

Structural studies on the microglia TREM2-DAP12 complex

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Triggering Receptor Expressed on Myeloid cells 2 (TREM2) is a transmembrane protein specifically expressed in the microglia of the brain. TREM2 is an immune receptor that binds anionic ligands. Ligand binding induces phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) within the intracellular domain of its adaptor protein DNAX activation protein 12 (DAP12), thereby activating downstream signaling pathways that regulate microglial survival, proliferation, phagocytosis, and motility. Among the risk factors of Alzheimer's disease (AD) found to date, the TREM2 rare variants are one of the most dangerous factors for AD development. TREM2 signaling stimulate microglial A̢ plaque clearing in the early-stage AD. However, over-activated TREM2 signaling can induces in uncontrolled neuronal death in the late-stage AD. Although the extracellular domain structure was revealed by X-ray crystallography, the structure of full length TREM2 and DAP12 protein complex remain to be elucidated.

We have successfully expressed TREM2 and DAP12 proteins in Hi5 insect cell infected with baculovirus, and purified human TREM2 and TREM2/DAP12 protein. Interestingly, When TREM2 was separated from Superdex 200 column using size-exclusion chromatography, TREM2 peak could be observed at about 13 mL, indicating that TREM2 should be assembled in a certain oligomeric state. Indeed, 2D classification of Cryo-EM imaging showed that TREM2 may form a dimeric architecture. However, the orientation of TREM2 particles observed in Cryo-EM imaging does not seem to be diverse, so to overcome this, we plan to increase the diversity of orientation by binding DAP12 and antibodies to TREM2 or making it in a lipid nanodisc state.

Introduction

Alzheimer's Disease

- Most common type of dementia.
- Neurodegenerative disease that impairs the normal function of neurons.
- Histopathological marker: Amyloid plaque accumulation and neurofibrillary tangles.
- Microglia, the resident immune cells of the central nervous system, are involved in the removal of amyloid plaques.

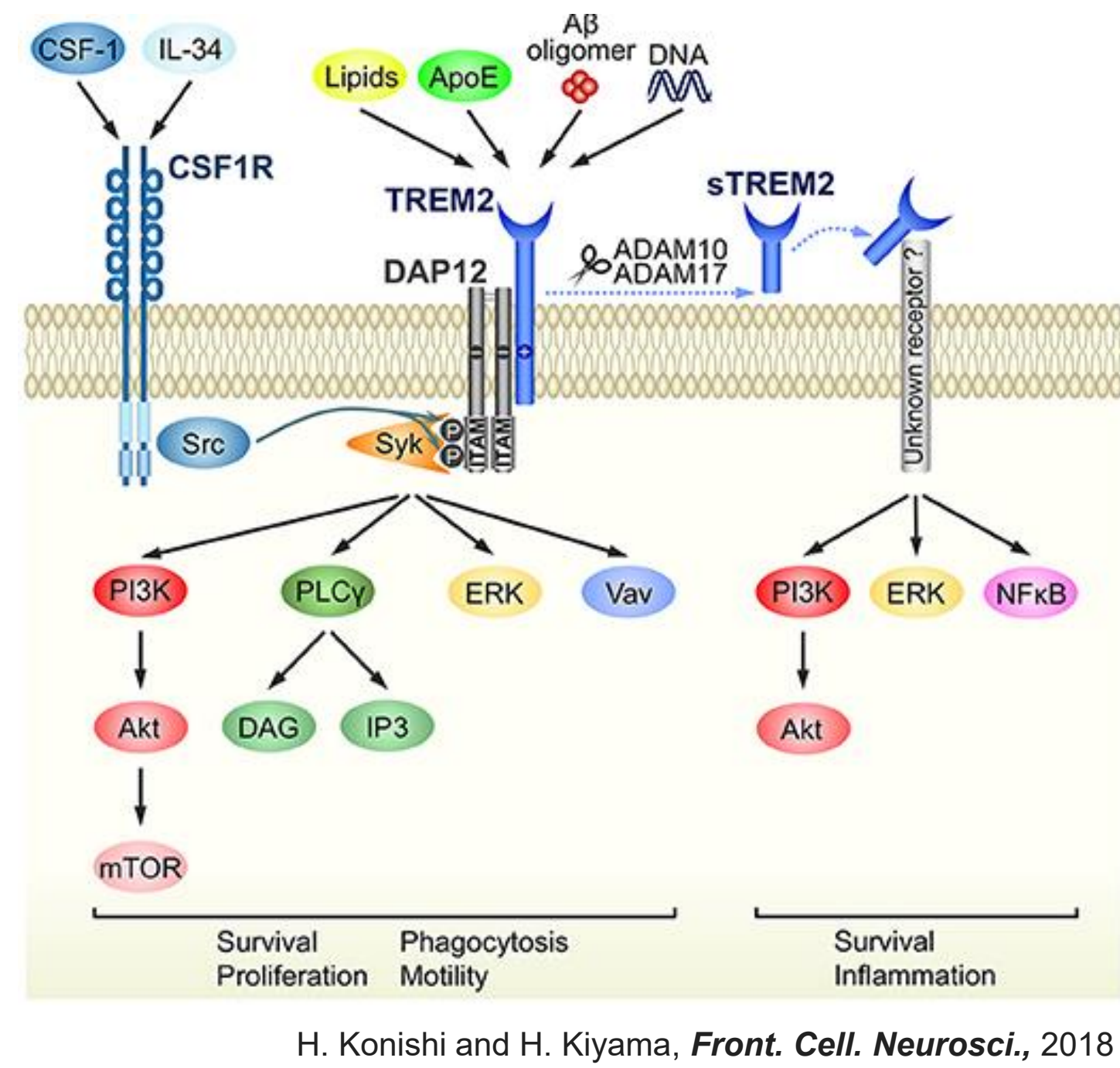
Microglia

- In neurodegenerative diseases, microglia undergo a transition into a disease-associated microglia (DAM) phenotype.
- DAM contribute to the clearance of amyloid plaques and modulation of inflammatory responses, thereby helping to protect neurons in neurodegenerative conditions.
- However, when excessively activated, it releases neurotoxic molecules that can damage neurons.
- TREM2 is essential for maintaining the balanced function of microglia.

TREM2

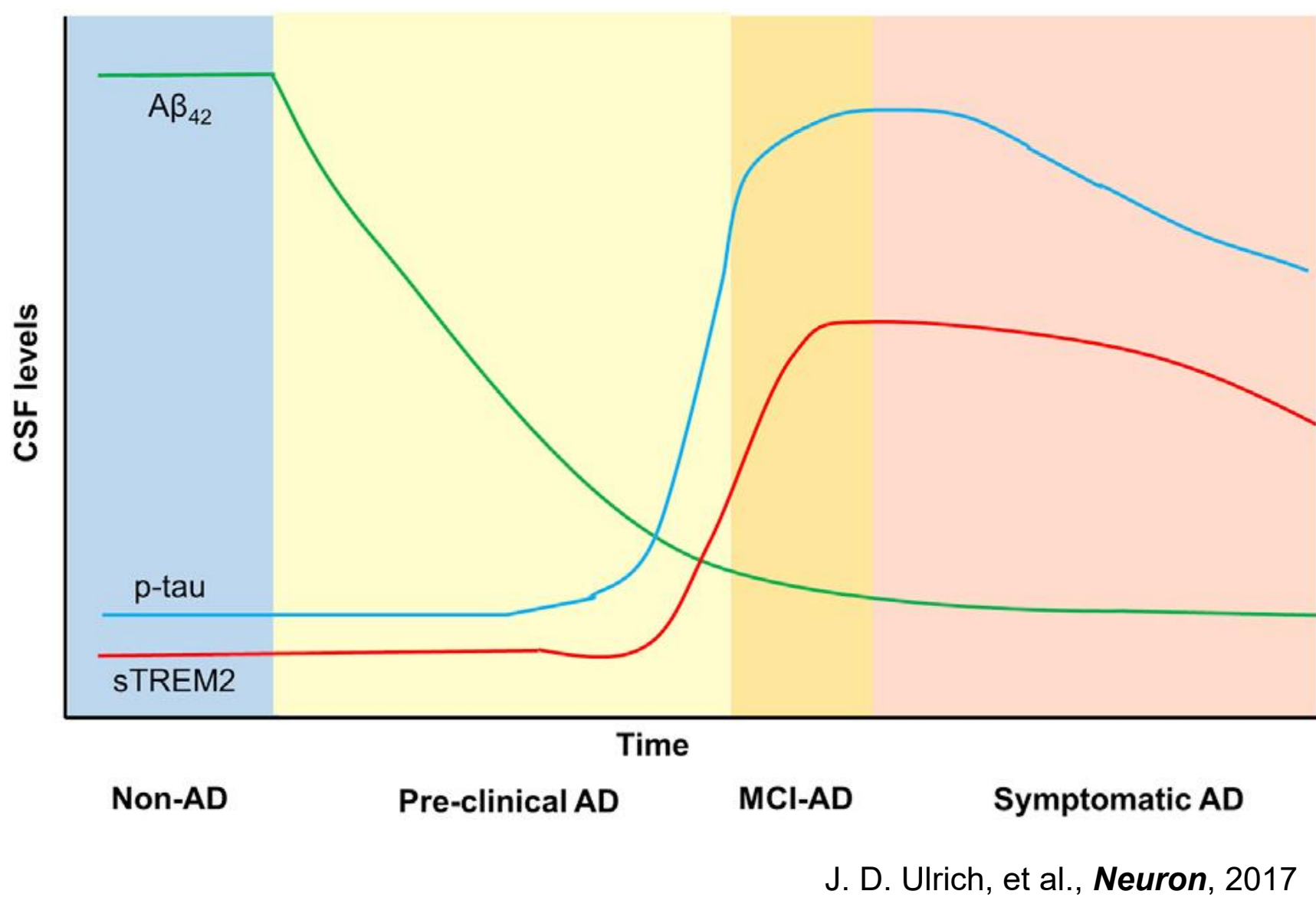
- Regulates microglial activation, survival, and phagocytosis.
- TREM2 gene variants are associated with a 2- to 4- fold increased risk of AD.
- Soluble TREM2 (sTREM2) is a promising biomarker for AD.

❖ TREM2 signaling in microglia



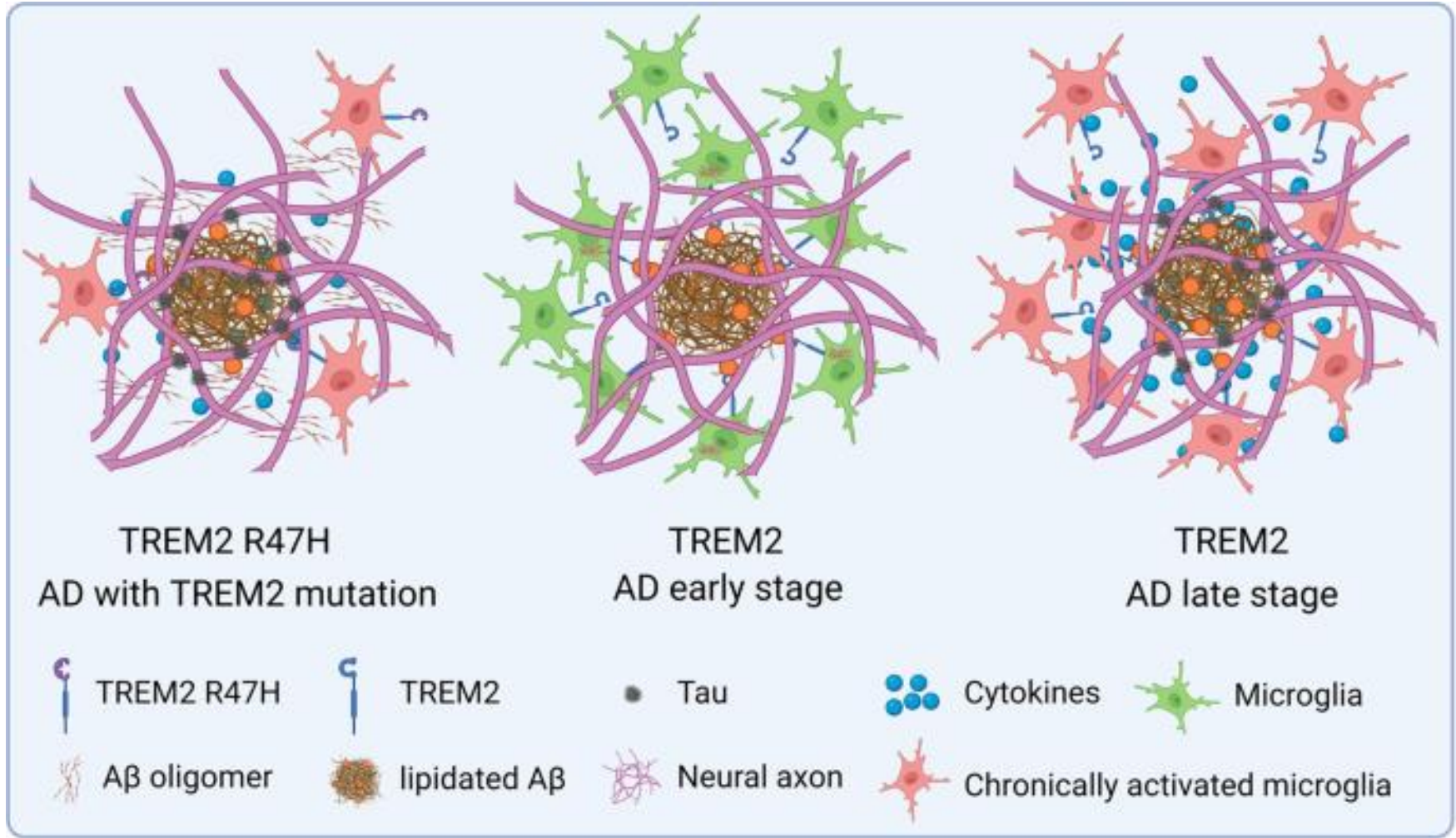
H. Konishi and H. Kiyama, *Front. Cell. Neurosci.*, 2018

❖ Elevation of sTREM2 in AD patients' CSF



J. D. Ulrich, et al., *Neuron*, 2017

Rationale



W. Zhang and H. Xia, et al., *Sig Transduct Target Ther.*, 2023

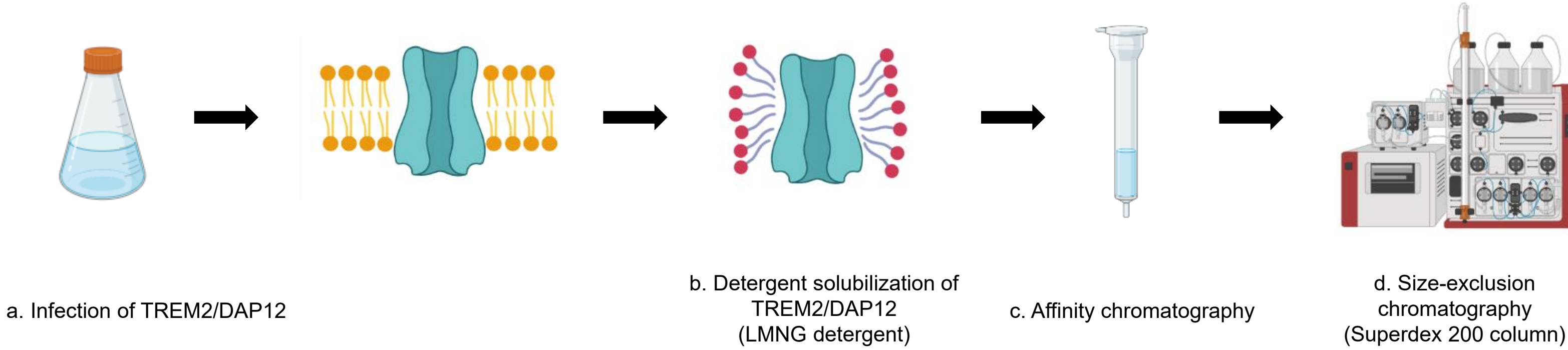
Since the role of TREM2 varies across different stages of AD progression, effective modulation of its activity may offer a promising therapeutic strategy. To enable such approaches, understanding the structure of the TREM2–DAP12 complex is essential, as it provides insight into TREM2's mechanism of action and how it might be therapeutically targeted.

TREM2 modulation

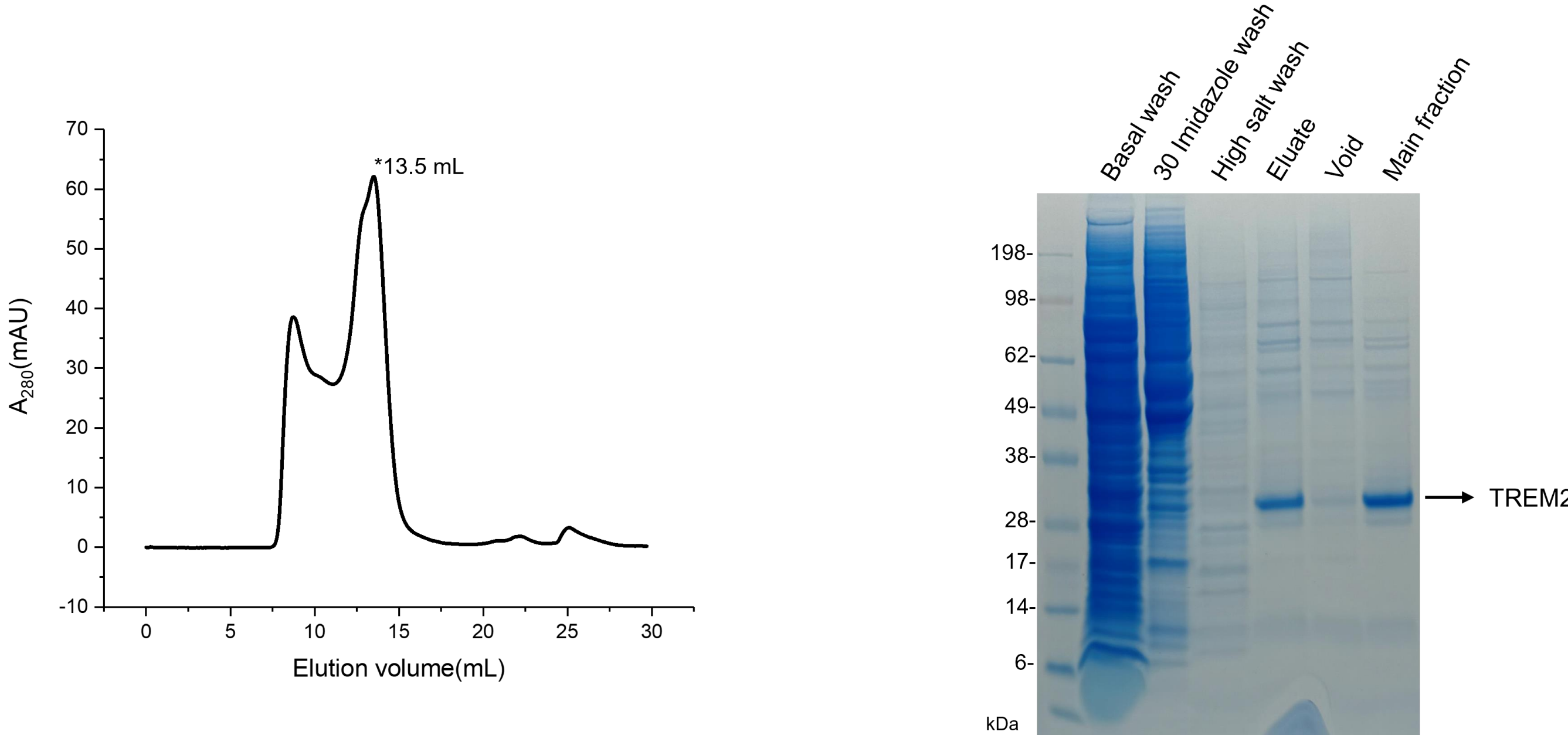
Early stage	Late stage
-Promotion of DAM	-Maintenance of DAM
-Amyloid-̢ clearance	-Limited amyloid-̢ clearance
-Suppresses early inflammation	-Attempts to suppress chronic inflammation with limited effect

Results

Purification process

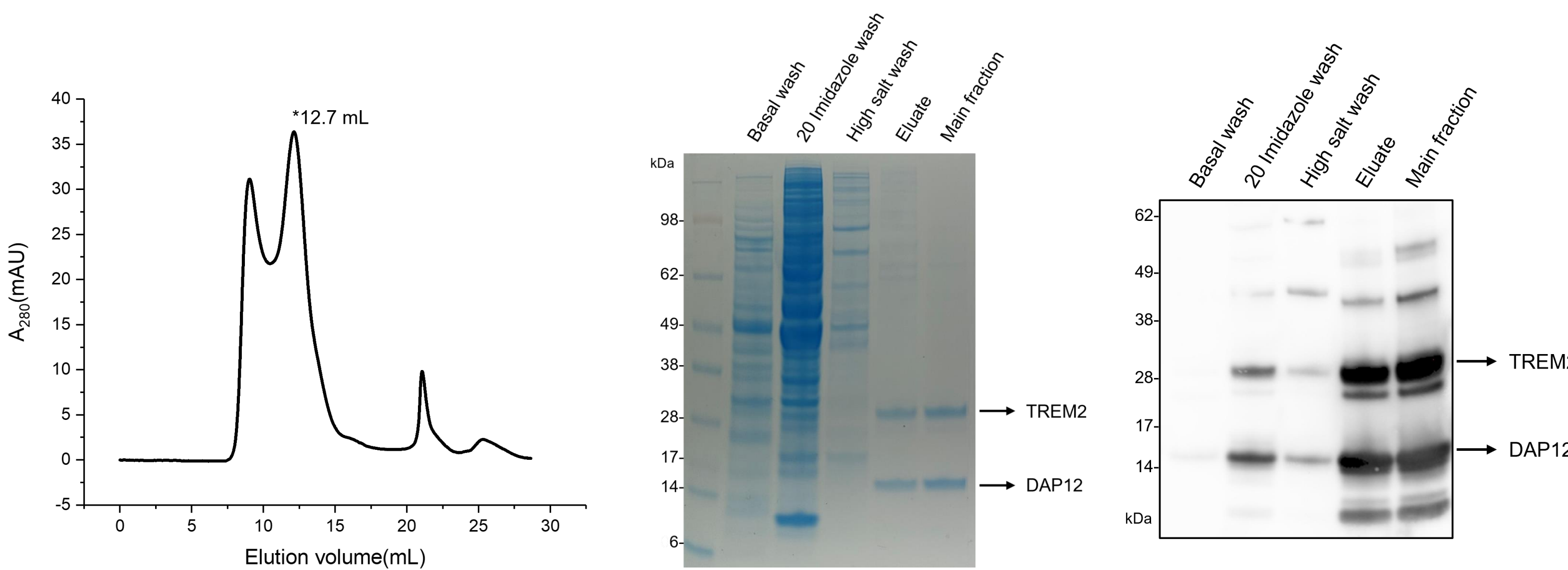


1. Purification of TREM2



Size-exclusion chromatography using a Superdex 200 column revealed a major peak at ~13.5 mL, indicative of a assembly with a molecular weight of ~100 kDa or higher, suggesting that TREM2 forms oligomers considering the estimated molecular weight of monomeric TREM2 is ~25 kDa. .

2. Purification of TREM2-DAP12 complex



Purification of the co-expressed TREM2-DAP12 complex in Hi5 cell. Size-exclusion chromatography (Superdex 200 column) showed that the main peak eluted ~0.8mL faster than TREM2 alone (above), indicating an increase in molecular weight due to DAP12. Coomassie blue staining and western blot analysis confirmed that both TREM2 and DAP12 were present in the main fraction, suggesting the formation of a stable complex.

On-going studies

1. We have screened and collected preliminary data for evaluating cryo-EM imaging conditions. 2D classification of Cryo-EM imaging showed that TREM2 may form a dimeric architecture.
2. However, the lack of orientation diversity limited structural determination. To address this, we are going to test TREM2-DAP12-mAb (Fab) complex and nanodiscs reconstituted protein complexes.
3. In addition, we are going to purify and determine structure of native TREM2–DAP12 complex from both WT and AD model mice brain using an in-house developed monoclonal antibody.

