

Nanoparticle Enrichment And AI In Silico Spectral Libraries Enhance Blood Proteomics in Mild Cognitive Impairment

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Blood-based proteomics may enable early detection of mild cognitive impairment (MCI), but plasma biomarker discovery is limited by incomplete peptide identification in DIA workflows. We analyzed plasma from individuals with MCI and normal controls (NC) using **nanoparticle enrichment and DIA mass spectrometry** on Orbitrap Exploris and Astral platforms, with and without **NeurOps, an AI-powered in silico spectral library tool**. **NeurOps improved peptide and protein identifications across both platforms, especially unique proteotypic peptides**. In *APOE4*-carrier MCI versus NC, 145 differentially expressed proteins formed a brain-enriched interaction network, and hub proteins showed stepwise abundance increases and strong ROC performance. **These findings suggest that this integrated workflow improves plasma proteome profiling and supports blood biomarker discovery for mild cognitive impairment.**

INTRODUCTION

METHODS

RESULTS

CONCLUSION

Blood-based proteomics is a promising approach for early detection of mild cognitive impairment (MCI), a prodromal stage of Alzheimer's disease, and for elucidating underlying pathophysiology. However, plasma biomarker discovery remains challenging due to the extreme dynamic range of protein abundances and incomplete or biased peptide detection in data-independent acquisition (DIA) workflows. Nanoparticle-based enrichment can increase access to low-abundance proteins, yet DIA identification and reproducibility remain constrained by spectral library quality. Therefore, AI-powered in silico spectral library generation tailored to DIA analysis is needed to improve sensitivity and robustness for plasma protein candidates discovery in early cognitive decline.

Plasma samples from individuals with MCI and normal controls (NC) were profiled using nanoparticle enrichment followed by DIA mass spectrometry on Orbitrap Exploris and Orbitrap Astral platforms. DIA data were analyzed with and without NeurOps, an AI-powered in silico spectral library generation tool. Proteome depth and identification quality were evaluated using six DIA metrics: total peptide-spectrum matches, total peptides, unique peptides, unique proteotypic peptides, unique proteins, and protein groups. Differentially expressed proteins (DEPs) were identified and used to build protein-protein interaction (PPI) networks. Networks were annotated and prioritized using a Clinical Knowledge Graph (CKG) linking protein-tissue-disease associations. Candidate panels were assessed by receiver operating characteristic (ROC) analyses for classification of MCI versus NC and *APOE4*-carrier MCI versus NC.

Across both DIA platforms, NeurOps increased identifications at the spectrum, peptide, and protein-group levels, with the significant improvement observed for unique proteotypic peptides, supporting improved protein-level confidence. From *APOE4*-carrier MCI versus NC, 145 DEPs generated a brain-region-informed PPI network containing a tightly connected brain-enriched module. CKG-assisted annotation prioritized hub proteins (VCL, FLNA, CYCS, PTGS1) with strengthened disease-linked connectivity to cognitive disorder and dementia profiles. These hubs showed stepwise abundance increases from controls to MCI to *APOE4*-carrier MCI and yielded strong ROC performance using multi-protein composite panels.

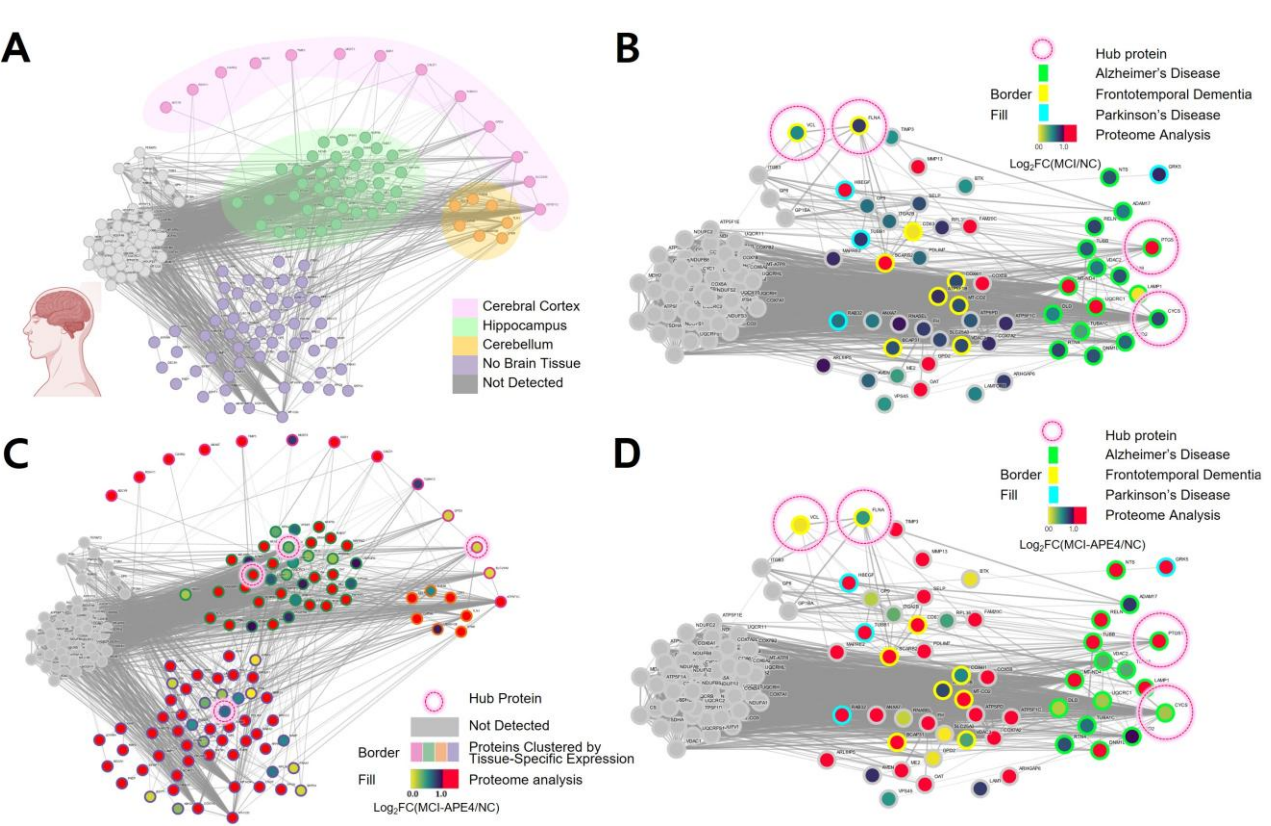


Figure 2. Protein-protein interaction (PPI) network and clinical knowledge graph (CKG)-assisted prioritization of candidate proteins for MCI classification. A PPI network was constructed from 145 differentially expressed proteins (DEPs) identified in *APOE4*-carrier MCI versus normal controls (NC) ($P \leq 0.05$; fold change, $FC \geq 1.0$) and used to prioritize candidate proteins for MCI classification. **(A)** Brain-region-informed PPI map in which proteins are grouped by tissue-specific expression patterns (cerebral cortex, hippocampus, and cerebellum), showing a tightly connected module of brain-derived proteins within the plasma interaction network; proteins without brain-tissue specificity or not detected are shown in gray. **(B)** CKG-based annotation of the PPI network using the MCI versus NC proteome contrast; node fill represents the proteomic effect size ($\log_2FC$), and colored node borders indicate disease-gene set associations (Alzheimer's disease, frontotemporal dementia, and Parkinson's disease). **(C)** The same network highlighting hub proteins (magenta labels), defined by high network centrality (e.g., degree and betweenness centrality) in the *APOE4*-carrier MCI versus NC groups. **(D)** CKG-annotated network using the *APOE4*-carrier MCI versus NC contrast, emphasizing strengthened effect sizes and disease-linked connectivity of key hubs (e.g., VCL, FLNA, CYCS, and PTGS1), supporting their prioritization as candidate biomarkers along the MCI-to-dementia progression axis.

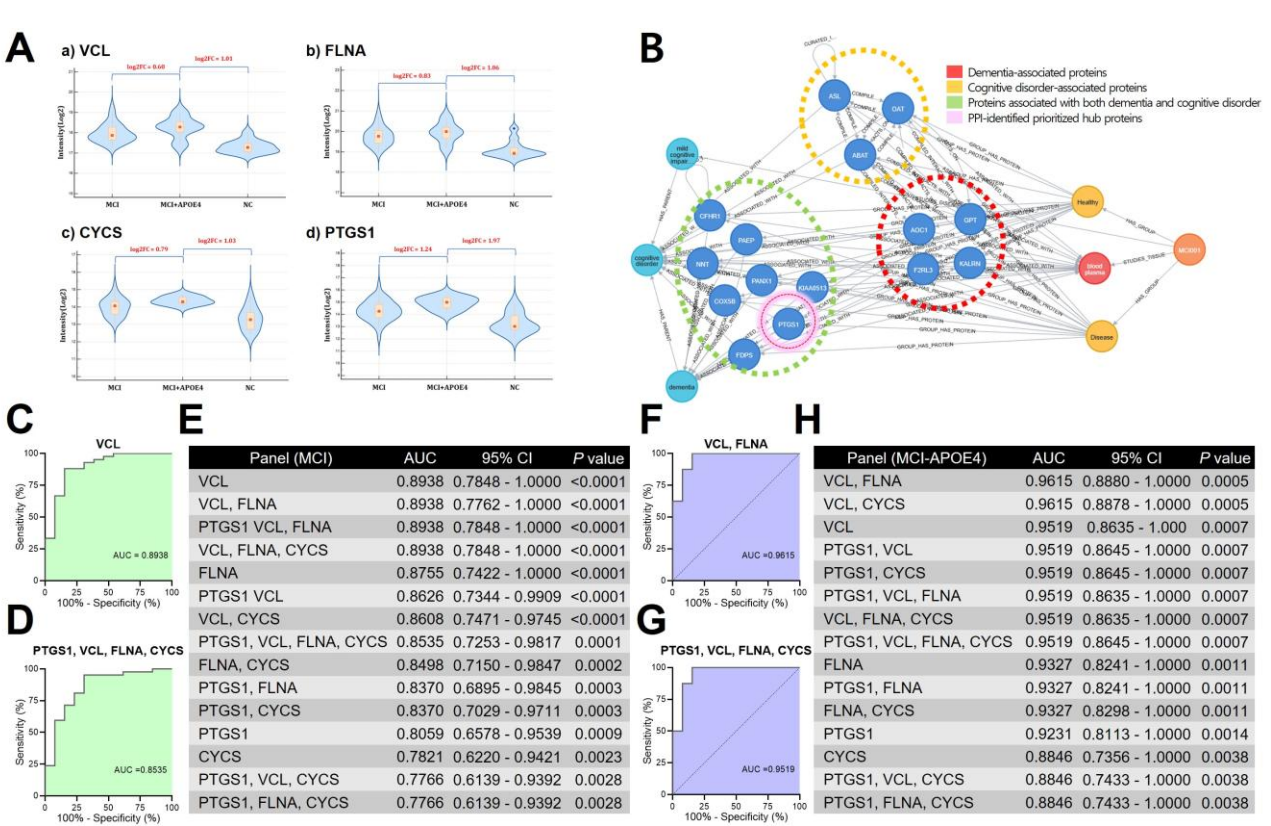


Figure 3. Prioritized candidate proteins discriminate MCI and *APOE4*-carrier MCI from normal controls (A) Violin plots of $\log_2$ -transformed plasma intensities for four PPI-derived hub proteins (VCL, FLNA, CYCS, and PTGS1) across normal controls (NC), overall MCI, and *APOE4*-carrier MCI, showing a stepwise increase in abundance ($NC < MCI < APOE4$ -carrier MCI), with annotated $\log_2FC$. **(B)** CKG-derived protein-tissue-disease network generated from normalized plasma proteomes after missing-value handling, highlighting 15 candidate proteins linked to cognitive disorder and/or dementia and stratified by disease connectivity: cognitive disorder only (ASL, OAT, ABAT); dementia only (AOC1, GPT, F2RL3, KALRN); both cognitive disorder and dementia (CFHR1, NNT, PAEP, COX5B, PANX1, KIAA0513, PTGS1, FDPS). **(C-E)** ROC analyses for MCI versus NC using composite panels based on all 15 combinations of the four hub proteins: (C) best-performing representative panel (highest AUC), (D) four-protein panel, and (E) summary of AUC values with 95% confidence intervals (CI) and P values for all composite panels. **(F-H)** Corresponding ROC analyses for *APOE4*-carrier MCI versus NC: (F) best-performing representative panel (highest AUC), (G) four-protein panel, and (H) summary of AUC values with 95% CI and P values for all composite panels. Abbreviations: NC, normal control; MCI, mild cognitive impairment; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval

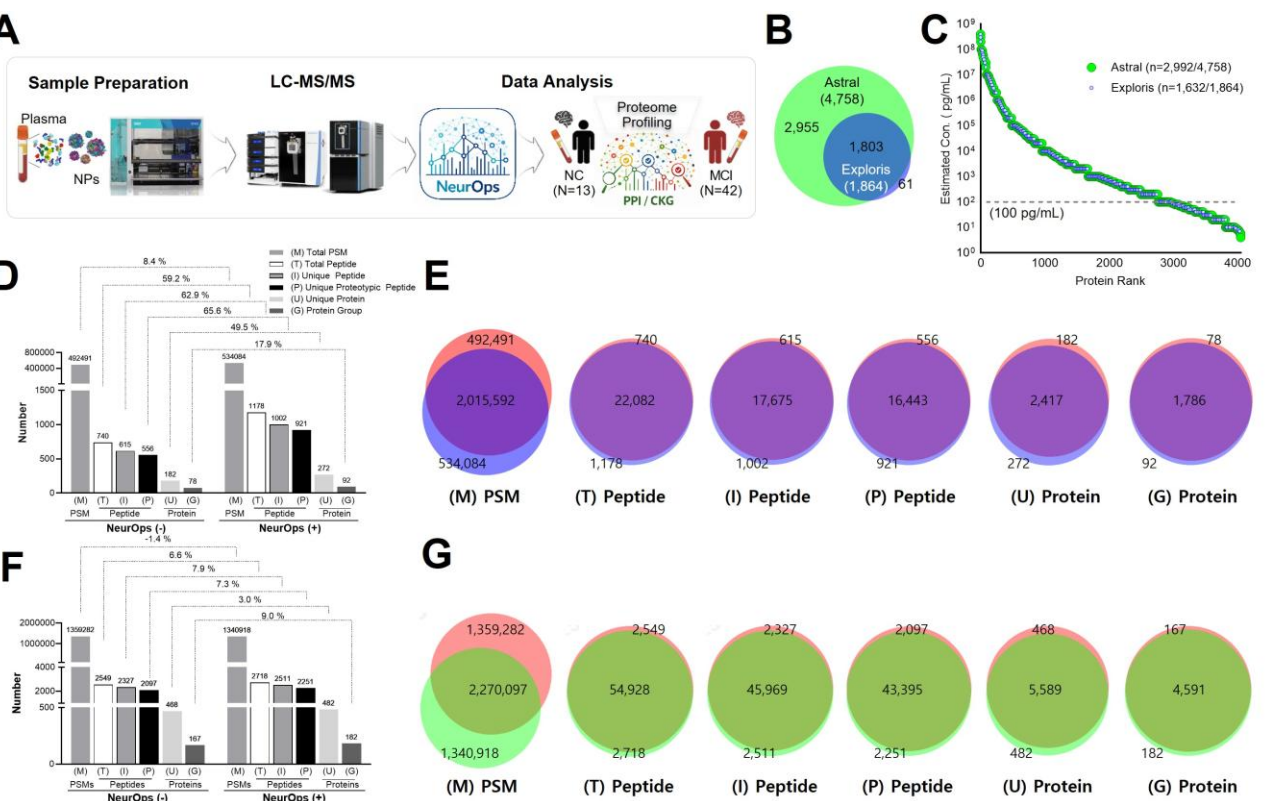


Figure 1. Improved peptide detection in DIA datasets using NeurOps AI-powered in silico spectral library generation (A) Overview of the nanoparticle-enriched mass spectrometry workflow for plasma proteome profiling in mild cognitive impairment (MCI), integrated with NeurOps, an AI-powered in silico spectral library generation tool. **(B-G)** Plasma proteome profiling and comparative performance acquired on Orbitrap Exploris and Orbitrap Astral mass spectrometry with or without NeurOps.

Integrating nanoparticle enrichment with AI-powered in silico spectral library generation mitigates spectral-library mismatch and incomplete peptide detection, thereby enhancing plasma proteome profiling. This workflow prioritizes clinically relevant, brain-linked candidate biomarkers and provides a practical framework for blood-based stratification of early cognitive impairment and *APOE4*-associated risk.

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