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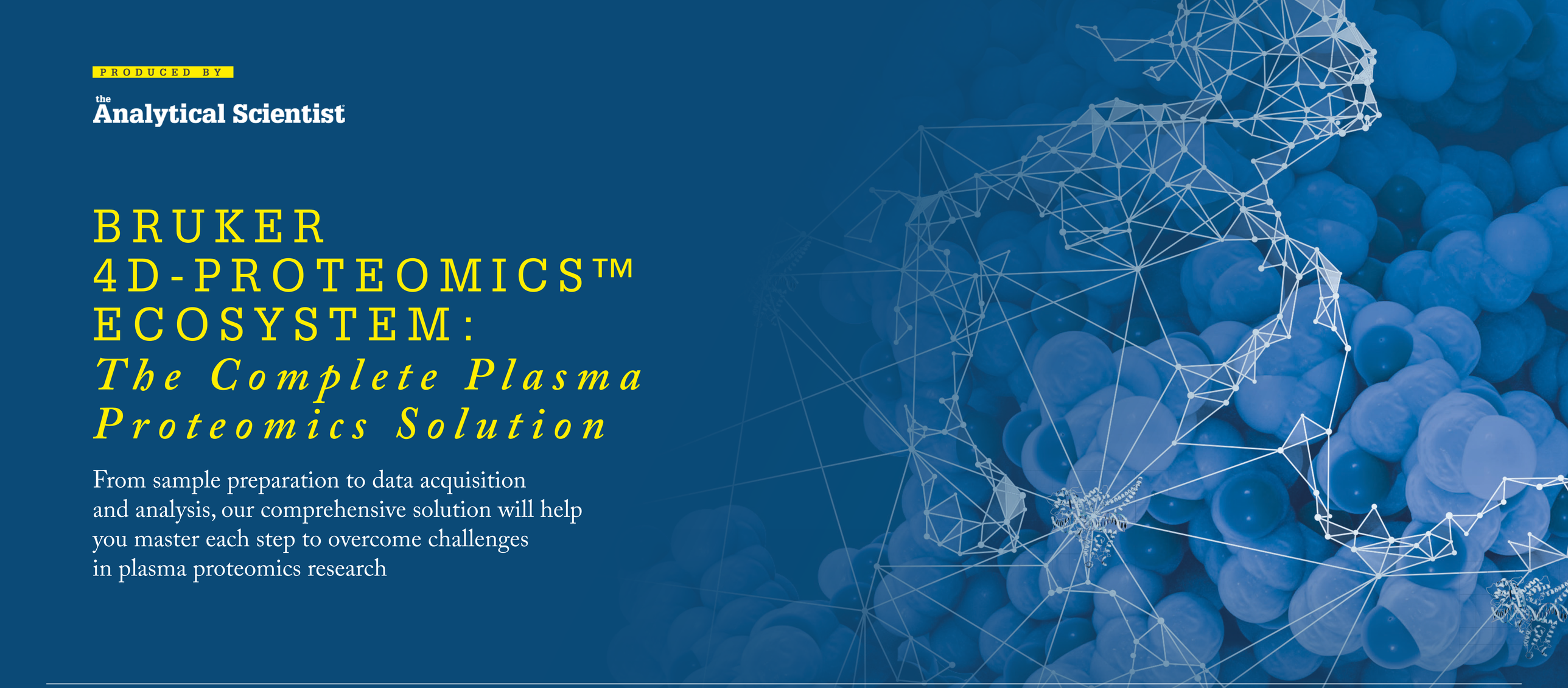
the Analytical Scientist

BRUKER 4D-PROTEOMICS™ ECOSYSTEM: *The Complete Plasma Proteomics Solution*

From sample preparation to data acquisition and analysis, our comprehensive solution will help you master each step to overcome challenges in plasma proteomics research



Application Note: The Sample Prep Solution: ENRICH-iST

Application Note: Deeper Coverage with ENRICH-iST

Application Note: dia-PASEF – Increased Throughput and Analytical Depth for Neat Plasma Analysis

Application Note: Rapid PTM Analysis with dia-PASEF

Case study: High-Throughput Proteomics at Oxford University

Research Paper: High-Confidence Discovery and Targeted Plasma Proteomics With PQ500

Summary: Bruker 4D-Proteomics Ecosystem: Discover More; Quantify More

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APPLICATION NOTE

The Sample Prep Solution: ENRICH-iST™

Blood plasma is easily obtainable and a valuable source of information regarding an individual's health status, which has led to its wide use in biomarker discovery research. Over the past decade, LC-MS-based proteomics has emerged as a powerful tool for identifying and quantifying proteins in plasma. However, the high dynamic range (up to 12 orders of magnitude) and the heterogeneity and complexity of plasma samples limit access to the full proteome, posing significant challenges for LC-MS-based proteomics. Consequently, results lack robustness and reproducibility, workflows are complex and tedious, and low abundance proteins are masked by high abundance proteins.

Various techniques have been developed for sample preparation of plasma to reduce the dynamic range, but they are either time-consuming, incompatible with high-throughput techniques, or lacking precision.

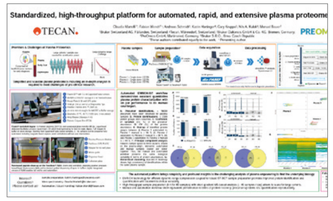
PreOmics' complete ENRICH-iST workflow – including proteomic sample preparation and peptide clean-up – is an easy-to-use, yet robust solution to the dynamic range challenge. It is based on biologically unbiased enrichment of low abundance proteins onto paramagnetic particles (EN-BEADS). This reduces the dynamic range while conserving analytical depth and proteome coverage.

APPLICATION PAGE

Smile! Optimizing Plasma and Serum Sample Preparation for Deeper, High-Throughput Proteome Profiling

APPLICATION NOTE

ENRICH-iST: Solving the Dynamic Range Challenge for Efficient High-Throughput Plasma Proteomics Analyes



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Standardized, High-Throughput Platform for Automated, Rapid, and Extensive Plasma Proteome Characterization

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Next:
Deeper Coverage with ENRICH-iST

APPLICATION NOTE

Deeper Coverage with ENRICH-iST

Introduction

Access to blood proteome information is limited by the highly dynamic nature of protein abundance in plasma, which spans approximately more than 10 orders of magnitude and with only 22 proteins accounting for 99 percent of the whole protein content. To address this challenge, researchers at Bruker, PreOmics, and Biognosys developed a novel workflow for LC-MS-based plasma proteomics that enriches low abundant proteins and enables an improved coverage of the plasma proteome.

Methods

Plasma samples from a clinical cohort of non-small cell lung cancer patients and healthy donors were obtained from Biognosys. Initially, samples were prepared with the iST sample preparation kit (PreOmics). Additionally, 10 µl of plasma were prepared with the ENRICH-iST kit and 300 ng of peptides were analyzed on the TimsTOF HT mass spectrometer coupled to a nanoElute LC system.

Results

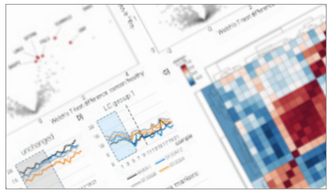
More than 1450 proteins were identified in ENRICH-iST samples in comparison to 650 protein IDs in neat plasma, increasing the plasma proteome by more than twofold. Moreover, using a sample specific library (~2200 entries) from high-pH fractionation of sample pools, more than 2000 proteins were identified and almost 1500 proteins were quantified in all samples of the dataset. Proteins identified in neat plasma generally showed an increase of precursors in ENRICH-iST, permitting more accurate quantitation.

The number of statistically significant proteins in the ENRICH-iST samples was higher than in the neat samples, corroborating the researchers' assumption that the enrichment step can preserve the inherent protein-abundance ratios. Overall, 20 proteins were significantly altered between the study groups in the ENRICH-iST dataset.

Based on the current data, the two patient groups could be distinguished. Proteins overexpressed in group 1 were S100A8, S100A9, S100A12, and Anxa1, of which the first are common markers for inflammation and immune response. These proteins are unaltered in group 2, for which TFRC, ORM1, A2M, and SerpinA6 had the highest difference. Based on 23 most altered protein profiles, most patient groups and healthy donors could be distinguished, however, additional patient data would be needed to allow further refinement. Calculating the coefficient of variation for all proteins in the healthy group (biological replicates) demonstrates a high reproducibility of protein abundance, with a median of 20 percent.

Summary

ENRICH-iST efficiently reduces the dynamic range in plasma increasing the number of detected proteins by at least two times. In addition, deeper plasma proteome coverage and more significant proteins are observed with ENRICH-iST while preserving a high reproducibility (CV ~20 percent). Finally, sample inherent protein abundances are retained upon ENRICH-iST sample preparation.



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Deeper Plasma Proteome Coverage Enables Identification of Novel Biomarkers and Classification of Diseases

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Previous: The Sample Prep Solution: ENRICH-iST

Next: dia-PASEF – Increased Throughput and Analytical Depth for Neat Plasma Analysis

APPLICATION NOTE

dia-PASEF® – Increased Throughput and Analytical Depth for Neat Plasma Analysis

Introduction

Proteomics analysis of neat plasma remains a challenge because of the huge dynamic range of the plasma proteins, and the current depletion technologies can be expensive. In this application note, researchers from the Necker Proteomics Platform, University Paris, France, and Bruker focus on workflow optimization of neat plasma analysis using the dia-PASEF approach to maximize the number of proteins groups identified and quantified, while minimizing both gradient time and missing value levels. Two workflows with variable LC settings and ion mobility (IM) ranges were compared, with data analyzed using DIA-NN.

Methods

Optimization tests were performed using healthy patient plasma. For the small-sized clinical study, plasma samples from 15 patients affected by a rare dermatological genetic disease (RDGD) and 18 age-matched controls patients (CP) were used.

Results and Discussion

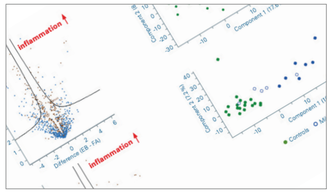
The results showed that dia-PASEF allowed for the identification of roughly 50 percent more protein groups from the same samples while also reducing variability: indeed, at any CV threshold chosen, dia-PASEF always allowed to quantify more proteins. Consequently, the number of proteins we can expect to quantify is higher with dia-PASEF.

Three different dia-PASEF methods schemes covering similar m/z but different mobility ranges were used. The broader IM range allows for the identification of up to 20 percent more proteins.

Analysis depth achievable using dia-PASEF was improved by working on the search strategy. Best results were achieved using dia-NN with the library free mode. In agreement with the high interpersonal variability of plasma protein abundance, an increase in the number of identified protein groups was reported as more sample files were searched simultaneously, in particular, when Match Between Run (MBR) was allowed.

Conclusion

In conclusion, both 60 Sample Per Day (SPD) and 24 SPD can deliver the main biological message, the slowest one giving access to a new relevant class of proteins not detected (or rarely detected) in the faster pipeline. Choice of depth over speed should be made as a function of the size of the cohort to analyze.



APPLICATION NOTE

Pushing DIA Proteomics Analyses of Neat Plasma Towards 1000 Protein Groups ID's/Injection

APPLICATION NOTE

Development of a Targeted Plasma Quantitation Assay Using dia-PASEF

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Previous: Deeper Coverage with ENRICH-iST

Next: Rapid PTM Analysis with dia-PASEF

APPLICATION NOTE

Rapid PTM Analysis with dia-PASEF

Introduction

Data-independent acquisition (DIA) has been shown to increase quantitative reproducibility and accuracy in MS-based phosphoproteomics. In dia-PASEF, DIA is combined with trapped ion mobility separation (TIMS), which allows for increased speed and sensitivity, as well as separation of signals from peptides that would otherwise be co-fragmentated. Phosphorylation can alter the gas phase structure of peptides; in addition, the encoding of each phosphopeptide with its specific Collisional Cross Section (CCS) value allows for more confident identification and characterization of the detected phosphopeptides.

Here, the Hela cell line phosphoproteome was analyzed with liquid chromatography gradients from 7 to 21 minutes to investigate whether the increased efficiency and selectivity of dia-PASEF could be used to increase both the throughput and depth of phosphoproteomics analyses.

Methods

Human epithelial cervix carcinoma cell lysates were prepared following standard protocolsand 200 µg proteome digest were used to enrich phosphorylated peptides according to the "EasyPhos" protocol (1). Peptides were separated using a nanoElute LC. The LC system was connected via a CaptiveSpray nano-electrospray source to a timsTOF Pro. The mass spectrometer was operated in dia-PASEF mode. The dia-PASEF raw files were processed with Biognosys' Spectronaut® software in library-free mode with the UniProt human reference proteome.

Results and Discussion

To assess the performance of dia-PASEF, amounts of HeLa phosphopeptides equivalent to the enrichment from 20 µg starting material (the protein mass of ~100,000 cells) were injected and separated using short gradients of 21- and 7-minutes. With directDIA, in total ~12,500 phosphopeptides from quadruplicate injections with a 21-minute gradient were detected. Out of these, ~9200 were localized by the Spectronaut software with a probability >0.75 to a specific site (class I sites) in one of 3025 protein sequences. Remarkably, with the 7-minute gradient, ~9300 phosphopeptides (~7200 class I sites) and 2627 phosphoproteins were identified.

While proteome analysis typically aggregates multiple peptide data points per protein, in phosphoproteomics, in principle, each peptide represents an individual modification pattern. When assessing the reproducibility on the peptide level, the data matrices were virtually complete (>99%, Figure 4A, B) and almost all phosphopeptides were detected in four out of four replicates. Intensity values were also highly correlated between technical replicates for both 7-minutes and 21-minutes gradients (R >0.99, Figure 4C, D). This result was attributed to the combination of reproducible data acquisition with dia-PASEF and the highly specific library-free data analysis.

Conclusion

dia-PASEF on the timsTOF platform is very well suited for high-throughput and high-sensitivity phosphoproteomics. More than 12,500 phosphopeptides, including ~9200 class I phosphosites could be reproducibly identified with short 21-minute gradients from low input material without the need for a project specific library. Even for three-times-shorter, 7-minute gradients, more than 9300 phosphopeptides were detected (75 percent or 21-minute gradient total) when applying a dia-PASEF scheme that takes precursor density distribution into account for optimal window placement. The results showed a high degree of reproducibility and data completeness for short gradients using low sample input material, making dia-PASEF ideally suited for application to large sample cohorts.

Reference

1. *SJ Humphrey et al., "High-throughput and high-sensitivity phosphoproteomics with the EasyPhos platform," Nat Protoc, 13, 1897–1916 (2018). DOI: 10.1038/s41596-018-0014-9.*

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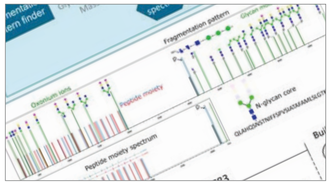
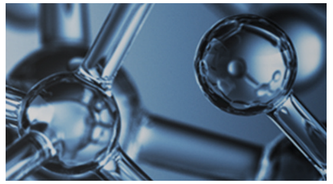


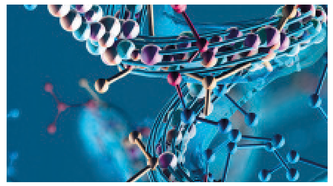
ARTICLE

The GlycoPaSER Prototype as a Real-Time N-Glycopeptide Identification Tool



WEBINAR OVERVIEW

Functional Phosphoproteomics Finds Resolutions with Ion Mobility



APPLICATION NOTE

Rapid Phosphoproteomics with dia-PASEF

Previous: dia-PASEF – Increased Throughput and Analytical Depth for Neat Plasma Analysis

Next: High-Throughput Proteomics at Oxford University

CASE STUDY

High-Throughput Proteomics at Oxford University

Roman Fischer and his team work in the Discovery Proteomics Facility at the Target Discovery Institute, University of Oxford, are studying protein function, dynamics, post-translational modifications and their effects on protein turnover, antigen presentation, and metabolic pathways. By using the Bruker timsTOF Pro, the researchers were able to significantly improve their ability to achieve high-throughput proteomics for clinical studies. In Fischer's own words:

"We wanted a throughput of 100 samples per day. Bruker returned excellent data to us and had no concerns relating to instrument robustness."

"The largest sample batch that we have run on the timsTOF Pro instrument was 4500 injections of non-depleted blood – which is one of the most challenging samples you can throw at it."

"Bruker is hugely supportive in helping us with our lipidomics analyses, which we just started. Bruker has a group of experts who are experienced in that field, and they are very helpful."

"These new developments are vital for the progression of clinical proteomics research, and I'm looking forward to seeing how our research continues to evolve with these new technological advancements."

Follow the below link to find out more about how researchers at the Target Discovery Institute are using the Bruker timsTOF Pro to develop new methodologies for the proteome characterization of clinical cohort samples.

CUSTOMER INSIGHT

Advances in Clinical Proteomics Research with High-Throughput Mass Spectrometry

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Previous: Rapid PTM Analysis with dia-PASEF

Next: High-Confidence Discovery and Targeted Plasma Proteomics With PQ500

RESEARCH PAPER

High-Confidence Discovery and Targeted Plasma Proteomics With PQ500

Robust detection and quantification of human plasma proteins are now possible with Biognosys' PQ500™ Reference Peptides. Containing 804 carefully selected stable isotope-labeled standard (SIS) peptides, PQ500 represents more than 500 human proteins found in plasma. This unique composition allows for the simultaneous measurement of hundreds of proteins in a single experiment.

PQ500 accommodates both targeted (MRM/PRM) and data-independent acquisition (DIA) workflows. While the kit seamlessly integrates with the traditional targeted approach, its application in DIA workflows provides high sensitivity and reproducibility. This is particularly relevant in addressing the challenging task of label-free relative quantification in plasma samples – key indicators for several diseases and physiological processes.

Moreover, when using PQ500, results are consistent and reproducible in multi-site studies. Fold changes and p-values remain stable across different platforms, making the kit a valuable tool for population-scale blood fluid analyses spread across multiple analytical platforms.

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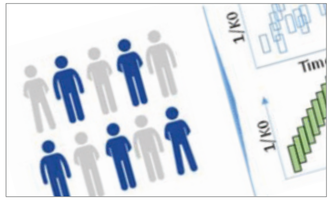

RESEARCH PAPER

Quantification of 782 Plasma Peptides by Multiplexed Targeted

APPLICATION NOTE

Development of a Targeted Plasma Quantitation

WEBPAGE

Kits

Previous: High-Throughput Proteomics at Oxford University

Next: Bruker 4D-Proteomics Ecosystem: Discover More; Quantify More

SUMMARY

Bruker 4D-Proteomics Ecosystem: Discover More; Quantify More

Benefit	Feature
Deep depth sample prep	Expand insights with PreOmics ENRICH-iST kits to gain high-depth analysis of plasma and serum proteomes, including low-abundant protein
Precision profiling	PQ500 from Biognosys enables absolute quantification of almost 600 proteins in plasma samples, compatible with a variety of acquisition types, including prm-PASEF and dia-PASEF modes
Enhanced discovery	Improve protein separation, coverage, throughput, sensitivity, and reproducibility discover new proteins, biomarkers, and understanding of the proteome with PepSep™ HPLC columns and the nanoElute® 2
Extended proteome	The combination of CaptiveSpray ion source, VIP-HESI ion source, TIMS, powered by PASEF provides a wide dynamic range of detection and allows researchers to map PTM's and quantify proteins across a wide range of concentrations, even in complex samples such as plasma
Enriched data analysis	Streamline real-time decisions and large-scale plasma proteomics analysis, saving time and eliminating bottlenecks with two powerful software solutions, Bruker ProteoScape™ with CCS-enabled 4D-Proteomics™ acquisitions using TIMScore™ and TIMS DIA-NN and Spectronaut® 18 library-free, directDIA+ workflow
Novel insights	Biognosys TrueDiscovery™ CRO service provides comprehensive, scalable, and unbiased insights into plasma biomarkers and protein profiles

TRANSFORM YOUR PLASMA PROTEOMICS RESEARCH WITH BRUKER 4D-PROTOEMICS™ ECOSYSTEM>

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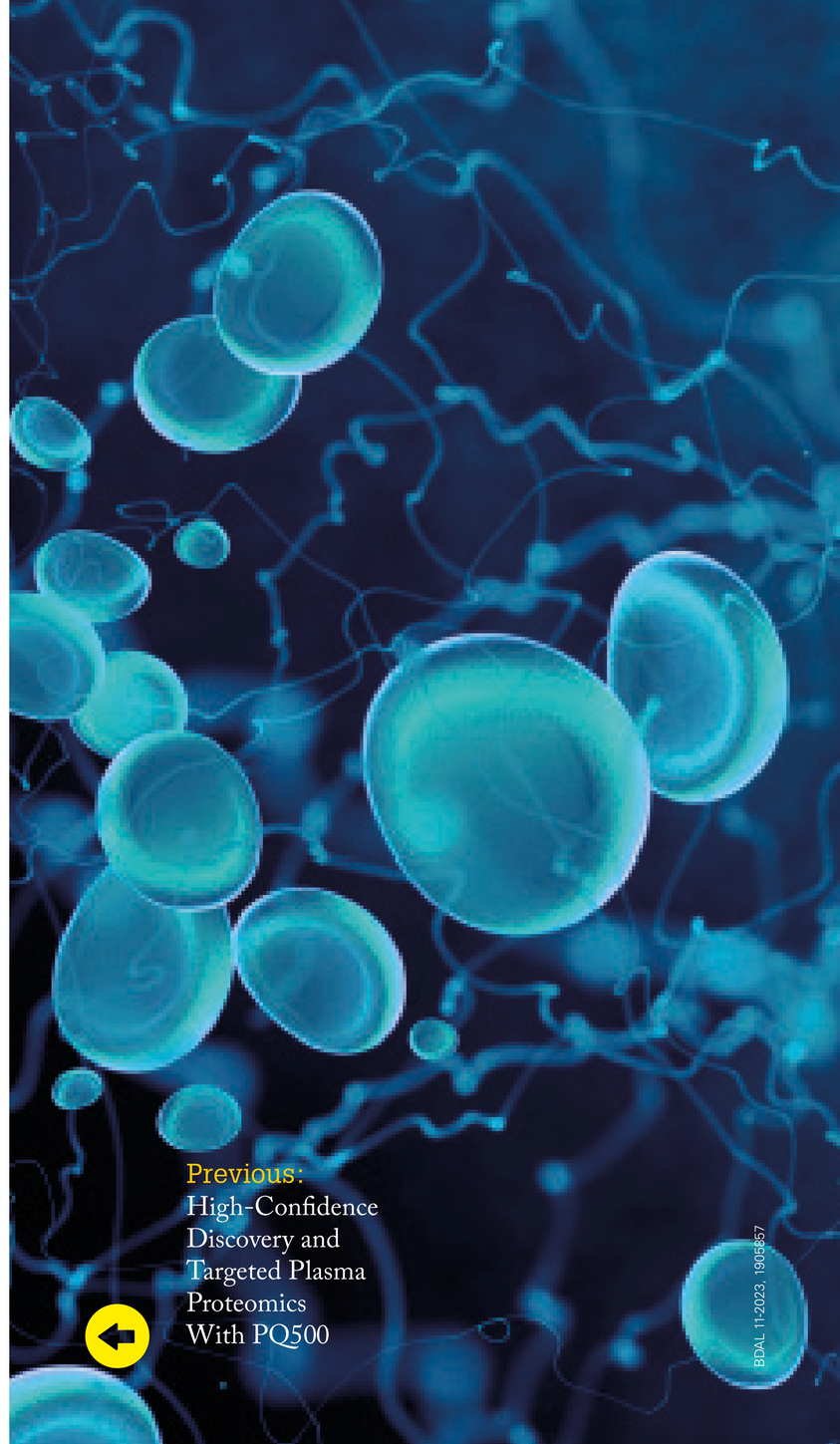

Previous:
High-Confidence Discovery and Targeted Plasma Proteomics With PQ500

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