FINAL SCRIPT: APPROVED FOR FILMING

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Title: Experimental Approaches for Biochemical Analysis of Glial Fibrillary Acidic Protein and its Disease-Associated Variants

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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, 200 m apart**

   If **Yes**, how far apart are the locations? Within 200 meters

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: 11
Number of Shots: 22

Introduction

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

INTRODUCTION:

1.1. **Ming-Der Perng:** My research focuses on GFAP changes in affect astrocytes and how their dysfunctions lead to Alexander disease. We share easy-to-follow methods to study GFAP.

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

1.2. **Ming-Der Perng:** Disease-related versions of GFAP are harder to study because they don't assemble properly, tend to clump together, and have unusual modifications.

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

CONCLUSION:

1.3. **Ming-Der Perng:** We still lack a clear picture of how mutant GFAP alters filaments, drives aggregation, and impairs cells. Our protocol directly investigates these mechanisms.

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

1.4. **Ming-Der Perng:** We have developed a standardized method to purify both normal and mutant GFAP, making it much easier to study the protein and its role in disease.

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

1.5. **Ming-Der Perng:** This protocol streamlines preparing normal and mutant GFAP to clarify how filament assembly, aggregation, and modifications contribute to Alexander disease.

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

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Life Sciences and Medicine at the National Tsing Hua University

2. Negative Staining and Transmission Electron Microscopy of Sample Assemblies

Demonstrator: Ni-Hsuan Lin

2.1. To begin, place glow discharge Formvar and carbon-coated copper grids in a Glow Discharge Cleaning system **[1].** Clean the grids for 45 seconds at 20 milliamperes **[2]**. Deliver the assembly mixtures onto the glow-discharged grid **[3-TXT].**

2.1.1. WIDE: Talent placing copper grids into a Glow Discharge Cleaning system.

2.1.2. Talent starting the discharge cycle.

2.1.3. Talent pipetting the assembly mixture onto the prepared grid and letting it sit undisturbed. **TXT: Allow samples to bind to the support film for 60 s**

2.2. Remove excess liquid by wicking the edge of the grid with a piece of blotting paper **[1]**. Then wash the grids with distilled water **[2]**. Stain the grid with 20 microliters of 1 percent uranyl acetate for 60 seconds **[3]**.

2.2.1. Talent gently touching the edge of the grid with blotting paper to wick away the liquid.

2.2.2. Talent washing the grid by gently pipetting distilled water over it and removing excess liquid.

2.2.3. Talent pipetting 20 microliters of 1 percent uranyl acetate to the grid and waiting.

2.3. Remove the excess staining solution **[1]** and allow the grid to air-dry for 30 seconds **[2]**.

2.3.1. Talent wicking away uranyl acetate using blotting paper.

2.3.2. Talent leaving the grid on a clean bench surface to air-dry.

2.4. Examine the prepared grids using a transmission electron microscope in high-resolution mode at an accelerating voltage of 100 kilovolts **[1]**.

2.4.1. Talent places the grid under a TEM and sets voltage.

3. Sequential Buffer Extraction and Ion Exchange Chromatography for GFAP Purification from Rat Brain Tissue

3.1. Extract brain tissues from Alexander disease rats using a Douce homogenizer containing 10 milliliters of TEN *(ten)* buffer **[1]**.

3.1.1. WIDE: Talent placing brain tissues into a Douce homogenizer and adding 10 milliliters of TEN buffer. **TXT: TEN buffer: 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 5 mM EDTA**

3.2. Centrifuge the brain homogenates at 76,000 *g* at 4 degrees Celsius for 20 minutes **[1]**. Then sequentially extract the resulting pellet with 10 milliliters of Triton X-100 buffer, sucrose buffer, high salt buffer and urea buffer **[2]**.

3.2.1. Talent placing the homogenate tubes into the ultracentrifuge.

3.2.2. Talent adding 10 milliliters of buffer to the pellet and mixing.
AUTHORS: Perform the addition of any 1 buffer. Keep the other buffers in labeled tubes in the background of the shot
AND
TEXT ON PLAIN BACKGROUND:
Triton X-100 buffer: 1% (v/v) Triton X-100 in TEN buffer

Sucrose buffer: 0.85 M sucrose and 0.5% (v/v) Triton X-100 in TEN buffer

High salt buffer: 1.5 M KCl and 0.5% (v/v) Triton X-100 in TEN buffer

Urea buffer: 8 M urea, 10 mM Tris-HCl, pH 7.4, and 5 mM EDTA
Video Editor: Please play both shots side by side in a split screen

3.3. Collect the supernatant fraction **[1]** and dialyze it against Q column buffer **[2-TXT]**.

3.3.1. Talent pipetting out the supernatant into a clean dialysis tubing.

3.3.2. Talent immersing the dialysis tubing in Q column buffer and sealing it. **TXT: Q column buffer: 6 M urea, 10 mM Tris-HCl, pH 8, 5 mM EDTA, and 14.4 mM β-mercaptoethanol**

3.4. Now, load the dialyzed sample onto an anion exchange column in an NGC Chromatography System **[1]**. Elute the bound proteins using a linear gradient of 0 to 0.5 molar sodium chloride in Q buffer at a flow rate of 1 milliliter per minute **[2]**.

3.4.1. Talent attaching the dialysis sample to the NGC Chromatography System and loading it into the anion exchange column.

3.4.2. Shot of the NGC system running the sodium chloride gradient from 0 to 0.5 molar at 1 milliliter per minute.
Videographer: Please capture the screen of the instrument for this shot

3.5. Pool the glial fibrillary acidic protein-containing fractions **[1]** and dialyze them against S column buffer **[2-TXT]**.

3.5.1. Talent combining the appropriate fractions into one container.

3.5.2. Talent placing the combined fractions into dialysis tubing and submerging it in S column buffer. **TXT: S column buffer: 6 M urea, 20 mM MES, pH 6, and 14.4**

mM β-mercaptoethanol

3.6. Now apply the dialyzed sample to a cation exchange column **[1]** and elute the bound proteins using a linear gradient of 0 to 1 molar sodium chloride in S buffer at a flow rate of 1 milliliter per minute **[2]**.

3.6.1. Talent connecting the dialysate to the cation exchange column on the NGC system.

3.6.2. Shot of the sodium chloride gradient being applied from 0 to 1 molar at 1 milliliter per minute in the S buffer.
Videographer: Please capture the screen of the instrument for this shot

3.7. Analyze the eluted fractions by SDS-PAGE and Coomassie blue staining **[1]**. Collect those fractions containing purified glial fibrillary acidic protein **[2]**.

3.7.1. Talent loading eluted samples onto SDS-PAGE gel and staining with Coomassie blue.

3.7.2. Talent identifying and collecting the purified glial fibrillary acidic protein bands from the gel.

Results

4. Results

4.1. Wild-type GFAP *(G-F-A-P)* formed uniform 10-nanometer filaments *in vitro* **[1]**, whereas the R239H *(R-Two-Three-Nine-H)* mutant produced dense, aggregated structures **[2]**.

4.1.1. LAB MEDIA: Figure 2A.

4.1.2. LAB MEDIA: Figure 2B.

4.2. Under low-speed centrifugation, most wild-type GFAP remained in the supernatant **[1]**, while the majority of R239H GFAP was found in the pellet fraction **[2]**. Under high-speed centrifugation, nearly all wild-type GFAP and R239H GFAP were found in the pellet fractions, confirming efficient sedimentation **[3]**.

4.2.1. LAB MEDIA: Figure 2C. *Video editor: Highlight lane 1 (S) and lane 2 (P) for "WT"*

4.2.2. LAB MEDIA: Figure 2C. *Video editor: Highlight lane 4 (P) for "R239H"*

4.2.3. LAB MEDIA: Figure 2D. *Video editor: Highlight lanes 2 and 4 labeled "P" for both WT and R239H.*

4.3. Following hydrogen peroxide treatment, wild-type GFAP formed multiple high molecular weight bands **[1]**, which were reduced to monomers by DTT **[2]**.

4.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight lane 2 under "WT GFAP"*

4.3.2. LAB MEDIA: Figure 3A. *Video editor: Highlight lanes 3 and 4 under "WT GFAP"*

4.4. In the absence of oxidative stress, R239H GFAP formed a high molecular weight band around 180 kilodaltons **[1]**, which required high concentrations of dithiothreitol *(D-T-T)* to convert to a monomeric form **[2]**.

4.4.1. LAB MEDIA: Figure 3A. *Video editor: Highlight lane 5 under "R239H GFAP" showing a band near 180 kDa.*

4.4.2. LAB MEDIA: Figure 3A. *Video editor: Highlight lane 8 showing a single prominent monomeric band at ~50 kDa.*

4.5. Quantification confirmed that a higher proportion of R239H GFAP remained in high molecular weight forms compared to wild-type GFAP **[1]**.

4.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the higher light gray bar values (HMG GFAP) for R239H lanes*

4.6. Immunoblotting confirmed that native GFAP from R237H rat brain was ubiquitinated, as shown by overlapping signals for GFAP and ubiquitin **[1]**.

4.6.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the merged image (lane 3)*

4.7. Electron microscopy revealed that GFAP from Alexander disease rat brain failed to form filaments **[1]**.

4.7.1. LAB MEDIA: Figure 5A.

4.8. In low-speed centrifugation, most GFAP from Alexander disease rat brains remained in the supernatant **[1]**, while under high-speed centrifugation it sedimented into the pellet fraction **[2]**.

4.8.1. LAB MEDIA: Figure 5B. *Video editor: Highlight lane 1 (S) for LS and lane 2 (P)*

4.8.2. LAB MEDIA: Figure 5B. *Video editor: Highlight lane 4 (P) for HS*

4.9. Quantification confirmed a shift in GFAP distribution from supernatant to pellet between low-speed and high-speed centrifugation **[1]**.

4.9.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the black bars labeled "P" in lanes 2 and 4*

1. Formvar
   Pronunciation link: No confirmed link found
   IPA: /ˈfɔːrmˌvɑr/
   Phonetic spelling: FORM-var
2. uranyl
   Pronunciation link: https://www.merriam-webster.com/dictionary/uranyl
   IPA: /jʊˈreɪnəl/
   Phonetic spelling: yoo-RAY-nuhl
3. acetate
   Pronunciation link: https://www.merriam-webster.com/dictionary/acetate
   IPA: /ˈæsəˌteɪt/
   Phonetic spelling: AS-uh-tayt
4. ultracentrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/ultracentrifuge
   IPA: /ˌʌltrəˈsɛntrəfjuːdʒ/
   Phonetic spelling: UL-truh-SEN-truh-fyoog
5. Triton
   Pronunciation link: https://www.merriam-webster.com/dictionary/triton
   IPA: /ˈtraɪtən/
   Phonetic spelling: TRY-tuhn
6. sucrose
   Pronunciation link: https://www.merriam-webster.com/dictionary/sucrose
   IPA: /ˈsuːˌkroʊs/
   Phonetic spelling: SOO-krohs
7. urea
   Pronunciation link: https://www.merriam-webster.com/dictionary/urea

IPA: /juˈriːə/
Phonetic spelling: yoo-REE-uh

8. β-mercaptoethanol
   Pronunciation link: No confirmed link found
   IPA (approx.): /ˌbeɪtə-mɜrkæpˌtoʊˈɛθənɒl/
   Phonetic spelling: BAY-tuh-mer-cap-toh-ETH-uhnol
9. chromatography
   Pronunciation link: https://www.merriam-webster.com/dictionary/chromatography
   IPA: /ˌkroʊməˈtɑːgrəfi/
   Phonetic spelling: kroh-muh-TAH-gruh-fee
10. anion
    Pronunciation link: https://www.merriam-webster.com/dictionary/anion
    IPA: /ˈeɪˌnaɪən/
    Phonetic spelling: AY-ny-ən
11. cation
    Pronunciation link: https://www.merriam-webster.com/dictionary/cation
    IPA: /ˈkæˌtaɪən/
    Phonetic spelling: KAT-y-ən
12. Coomassie
    Pronunciation link: https://www.merriam-webster.com/dictionary/coomassie
    IPA: /ˈkuːməˌʃiː/
    Phonetic spelling: KOO-muh-shee
13. glial
    Pronunciation link: https://www.merriam-webster.com/dictionary/glial
    IPA: /ˈglaɪəl/
    Phonetic spelling: GLY-uhl
14. fibrillary
    Pronunciation link: https://www.merriam-webster.com/dictionary/fibrillary
    IPA: /fəˈbrɪlәri/
    Phonetic spelling: fuh-BRIH-luh-ree
15. acidic
    Pronunciation link: https://www.merriam-webster.com/dictionary/acidic
    IPA: /əˈsɪdɪk/
    Phonetic spelling: uh-SID-ik
16. protein
    Pronunciation link: https://www.merriam-webster.com/dictionary/protein
    IPA: /ˈproʊˌtiːn/
    Phonetic spelling: PROH-teen
17. kilodalton
    Pronunciation link: https://www.merriam-webster.com/dictionary/kilodalton
    IPA: /ˌkaɪloʊˈdɑltən/
    Phonetic spelling: KY-loh-DAL-tuhn
18. dithiothreitol
    Pronunciation link: No confirmed link found

IPA (approx.): /ˌdaɪθaɪoʊˈθriːtɒl/
Phonetic spelling: dye-THY-oh-THREE-tol

19. ubiquitinated
Pronunciation link: No confirmed link found
IPA (approx.): /juːˌbɪkwɪtɪˈneɪtɪd/
Phonetic spelling: yoo-BIK-wi-tuh-NAY-tid

20. supernatant
Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˌsuːpərˈneɪtənt/
Phonetic spelling: soo-per-NAY-tuhnt