Standardized and fully automated deep plasma profiling of plasma integrated with Evosep One enables large scale clinical cohort analysis

Joel Vej-Nielsen[1], Deanna Plubell[2], Christine C. Wu[2], Magnus Huusfeldt[1], Andrea Ellero[3], Stoyan Stoychev[1,3], Dorte B. Bekker-Jensen[1], Michael J. MacCoss[2], Nicolai Bache[1]

[1] Evosep Biosystems, Denmark. [2] Department of Genome Sciences, University of Washington, Seattle, USA. [3] Resyn Bioscieces, South Africa

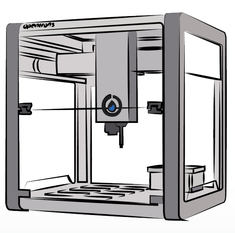
Highlights

- Complete end-to-end workflow allowing deep plasma profiling in a single analysis.
- Standardized and fully automated enrichment of membrane-bound vesicles and depletion of abundant proteins from just 4 µl of plasma.
- Coupled to SCIEX ZenoTOF, the workflow reproducibly identifies >3,500 protein groups at a throughput of 30 samples per day by the push of a button.

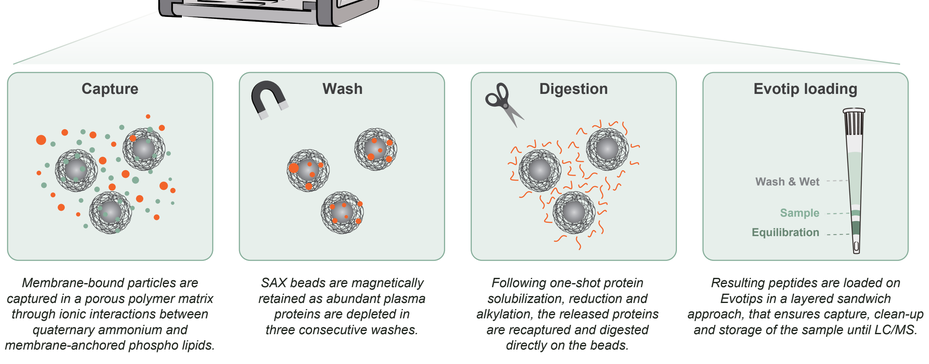
Rapid, fully automated membrane vesicle enrichment

Push-button solution - from plasma to peptides on Evotips

Here we present an end-to-end workflow on the Opentrons (OT-2) for the analysis of undepleted plasma and implementation of the Mag-Net protocol with PAC digestion and automated peptide cleanup for complete hands-free deep plasma proteome profiling. By simultaneously enriching vesicle-cargo proteins and depleting abundant plasma proteins using MagReSyn SAX beads, this workflow significantly increases plasma proteome depth in a standardized easy-to-use workflow. With just 4 µl of plasma input this method presents a cost-effective and sample conserving strategy for robust analysis of large sample cohorts. The workflow is based on this pre-print *https://www.biorxiv.org/content/10.1101/2023.06.10.544439v2*



96 samples
~10 hours



Membrane-bound particles are captured in a porous polymer matrix through ionic interactions between quaternary ammonium and membrane-anchored phospho lipids.

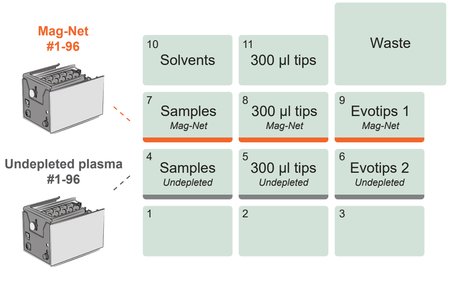
SAX beads are magnetically retained as abundant plasma proteins are depleted in three consecutive washes.

Following one-shot protein solubilization, reduction and alkylation, the released proteins are recaptured and digested directly on the beads.

Resulting peptides are loaded on Evotips in a layered sandwich approach, that ensures capture, clean-up and storage of the sample until LC/MS.

High-throughput and ease-of-use

After deposition of 4 µl plasma and 1 µl MagResyn SAX beads, the protocol efficiently prepares 96 enriched plasma samples in 10 hours. To facilitate more comprehensive analysis, an additional set of 96 paired undepleted plasma samples can be prepared using just one additional µl of plasma and an additional 2 hours of running time.

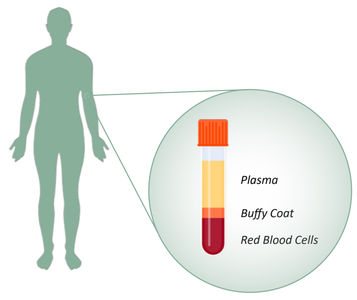


Layout of the OT-2 deck for 192 paired undepleted and Mag-Net enriched samples.

Validation of enrichment and depletion

Different approaches to plasma preparations

Plasma preparation protocols vary between labs and we tested three procedures to assess their impact on the achievable proteome depth using the 100 samples per day method and the ZenoTOF 7600. The analysis of undepleted plasma samples revealed similar proteomic profiles across all three plasma preparation methods, while the Mag-Net enriched samples showed clear differences. All preparation strategies resulted in similar levels of depletion, but the 1x1200g prep resulted in the highest level of enrichment.



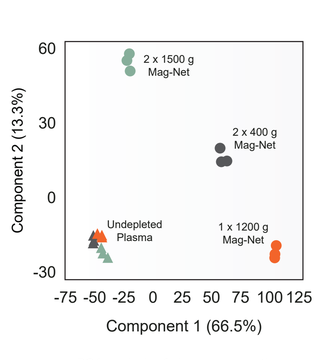
Different procedures for collection of plasma from whole blood

1 healthy donor

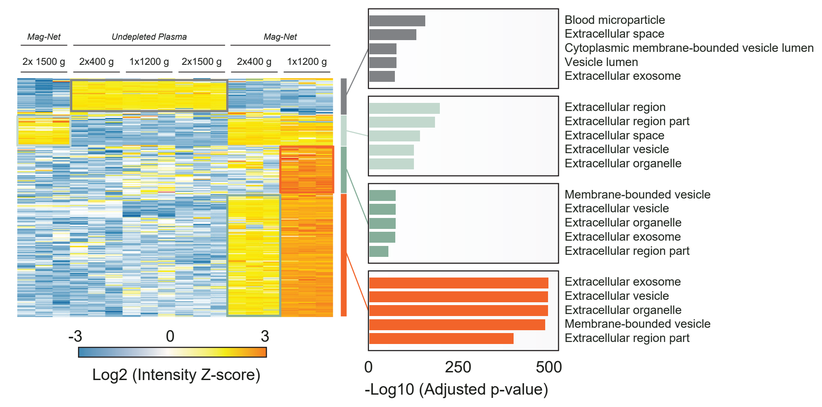
Early Detection Research Network SOP
1 x 1200 g for 15 min (1x1200g)

Platelet Poor Plasma
2 x 1500 g for 10 min (2x1500g)

Platelet Rich Plasma
2 x 400 g for 10 min (2x400g)



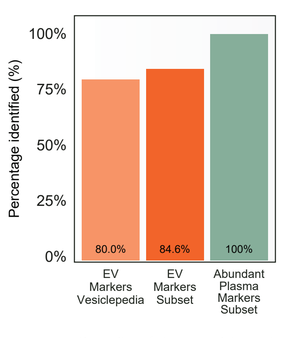
PCA analysis of plasma preparations



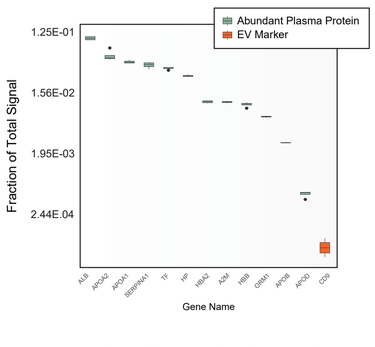
Hierarchical clustering of protein intensities and enrichment analysis of the proteins that were depleted or enriched in the Mag-Net protocol using the Enrichr tool within Jensen COMPARTMENTS.

Good enrichment of vesicles across plasma preparations

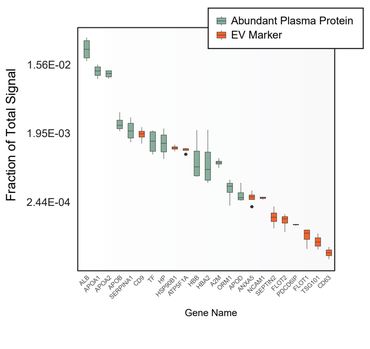
Significant enrichment was achieved in Mag-Net 1x1200g detecting 80% of the EV marker proteins listed in Vesiclepedia. This enrichment was accomplished through efficient reduction of the dynamic range within the sample.



Proportion of plasma proteins and EV markers in Mag-Net (1x1200g) plasma.



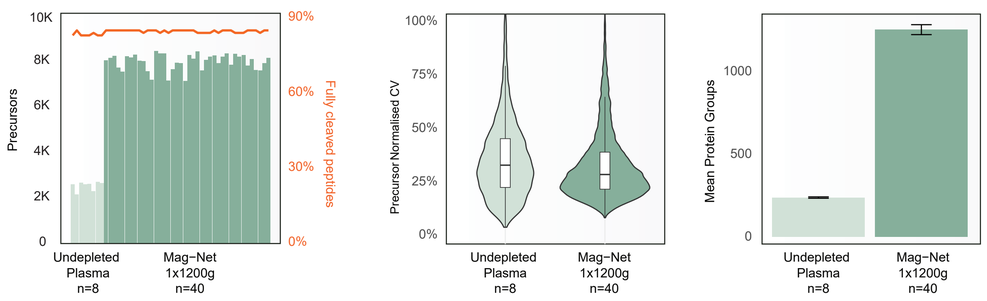
Fraction of total signal intensities of selected abundant proteins in undepleted plasma (1x1200g).



Fraction of total signal intensities of selected abundant proteins in Mag-Net plasma (1x1200g).

Rapid and robust plasma analysis

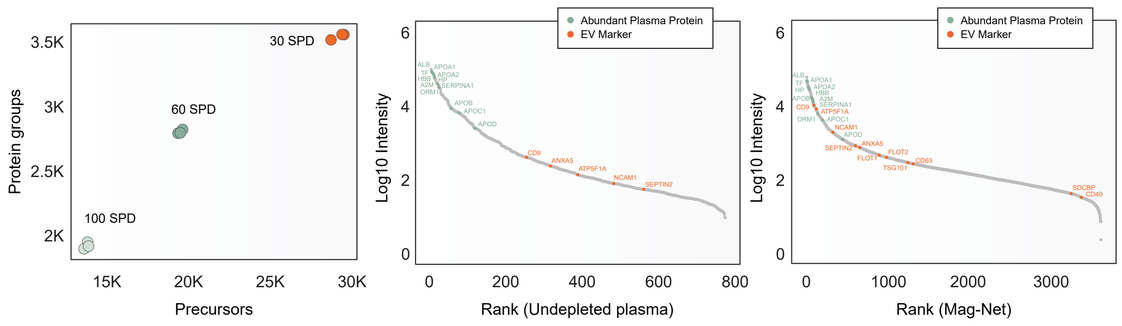
The reproducibility of the protocol was evaluated by preparing 40 Mag-Net samples and 8 samples of undepleted plasma (1x1200g). The analysis reproducibly resulted in the identification of 2,500 precursors in undepleted plasma and 7,500 precursors in Mag-Net samples with a digestion efficiency of ~85% using the 200 SPD method in combination with the SCIEX ZenoTOF 7600 and analyzed in library-free mode using DIA-NN. This resulted in the identification of 250 and 1,250 protein groups in undepleted plasma and Mag-Net samples, respectively. Finally, Mag-Net samples showed improved precursor precision compared to undepleted plasma.



Identifications, digestion efficiency and reproducibility of Mag-Net 1x1200g samples loaded on Evotips and analysed with 200 SPD on the ZenoTOF 7600.

More than 3,500 proteins identified in plasma

In combination with our standard methods the protocol allows for high-throughput application and in-depth plasma analysis. This resulted in the identification of 1,700 and 2,500 protein groups with the 100 and 60 samples per day methods respectively, when coupled to the SCIEX ZenoTOF 7600. This was increased to 30,000 precursors leading to more than 3,500 protein groups with the 30 samples per day method in library-free mode using DIA-NN. The protocol shows an effective reduction of the dynamic range and a 4-fold increase in the amount of identified protein groups relative to undepleted plasma.



Identifications and dynamic range for Mag-Net enriched and undepleted 1x 1200g plasma, each condition was searched separately with match between runs.