Nanomics

Application Note

Use of the Proteonano™ Ultraplex platform for deep proteomic studies in mouse plasma samples

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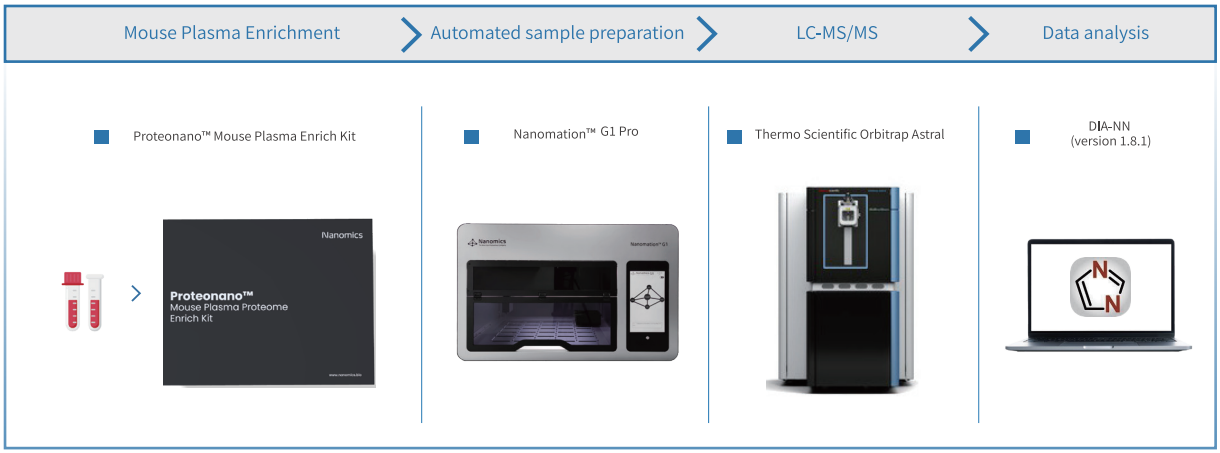
Keywords:
Mouse models, plasma sample, proteomics, mass spectrometry, Proteonano™ Mouse Plasma Enrich Kit, Proteonano™ Pro Suite

Objective:
To develop a high throughput and reliable workflow that incorporates proteonano™ ultraplex proteomics platform based sample processing to enable deep proteomic analysis of mouse blood samples.

Introduction

Proteomics technology is capable of qualitative and quantitative analysis of proteins expressed by the genome [1]. It offers as a complementary approach to genomic and transcriptomic analysis by probing the expression and modifications of proteins that directly controls biological processes. It is widely used in research areas such as the discovery of potential protein biomarkers for diseases, drug targets, and mechanisms of protein interactions. Proteomics usually employs cells, tissues, blood samples and other biological fluids as research materials. Among these, blood samples are more convenient to collect, less costly, and easier to obtain in large quantities for analysis compared to other tissue samples. Additionally, blood samples can reflect the overall biological status and metabolic condition of an organism, making them widely applicable in various biological studies [2].

In recent years, the widespread application of model organisms has brought significant convenience and progress to the study of human diseases. The use of organisms such as rats, mice, pigs, and monkeys has become an important means of exploring disease mechanisms and physiological processes. Kumawat et al. [3] administered αIL17A Affibody molecules in mice to study the levels of pro-inflammatory



Figuer 1: MS Combined with proteonano™ ultraplex proteomics platform for mouse blood sample proteomics analysis

proteins (APOE) in mouse plasma, thereby comparing the levels of inflammatory mediators and the development of atherosclerosis between treatments; Ker zeli et al. [4] established a progressive NMIBC (non-muscle invasive bladder cancer) mouse model to analyze the differential changes in mouse plasma proteomics during the carcinogenic process of bladder cancer.

Mouse models have been used as primary preclinical models for human diseases and are widely used for drug efficacy and toxicology studies, due to their accessibility, relative low cost, and existence of a multitude of mouse disease models. Use of bottom-up untargeted proteomic analysis can facilitate characterization of changes during disease development and therapeutic response. This approach provides a unbiased methods to identify differentially expressed blood proteins, and can also discover protein modifications such as phosphorylation and acetylation. However, there are still issues with LC-MS/MS based untargeted proteomic workflows for tissue or blood sample analysis, including lack of LC-MS assay standardization, inconsistent preprocessing procedures, and varying data analysis pipeline, which can result in incomplete experimental data, inability to replicate experimental data, and difficulties to compare results obtained from different studies. This, in turn, affects the accuracy and reliability of the proteomic data.

Another major challenge for proteomic analysis of blood samples is the existence of high abundance protein. These proteins, including albumin and globulins, limits detection of low abundant blood proteins, limiting detection depth of LC-MS/MS based untargeted proteomic analysis.

The Proteonano™ Ultraplex Proteomics Platform is a highly automated and standardized proteomics sample preparation and analysis system launched by Nanomics Biotech. It consists of the Proteonano™ Kit and the Nanomation™ automated sample processing suite. The Proteonano™ Kit series is developed leverage surface-modified, monodisperse magnetic nanoparticles（S3MNPs）, and the Nanomation™ automated sample processing suite affords consistent sample processing of large sample cohorts across different sample processing batches. Combination of both components addresses the bottleneck of detecting low-abundance proteins in mass spectrometry in a reliable fashion.

In this note, we utilized Nanomics Biotech ' s Proteonano™ Ultraplex Proteomics Platform, in conjunction with high resolution mass spectrometry technology, to conduct untargeted proteomic analysis on different mouse blood samples. This provides application scenarios for mouse blood samples and establish a standardized analysis workflow.

Results and Discussion

Enrichment of Proteins from Mouse Plasma Samples: Proteonano™ Ultraplex Proteomics Platform

The Proteonano™ Ultraplex Proteomics Platform is a discovery platform specifically designed to address the bottlenecks in mass spectrometry based protein detection for blood samples. It consists of the Proteonano ™ Mouse Plasma Proteome Enrich Kit and the Nanomation ™ G1 Pro Suite. The Proteonano™ proteomics kit employs the surface-modified, monodisperse magnetic nanoparticles technology developed by Nanomics Biotech to enrich proteins in biological samples [5].

Proteonano™ Mouse Plasma Proteome Enrich Kit from Nanomics Biotech and the Nanomation ™ G1 pro suite were used for automated processing of blood samples, including mouse serum, mouse plasma, and mouse whole blood. samples that were not processed with the Proteonano™ Ultraplex Proteomics Platform were used as control groups.

Performance of the Proteonano Platform for Different Types of Mouse Blood Samples

1.Number of Protein Identifications and Dynamic Range in Mouse Blood Samples

Using the Proteonano™ Mouse Plasma Proteome Enrich Kit and the Proteonano™ Ultraplex proteomics platform, coupled with a high resolution mass spectrometer (Thermo Fisher Orbitrap Astral system), deep coverage of proteins can be achieved with only ng-level protein sample input. Peptides are dissolved in a buffer solution for sample loading, and after 300 ng of lyophilized peptides were dissolved and injected to LC-MS/MS system with a 5 μ m*150 mm, C18, 2 μm, 100 Å chromatography column for separation. A 24-minute analytical gradient is established using two mobile phases (mobile phase A: 0.1%

formic acid and mobile phase B: 0.1% formic acid, 80% ACN). The flow rate of the liquid chromatography is set at 1.8 µ L/min, and the mass spectrometry results are collected in Data-Independent Acquisition (DIA) mode (Figure 1, Table 1) followed by protein library searching using the DIA-NN software.

Using this sample processing, data acquisition, and data analysis pipeline identified 3791 protein groups from mouse serum samples, 5406 protein groups from mouse plasma samples, and 6585 protein groups from mouse whole blood samples. Sample processing by the Proteonano™ Mouse Plasma Proteome Enrich Kit provided about two-fold increase in protein groups detected for each of the sample type relative to samples processed without using the kit, thus significantly increasing proteomic detection depth (Figure 2). When mouse proteins identified in the study were mapped to the reported plasma concentrations of their protein counterpart in human plasma protein project (HPPP), their concentration span nine orders of magnitude, indicating efficient detection of low abundance proteins (Figure 3). When compared to samples processed by conventional methods, which only cover 3-5 orders of magnitude, the depth of identification has been enhanced by nearly 10,000 times.

MS Platform	Kit	Sample	Injection Volume (ng)	Gradient (min)
Orbitrap Astral	Proteonano™ Mouse Plasma Proteome Enrich Kit	mouse serum mouse plasma mouse whole blood	300	24

Table 1: MS experimental parameters for different types of mouse blood samples

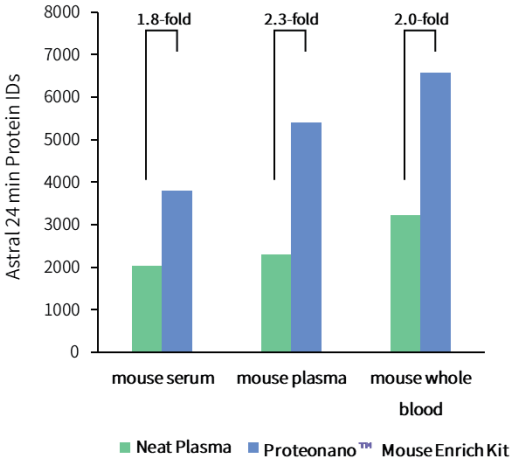


Figure 2: Number of protein identifications in different types of mouse plasma samples

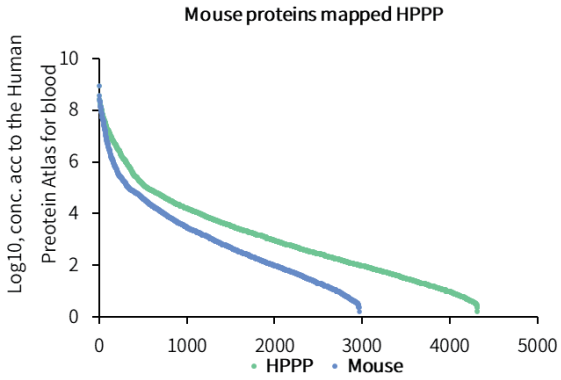


Figure 3: The protein identification depth (dynamic range) in mouse whole blood

2. Comparison of Protein Group Identified in Mouse Blood Samples Between Proteonano™ and Olink Target

Olink is a commonly used platform that can detect abundance of multiple proteins simultaneously in the same sample in a targeted fashion. This methods uses ELISA-like binding of antibody pairs to protein targets. While higher multiplexity Olinks panels are marketed, they are less commonly used than Olink' s 48 and 96 panel kits. When the 6931 protein groups identified in mouse blood samples were compared with Olink Mouse Target 48&96 panels, it was found that the mouse plasma samples processed by all three platforms could detect Q08048 (Hepatocyte growth factor) and P18340 (C-X-C motif chemokine 9). Proteonano™ and Olink Mouse 48 shared detection of 5 proteins, while Proteonano™ and Olink Mouse 96 shared detection of 29 proteins (Table 2).

Uniprot ID	Protein name	Uniprot ID	Protein name	Uniprot ID	Protein name
P40224	Stromal cell-derived factor 1	Q99KJ8	Dynactin subunit 2	Q9R1E0	Forkhead box protein O1
Q9WUZ6	C-C motif chemokine 17	P07091	Protein S100-A4	Q00493	Carboxypeptidase E
P48298	Eotaxin	Q9Z0T9	Integrin beta-6	Q80UG2	Plexin-A4
O88430	C-C motif chemokine 22	P31240	Platelet-derived growth factor subunit B	P47713	Cytosolic phospholipase A2
O54824	Pro-interleukin-16	P18406	CCN family member 1	P97326	Cadherin-6
P18340	C-X-C motif chemokine 9	O89023	Tripeptidyl-peptidase 1	P48787	Troponin I, cardiac muscle
Q08048	Hepatocyte growth factor	P70677	Caspase-3	Q9ERB0	Synaptosomal-associated protein 29
Q8VCF1	Soluble calcium-activated nucleotidase 1	Q61982	Neurogenic locus notch homolog protein 3	P99029	Peroxiredoxin-5, mitochondrial
P70236	Dual specificity mitogen activated protein kinase kinase 6	O88393	Transforming growth factor beta receptor type 3	Q8CGN5	Perilipin-1
P26323	Friend leukemia integration 1 transcription factor	P04202	Transforming growth factor beta-1 proprotein	P17183	Gamma-enolase
Q8BTW9	Serine/threonine-protein kinase PAK 4	Q8BVI4	Dihydropteridine reductase	P11152	Lipoprotein lipase
Q8R5A3	Amyloid beta A4 precursor protein-binding family B member 1-interacting protein	O35625	Axin-1	P11103	Poly polymerase 1

Table 2: Proteonano™ mouse kit and olink mouse panels detecting differential protein information in mouse

The Proteonano™ platform demonstrates exceptional advantages in the detection of proteins in mouse plasma samples, being able to identify 6895 proteins that were not detected by Olink Mouse 48 and Olink Mouse 96. This further confirms Proteonano™ ability to provide more comprehensive, accurate, and in-depth proteomic data.

By supplementing the protein information missed by the Olink Target Mouse Panel, researchers can enhance their systematic understanding of the protein composition in mouse blood, which facilitates the revelation of potential biological mechanisms and the discovery of new biomarkers (Figure 4)

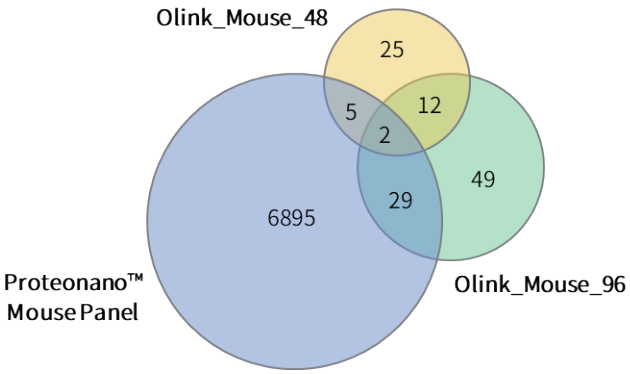


Figure 4: Number of protein identifications in different types of mouse plasma samples

Conclusion

We evaluated the performance the Nanomics Biotech' s Proteonano™ Ultraplex Proteomics Platform for proteomics in different mouse blood samples.

(1) The Proteonano™ platform displays high analytical performance: Protein identification results obtained from mouse blood samples processed with the Proteonano™ Mouse Plasma Proteome Enrich Kit and the Proteonano™ Ultraplex Proteomics Platform show a significant improvement compared to direct digestion experiments.

(2) The Proteonano™ platform exhibits superior sensitivity: In the comparison of similar products for protein detection in mouse blood samples, the Proteonano™ Mouse Plasma Proteome Enrich Kit stands out for its exceptional protein detection capabilities.

(3) The Proteonano™ platform offers high standardization: For different mouse blood samples, an automated, rigorous database (Swiss-Prot) and data analysis process are employed, ensuring the reliability and standardization of the results

In summary, the Proteonano™ ultraplex platform offers an important technical means for untargeted proteomic analysis of mouse blood samples. This platform demonstrates outstanding accuracy and sensitivity, ensuring an accurate and comprehensive proteomic analysis platform for mouse blood sample analysis and provides a feasible solution for the discovery and translational study of multiple protein biomarkers.

Materials and Methods

Protein enrichment reagents

- Proteonano™ Mouse Plasma Proteome Enrich Kit (Nanomics Biotech, Hangzhou, China)
- Proteonano™ Ultraplex Proteomics Platform (Pro Suite) (Nanomics Biotech, Hangzhou, China)
- Trypsin (V5111, Promega Corporation, Madison, WI, USA)

Mass Spectrometer & Liquid Chromatography

-Vanquish Neo coupled with Orbitrap Astral (Thermo Fisher Scientific Waltham, MA, USA)

Data Analysis Software

- DIA-NN (version 1.8.1)
- Uniprot Reviewed (Swiss-Prot) (20,422 Entries)

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