Enhanced biomarker discovery in blood plasma through a unique single-particle workflow for deeper proteome coverage

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INTRODUCTION

LC-MS-based plasma proteomics offers great potential for disease biomarker discovery and advancing our understanding of underlying pathophysiology. However, it faces challenges posed by the high dynamic range of plasma protein concentrations. The ENRICHplus technology addresses this limitation by enriching low-abundance proteins using paramagnetic beads, significantly increasing protein identification and enhancing biomarker discovery in diseases such as colorectal cancer (CRC). By enabling deeper proteome coverage, ENRICHplus improves early diagnosis, patient stratification, and personalized treatment in CRC research.

MATERIALS

Human EDTA plasma samples from a small clinical cohort of CRC patients (N=5) and matched healthy donors (N=5) were obtained from Biognosys. Pooled samples were applied for technical evaluation (n=3).

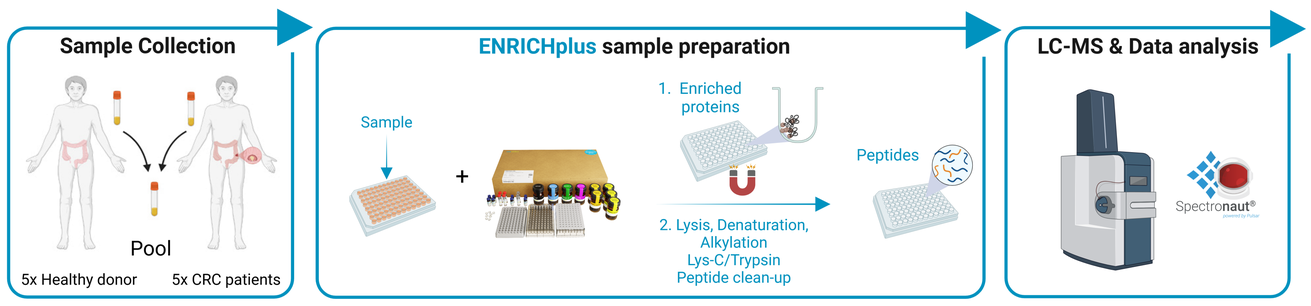
METHODS

Sample preparation: For ENRICHplus samples, 50 µL of plasma was processed with the ENRICHplus workflow. For neat samples, 2 µL of plasma was prepared following the PAC protocol.

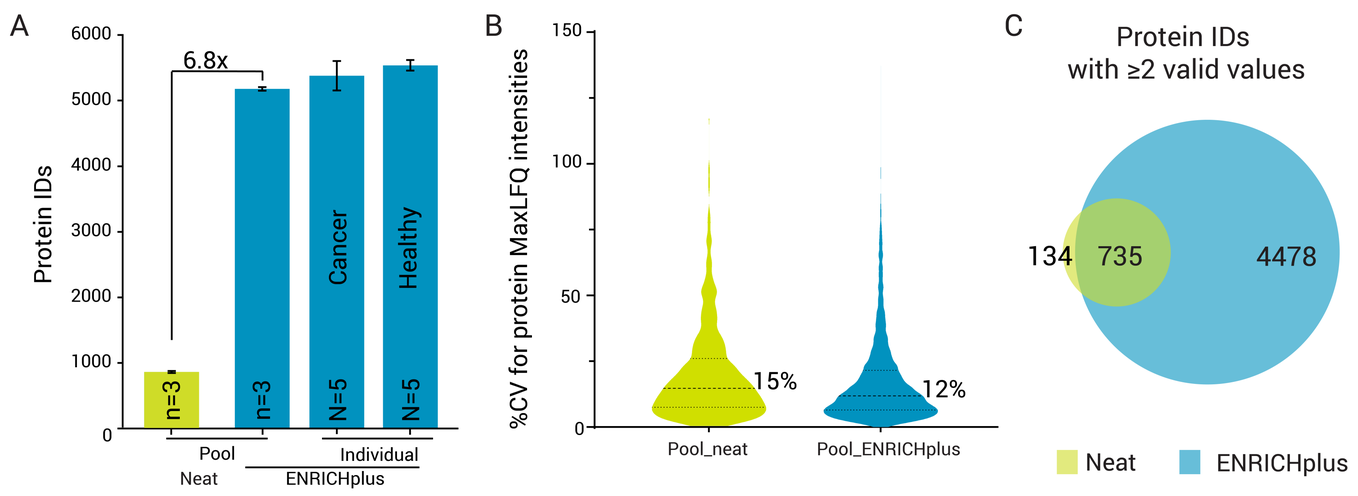
LC-MS analysis: 800 ng of peptides were analyzed using an Easy nLC 1200 (Thermo Fisher Scientific) equipped with an Aurora™ Ultimate CSI 25×75 C18 UHPLC column (IonOpticks) using a 50-minute gradient, coupled to a timsTOF HT (Bruker) in dia-PASEF® mode.

Data processing: Spectronaut® 19 using directDIA™+ mode. Gene ontology (GO) enrichment was analyzed using STRING-DB.

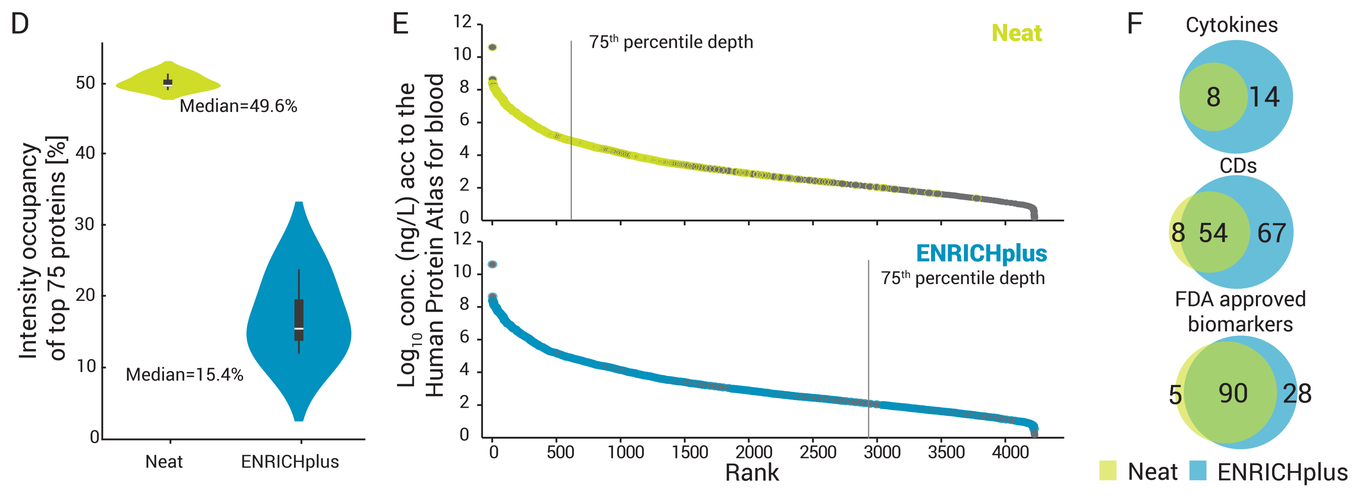
RESULTS



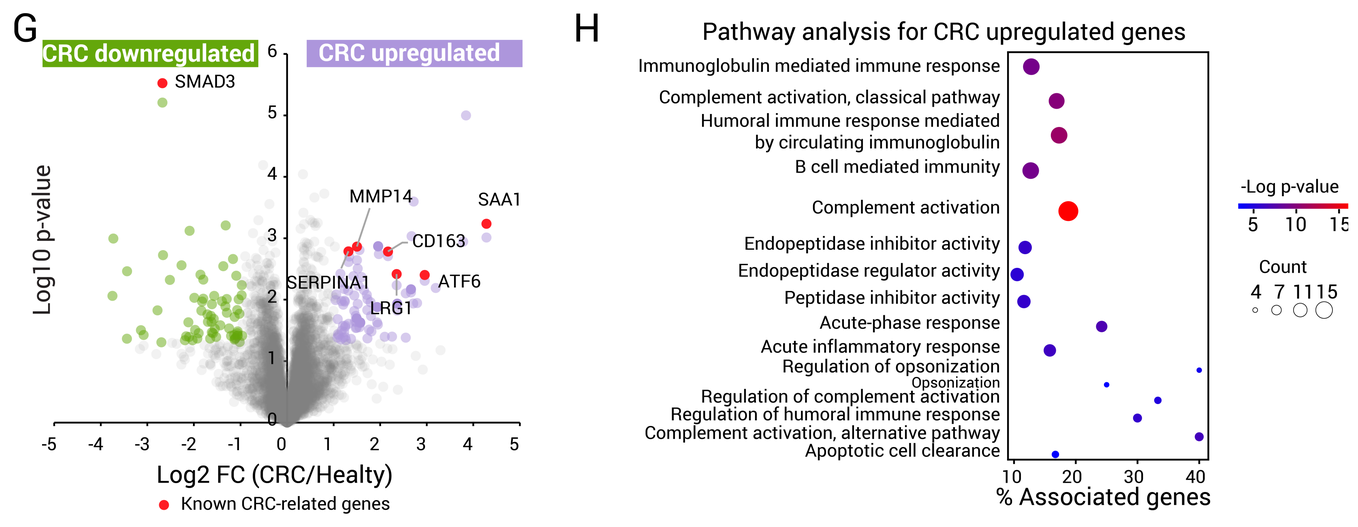
Workflow overview for ENRICHplus applied to plasma from healthy donors and CRC patients.



Increased protein identifications by ENRICHplus. A) ENRICHplus enhanced protein identifications by 6.8-fold compared to neat samples. B) ENRICHplus exhibited reduced technical variation in pooled plasma samples, with a coefficient of variation (CV) of 12% for protein MaxLFQ intensities, compared to 15% for neat plasma samples. C) While 85% of the proteins found in neat plasma were also detected with ENRICHplus, additional 4,478 proteins were identified using the enrichment workflow.



Enhanced proteome coverage by ENRICHplus in CRC study. D) ENRICHplus compressed the dynamic range of the plasma proteome by reducing the intensity of the top 75 detected proteins in neat plasma from 50% to 15%. E) ENRICHplus efficiently improved proteome coverage by spanning a protein concentration range of 10 orders of magnitude. F) ENRICHplus enabled the detection of significantly more cytokines, cluster of differentiation (CD) proteins, and FDA-approved biomarkers.



Expanded potential for CRC-related biomarker discovery. G) Principal component analysis (PCA) effectively separated the healthy donors and CRC patients (not shown). Volcano plot analysis of plasma processed with ENRICHplus revealed 7 known CRC-associated genes, including 3 potential CRC biomarkers, distinguishing CRC from healthy cohorts. H) For CRC-upregulated proteins, 16 GOBP pathways were enriched, which are directly linked to cancer immune evasion, as well as tumor invasion, metastasis, and progression.

KEY TAKEAWAYS

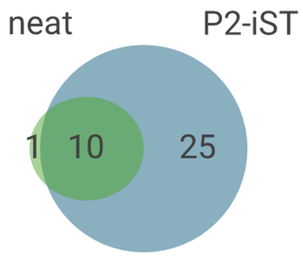
Enhanced protein detection:
Up to **6.8-fold increase** in plasma protein identifications with high reproducibility.

Improved low-abundance protein coverage:
10 orders of magnitude dynamic range compression enables detection of cytokines, CD proteins, and pharmacogenomic biomarkers.

Expanded biomarker profiling:
71 upregulated and **65 downregulated** proteins identified in CRC samples, including established CRC biomarkers.

Scalable and reproducible workflow:
Fully **automatable, high-throughput** method supporting large-scale plasma proteomics studies.

PREVIEW: P2-iST Plasma



Improved sensitivity for low-abundance proteins.

New **P2-iST Plasma workflow** improves protein identification and quantification of biomarker (Sample: human EDTA plasma; LC-MS settings: nLC–diaPaSEF–timsTOF HT; Data processing: Spectronaut® 20).

Visit our booth (#703) to learn more.

CONTACT & MORE

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Conflict of Interest Disclosure
Wohlfahrt, J. is employed by PreOmics Inc.
Hu, Z., Hartinger, K., Pan, K. and Kulak, N.A. are employed by PreOmics GmbH.
Räss, L., Schär, S., Below, C. and Bruderer, R. are employed by Biognosys AG.

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