Development of proteomic-biomarker landscape of AY9944-treated mouse brain tissues mimicking Smith-Lemli-Opitz syndrome (SLOS) by using DIA-PASEF and DIA-FAIMS mass spectrometry

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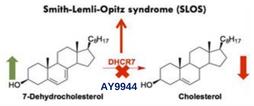
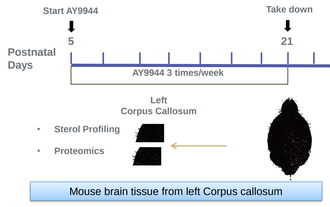
Goal: Comparison of two very recent DIA mass spectrometry techniques - DIA-PASEF & DIA-FAIMS - in analyzing proteomic biomarkers related to the compound AY9944-treated mouse tissues mimicking Smith-Lemli-Opitz syndrome (SLOS).

SLOS: significant unmet need

- **A Rare disease** - An autosomal recessive inherited disorders with an incidence of **1 in 10,000–70,000** and a carrier frequency as high as **1 in 30,** and is caused by 7-dehydrocholesterol reductase (DHCR7) deficiency in cholesterol biosynthesis pathway
- **Hypomyelination is one of hallmarks**-CNS defects in white matter, often involving corpus callosum absence or hypoplasia and reported to involve absence of myelin and demyelination
- **Pathogenesis** –Multiple congenital malformation, mental retardation with behavioral phenotype
- **Biological target** – Lost of function of DHCR7 enzyme activity
- **Biochemical biomarkers-** Accumulation of 7DHC (2.7~470 ug/ml; 10~2000-fold normal) and a deficiency of endogenous cholesterol



Sample information



Take down: 21 days (21 days-treated)	Take down: 21 days (ctrl)	Take down: 35 days (21-days treated)	Take down: 35 days (Ctrl)
Mom1-Pup1	Mom2-Pup1	Mom3-Pup1	Mom4-Pup1
Mom1-Pup2	Mom2-Pup2	Mom3-Pup2	Mom4-Pup2
Mom1-Pup3	Mom2-Pup3	Mom3-Pup3	Mom4-Pup3
Mom1-Pup4	Mom2-Pup4	Mom3-Pup4	Mom4-Pup4
Mom1-Pup5	Mom2-Pup5	Mom3-Pup5	Mom4-Pup5
Mom1-Pup6	Mom2-Pup6	Mom3-Pup6	Mom4-Pup6
Mom1-Pup7	Mom2-Pup7	Mom3-Pup7	Mom4-Pup7
		Mom3-Pup8	QC: (pool)

LC/MS acquisitions
DDA-MS (spectral library generation)
DIA-FAIMS MS (Orbitrap Exploris 480 – in house)
DIA-PASEF MS (Bruker timsTOF – Bruker facility)

Total 29 samples in 4 groups
Two treatment groups, two control groups in two timepoints.
Samples across time-points are independent.

QC: mixture of all samples to monitor sample preparation and LC/MS acquisition

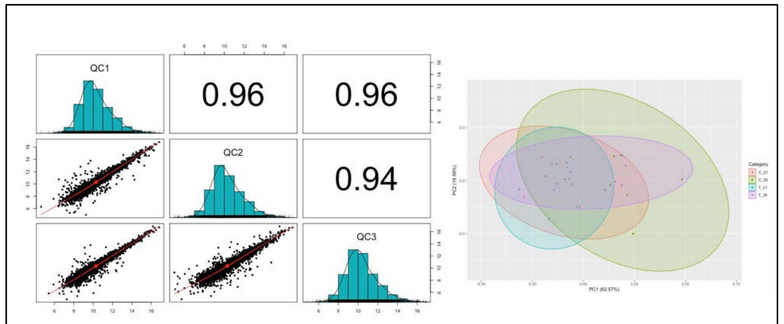


Fig. 2. (left-side) QC (sample pool) performances are satisfactory. **(right-side)** Ctrl- and treated-samples of 21- and 35-days are well segregated in 2D PCA plot.

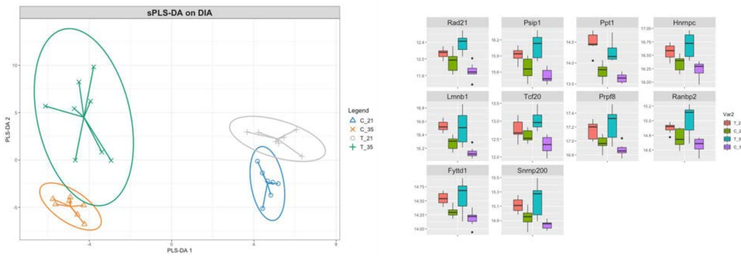
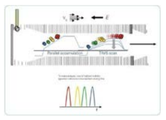


Fig. 3. (left-side) PLS (partial least square regression) for biomarker selection of Thermo Exploris480 mass spectrometer acquired samples. Component 1: 21-days vs. 35-days; Component 2: Treated vs. Ctrl. **(right-side)** Top 10 proteins in component 2



timsTOF Pro Mass Spectrometer (Bruker)

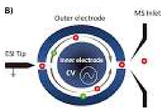
Mouse brain homogenization with
2 mm Zirconia bead
N=29 [Mom#1: n=7; Mom#2: n=7; Mom#3: n=8; and Mom#4: n=7

Total protein measurement by BCA assay for normalization

[40 ug protein] Reduction, and alkylation, & digestion (Lys-C/Trypsin) with RapiGest SF

nanoLC-DIA-MS

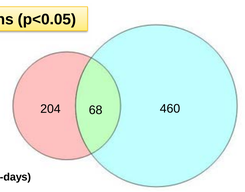
Data analysis (Frag-Pipe & DIA-NN)



Orbitrap Exploris™ 480 Mass Spectrometer (Thermo Scientific)

Fig. 1. Experimental workflow

Differential proteins (p<0.05)



Top Canonical Pathways

Name	p-value	Overlap
Synaptogenesis Signaling Pathway	5.89E-07	5.1 % 16/314
Mechanisms of Viral Exit from Host Cells	9.21E-05	12.2 % 5/41
Spliceosomal Cycle	2.18E-04	10.2 % 5/49
Superpathway of Cholesterol Biosynthesis	2.95E-04	13.8 % 4/29
Cholesterol Biosynthesis I	3.67E-04	23.1 % 3/13

Fig. 4. (top) t test on differential proteins. **(bottom)** Top canonical pathways derived from IPA (Integrated Pathway Analysis) of significant proteins from Exploris480 acquisition of 21-days samples

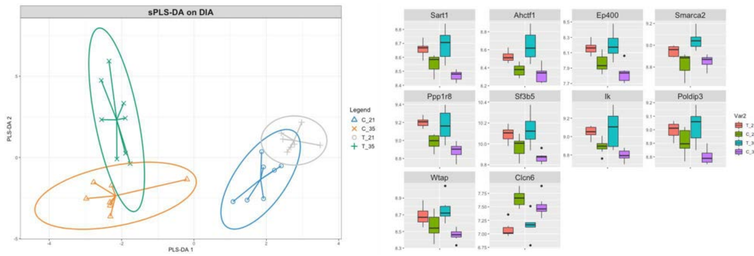
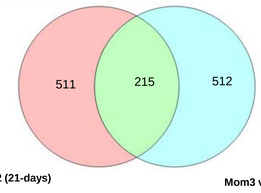


Fig. 5. (left-side) PLS (partial least square regression) for biomarker selection of Bruker timsTOF Pro mass spectrometer acquired samples. Component 1: 21-days vs. 35-days; Component 2: Treated vs. Ctrl. **(right-side)** Top 10 proteins in component 2

Differential proteins (p<0.05)



Top Canonical Pathways

Name	p-value	Overlap
Spliceosomal Cycle	9.04E-11	28.6 % 14/49
Huntington's Disease Signaling	2.40E-08	10.0 % 28/281
Superpathway of Cholesterol Biosynthesis	1.40E-06	27.6 % 8/29
Synaptogenesis Signaling Pathway	2.68E-06	8.3 % 26/314
Cholesterol Biosynthesis I	2.37E-05	38.5 % 5/13

Fig. 6. (top) t test on differential proteins. **(bottom)** Top canonical pathways derived from IPA (Integrated Pathway Analysis) of significant proteins from timsTOF Pro acquisition of 21-days samples

CONCLUSIONS

- A total of 6,811 and 5,516 unique proteins have been identified from DIA-PASEF (Bruker nanoElute LC/Bruker timsTOF Pro MS) and DIA-FAIMS methods (Waters nanoAcquity UPLC/Thermo Exploris480 MS), respectively.
- Comparing the AY9944-treated samples versus control, a total of 204 and 460 differentially expressed proteins with a p<0.05 (an overlap of 68 proteins) were identified from 21- and 35-days samples, respectively, in DIA-FAIMS study. On the other hand, 511 and 512 proteins with an overlap of 215 proteins were identified from 21- and 35-days samples, respectively, with a significant p value (p<0.05) from DIA-PASEF study.
- Top canonical pathways related to differentially expressed proteins in both methodologies includes Superpathway of Cholesterol Biosynthesis, Synaptogenesis Signaling Pathway, Spliceosome Cycle, and Cholesterol Biosynthesis I which are related to neurodegeneration.
- Overall, DIA-PASEF data provides better performance compared to DIA-FAIMS in this study.

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