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Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21920398>

Title: A Split-Luciferase Complementation Assay for Temporally Resolved Measurement of Tau Clearance in Microglia

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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**, shots 3.10.1, 3.10.2, 3.11.1, 3.11.2, and 3.11.3 **(Authors: We have modified this response, as there are SCREEN-labeled shots in the 3rd section.)**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](#). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

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

- 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: 13

Number of Shots: 37

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Daya Mena:** Our research investigates the unknown cellular mechanisms regulating tau clearance and spread in the brain, a major driver of neurodegenerative disease.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Daya Mena:** This protocol measures tau clearance across various cellular systems, specifically interrogating tau fibril and monomer clearance in microglia.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Daya Mena:** Using this protocol, researchers can study cellular pathways that regulate tau clearance or degradation across different cell types and conditions.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Daya Mena:** Complete trypsinization to fully remove extracellular tau is critical; incomplete removal or cell loss distorts the clearance signal.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Daya Mena:** Future work could extend this assay to additional cell types like astrocytes and oligodendrocytes, or tag endogenous tau via CRISPR at the MAPT locus.

- 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.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Tau-SLP Protein and iPSC-Derived iTF-Microglia Culture

Demonstrator: Daya Mena

2.1. To begin, use a standard cloning method, such as Gibson assembly, to append the split-luciferase peptide, or SLP (*S-L-P*) tag, onto a Tau (*Tow*) construct to create a tagged Tau, or Tau-SLP construct [1-TXT]. After Tau-SLP expression and purification, verify the purity by SDS-PAGE (*S-D-S-Page*) [2]. Then, remove the bacterial endotoxin before using it with microglial cells [3]. Quantify the concentration of the tagged Tau construct using a standard BCA assay [4].

2.1.1. WIDE: Talent assembling the tagged Tau construct using the cloning reagents. **TXT: SLP tag Sequence: VSGWRLFKKIS** ***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Representative shot of talent checking Tau-SLP purity by SDS-PAGE.

2.1.3. Talent performing bacterial endotoxin removal from the purified protein.

2.1.4. Representative shot of talent measuring the protein concentration using a bicinchoninic acid assay. **NOTE: 2.1.4 is slated as 2.2.1**

2.2. Validate the functional luminescence of the Tau-SLP construct by creating a standard curve using a commercially available luciferase substrate and a complementation reagent, following the manufacturer's instructions [1]. Differentiate an induced pluripotent stem cell, or iPSC (*I-P-S-C*) line harboring 6 doxycycline-inducible transcription factors to produce induced transcription-factor microglia-like, or iTF-Microglia (*I-T-F-Microglia*) cells using a validated protocol [2], culturing the cells in the appropriate medium [3] at 37 degrees Celsius with 5 percent carbon dioxide [4].

2.2.1. Talent preparing the standard curve using the luciferase substrate and complementation reagent.

2.2.2. Talent adding culture medium to the microglial cells for culturing.

2.2.3. TEXT ON A PLAIN BACKGROUND

Culture Media:

- | | |
|---|----------|
| • Advanced DMEM/F12 with Antibiotic-Antimycotic | 1X |
| • GlutaMAX | 1X |
| • Doxycycline | 2ug/ml |
| • Human IL-34 | 100ng/mL |

- Human GM-CSF 10ng/mL
- M-CSF 50ng/mL
- Human TGF-B1 50ng/mL

2.2.4. Talent placing the culture dish containing microglial cells into the incubator.

3. Tau Clearance Assay

Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up

3.1. Seed 100,000 cells per well using 250 microliters of medium in a 24-well plate in triplicate per condition [1]. Incubate the plate overnight at 37 degrees Celsius and 5 percent oxygen [2].

3.1.1. Talent plating the cells into the 24-well plate.

3.1.2. Talent placing the plate into the incubator.

3.2. Remove the culture medium [1], wash the cells once with 250 microliters of PBS [2], and replace it with 250 microliters of fresh culture medium [3].

3.2.1. Talent aspirating the culture medium from the wells.

3.2.2. Talent washing the cells with phosphate-buffered saline.

3.2.3. Talent adding fresh culture medium to the wells.

3.3. To measure Tau-SLP clearance temporally, expose the cells to the Tau-SLP monomer or fibril construct-containing media in a staggered manner [1] so that all wells are exposed for one hour and are harvested simultaneously while differing only in the clearance time over a 5-hour timeline [2]. Include wells that receive no treatment with the tagged Tau construct for background subtraction [3].

3.3.1. Talent arranging the experimental groups for staggered treatment.

3.3.2. LAB MEDIA: Figure 1B. *Video Editor: Please emphasize the text "Tau-SLP Incubation (1h)" sequentially across 4h, 2h, 0h timepoints on the vertical axis.*

3.3.3. LAB MEDIA: Figure 1C. *Video Editor: Please emphasize the top row grey color wells.*

3.4. Add 100 nanomolar Tau-SLP-containing medium to the 4-hour clearance group at Hour 0 of treatment initiation [1]. At the end of the 1-hour internalization period, wash the treated cells 3 times with PBS to remove the extracellular Tau-SLP [2]. Then add Tau-SLP-free culture medium and incubate for the designated clearance period [3]. Next, at Hour 2, add the construct-containing medium to the 2-hour clearance group, then replace it with Tau-SLP-free medium at Hour 3, as previously demonstrated [4]. Finally,

at Hour 4, add the construct-containing medium to the 0-hour clearance group and incubate for 1 hour prior to simultaneous harvest [5].

3.4.1. Talent adding the tagged Tau construct-containing medium to the 4-hour clearance group.

3.4.2. Talent washing the cells with PBS.

3.4.3. Talent replacing the Tau-SLP media with fresh media.

3.4.4. Talent adding the tagged Tau construct-containing medium to the 2-hour clearance group.

3.4.5. Talent placing the treated plate in the incubator. NOTE: 3.4.5 did not shoot, same as 3.1.2

3.5. Following all clearance time points, remove the medium from each well [1] and wash with 250 microliters of PBS [2].

3.5.1. Talent aspirating the medium from the wells.

3.5.2. Talent washing the wells with phosphate-buffered saline.

3.6. Treat each well with 250 microliters of 0.25 percent Trypsin-EDTA for 5 minutes at 37 degrees Celsius [1]. Neutralize each well with 250 microliters of trypsin inhibitor at 5 milligrams per milliliter [2].

3.6.1. Talent adding Trypsin-EDTA to the wells.

3.6.2. Talent adding the trypsin inhibitor to each well.

3.7. Collect the contents of each well into an appropriately labeled 1.5-milliliter microcentrifuge tube [1]. Then, pellet the cells by centrifugation at 500 *g* for 5 minutes [2].

3.7.1. Talent transferring each sample into a labeled microcentrifuge tube.

3.7.2. Talent placing the tubes into the centrifuge.

3.8. Prepare a sufficient volume of lysis buffer containing the luciferase substrate and complementation components [1]. Use an aspirating pipette to remove the supernatant [2]. Resuspend the cell pellet in 50 microliters of the prepared lysis buffer to produce the split-luciferase complementation complex that generates luminescence [3].

3.8.1. Talent preparing the lysis buffer.

3.8.2. Talent removing the supernatant with an aspirating pipette.

3.8.3. Talent resuspending the cell pellet in the lysis buffer. NOTE: 3.8.3 was shot as part of 3.8.1

3.9. Immediately, transfer the lysed cells to a white half-volume 96-well plate [1]. Read the luminescence on a microplate reader after orbital shaking for 10 seconds at room

temperature [2].

3.9.1. Talent transferring the lysed cells into the white half-volume 96-well plate.

3.9.2. Talent placing the plate into the microplate reader.

3.10. Determine the normalized Tau-SLP abundance by subtracting the mean luminescence value obtained from untreated wells [1]. Normalize each value to the mean background-subtracted signal of the 0-hour clearance group [2].

3.10.1. SCREEN: Screen-Recording-2026-07-21-at-3.42.04-PM.mov: 00:04-00:16

3.10.2. SCREEN: Screen-Recording-2026-07-21-at-3.42.04-PM.mov: 00:20-00:27

3.11. Plot the normalized Tau-SLP abundance as a function of clearance time. Then, calculate the Tau-SLP percentage clearance [1].

3.11.1. SCREEN: Screen-Recording-2026-07-21-at-3.42.04-PM.mov: 00:28-00:35

Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up

Results

4. Results

- 4.1. Monomeric Tau-SLP conjugates were completely cleared in iTF-Microglia within 60 minutes [1], while fibrillar Tau-SLP conjugates persisted over 240 minutes, indicating the sensitivity of the luminescence-based assay to detect differences in handling of Tau-SLP species by microglia [2].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video Editor: Please emphasize the blue line ending at X-axis value 60.*
 - 4.1.2. LAB MEDIA: Figure 2A. *Video Editor: Please emphasize the red line ending at X-axis value 240.*
- 4.2. Additional assessment by comparing the clearance of monomeric Tau-SLP conjugate against a mutant tau-SLP conjugate showed significantly reduced clearance kinetics of mutant Tau-SLP at only short clearance time points of 15 minutes [1].
 - 4.2.1. LAB MEDIA: Figure 2B. *Video Editor: Please emphasize the height of the green bar compared to the blue bar at X-axis values t=15m*
- 4.3. Cross-validation of Tau-SLP clearance using lysosomal disruptor, L-Leucyl-L-Leucine methyl ester showed significantly increased steady state levels of Tau-SLP at zero-minute clearance time [1], alongside a significantly reduced rate of degradation, characterized by a 4-fold increase in Tau-SLP remaining after a 60-minute clearance window [2].
 - 4.3.1. LAB MEDIA: Figure 3A. *Video Editor: Please emphasize the height of violet color bar against the blue bar.*
 - 4.3.2. LAB MEDIA: Figure 3B. *Video Editor: Please emphasize the heights of blue and violet bars at t=60m X-axis value.*
- 4.4. Comparison of Tau-SLP clearance between iPSC-derived neurons and microglia revealed reduced clearance kinetics for both monomeric [1] and fibrillar Tau-SLP conjugates in neuronal cells [2].
 - 4.4.1. LAB MEDIA: Figure 4A. *Video Editor: Please emphasize the height of the violet line at X-axis values 30 and 60 corresponding to the blue line.*
 - 4.4.2. LAB MEDIA: Figure 4B. *Video Editor: Please emphasize the height of the red line at X-axis values 120 and 240 corresponding to the pink line.*

1. Gibson assembly
Pronunciation link: No confirmed link found
IPA: /'gɪb.sən ə'sem.bli/
Phonetic Spelling: gib·suhn uh·sem·blee
2. Split-luciferase
Pronunciation link: No confirmed link found
IPA: /splɪt lu:'sɪf.ə'reɪs/
Phonetic Spelling: split loo·sif·uh·rays
3. Luciferase
Pronunciation link: <https://www.merriam-webster.com/dictionary/luciferase>
IPA: /lu:'sɪf.ə'reɪs/
Phonetic Spelling: loo·sif·uh·rays
4. Peptide
Pronunciation link: <https://www.merriam-webster.com/dictionary/peptide>
IPA: /'pep.tɪd/
Phonetic Spelling: pep·tyde
5. Tau
Pronunciation link: <https://www.merriam-webster.com/dictionary/tau>
IPA: /taʊ/
Phonetic Spelling: tow
6. SDS-PAGE
Pronunciation link: No confirmed link found
IPA: /,es.di:es 'peɪdʒ/
Phonetic Spelling: ess·dee·ess payj
7. Endotoxin
Pronunciation link: <https://www.merriam-webster.com/dictionary/endotoxin>
IPA: /,en.doʊ'tɑ:k.sɪn/
Phonetic Spelling: en·doh·tok·sin
8. Microglial
Pronunciation link: <https://www.merriam-webster.com/dictionary/microglia>
IPA: /maɪ'kra:g.li.əl/
Phonetic Spelling: my·krog·lee·uhl
9. Bicinchoninic acid
Pronunciation link: No confirmed link found
IPA: /,baɪ.sɪn.kə'nɪn.ɪk 'æs.ɪd/
Phonetic Spelling: bye·sin·kuh·nin·ik ass·id
10. Luminescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/luminescence>
IPA: /,lu:.mə'nes.əns/
Phonetic Spelling: loo·muh·ness·uhns
11. Complementation
Pronunciation link: <https://www.merriam-webster.com/dictionary/complementation>
IPA: /,kɑ:m.plə.men'teɪ.ʃən/
Phonetic Spelling: kom·pluh·men·tay·shuhn

12. Pluripotent
Pronunciation link: <https://www.merriam-webster.com/dictionary/pluripotent>
IPA: /ˌplʊr.əˈpʊ.tənt/
Phonetic Spelling: plur·uh·poh·tuht
13. Doxycycline
Pronunciation link: <https://www.merriam-webster.com/dictionary/doxycycline>
IPA: /ˌdɒ.ki.siˈsaɪ.kliːn/
Phonetic Spelling: dok·see·sy·kleen
14. Transcription factor
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription%20factor>
IPA: /trænˈskɹɪp.ʃən ˈfæk.tər/
Phonetic Spelling: tran·skrip·shuhn fak·ter
15. Antibiotic-antimycotic
Pronunciation link: No confirmed link found
IPA: /ˌæn.ti.baɪˈɑː.tɪk ˌæn.ti.maɪˈkɑː.tɪk/
Phonetic Spelling: an·tee·bye·ot·ik an·tee·my·kot·ik
16. Triplicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/triplicate>
IPA: /ˈtrɪp.lɪ.kət/
Phonetic Spelling: trip·lih·kuht
17. Phosphate-buffered saline
Pronunciation link: No confirmed link found
IPA: /ˈfɑːs.feɪt ˈbʌf.əd ˈseɪ.liːn/
Phonetic Spelling: foss·fayt buf·erd say·leen
18. Monomer
Pronunciation link: <https://www.merriam-webster.com/dictionary/monomer>
IPA: /ˈmɑː.nə.mər/
Phonetic Spelling: mon·uh·mer
19. Fibril
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibril>
IPA: /ˈfaɪ.brəl/
Phonetic Spelling: fy·bruhl
20. Internalization
Pronunciation link: <https://www.merriam-webster.com/dictionary/internalization>
IPA: /ɪnˈtɜː.nəl.əˈzeɪ.ʃən/
Phonetic Spelling: in·ter·nuh·luh·zay·shuhn
21. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /ˈtrɪp.sɪn/
Phonetic Spelling: trip·sin
22. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /ˌmaɪ.kroʊˈsen.trəˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj

23. Lysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>
IPA: /'laɪ.sɪs/
Phonetic Spelling: ly·sis
24. Fibrillar
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrillar>
IPA: /'faɪ.brə.lə/
Phonetic Spelling: fy·bru·ler
25. Lysosomal
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysosomal>
IPA: /,laɪ.sə'soʊ.məl/
Phonetic Spelling: ly·suh·soh·muhl
26. L-Leucyl-L-Leucine methyl ester
Pronunciation link: No confirmed link found
IPA: /el 'luː.sɪl el 'luː.sɪn 'meθ.əl 'es.tə/
Phonetic Spelling: el loo·sil el loo·seen meth·uhl es·ter
27. Degradation
Pronunciation link: <https://www.merriam-webster.com/dictionary/degradation>
IPA: /,deg.rə'deɪ.ʃən/
Phonetic Spelling: deg·ruh·day·shuhn
28. Neuronal
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuronal>
IPA: /nʊ'roʊ.nəl/
Phonetic Spelling: noo·roh·nuhl