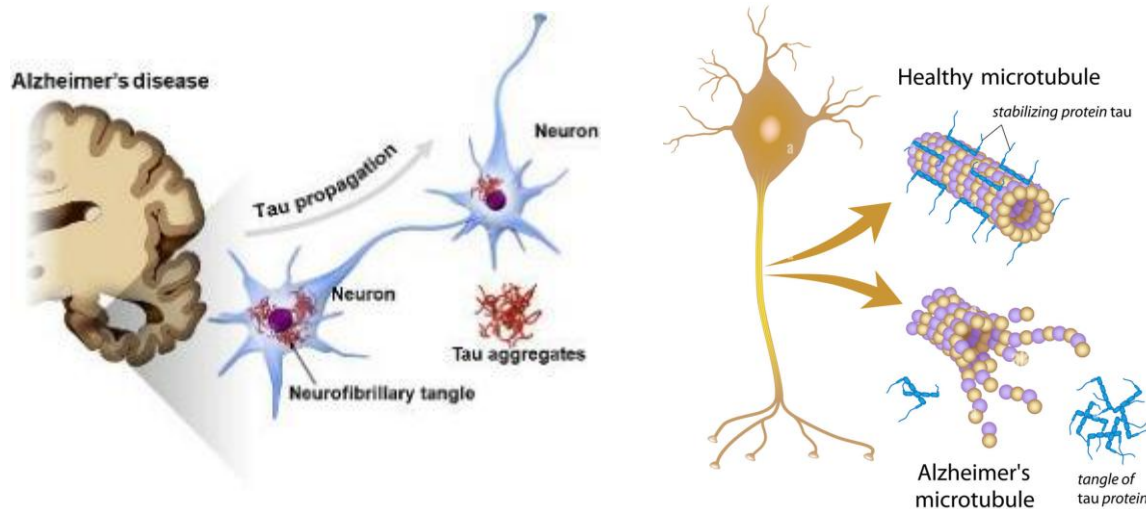


Synthesis and Purification of Peptide Trimers for Tau R3 Inhibition and Disaggregation

Fall 2025, Undergraduate Laboratory Internship
Laboratory of Chemical Biology (Prof. YoungSoo Kim)
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Experimental Objectives

- **Synthesis and Purification:** Synthesize five candidate peptide trimers designed to inhibit and disaggregate TauR3 protein—a primary pathological feature of Alzheimer's Disease—using Solid-Phase Peptide Synthesis (SPPS), followed by HPLC purification.
- **Efficacy Testing:** Conduct Inhibition and Disaggregation Assays on the synthesized peptide trimers to evaluate their impact on TauR3.



Theoretical Background: TauR3 Protein

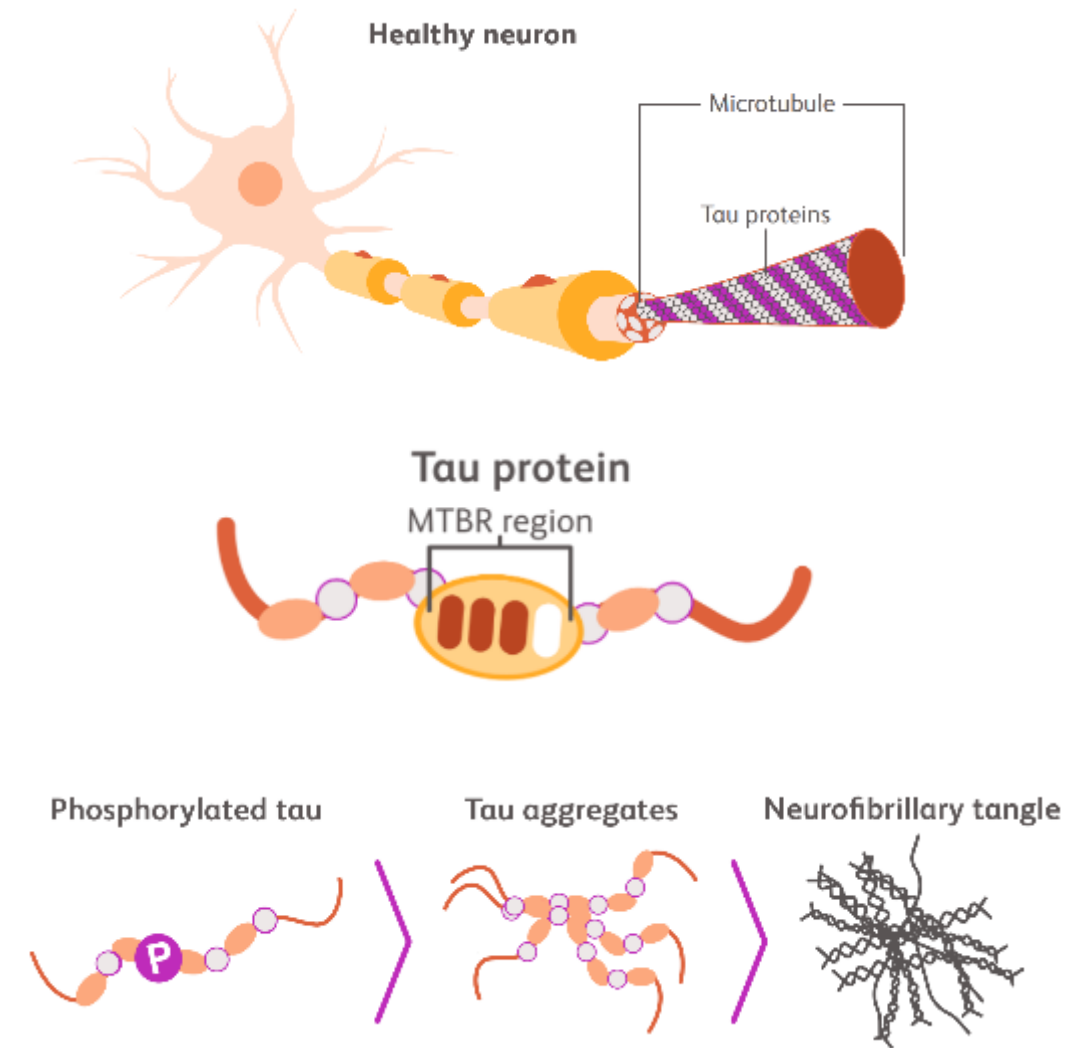
Function: Tau proteins play a critical role in stabilizing microtubules within brain cells (neurons).

Structure (MTBR): The region where the tau protein binds to microtubules is called the Microtubule Binding Repeat (MTBR). Tau variants are classified based on these repeats:

- **3R Tau** (Tau R3): Contains three binding repeats.
- **4R Tau**: Contains four binding repeats.

In Alzheimer's Disease, 3R and 4R tau proteins typically aggregate in an approximate 1:1 ratio.

Pathology: The Neurofibrillary Tangles (NFTs) found in the brains of Alzheimer's patients consist of "Paired Helical Filaments" (PHFs) formed by the co-aggregation of TauR3 and 4R tau.



Pathological Mechanism

Normal **TauR3** is essential for brain function; however, in the environment of Alzheimer's Disease, the following degenerative processes occur:

1. **Hyperphosphorylation:** Triggered by factors such as Amyloid-beta ($A\beta$), phosphate groups excessively attach to the tau protein.
2. **Microtubule Dissociation:** Hyperphosphorylated TauR3 detaches from the microtubules. As a result, the microtubules—which serve as the structural scaffold of the neuron—collapse, leading to the failure of the intracellular transport system.
3. **Formation of Toxic Aggregates:** The dissociated Tau R3 proteins entangle to form oligomers, which eventually develop into neurofibrillary tangles (NFTs), ultimately causing neuronal cell death.

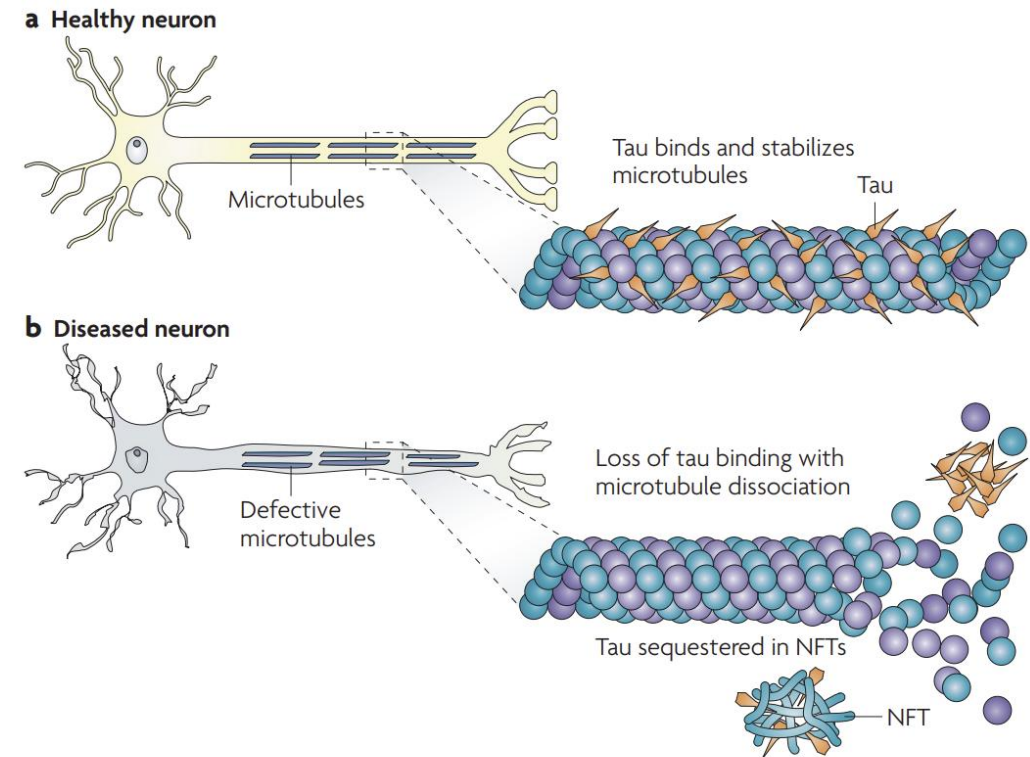


Figure 2 | **Tau in healthy neurons and in tauopathies.** **a** | Tau (also known as MAPT) facilitates microtubule stabilization within cells and is particularly abundant in neurons. Microtubules serve as 'tracks' that are essential for normal trafficking of cellular cargo along the lengthy axonal projections of neurons. **b** | It is thought that tau function is compromised in Alzheimer's disease and other tauopathies. This probably results from both tau hyperphosphorylation, which reduces the binding of tau to microtubules, and the sequestration of hyperphosphorylated tau into neurofibrillary tangles (NFTs), which reduces the amount of tau that is available to bind microtubules. The loss of tau function leads to microtubule instability and reduced axonal transport, which could contribute to neuropathology.

Research Rationale

- 1. D-Amino Acid Peptides:** Short D-amino acid peptides can bind to tau fibrils, facilitating their disaggregation and inhibition.
- 2. Proteolytic Stability:** Unlike standard L-amino acid peptides, D-amino acid peptides are not easily degraded by endogenous enzymes. This high metabolic stability allows them to remain in the bloodstream or brain for longer durations, effectively targeting specific sites on the tau protein.
- 3. Therapeutic Advantages:** Compared to antibody-based therapeutics, D-peptides are smaller in molecular size, which reduces the risk of an immune response and potentially increases the efficiency of crossing the Blood-Brain Barrier (BBB).

Hou K, Ge P, Sawaya MR, Lutter L, Dolinsky JL, Yang Y, Jiang YX, Boyer DR, Cheng X, Pi J, Zhang J. How short peptides disassemble tau fibrils in Alzheimer's disease. *Nature*. 2025 Aug 28;644(8078):1020-7.

Aggidis A, Devitt G, Zhang Y, Chatterjee S, Townsend D, Fullwood NJ, Ortega ER, Tarutani A, Hasegawa M, Cooper A, Williamson P. A novel peptide-based tau aggregation inhibitor as a potential therapeutic for Alzheimer's disease and other tauopathies. *Alzheimer's & Dementia*. 2024 Nov;20(11):7788-804.

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How short peptides disassemble tau fibrils in Alzheimer's disease

[Ke Hou](#) , [Peng Ge](#), [Michael R. Sawaya](#), [Liisa Lutter](#), [Joshua L. Dolinsky](#), [Yuan Yang](#), [Yi Xiao Jiang](#), [David R. Boyer](#), [Xinyi Cheng](#), [Justin Pi](#), [Jeffrey Zhang](#), [Jiahui Lu](#), [Romany Abskharon](#), [Shixin Yang](#), [Zhiheng Yu](#), [Juli Feigon](#) & [David S. Eisenberg](#) 



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A novel peptide-based tau aggregation inhibitor as a potential therapeutic for Alzheimer's disease and other tauopathies

[Anthony Aggidis](#) , [George Devitt](#), [Yongrui Zhang](#), [Shreyasi Chatterjee](#), [David Townsend](#), [Nigel J. Fullwood](#), [Eva Ruiz Ortega](#), [Airi Tarutani](#), [Masato Hasegawa](#), [Amber Cooper](#) ... See all authors ▾

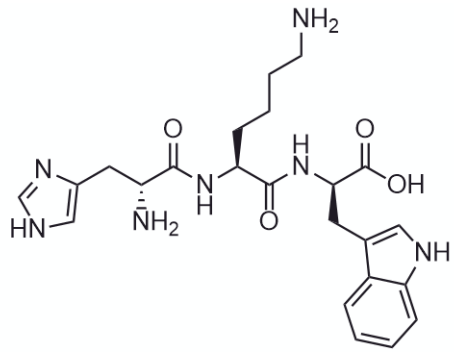
First published: 03 October 2024 | <https://doi.org/10.1002/alz.14246> |  VIEW METRICS

Co-authors David Allsop and Nigel J. Fullwood are now deceased.

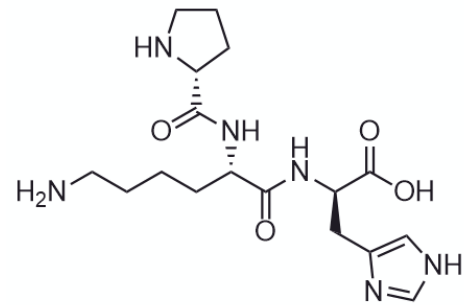


Experimental Procedure

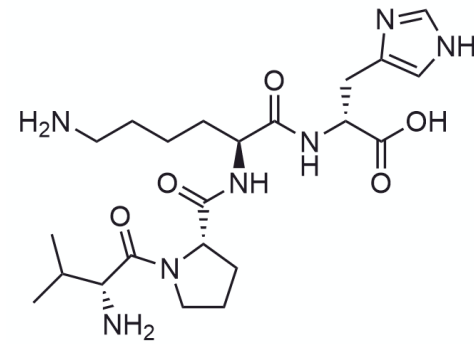
Five different short peptide trimers will be synthesized using the SPPS (Solid-Phase Peptide Synthesis) method, followed by Tau R3 Inhibition and Disaggregation Assays to evaluate their efficacy.



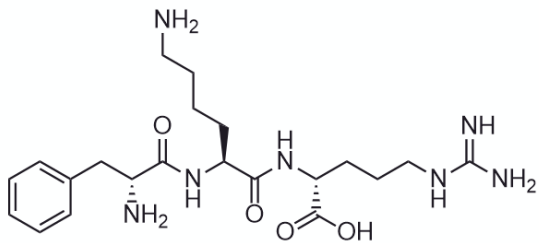
D-His-Lys-D-Trp



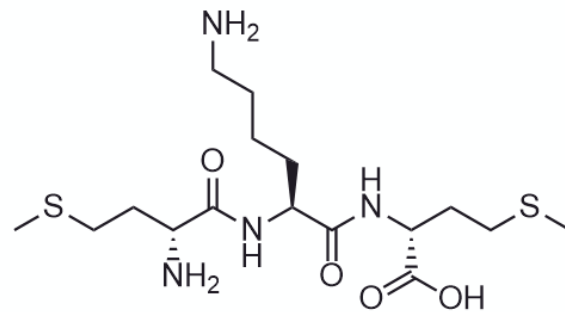
D-Pro-Lys-D-His



D-Val-Pro-Lys-D-His



D-Phe-Lys-D-Arg



D-Met-Lys-D-Met

X_DK(X_D) Peptide Trimer Synthesis

- Scale : 0.5 mmol
- Resin : Wang Resin (0.85 mmol/g scale)
- L form amino acid

1. Resin swelling (4 hr)

- Wang resin (0.85 mmol/g), Fmoc-Lys(Alloc)-OH
- 0.5 mmol

2. Fmoc-Lys(Alloc)-OH (symmetric anhydride) (2 hr)

- Amino acid 10 eq. + DIC 5 eq. + DMAP (catalyst)

3. Fmoc deprotection (5 min + 10 min)

- 20% piperidine (0.1 M oxyma), double deprotection

4. Amino acid (aa1) coupling (40 min)

- Amino acid 5 eq. (Dissolve in 10 mL DMF) + DIC 5 eq. + Oxyma (DIEA) 5 eq. (10 mL)

5. Fmoc deprotection (5 min + 10 min)

- 20% piperidine (0.1 M oxyma), double deprotection



X_DK(X_D) Peptide Trimer Synthesis

- Scale : 0.5 mmol
- Resin : Wang Resin (0.85 mmol/g scale)
- L form amino acid

6. Boc₂O protection (18 hr)

- Boc₂O 1 mL + DIEA 0.01 mL + DMF 2 mL

7. Alloc deprotection (2 hr)

- 0.25 eq. Pd(PPh₃)₄ + 24 eq. DMBA (135.21) in 20 mL DCM

8. 20% Piperidine washing (2 min)

9. AA2 Coupling (40 min)

10. Fmoc deprotection (5 min + 5 min)

11. Cleavage & Ether precipitation (3.5 hr) total 4 mL

- TFA : TIS : DODT : DW = 92.5 : 2.5 : 2.5 : 2.5



HPLC Peptide Purification

1. Solvent, Sample Preparation

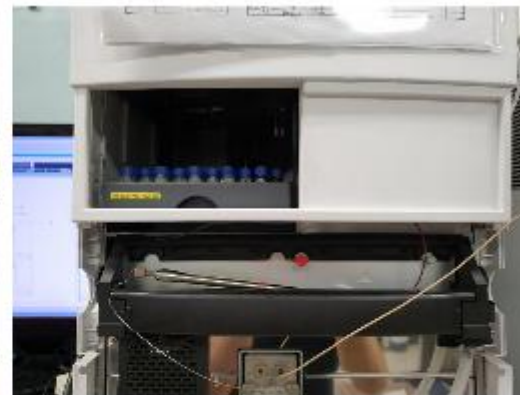
- Solvent A: HPLC-grade H₂O + 0.1% Trifluoroacetic acid (TFA)
- Solvent B: HPLC-grade Acetonitrile (ACN) + 0.1% TFA
- Sample: Dissolve crude peptide in Solvent A (use <10% ACN or DMSO if needed for solubility).
- Filtration: Filter sample through a 0.22 µm PVDF membrane filter.

2. Column Equilibration (Pre-Injection)

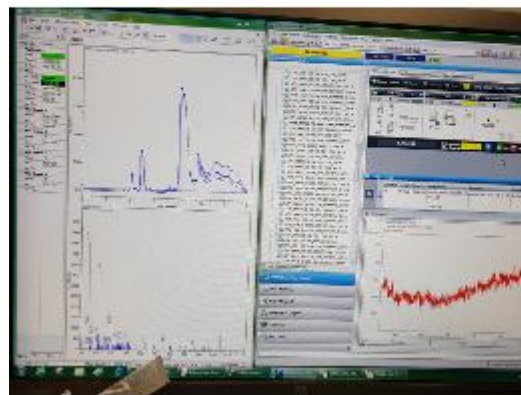
- Set pump to isocratic mode at method starting condition (95% A / 5% B)
- Flush 10-20 column volumes of starting mobile phase

3. Analytical Scouting (Identify peak retention time)

- Column: C18, 4.6 x 250 mm, 5 µm
- Flow Rate: 1.0 mL/min
- Detection: 220 nm (Backbone), 280 nm (Trp/Tyr/Phe)
- Gradient:
 - 0.0 min → 5% B
 - 30.0 min → 95% B
- Objective: Note the retention time (t_R) of the target peak.



Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	0.0	5.0	95.0	0.0	5.000	200.00
8.00	0.0	5.0	95.0	0.0	5.000	
21.00	0.0	12.6	87.4	0.0	5.000	
25.10	0.0	60.0	40.0	0.0	5.000	
30.00	0.0	95.0	5.0	0.0	5.000	
32.10	0.0	5.0	95.0	0.0	5.000	
35.00	0.0	5.0	95.0	0.0	5.000	



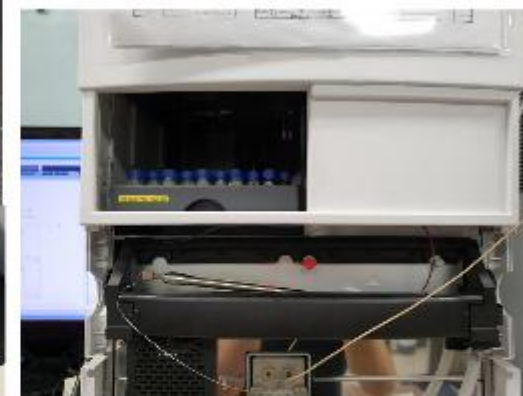
HPLC Peptide Purification

4. Preparative Run (Purification)

- Column: C18, 4.6 x 250 mm, 5 μ m
- Focused Gradient (i.e. peak flattening):
 - Start: (%B at tR) - 10%
 - End: (%B at tR) + 10%
 - Duration: 20-30 minutes
- Fraction Collection (Agilent 1260):
 - Mode: Peak-based (Slope/Threshold)
 - Threshold: 50-100 mAU (adjust based on sample concentration)

5. Fraction Analysis & Recovery

- Purity Check: Re-inject 10 μ L of collected fractions into the Analytical Method.
- Pooling: Combine fractions with >95% peak area integration.
- Evaporation: Rotovap at <30°C to remove Acetonitrile.
- Lyophilization: Freeze-dry the aqueous remainder to obtain peptide + TFA salt powder



Time (min)	A [%]	B [%]	C [%]	D [%]	Flow (mL/min)	Max. Pressure Limit (bar)
0.00	0.0	5.0	95.0	0.0	5.000	200.00
8.00	0.0	5.0	95.0	0.0	5.000	
21.00	0.0	12.6	87.4	0.0	5.000	
25.10	0.0	60.0	40.0	0.0	5.000	
30.00	0.0	95.0	5.0	0.0	5.000	
32.10	0.0	5.0	95.0	0.0	5.000	
35.00	0.0	5.0	95.0	0.0	5.000	

