Integrated Metabolomics and Proteomics: Mapping the Metabolic Landscape of the Mouse Placenta

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Multi-omics on timsMetabo™

Modern LC-MS/MS systems are typically dedicated to either proteomics or metabolomics. However, these data types are inherently complementary: metabolomics provides a direct readout of pathway activity, while proteomics clarifies the enzymatic drivers of the observed shifts and helps understand the direction of change.

As a metabolically active organ essential for fetal development, the placenta serves as an ideal model for testing the timsMetabo platform's ability to map complex metabolic reprogramming. In this proof-of-concept study, we explore the discovery potential of an integrated approach: we define metabolic signatures in the mouse placenta using metabolomics and proteomics datasets acquired in a parallel split-sample design. By leveraging this integrated workflow, we demonstrate that even placentas from the same mother exhibit unique molecular signatures and begin mapping enzymatic drivers of inter-placental heterogeneity.

Fig. 1 | Separating mouse fetus from the placenta.

Metabolomics

Placentas (n = 9; 3 per mouse) were extracted with hot ethanol spiked with 17 $^{13}C/^{15}N$-labeled amino acids. Analytes were separated on a Waters T3 HSS column (100 x 2.1 mm) using 0.1% formic acid in water/acetonitrile. TIMS-LC-MS/MS data were acquired in PASEF and MoRE modes (Fig 1a). Data were processed using MetaboScape.

Samples clustered by maternal origin (Fig 2b), though significant intra-litter variability was observed. Differential analysis identified lipids as the primary drivers of metabolic reprogramming between genotypes (Fig 2c).

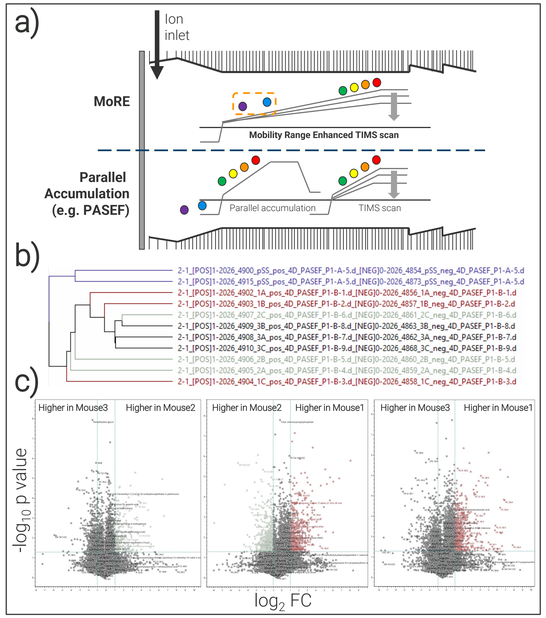
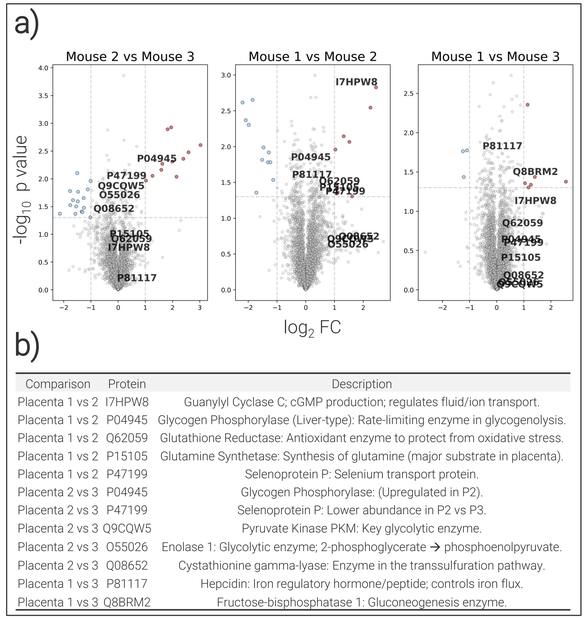


Fig. 2 | Metabolomics data acquisition and data exploration. a) Leveraging trapped ion mobility separation for DDA analysis using MoRE and PASEF scan modes. b) Clustergram of placenta from the same mice (same colors) compared to placenta from different mice. c) Pairwise differential analysis between mice n = 3 placentas per mouse).

Proteomics

Proteins were recovered from lyophilized tissue, denatured in the presence of protease inhibitors, reduced, alkylated, and cleaned with SP3 beads prior to overnight trypsin digestion and C18 cleanup. Peptides (150 ng) were separated on a PepSep column (100 x 0.15 mm) using a 0.1% formic acid water/acetonitrile gradient. TIMS-LC-MS/MS data were acquired in DIA-MoRE mode and processed using DIA-NN.

We identified 32,708 peptides mapping to 4,773 protein groups. Comparative analysis revealed 12 differentially abundant metabolic enzymes linked to energy homeostasis, showing significant variation between placentas from different mothers (Fig 3 a-b).



b)

Comparison	Protein	Description
Placenta 1 vs 2	I7HPW8	Guanylyl Cyclase C; cGMP production; regulates fluid/ion transport.
Placenta 1 vs 2	P04945	Glycogen Phosphorylase (Liver-type): Rate-limiting enzyme in glycogenolysis.
Placenta 1 vs 2	Q62059	Glutathione Reductase: Antioxidant enzyme to protect from oxidative stress.
Placenta 1 vs 2	P15105	Glutamine Synthetase: Synthesis of glutamine (major substrate in placenta).
Placenta 1 vs 2	P47199	Selenoprotein P: Selenium transport protein.
Placenta 2 vs 3	P04945	Glycogen Phosphorylase: (Upregulated in P2).
Placenta 2 vs 3	P47199	Selenoprotein P: Lower abundance in P2 vs P3.
Placenta 2 vs 3	Q9CQW5	Pyruvate Kinase PKM: Key glycolytic enzyme.
Placenta 2 vs 3	O55026	Enolase 1: Glycolytic enzyme; 2-phosphoglycerate → phosphoenolpyruvate.
Placenta 2 vs 3	Q08652	Cystathionine gamma-lyase: Enzyme in the transsulfuration pathway.
Placenta 1 vs 3	P81117	Hepcidin: Iron regulatory hormone/peptide; controls iron flux.
Placenta 1 vs 3	Q8BRM2	Fructose-bisphosphatase 1: Gluconeogenesis enzyme.

Fig. 3 | a) Pairwise differential analysis between individual mouse placentas (n = 3 mothers; 3 placenta per mother). Volcano plots highlight proteins with significant differential abundance (Log2 fold change > 1, -log10 p-value > 1.3). b) Targeted identification of differentially abundant metabolic enzymes. Table summarizes key enzymes involved in energy homeostasis, nutrient transport, and iron regulation identified from the proteomic comparisons in (a).

Broad metabolic pathway coverage via integrated multiomics

All identified metabolites and proteins were visualized over core metabolic pathways (Fig 4). We observed that many pathways, such as the TCA cycle, urea cycle, and nucleotide synthesis, have complementary metabolomics and proteomics coverage, allowing for the determination of differential pathway activity in future comparative studies.

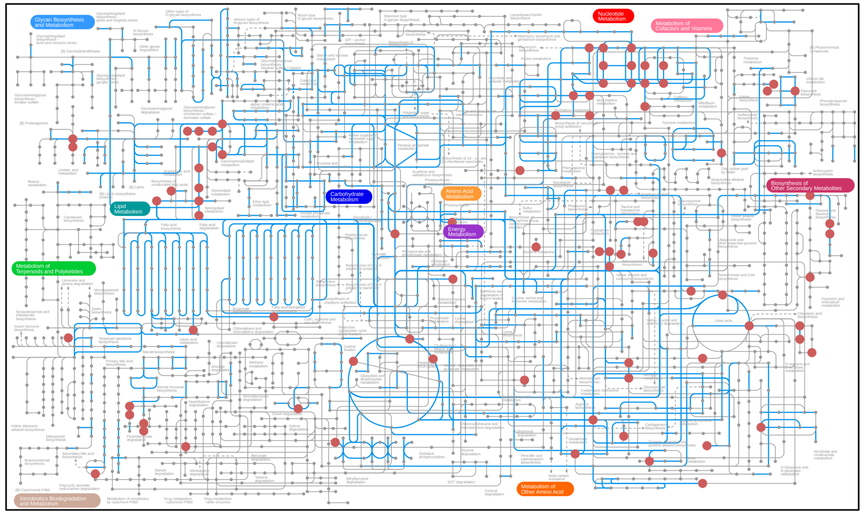


Fig. 4 | Metabolomics and proteomics data combined into an iPath3 metabolic pathway map.

- timsMetabo workflow enables multiomics analyses with minimal downtime between acquisition modes, completing total data collection in under two weeks.
- Significant biological variation exists between placentas from the same mother; each placenta functions as a highly individual organ.
- Proteomics analysis recovers metabolic enzymes known to be essential to placental function.
- Combining metabolomics and proteomics provides a synergistic biological view that is more informative than the sum of its individual parts.
- Outlook: Apply this methodology to investigate placental and fetal disease and increase metabolome and lipid coverage by employing complementary LC methods.

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