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Expanding multi-omics capabilities on the timsMetabo with deep bottom-up proteome analysis

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Introduction

Comprehensive investigation of biochemical and medical systems necessitates robust and scalable analysis of diverse molecular classes across a broad dynamic range of sample amounts. The timsMetabo mass spectrometer was designed to deliver unprecedented TIMS separation power for small molecules, while maintaining the established, high performance for bottom-up proteome sample analysis. We demonstrate that the timsMetabo can address complex cell lysate samples from hundreds of nanograms to single cell levels, as well as comprehensively covering the plasma proteome for neat plasma preparations and enriched plasma protein fractions (ENRICH-iST). Furthermore, we present a new TIMS-enabled MS/MS mode which unlocks a larger dynamic loading range for complex proteomics samples.

Dia-MoRE enhances TIMS separation of peptide precursors

The recently launched timsMetabo system features enhanced transmission efficiency for low m/z analytes, which is particularly important for 4D-Metabolomics. Ion transmission from the ion source into the TIMS region was optimized for higher gas flows, improving sensitivity under low sample input conditions. Synergistic operation of the TIMS-MX cartridge and novel Athena ion processor (AIP) enables uncompromised high-throughput time-of-flight analysis for a wide *m/z* range, improving MS sensitivity and established PASEF data collection[3]. Furthermore, the timsMetabo can be operated in the novel Mobility Range Enhancement (MoRE) mode which increases the tims separation space and thereby facilitates 4D-analysis of labile molecules and is compatible with dda- and dia- acquisition schemes.

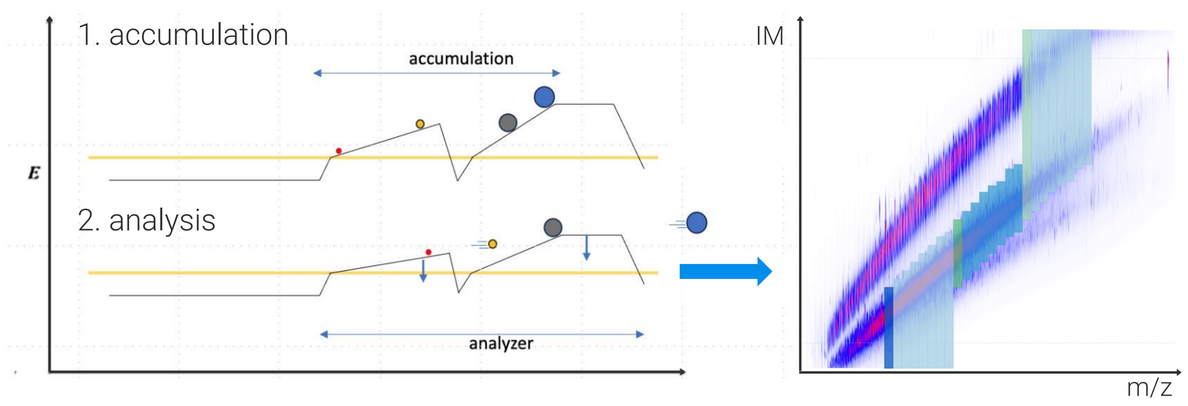

Figure 1 dia-MoRE acquisition on timsMetabo.

Proteome coverage by dia-PASEF and dia-MORE acquisition

First, we evaluated the proteomics performance of the timsMetabo mass spectrometer for a wide range of sample amounts of whole cell K562 lysates (Promega) from single cells (250 pg) to 100 ng. Peptides were separated by nano-RP LC on the nanoElute HPLC using an IonOpticks Aurora column (25 cm x 75 µm, C18) in 22 in and directly infused into the mass spectrometer via the captive spray ionization source. For low sample amounts, dia-PASEF acquisition was performed to achieve highest sensitivity, higher sample amounts were acquired with dia-MoRE to enhance ion mobility separation for precursor ions. We observed 3300 protein groups (~17k peptides) in 250 pg K562 samples and more than 7000 protein groups (~63k peptides) for 100 ng injection amount (Fig. 2A). For all injection amounts, we observed excellent reproducibility (Fig. 2B). Due to the sequential accumulation/elution steps occurring in dia-MoRE acquisition, the number of datapoints was slightly reduced from 12 to 8 (Fig. 2C). For dia-PASEF and dia-MoRE acquisition, we recorded excellent relative fold change values between different injection amounts; for low injection amounts, the protein abundance relative to 250 pg was 3.66 for 1 ng injections and 19 for 5 ng injections, compared to theoretical FC of 4 and 20 respectively (Fig. 2D). In dia-MoRE, a similar reproducibility of expected fold changes was observed relative to 10 ng K562 injections for 20 ng, 50 ng and 100 ng peptides (Fig. 2E).

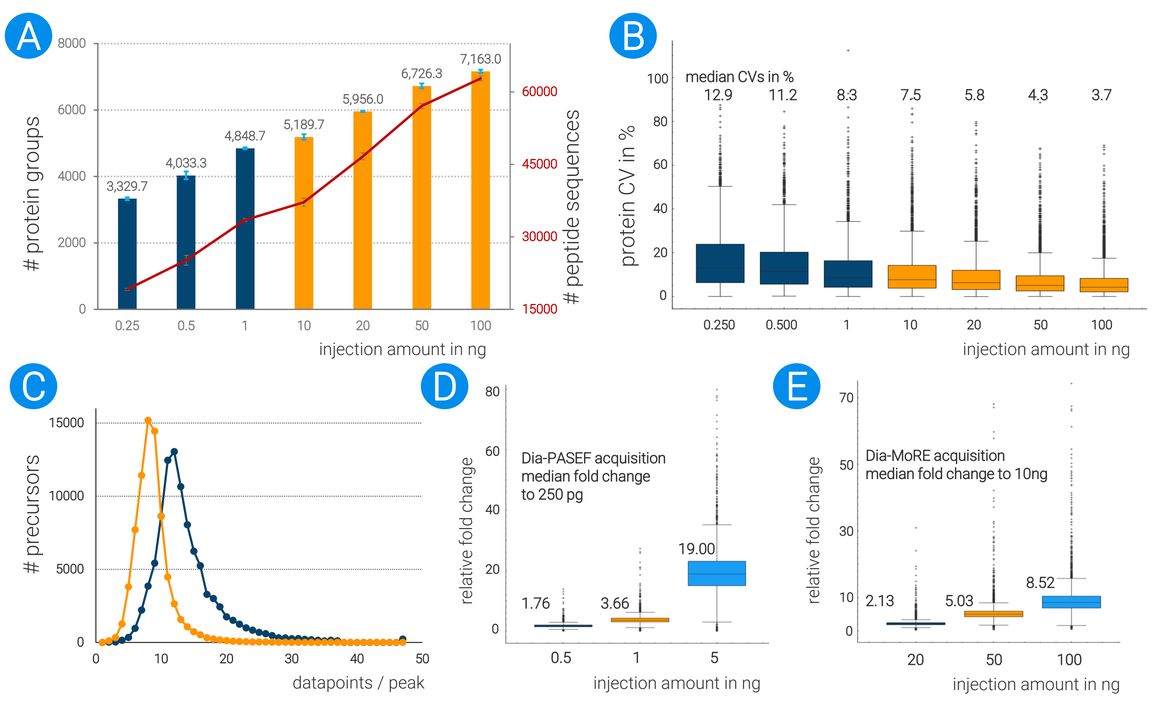

Figure 2 Proteomics metrics of timsMetabo. A) Protein group identifications for dia-PASEF acquisition for low injection amounts of K562 digest (average from 3 replicates, dark blue) and dia-MoRE acquisition for high injection amounts (orange). Corresponding peptide identifications are shown in red (right axis) B) Protein group CVs for dia-PASEF (dark blue) and dia-MoRE acquisition (orange) for tryptic K562 cell digest. C) Separate ion accumulation in dia-MoRE acquisition (orange) reduces the number of datapoints for precursor ion by 30-40% for 30 ms accumulation time in comparison to dia-PASEF acquisition (dark blue) D) Relative fold change to 250 pg data for 3300 common protein groups acquired with dia-PASEF acquisition. E) Relative fold change to 10 ng K562 for >4800 common protein groups acquired with dia-MoRE acquisition.

Plasma proteomics with timsMetabo

Human plasma was prepared by tryptic digestion and with the PreOmics ENRICH-iST kit. 200 ng of neat plasma peptides and 100 ng of enriched plasma were analyzed on the timsMetabo mass spectrometer set up in dia-MoRE acquisition mode. For neat and enriched plasma samples, 540 and 1600 protein groups were identified (Fig. 3A), respectively, using a 22 min nano-RP peptide separation. This confirms the previously reported enrichment factor of ~3x protein IDs. Both samples reported excellent CV values and reproducibility over the whole protein abundance range (Fig. 3B/C). Although proteins covered over 5 orders of magnitude in both samples, additional enrichment extended the coverage of low abundant proteins significantly. (Fig. 3D)

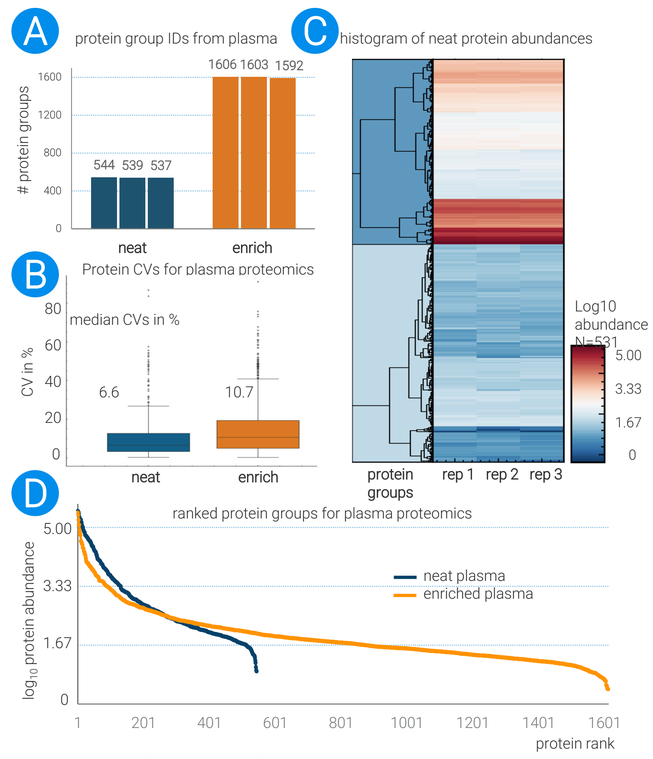

Figure 3 Plasma Proteomics. A) Protein group identifications from neat and enriched plasma fractions B) reproducibility of protein quantitation in 3 replicates of plasma preparations C) heatmap of protein abundances in 3 replicates of neat plasma digests D) distribution of protein abundances (as log10) in neat and enriched plasma fractions.

Protein quantitation in complex proteome samples

To monitor changes in biological systems reliable protein quantification is important. After determining excellent precision of the timsMetabo (Fig. 2B), we evaluated foldchange accuracy using two mixtures of tryptic whole cell peptides of K562 (human), yeast and E.coli. Whereas the amount of K562 was unchanged at 50% in both mixtures, the yeast content was reduced from 30% to 20% in the second sample and the E.coli fraction was increased from 20% to 30%, accordingly. Comparing both samples, we would expect a similar effect of regulation for E.coli and yeast proteins however in different direction. We monitored more than 10k protein groups consisting of 5900 proteins matching to human genes, 2600 yeast matches and 2000 E.coli proteins (Fig. 4A). Abundance ratios were calculated by dividing the average protein abundance in sample 2 by sample1, human proteins cluster around an abundance ratio of 1, confirming that the content of human peptides was not altered. Yeast protein groups are clustering narrowly at a ratio of 1.29, very close to the expected 1.33 abundance difference. E.coli protein groups are shifted downwards to a ratio of 0.73 (expected ratio 0.67) (Fig. 4B). Across all quantified proteins, more than 90% reported a CV of 20% or lower and 70% of protein were quantified with a CV of 10% or lower confirming the capability for precise quantitative proteomics in complex sample mixtures (Fig. 4C).

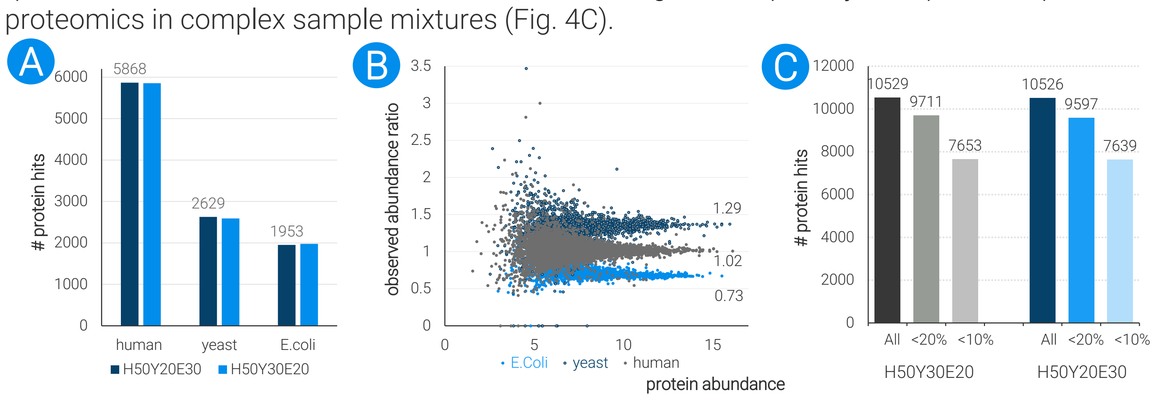

Figure 4 Protein quantitation from complex proteomics samples. A) More than 10,500 protein groups were detected in mixed proteome samples and the two mixtures reported only minor differences in protein counts for individual species. B) Abundance ratios of protein groups matching to specific genomes closely recover the theoretical mixing ratios (human 1, yeast 1.33, E.coli 0.67). C) From 3 biological replicates, more than 90% of protein groups were quantified with a CV <20% and about 75% were at 10% or lower CV.

Summary

The timsMetabo platform was designed to provide excellent 4D metabolomics and lipidomics analysis, however it also retains the outstanding proteomic performance of the timsTOF platforms. Our results on proteome coverage and quantitation in complex proteome mixture as well as depth in clinically relevant blood plasma samples, demonstrates the true multi-omics capability of the platform.

Conclusion

- timsMetabo provides two ion mobility separation modes for proteomics analysis: dia-PASEF offers high sensitivity and reproducibility for low sample amounts; dia-MoRE offers high protein coverage and reproducibility for high amounts.
- Analysis of neat and enriched plasma samples confirmed the high reproducibility of protein quantitation with CVs in the range of 6-10% on protein level. Plasma proteomics covered more than 500 and 1600 protein groups in neat and enriched fractions, respectively.
- Complex biological samples revealed accurate protein quantitation for proteins from different organisms.

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