



Mass Spectrometry Profiling of PNS-Tau Reveals Tissue-Specific Signatures in Heart and Kidney

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Background

PNS-tau differs from brain-derived tau (BD-tau) by including two extra exons (4a and 6) (**Figure 1**). Co-morbid conditions like cardiovascular and kidney disease (CVD and CKD) can affect the accuracy of tau-based Alzheimer's disease (AD) biomarkers, likely due to PNS-tau from peripheral tissues. To better understand PNS-tau and identify co-morbidity-specific markers that could improve AD biomarker performance, we combined in vitro studies of recombinant PNS-tau and BD-tau with in vivo profiling using co-immunoprecipitation and mass spectrometry (Co-IPMS) on heart and kidney tissues from groups with and without co-morbidities.

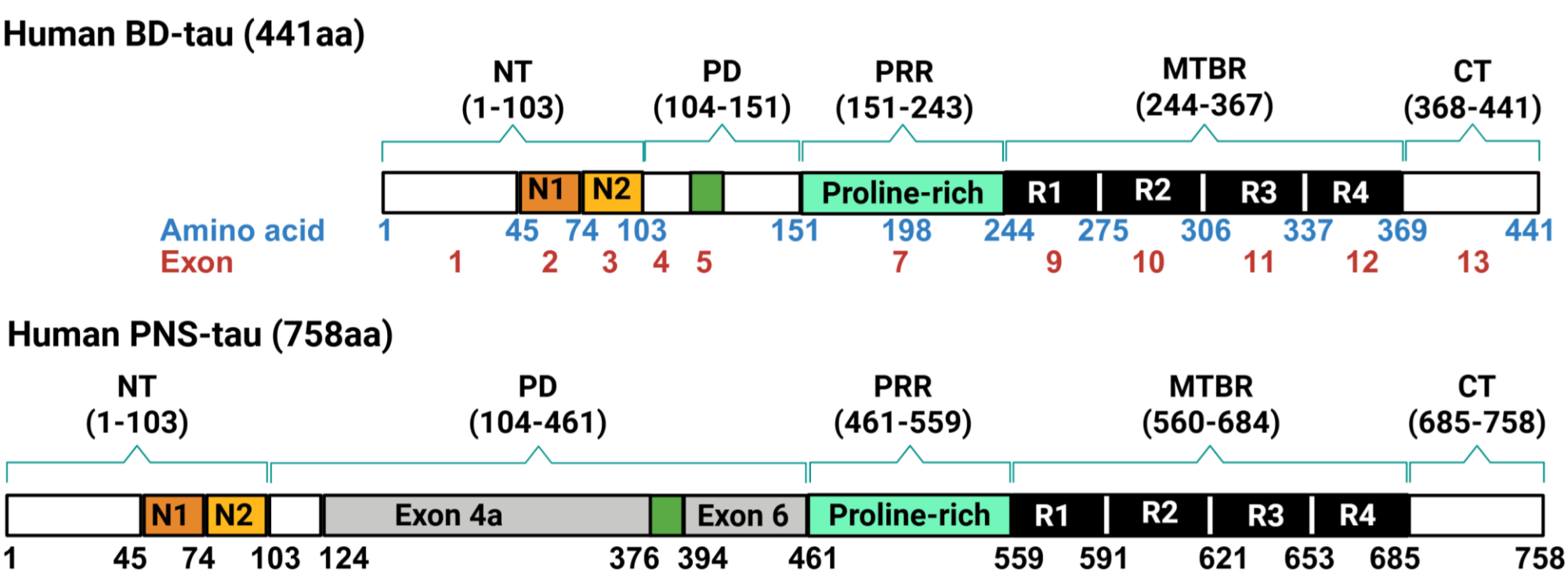


Figure 1. Schematic illustration for the structural difference between BD-tau and PNS-tau.

Methodology

Recombinant human PNS-tau and BD-tau proteins were incubated at 37 °C for up to 7 days and analyzed by ELISA and shotgun mass spectrometry for degradation profile analysis. For tissue experiments, human kidney (n=10, 5 healthy and 5 with CKD) and heart (n=10, 5 healthy and 5 with CVD) tissues obtained from BioIVT, LLC (Westbury, NY), were cryo-ground, homogenized in T-PER buffer, and total tau was captured using Dynabeads coated with tau antibody cocktail (**Figure 2**). The samples were digested with trypsin and analyzed by high-resolution LC-MS/MS. Database searching was performed with Peaks Studio, and tau peptide areas were extracted from raw files for accurate quantification.

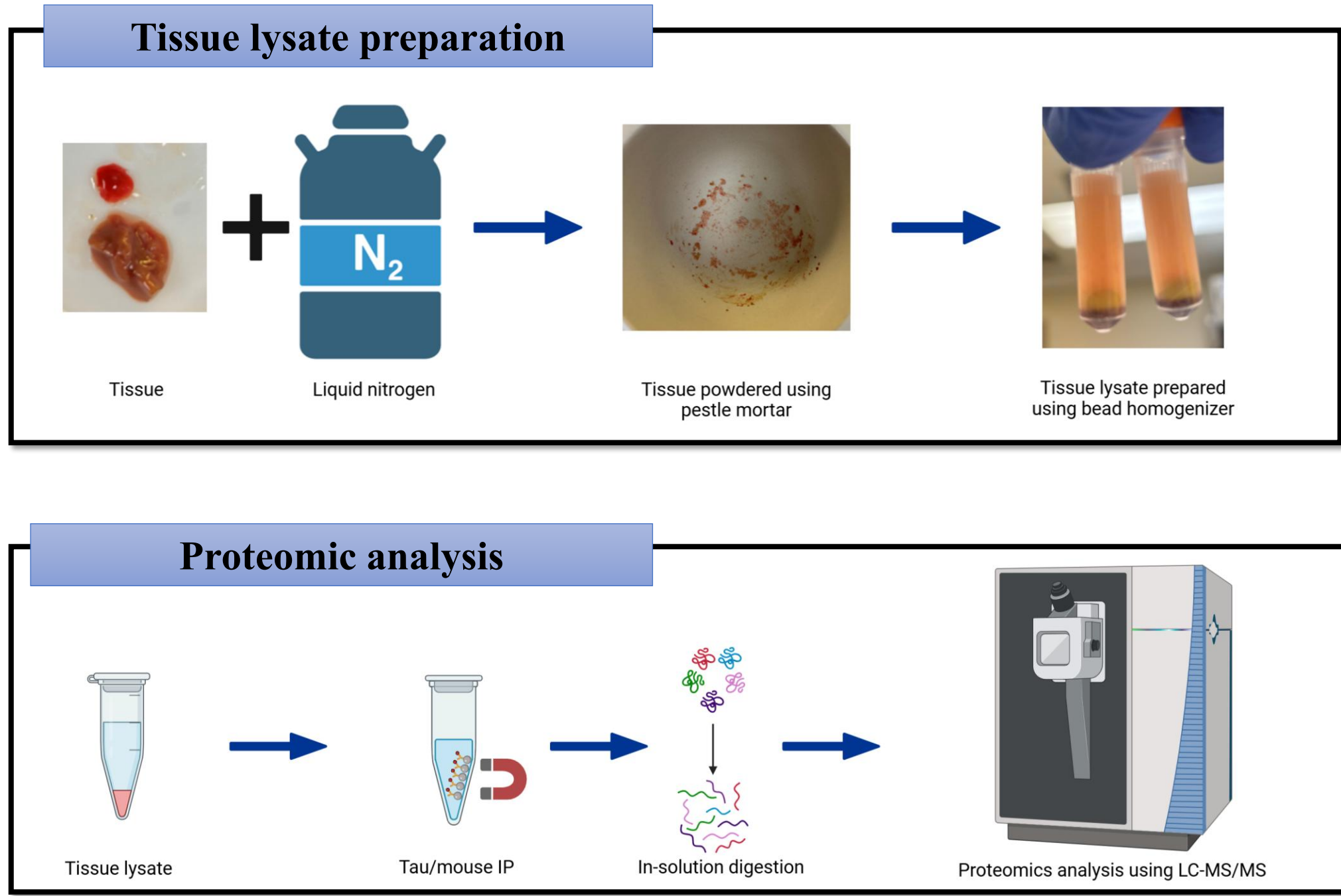


Figure 2. Flow Diagram of the research.

Results

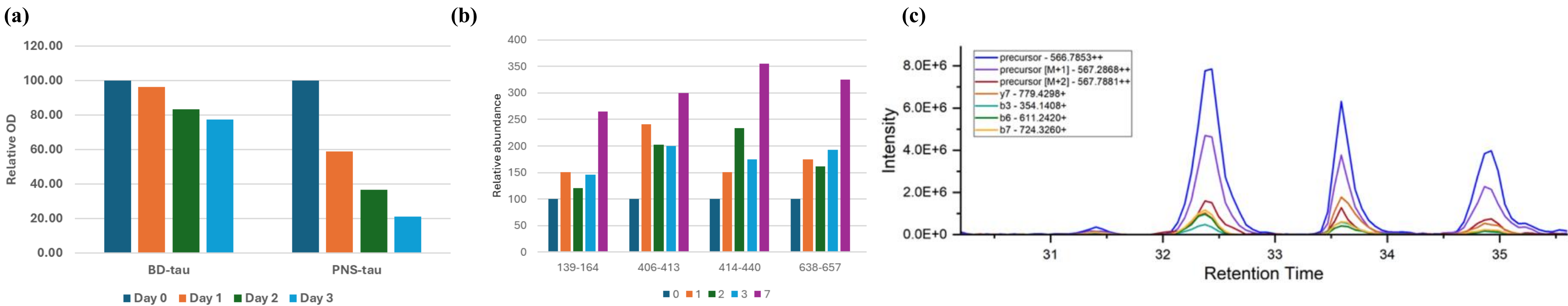
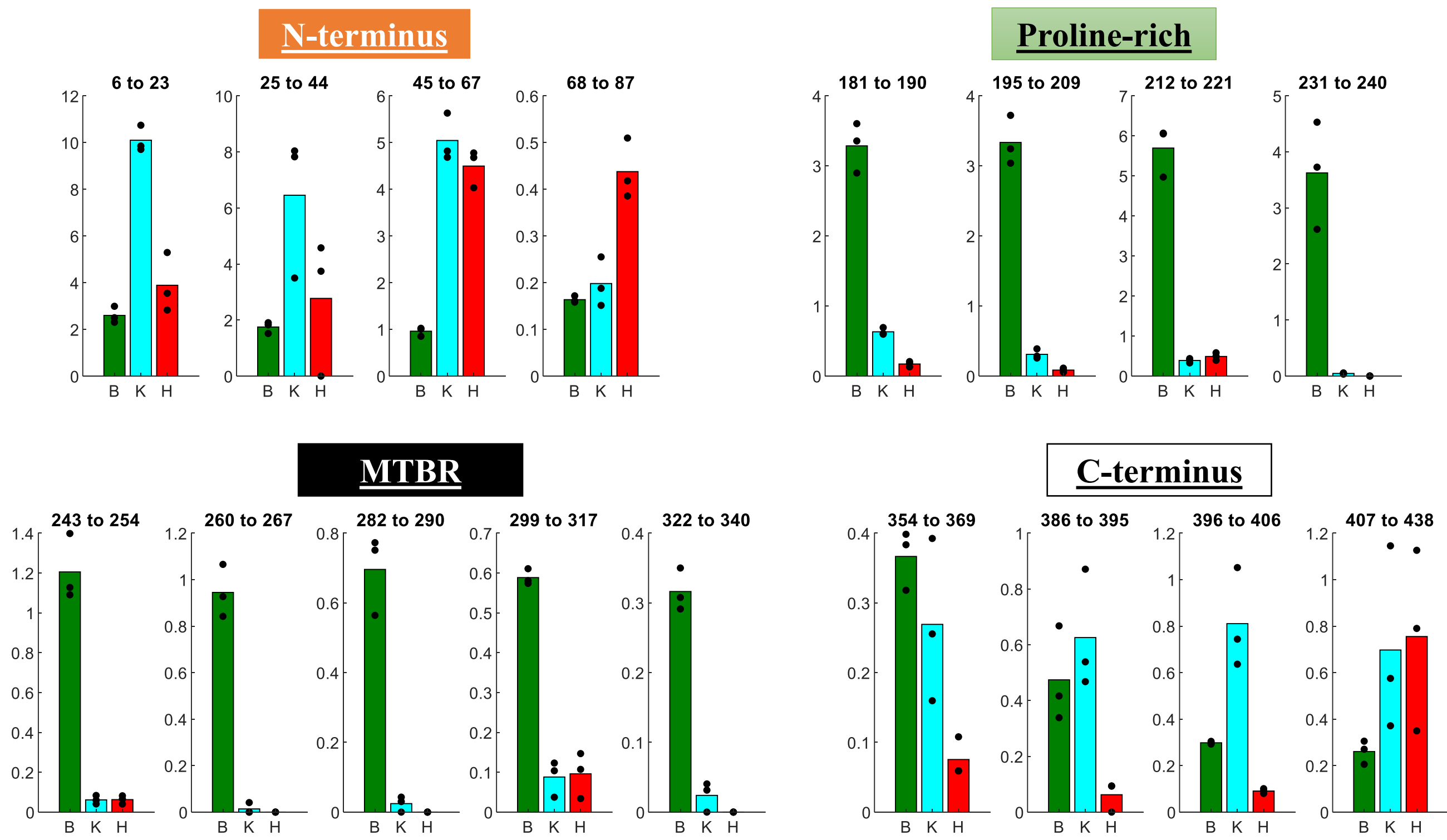


Figure 3. In vitro stability assay reveals intrinsic instability of PNS-tau. (a) Sandwich ELISA showed a more rapid decline in the N-terminal tau fragment during prolonged incubation at 37 °C. (b) Quantitative mass spectrometry detected several non-tryptic peptides with increased abundance over time. (c) Tau isomerization was evidenced by the appearance of multiple chromatographic peaks corresponding to the same peptide (residues 703–712: TDHGAEIVYK).

(a) Peptides shared by BD- and PNS-tau



(b) Unique PNS-tau peptides

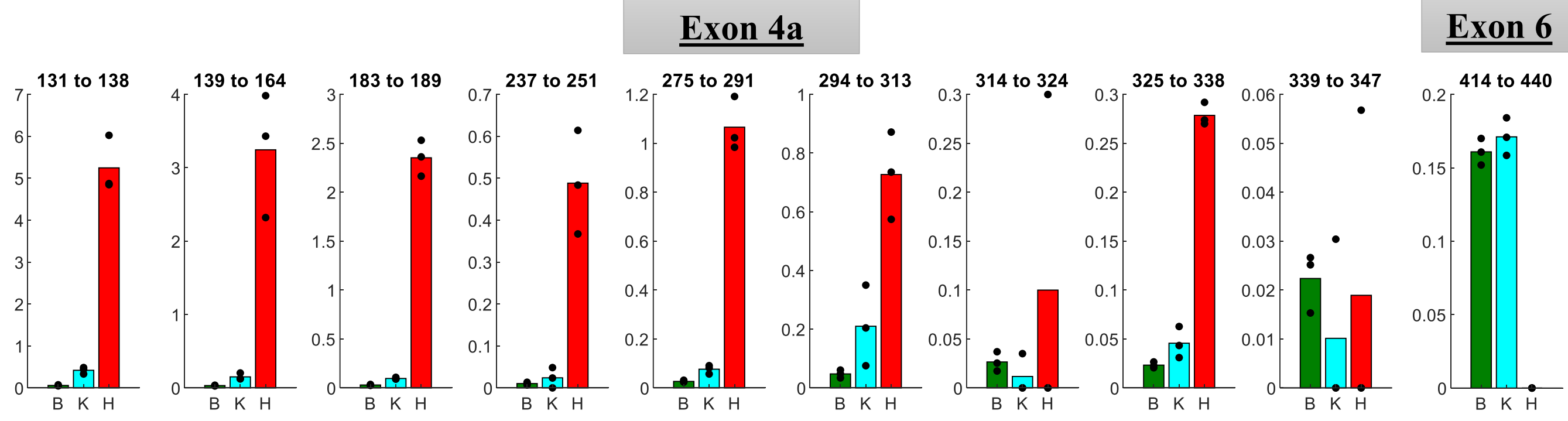


Figure 5. Venn diagram showing distribution of proteins from human kidney lysate tau and mouse co-IPMS.

Conclusion

Our study reveals key differences between PNS-tau and BD-tau and lays the groundwork for developing PNS-tau biomarkers to improve the accuracy of AD diagnosis.

Key findings

- PNS-tau accelerated instability and less prone to form tau aggregates.
- Tissue-specific signatures:
 - Exon-4a higher in heart
 - Exon 6 higher in kidney
 - MTBR higher in brain
- Protein-protein interactions:
 - Monoamine oxidase B (MAOB)
 - Alpha-synuclein (SNCA)

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ELISA results demonstrated a more rapid decline in signal for PNS-tau, indicating lower stability compared to BD-tau (**Figure 3**). Mass spectrometry profiles revealed potential cleavage hotspots in PNS-tau (139-164, 406-413, 414-440, 638-657), as well as evidence of isomerization (703-712), which may contribute to its accelerated instability. Preliminary analysis showed tissue-specific expression patterns: heart tissue exhibited higher levels of tau exon 4a, whereas kidney tissue displayed increased expression of exon 6 (**Figure 4**). Both had low abundance of the microtubule-binding region. Co-IPMS analysis identified potential PNS-tau interactors, including monoamine oxidase B and alpha-synuclein, which may help elucidate the unique functional characteristics of PNS-tau (**Figure 5**).