talent mixing reagents in a beaker with all the required reagents placed in front of him.

2.1.2. Talent adding protease and phosphatase inhibitors to the buffer immediately before use.

2.1.3. Talent placing the prepared buffer on ice.

Videographer's Note: 2.1.1-2.1.3 is included in clip C112C025 - 2.1.3 is not mentioned in the slate but it was filmed in the clip.

2.2. Place the frozen tissue sample into a micro tissue homogenizer tube fitted with a pestle **[1-TXT]**. Add 500 to 700 microliters of the native lysis buffer, adjusting the volume based on tissue compactness and ease of homogenization **[2]**. Then homogenize the sample on ice by gently moving the pestle up and down and against the abrasive walls until a uniform suspension is obtained **[3]**.

2.2.1. Talent placing a small frozen tissue sample into a micro tissue homogenizer tube. **TXT: Post-mortem brain tissue samples are used**

2.2.2. Talent pipetting native lysis buffer into the tube.

2.2.3. Talent gently moving the pestle up and down to homogenize the tissue on ice.

2.3. Incubate the lysates at 4 degrees Celsius for 30 minutes by placing the vial on a rotation unit **[1]**. Gently mix the samples during incubation through rotation **[2]**.

2.3.1. Talent placing the homogenized lysate vials on a rotation unit inside a cold room or refrigerated incubator.

2.3.2. Close-up shot showing the rotation unit gently rotating the vials during incubation.

2.4. Using a benchtop centrifuge, centrifuge the samples at 13,000 *g* for 10 minutes at 4 degrees Celsius to remove cellular debris **[1]**. Carefully collect the supernatants and transfer them into clear 1.5-milliliter microcentrifuge tubes **[2-TXT]**.

2.4.1. Talent loading the lysate tubes into the centrifuge.

2.4.2. Talent using a pipette to transfer the clear supernatant into fresh microcentrifuge tubes. **TXT: Avoid disturbing the pellet during transfer**

2.5. Determine the total protein concentration in the supernatant using the BCA assay kit according to the manufacturer's instructions **[1]**.

2.5.1. Talent loading of samples into a microplate and reading of absorbance.
Added shot: 2.5.2: Getting reading of the plate

Videographer's Note: CU of 2.5.2 was also shot

2.6. Next, take an aliquot of polyurethane beads directly from the isopropanol stock **[1-TXT]**.

2.6.1. Talent taking the polyurethane or control beads from the stock. **TXT: Add ~30% extra beads to offset loss during washing/handling**

Videographer's Note: Please disregard the clips C112C035 through C037. It is the 1st version of this step but it is not correct. Please use C112C038

2.7. Allow the beads to settle to remove the storage solvent **[1]**. Carefully aspirate the isopropanol **[2]**, then add native lysis buffer **[3]** and fully resuspend the beads by gentle pipetting or inversion **[4]**.

2.7.1. Talent placing the tube on the bench and allowing the beads to settle.

2.7.2. Talent aspirating the isopropanol using a pipette.

2.7.3. Talent adding the appropriate volume of native lysis buffer to the beads.

2.7.4. Talent gently pipetting or inverting the tube to fully resuspend the beads.

2.8. To wash and equilibrate the beads, vortex the tube **[1],** then centrifuge it **[2]**. After that, aspirate the supernatant with a vacuum line fitted with a pipette tip, taking care not to disturb the pellet **[3-TXT]**.

2.8.1. Talent vortexing the bead suspension in the tube.

2.8.2. Talent placing the tube into the centrifuge.

2.8.3. Talent aspirating the supernatant using a vacuum line with a pipette tip, showing care to avoid disturbing the bead pellet. **TXT: Repeat 3x**

Added shot: 2.8.3 CU as a close up version of 2.8.3

2.9. Add binding buffer to the washed beads in an equal ratio to create a uniform working bead slurry **[1]**. Aliquot 40 microliters of polyurethane bead slurry into 1.5-milliliter microcentrifuge tubes using a cut pipette tip for smooth dispensing **[2]**.

2.9.1. Talent pipetting an equal volume of binding buffer into the washed bead pellet and mixing gently.

2.9.2. Talent dispensing 40 microliters of bead slurry into labeled microcentrifuge tubes.

2.10. Then wash the beads three times with native lysis buffer by adding 1 milliliter of buffer to each tube **[1]**. Vortex the tube to resuspend the beads **[2]**, then centrifuge at 10,000 *g* for 1 minute **[3]**, and discard the supernatant by aspiration **[4-TXT]**.

2.10.1. Talent pipetting 1 milliliter of native lysis buffer into the bead-containing tube.

2.10.2. Talent vortexing the tube to resuspend the beads evenly.

2.10.3. Talent placing the tube in the centrifuge.

2.10.4. Talent aspirating the supernatant carefully using a pipette. **TXT: Ensure that the beads are fully resuspended between each wash**

2.11. After the final wash, remove most of the remaining liquid from the tubes, ensuring that the bead pellet remains undisturbed **[1]**.

2.11.1. Talent carefully aspirating the liquid above the pellet with a pipette.

2.12. Add the normalized protein extracts to individual 1.5-milliliter microcentrifuge tubes containing 40 microliters of control bead slurry **[1]**. Adjust the final volume to 250 microliters by adding the appropriate amount of native lysis buffer **[2]**.

2.12.1. Talent pipetting the normalized protein extract into labeled microcentrifuge tubes containing the control bead slurry.

2.12.2. Talent adding native lysis buffer to bring the total volume to 250 microliters.

2.13. Incubate the samples at 4 degrees Celsius for 30 minutes with rotation on an end-over-end rotator operating at 10 to 15 rpm **[1]**.

2.13.1. Talent placing the tubes onto the end-over-end rotator and starting rotation.

2.14. Centrifuge the tubes at 10,000 *g* for 1 minute at 4 degrees Celsius to pellet the control

beads along with aggregated or insoluble proteins **[1]**.

2.14.1. Talent loading the incubated tubes into a centrifuge, setting the speed and temperature, and starting the run.

2.15. Carefully collect the supernatant from the control beads using a 1-milliliter pipette **[1]** and transfer it into fresh 1.5-milliliter tubes containing 40 microliters of washed polyurethane bead slurry **[2]**.

2.15.1. Talent pipetting the supernatant from the control bead tube without disturbing the pellet.
Videographer's Note: Combined 2.15.1 and 2.15.2 into 1 step

2.15.2. Talent transferring the collected supernatant into tubes containing washed polyurethane beads.

2.16. Incubate the samples at 4 degrees Celsius for 3 hours with rotation on an end-over-end rotator **[1]**.

2.16.1. Talent placing the tubes onto the rotator and ensuring continuous gentle rotation.

2.17. After centrifuging the tubes carefully, aspirate the supernatant and wash the beads four times as demonstrated earlier **[1].**

2.17.1. Talent aspirating the supernatant with a pipette.

3. Protein Identification by LC-MS/MS with On-bead Protein Digestion
Demonstrator: Ciara O'Sullivan

3.1. Remove any residual PBS from the tube **[1].** Resuspend the washed beads in 80 microliters of 2 molar urea freshly prepared in 50 millimolar ammonium bicarbonate at pH 8.5 by pipetting or brief vortexing **[2].**

3.1.1. Talent aspirating the remaining phosphate-buffered saline from the tube.

3.1.2. Talent pipetting 80 microliters of freshly prepared 2 molar urea solution into the beads and pipetting it.

3.2. Then, add dithiothreitol to achieve a final concentration of 1 millimolar **[1].**

3.2.1. Talent adding an appropriate amount of DTT solution into the tube containing the resuspended beads.

3.3. After capping the tube, incubate at 37 degrees Celsius for 30 minutes with shaking at

1,100 rpm on a heated orbital shaker **[1-TXT].**

3.3.1. Talent placing the tubes on a heated orbital shaker set to 37 degrees Celsius. **TXT: Ensure the lids are sealed tightly to minimize evaporation**

3.4. Add iodoacetamide to reach a final concentration of 3.67 millimolar **[1].** Incubate the tubes in the dark at room temperature for 45 minutes with shaking at 1,100 rpm **[2].**

3.4.1. Talent adding iodoacetamide solution into the tube.

3.4.2. Talent placing the covered tubes on the shaker.

3.5. Now, add additional dithiothreitol to quench any unreacted iodoacetamide, ensuring a final concentration of 3.67 millimolar and mix gently by pipetting **[1].**

3.5.1. Talent adding the appropriate volume of dithiothreitol solution into the sample tube and mixing it.

3.6. Add 750 nanograms of 0.5 milligram per milliliter mass spectrometry–grade Lys-C *(Lice-Cee)* protease to the sample **[1]**. Incubate the mixture at 37 degrees Celsius for 1 hour with shaking at 1,150 rpm **[2]**.

3.6.1. Talent pipetting the calculated volume of Lys-C protease into the sample tube.

3.6.2. Talent placing the tube on a heated orbital shaker set to 37 degrees Celsius.

3.7. Next, add 750 nanograms of freshly prepared 0.5 milligram per milliliter sequencing-grade trypsin to the sample **[1]**. Incubate the mixture overnight at 37 degrees Celsius with shaking at 1,150 rpm **[2]**.

3.7.1. Talent adding trypsin to the sample.

3.7.2. Talent places the tube in the incubator.

3.8. On the following day, centrifuge the sample at 1,000 to 5,000 *g* for 1 to 5 minutes at room temperature **[1]**. Carefully transfer the supernatant into a fresh 1.5-milliliter microcentrifuge tube using a pipette and discard the beads **[2]**.

3.8.1. Talent placing the tubes in the centrifuge .

3.8.2. Talent transferring the clear supernatant into a new labeled microcentrifuge tube.

Added shot: 3.8.3B to shot final color of samples

3.9. Adjust the pH of the digest to below 3 by adding 50 percent trifluoroacetic acid dropwise **[1]**. Verify the pH using indicator strips **[2]**.

3.9.1. Talent carefully adding trifluoroacetic acid one drop at a time while gently swirling.

3.9.2. Talent using pH indicator strips confirming the solution is below pH 3.

Results

4. Results

4.1. PU-beads captured HSP90 *(H-S-P-Ninety)*, HSC70 *(H-S-C-Seventy)*, and HOP *(Hop)* proteins strongly from the epichaperome-high lysate **[1],** with minimal signal from the epichaperome-low lysate, confirming biological specificity of the probe **[2].**

4.1.1. LAB MEDIA: Figure 2A (middle panel). *Video editor: Highlight the three bands (HSP90, HSC70, HOP) in lane 1 of both "Batch 1 (new)" and "Batch 2 (old)"*

4.1.2. LAB MEDIA: Figure 2A (middle panel). *Video editor: Highlight the three bands (HSP90, HSC70, HOP) in lane 2 of both "Batch 1 (new)" and "Batch 2 (old)"*

4.2. The PU-beads cargo profile showed a rich, high-molecular-weight signal in the PU-beads lane and minimal background in the control-bead lane, confirming successful probe activity **[1].**

4.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the broad and dense banding pattern in the PU-beads lane, and the faint or sparse pattern in the Control-beads lane.*

4.3. Coomassie-stained SDS–PAGE gels showed consistent band distribution across four in-gel–processed samples, confirming successful enrichment of protein complexes from native lysates before mass spectrometry **[1].**

4.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the four lanes labeled PT1 to PT4, showing similar band patterns and intensities.*

4.4. Technical reproducibility was confirmed by principal component analysis, where replicate samples clustered tightly while different samples separated cleanly **[1].**

4.4.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the cluster of duplicate points for each sample, showing minimal distance between paired replicates*.

4.5. Precursor ion intensity distributions showed that most features had coefficients of variation below 20%, with median values between 9.7% and 11.9% across samples, confirming consistent peptide detection and recovery **[1].**

4.5.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the overlaid density plots and the labeled median values for each sample on the left side of the graph.*

4.6. Hierarchical clustering of log-transformed protein abundances revealed strong sample separation and preserved inter-sample variation, with protein intensities spanning from low-abundance to highly enriched proteins **[1].**

4.6.1. LAB MEDIA: Figure 3D. *Video editor: Show color gradient bar; emphasize the range from blue to yellow across samples.*

4.7. Pathway enrichment analysis demonstrated broad annotation coverage across multiple ontologies, including Gene Ontology categories and curated databases such as Reactome, KEGG *(Kegg)*, and WikiPathways *(Wiki-Pathways)* **[1].**

4.7.1. LAB MEDIA: Figure 3E. *Video editor: Pan across the colored sections of the dot plot labeled GO-BP, GO-MF, GO-CC, Reactome, KEGG, and WikiPathways.*

Pronunciation Guide:
Tuberous
Pronunciation link: https://www.merriam-webster.com/dictionary/tuberous
IPA: /ˈtuːbərəs/
Phonetic Spelling: TOO-buh-rus
Sclerosis
Pronunciation link: https://www.merriam-webster.com/dictionary/sclerosis
IPA: /sklə'roʊsɪs/
Phonetic Spelling: skleh-ROH-sis
Interactome
Pronunciation link: https://www.howtopronounce.com/interactome
IPA: /ɪn.tərˈæk.toʊm/
Phonetic Spelling: in-ter-RAK-tohm
Interactomics
Pronunciation link: No confirmed link found
IPA: /ˌɪn.tə.rækˈtɒm.ɪks/ or /ˌɪn.təˌrækˈtoʊmɪks/
Phonetic Spelling: in-ter-rak-TOH-miks
Proteomics
Pronunciation link: https://www.merriam-webster.com/dictionary/proteomics
IPA: /ˌproʊ-tiːˈoʊmɪks/
Phonetic Spelling: proh-tee-OH-miks
Epichaperome
Pronunciation link: https://www.howtopronounce.com/epichaperome
IPA: /ˌɛpɪˈtʃæpəˌroʊm/
Phonetic Spelling: epi-CHAP-uh-rohm
Chaperome
Pronunciation link: No confirmed link found
IPA: /ˈʃæpəˌroʊm/
Phonetic Spelling: SHAP-uh-rohm
Affinity
Pronunciation link: https://www.merriam-webster.com/dictionary/affinity
IPA: /əˈfɪnɪti/
Phonetic Spelling: uh-FIN-i-tee
Capture (as in affinity capture)
Pronunciation link: https://www.merriam-webster.com/dictionary/capture
IPA: /ˈkæp.tʃər/
Phonetic Spelling: KAP-chur
Homogenize
Pronunciation link: https://www.merriam-webster.com/dictionary/homogenize
IPA: /həˈmɑːdʒəˌnaɪz/
Phonetic Spelling: huh-MAH-juh-nize
Incubate
Pronunciation link: https://www.merriam-webster.com/dictionary/incubate

IPA: /ˈɪŋkjəˌbeɪt/
Phonetic Spelling: IN-kyuh-bait
Centrifuge
Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛn.trəˌfjuːʒ/
Phonetic Spelling: SEN-truh-fyoohj