Advancing plasma proteomics through a next-generation single-particle enrichment workflow for deeper and more quantitative biomarker discovery

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INTRODUCTION

Human plasma represents an exceptional yet analytically demanding matrix for proteomic biomarker discovery. Its extensive dynamic range and compositional heterogeneity limit the detection of low-abundance proteins, which often provide critical pathophysiological insights. To address these challenges, we present a workflow that integrates P2 plasma enrichment technology with iST sample preparation and advanced Spectronaut® 20 data processing. The combined platform leverages the selective enrichment power of P2 and the reproducibility of iST-based digestion and cleanup, enabling deep proteome coverage from human EDTA plasma samples.

MATERIALS & METHODS

Samples: Human EDTA plasma samples from a small pilot cohort of colorectal cancer (CRC) patients (n = 6) and healthy donors (n = 6) were obtained from Biognosys.

Sample preparation: Neat plasma samples (2 µL input) were processed using the iST-BCT protocol (PreOmics). P2-iST Plasma samples were prepared from 100 µL plasma on a KingFisher™ Flex system using the P2-iST protocol, including protein enrichment, lysis, digestion, and peptide cleanup.

LC–MS analysis: 800 ng of peptides were analyzed on an Evosep™ One system using an Aurora Elite CSI 75 µm × 150 mm C18 column, coupled to a timsTOF HT (Bruker). Peptides were separated using the Whisper™ Zoom 40 SPD method and acquired in diaPASEF® mode.

Data processing: Raw data were analyzed in Spectronaut® 20 (Biognosys) using directDIA+™ mode. Gene ontology (GO) enrichment analysis was performed using STRING-DB.

KEY TAKEAWAYS

Deeper plasma proteome: P2-iST Plasma enables ~6× increased plasma proteome coverage compared with neat plasma.

Low-abundance protein access: Enrichment expands detection of low-abundance proteins relevant to CRC biology.

Expanded differential signal: Increased depth yields more CRC-associated differentially expressed proteins.

Discovery-scale bio-signatures: Correlation-based analysis enables identification of CRC-associated multi-protein signatures.

Complementary diagnostic markers: Established plasma IVD markers provide robust quantitative anchors alongside discovery findings.

CONTACT & MORE

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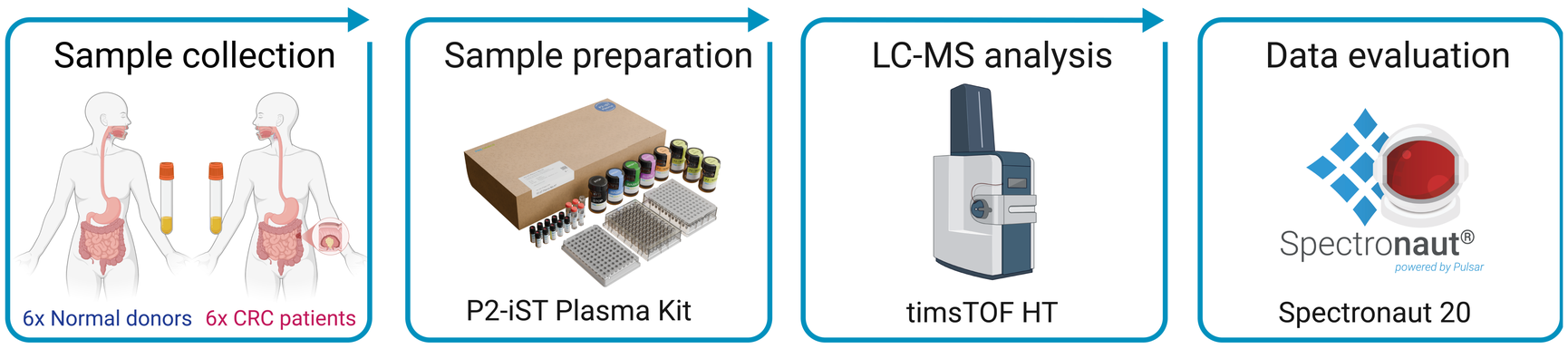
Conflict of Interest Disclosure
Abreha, M. is employed by PreOmics Inc.
Hu, Z., Limm, K., Kulak, N.A. are employed by PreOmics GmbH.
Schär, S., Arthur, V., Bruderer, R. are employed by Biognosys AG.

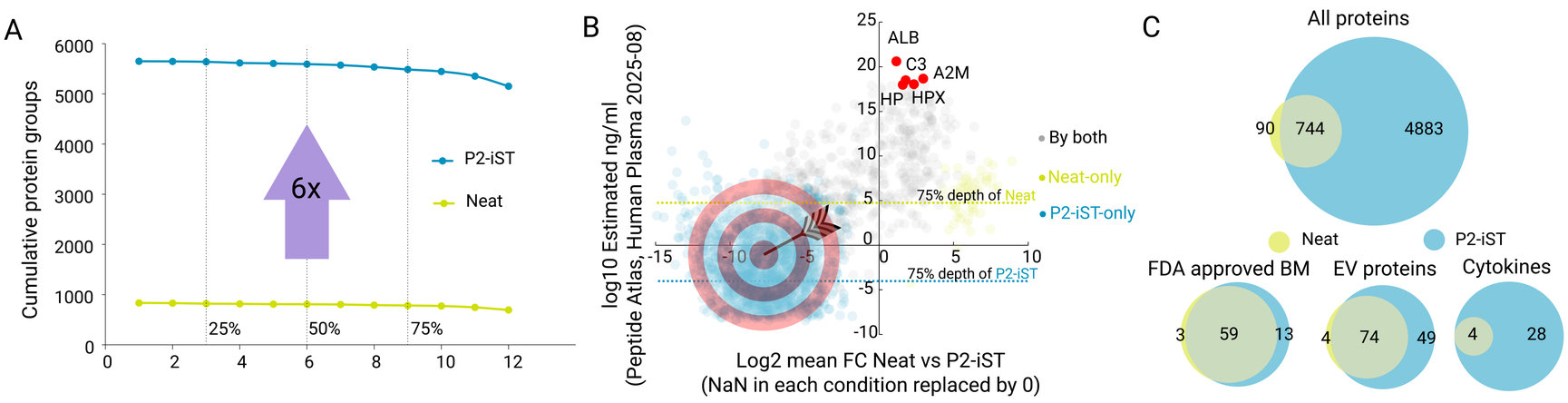
References:

- McQueeney KE, et al. *FASEB J.* **2008,** 32(10), 5661–5673.
- Anderson, N. L, et al. *Clin Chem.* **2010**, *56* (2), 177–185.
- PeptideAtlas - Human Plasma 2025-08.
- IVD status was assigned according to FDA-cleared assays and international clinical diagnostics guidelines.

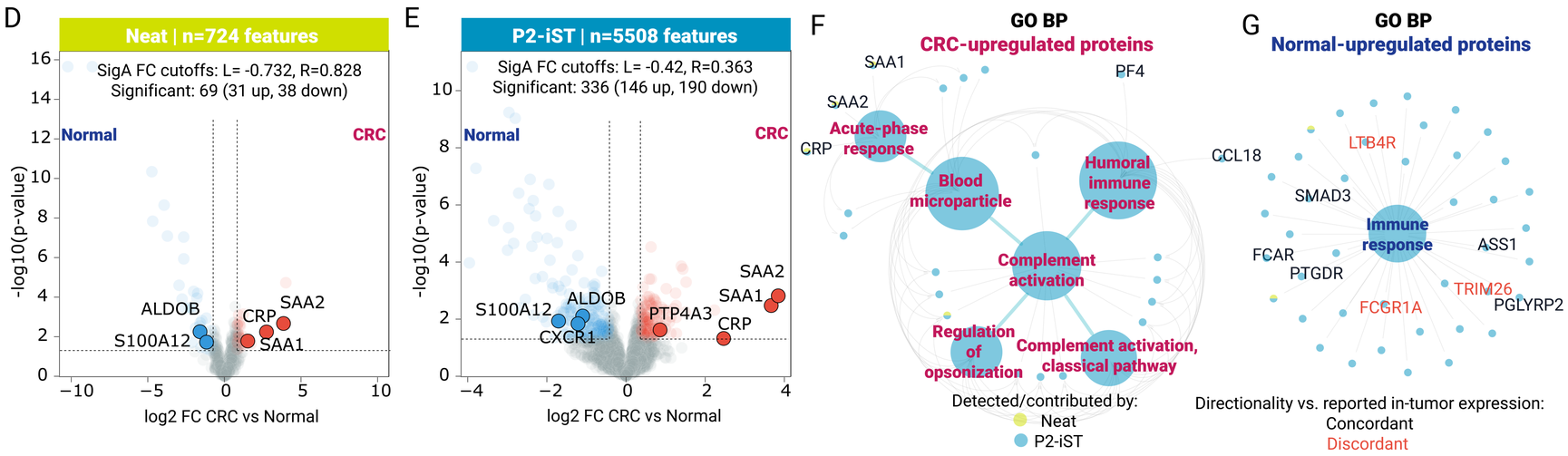
RESULTS



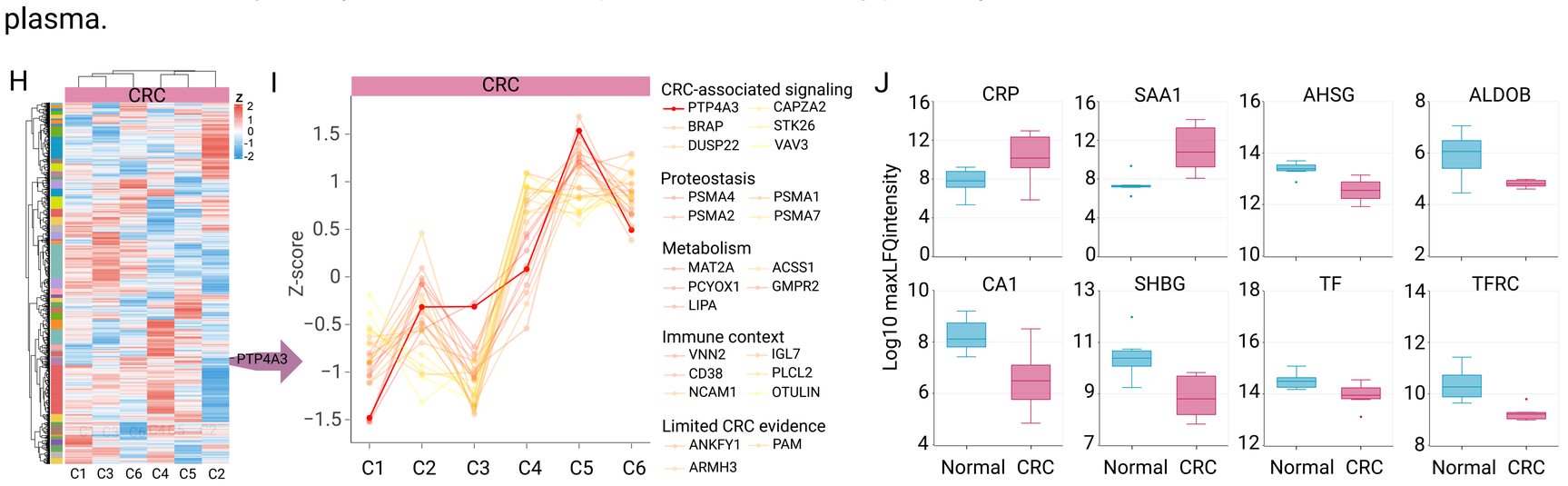
Experimental design: Human EDTA plasma from CRC patients (n = 6) and healthy donors (n = 6) were analyzed using neat plasma and P2-iST Plasma workflows followed by diaPASEF acquisition and directDIA+ processing in Spectronaut.



P2-iST Plasma expands plasma proteome depth and access to low-abundance proteins. (A) Cumulative protein group identifications demonstrate an approximately six-fold increase in plasma proteome depth achieved by P2-iST Plasma compared with neat plasma across the abundance distribution. **(B)** Comparison of mean protein fold change versus estimated plasma concentration shows preferential enrichment and detection of low-abundance proteins by P2-iST Plasma, extending coverage beyond the depth achieved by neat plasma. **(C)** Overlap analysis of detected proteins highlights complementary coverage between workflows, with P2-iST Plasma uniquely contributing thousands of additional proteins, including FDA-approved biomarkers, extracellular vesicle proteins, and cytokines.



Deeper coverage reveals expanded CRC-associated differential proteome and pathways. (D) Volcano plot of CRC versus normal plasma using neat plasma shows a limited number of differentially expressed proteins due to restricted proteome depth. **(E)** Volcano plot of CRC versus normal plasma using P2-iST Plasma reveals a substantially expanded differential proteome, enabling detection of additional CRC-associated proteins. **(F)** Gene ontology biological process enrichment of CRC-upregulated proteins identified by P2-iST Plasma highlights immune response, complement activation, and acute-phase pathways. **(G)** Gene ontology biological process enrichment of normal-upregulated proteins reveals immune regulatory and homeostatic processes, reflecting pathway-level differences between CRC and normal plasma.



From differential proteins to CRC-associated plasma bio-signatures. (H) Heatmap of quantified proteins shows unsupervised clustering patterns across CRC samples, highlighting correlation with the known CRC-associated marker PTP4A3. **(I)** Z-score–based intensity profiles of proteins co-varying with PTP4A3 reveal coordinated expression patterns, including proteins reported to be associated with CRC-related signaling, metabolism, and immune context. **(J)** Boxplots of selected IVD-approved plasma markers demonstrate consistent CRC–normal intensity differences with high quantitative confidence, providing robust quantitative anchors alongside discovery-scale signatures.