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IN VIVO MICRODIALYSIS EXPERIMENTS UNVEILED TAUOPATHY-ASSOCIATED PROTEOMIC CHANGES IN BRAIN EXTRACELLULAR FLUIDS

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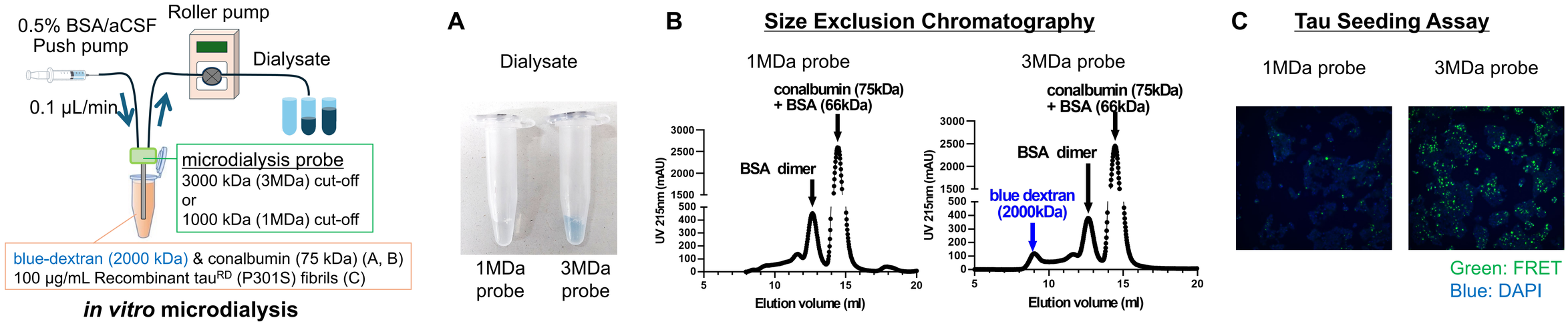
【Introduction】

The brain is supported by two types of extracellular fluids: interstitial fluid (ISF) and cerebrospinal fluid (CSF). Throughout the progression of neurodegenerative diseases such as tauopathies, various disease-associated alterations manifest in these brain extracellular fluids. ISF serves as the primary compartment for receiving secretions from brain cells. Therefore, ISF is expected to reflect brain changes such as tau aggregation or other cellular responses most accurately.
To gain comprehensive understanding of the specific proteomic alterations that occur in ISF, we utilized in vivo microdialysis and analyzed how tau seeding activity and the proteome in ISF alter with age or during the progression of tau pathology.

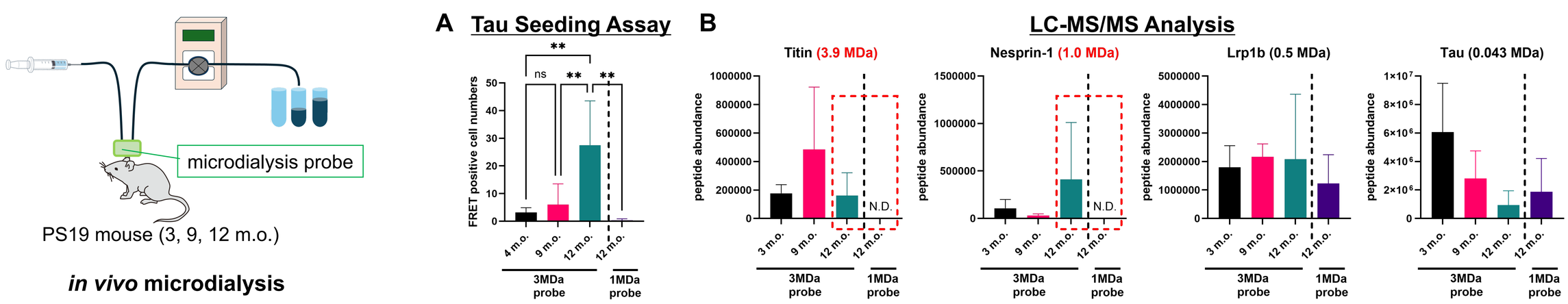
【Methods & Results】

Figure1. Microdialysis with 3MDa Cut-Off Probes Enhanced Recovery of High Molecular Weight Proteins and Seed-Competent Tau *in vitro*.



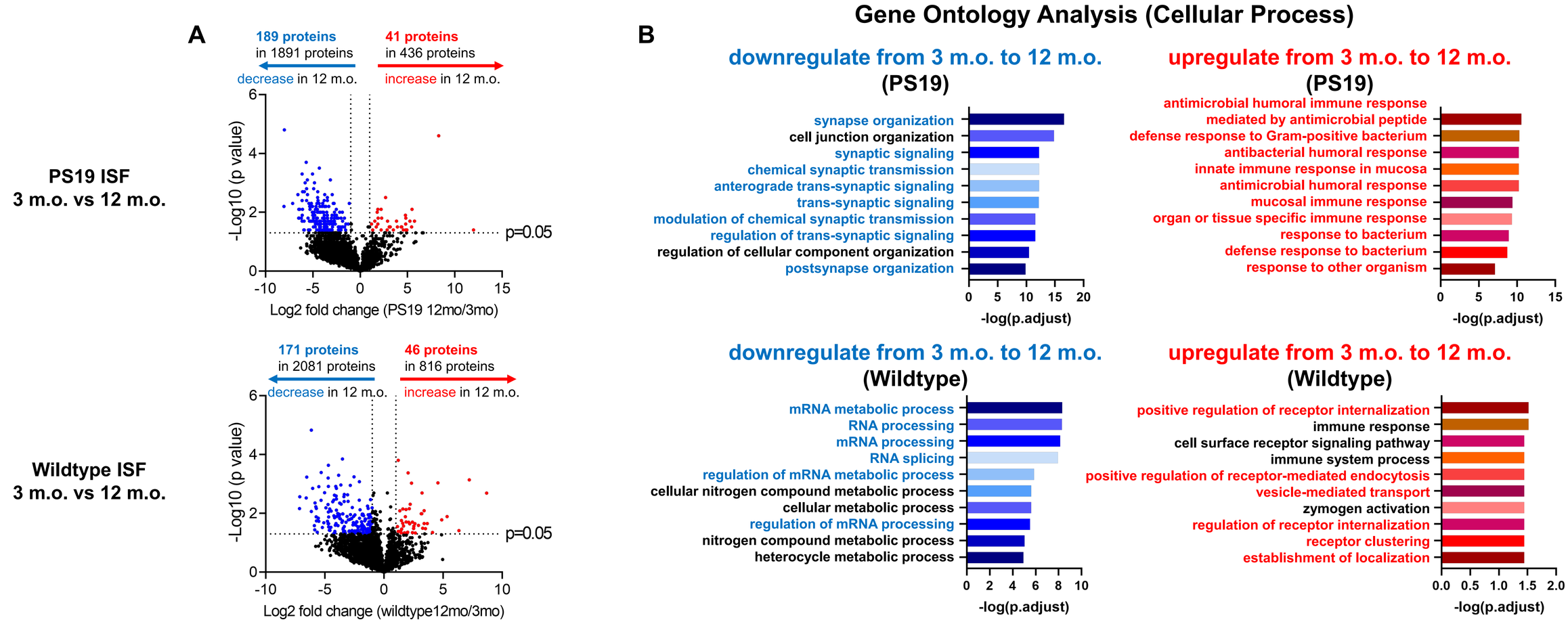
A. A representative picture of dialysate collected through 1MDa and 3MDa probes.
B. Size-exclusion chromatography profile of in vitro dialysates.
C. In vitro dialysates were applied with Lipofectamine 3000 to tau biosensor cells and images were obtained at 72 hours from transduction.

Figure 2. High Molecular Weight Proteins (>1MDa) and Seed-Competent Tau in ISF Were Only Recovered with 3MDa Cut-Off Probes.



A. ISF samples from PS19 at different ages were applied with Lipofectamine 3000 to tau biosensor cells.
B. ISF samples from PS19 at different ages were digested by trypsin and analyzed by LC-MS/MS for label free quantification. The raw data were searched against in silico predicted spectral library using DIA-NN. (version:1.8.1, https://github.com/vdemichev/DiaNN). The peptide abundance of representative proteins in each molecular weight range was quantified and compared (n=3).

Figure 3. Age- or Tau Pathology-Dependent Proteomic Alterations in ISF by LC-MS/MS Analysis



A. Volcano plots showing increased (red) and decreased (blue) proteins in ISF of 3-momnth-old vs 12-month-old PS19 or wildtype mice. (n=3)
B. Gene Ontology enrichment analysis were performed using the geneXplain platform on the proteins with significant differences.

【Summary & Conclusions】

- We established a new microdialysis method that enhances the recovery of high molecular weight proteins and successfully identified more than 2,000 proteins in ISF.
- Tau seeding activity was increased with age while total tau levels were decreased in ISF of PS19 mice.
- Proteins related to synaptic signaling were downregulated, while those related to immune responses were upregulated with age in PS19 mice.
- Proteins related to RNA metabolism were downregulated, while those related to receptor internalization were upregulated with age in wildtype mice.

This study revealed dynamic proteomic changes in ISF and suggests that ISF reflect distinct protein profiles that alter with age and tau pathology in brain.