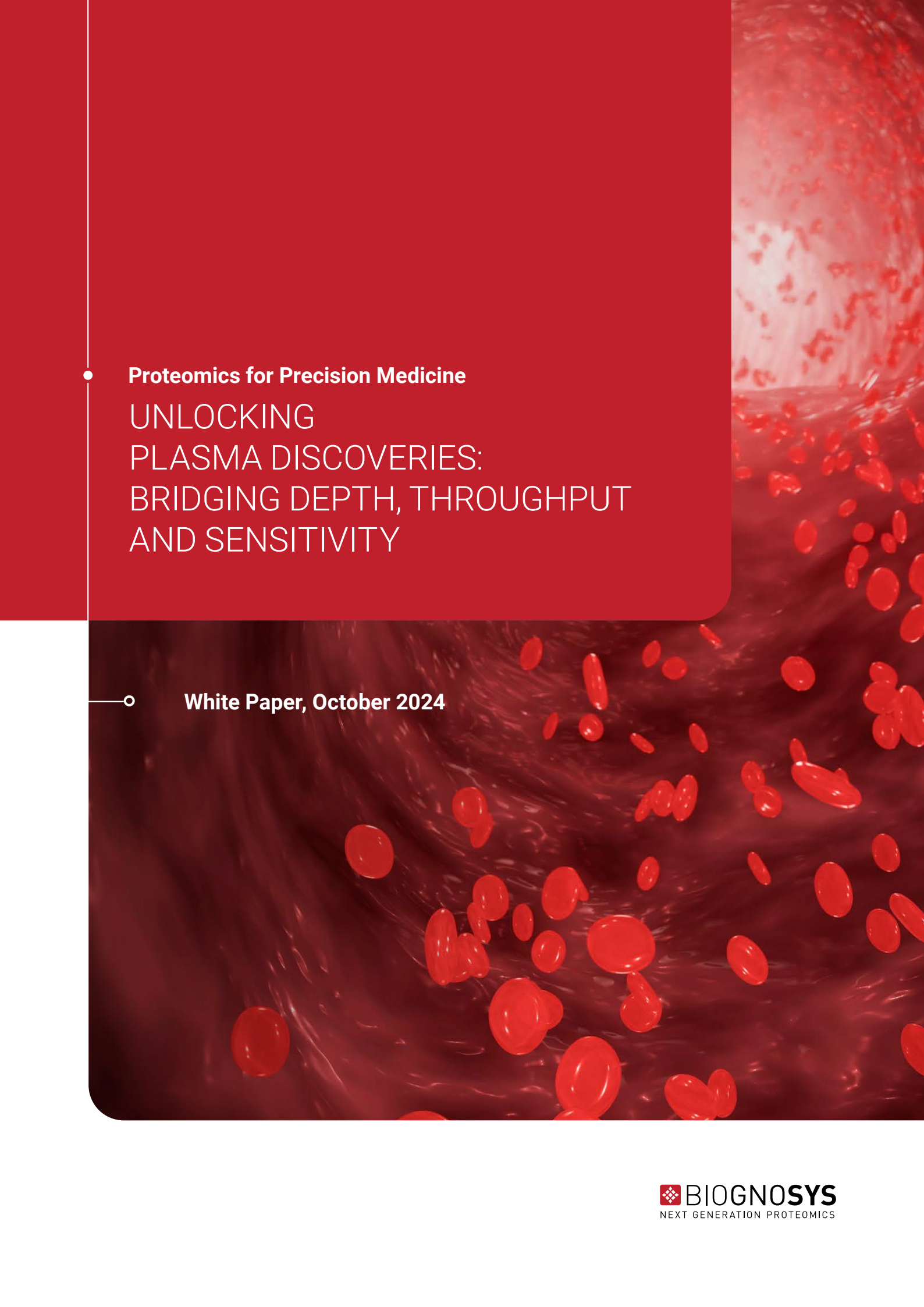




• **Proteomics for Precision Medicine**

UNLOCKING
PLASMA DISCOVERIES:
BRIDGING DEPTH, THROUGHPUT
AND SENSITIVITY

—○ **White Paper, October 2024**

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THE PROMISE OF PLASMA

There is an undeniable lack of affordable, robust methods for studying the plasma proteome in depth – mass spectrometry holds the key to unlocking plasma's potential.

Plasma is an inherently rich source of biological information, containing proteins from every tissue and organ. Providing a dynamic and comprehensive snapshot of an individual's biology, the plasma proteome has the power to uncover transformative insights across a wide range of applications.

Mass spectrometry (MS) is the key to unlocking this potential. MS-based proteomics has the ability to analyze complex samples with precision and at high throughput, making it the ideal tool for exploring the plasma proteome. By identifying and quantifying thousands of proteins, MS-based proteomics is revolutionizing how we diagnose diseases, monitor treatments, and develop new therapies.

Over the past decade, the field of plasma proteomics has seen remarkable progress due to breakthroughs in MS technology, innovative enrichment and separation techniques, and refined quantitative strategies.

These developments have collectively expanded proteome coverage, increased throughput, and improved depth and precision (Figure 1).

At Biognosys, we have been at the forefront of proteomics innovation for over 15 years. In this White Paper, we will explore the benefits of our proprietary P2 Plasma Enrichment System for increasing the depth by up to 10-fold. We will also cover how TrueDiscovery®, powered by P2, can unlock the full potential of your plasma samples, and how combining this with Alamar Bioscience's high-sensitivity NULISA™ assays enables the most comprehensive coverage of the plasma proteome to date.

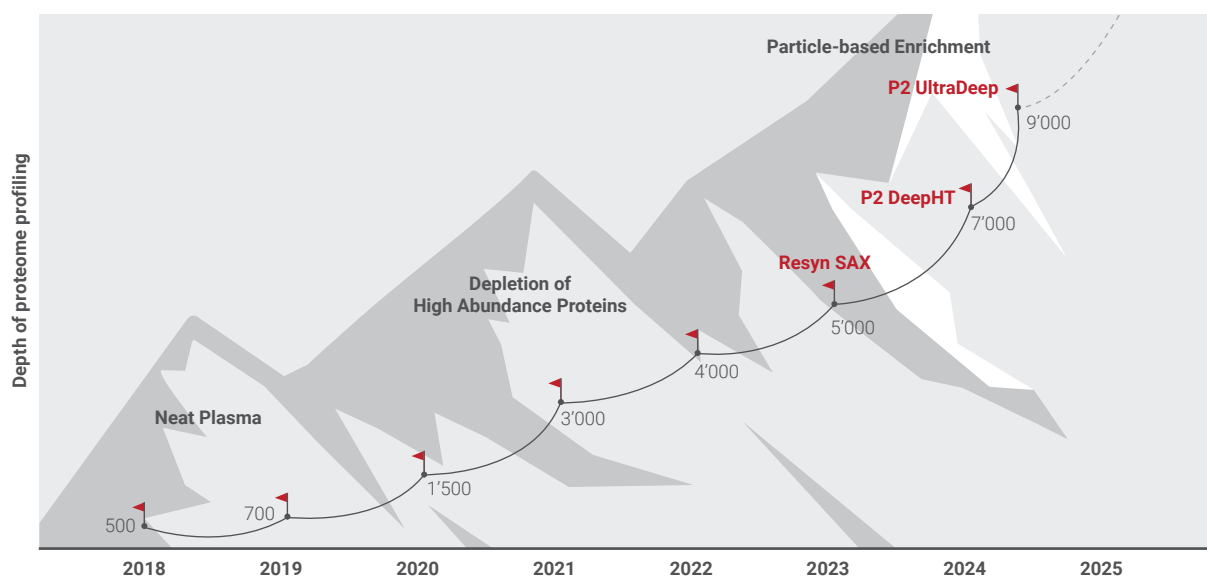


Figure 1. Technical advances at Biognosys have enabled us to reach new peaks in plasma profiling

THE CHALLENGE OF PLASMA PROTEOMICS

The large dynamic range in plasma samples, alongside their dynamic and complex nature, has made comprehensive analysis of the plasma proteome challenging.

The same complexity that makes plasma such a rich source of biological information, also makes plasma proteomics a challenge. Plasma samples contain thousands of proteins, ranging from highly abundant molecules like albumin to trace proteins, such as cytokines, present in picogram per milliliter quantities. This vast dynamic range hinders the detection and quantification of biologically relevant low-abundance proteins.

Furthermore, the multitude of potential proteoforms arising from alternative splicing and post-translational modifications compounds the challenge. Existing technologies frequently struggle to comprehensively characterize these proteoforms, limiting the identification of functionally relevant proteins and their modifications.

As a result, getting robust and reproducible data in plasma proteomics has been particularly challenging. To overcome these challenges, mass spectrometry has emerged as the gold standard for protein quantification. Unlike antibody-based methods that rely on the specificity of affinity reagents, MS can directly detect and quantify a wide range of proteins without the need for prior knowledge of their identity or modifications. This eliminates the bias associated with antibody-based methods, providing a more comprehensive and unbiased assessment of the proteome.

Our TrueDiscovery® platform is built on the most advanced MS technology available for proteomics profiling. It is powered by Data-Independent Acquisition (DIA), an MS technology co-invented by Biognosys. TrueDiscovery delivers high sensitivity and precision by capturing all detectable peptides within a mass-to-charge ratio window, ensuring consistent and accurate quantification.

Over the years, we have been on a mission to continuously improve the robustness, depth, and reproducibility of the TrueDiscovery platform to tackle the challenge posed by the large dynamic range of plasma. Our latest innovation, the P2 Plasma Enrichment System, solves this issue through advanced particle enrichment technology, effectively achieving a 10-fold increase in plasma proteome quantification depth - the highest enrichment ever reported.

INTRODUCING THE P2 PLASMA ENRICHMENT SYSTEM

Our proprietary P2 Plasma Enrichment System **builds on the protein corona effect** to selectively isolate and enrich low-abundance proteins from complex plasma samples.

When particles are introduced into a biological environment, such as plasma, a dynamic, multi-layered protein coat (the protein corona) forms around them. This corona is primarily composed of two layers: an inner 'hard' corona of tightly bound proteins and an outer 'soft' corona of loosely associated proteins.

Our system leverages this natural phenomenon. By carefully designing particles that attract and retain low-abundance proteins within the corona, we have developed a highly effective enrichment method (Figure 2). Combining our specially engineered particle surface with an optimized buffer environment stabilizes the protein corona, effectively trapping low-abundance proteins, while deriching abundant ones.

The result: exceptional performance. The P2 Plasma Enrichment System achieves the highest reported proteome enrichment, with greater reproducibility than any other corona-based workflow.

Our P2 technology is optimized for two applications: high throughput and in-depth studies. This flexibility allows researchers to tailor their proteomic analysis to their specific research goals, whether they require rapid turnaround times for large-scale studies or comprehensive coverage of the proteome for detailed investigations.

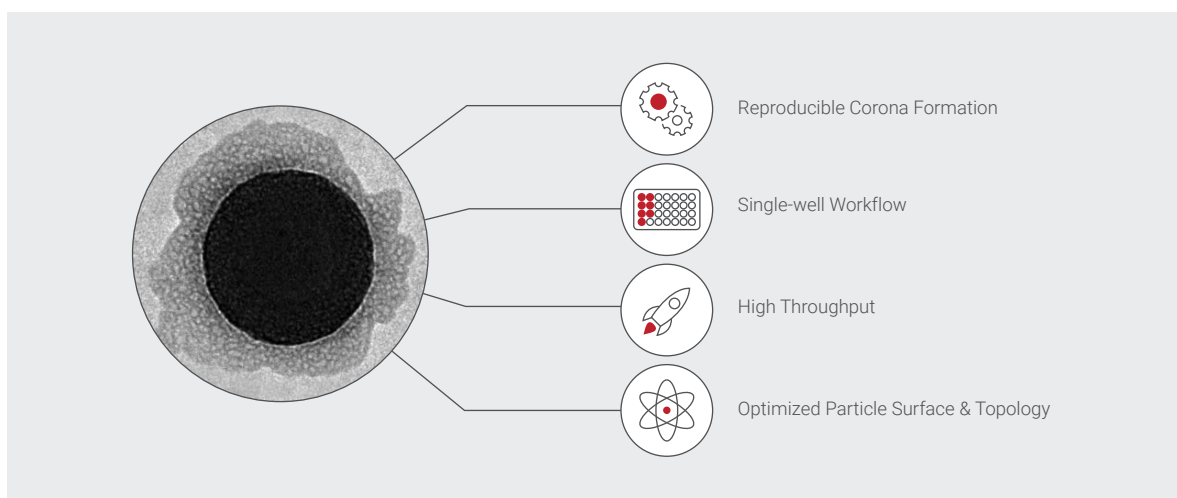


Figure 2. **Optimized corona formation effect with P2 Plasma Enrichment System.** Electron microscopy image of hard and soft protein corona adapted from Gopinath, 2019 Sci. Rep

OPTIMIZATION AT EVERY STEP

We have led the way in developing innovative solutions that deliver both depth and throughput, along with robust quantification.

Plasma proteomics has traditionally been a complex and inaccessible field, requiring specialized equipment and expertise. Our TrueDiscovery® plasma proteomics platform, powered by our innovative P2 Plasma Enrichment System, makes large-scale, in-depth, and high-throughput analysis accessible to researchers across biopharma and academia at an affordable price (Figure 3).

What's more, our P2 technology is available as two different offerings to suit your specific research needs:

High-Throughput Offering

Identify up to 7,000 protein groups with robust quantification. Leveraging the power of Bruker timsTOF technology, this offering enables the study of large sample sets with a throughput of 40 samples per day, per instrument.

UltraDeep Offering

Identify up to 9,000 proteins, ideal for studies involving several hundred samples to uncover novel biological

insights via ultra deep proteomics at a throughput of 8 samples per day.

When it comes to data analysis and interpretation, we deploy our proprietary Spectronaut® software for unbiased DIA-MS data analysis, while our Proteoverse™ data visualization and exploration platform allows researchers to easily extract biological insights.

Partnering with us doesn't just give you access to the world's largest high-end mass spectrometry facility, but also to our team of experts. With a deep understanding of the human proteome, we collaborate closely with our clients to tailor solutions for each project, ensuring a seamless experience from start to finish. We offer both Contract Research Organization (CRO) services and licensing options, allowing you to either outsource plasma proteomics projects or adopt the P2 enrichment technology in-house, depending on your specific research and development needs.

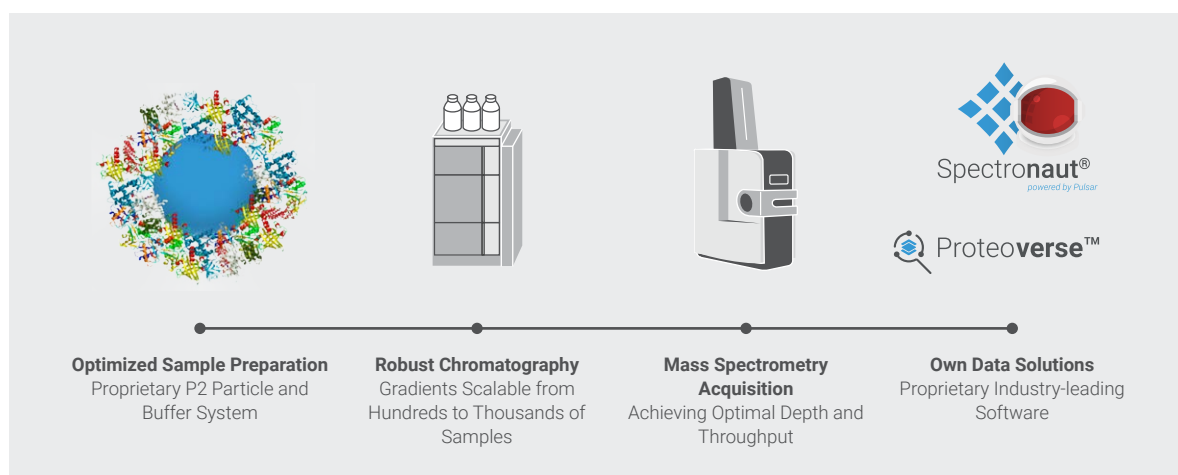


Figure 3. TrueDiscovery and P2 Plasma Enrichment System workflow

UNPARALLELED DEPTH AND PRECISION

With its exceptional depth, precision and reproducibility, the P2 Plasma Enrichment System is the ideal solution for large-scale proteomic studies, as well as in-depth investigations.

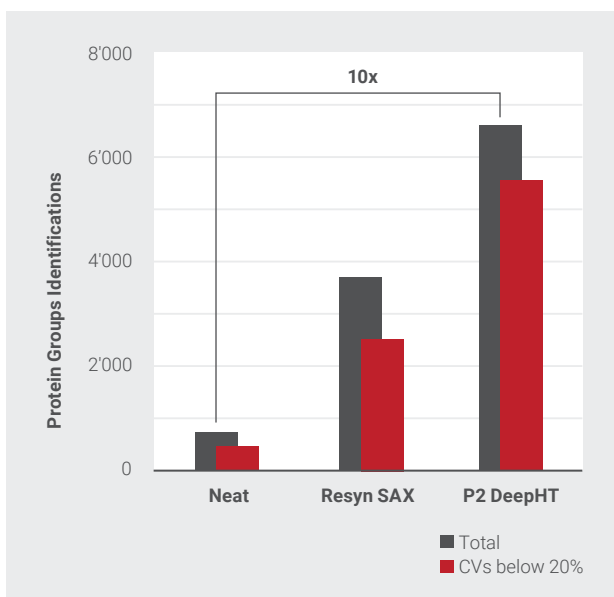


Figure 4. **High-throughput (DeepHT) P2 enrichment offering achieves an enrichment of 10-fold over neat plasma, the highest enrichment factor ever reported**

The P2 Plasma Enrichment System delivers a 10-fold increase in depth and can identify up to 7,000 proteins at high throughput across broad spectrum of protein classes (Figure 4).

Our P2 technology ensures precise quantification. Controlled Quantitative Experiments (CQEs) have demonstrated remarkable preservation of ratios (Figure 5).

This robust performance enables accurate measurement of fold changes, making the P2 technology ideal for biomarker discovery in clinical studies or across large-scale population cohorts.

Leveraging our decades of expertise as a leading CRO, Biognosys has developed the P2 Plasma Enrichment System as a highly optimized solution for plasma proteomics. With coefficients of variation consistently below 10%, the P2 technology delivers very high reproducibility and robustness.

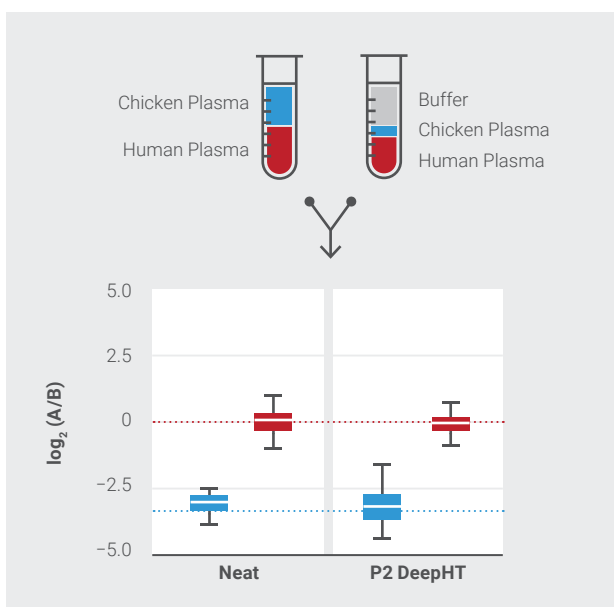


Figure 5. **Quantitative precision is preserved with enrichment as shown by Controlled Quantitative Experiments with neat and enriched multi-species mixtures of plasma**

CASE IN POINT

DEEP PROTEOME COVERAGE DELIVERS CLINICAL INSIGHTS IN ONCOLOGY

By going very deep into the plasma proteome, the P2 enrichment generates **key biological insights for clinical research**.

The P2 Enrichment System provides unmatched depth and precision in plasma proteome analysis at high throughput. But its value ultimately lies in its ability to generate meaningful biological insights.

To evaluate this, we investigated whether P2 could identify protein signatures distinguishing responders from non-responders in a clinical trial of immunotherapy in combination with chemotherapy for lung cancer.

We analyzed baseline samples collected before treatment using the High-Throughput P2 offering. This approach yielded deep proteome coverage, quantifying more than 6,500 proteins. The precision and reproducibility of this coverage enabled the identification of protein signatures that may predict therapeutic responses in clinical setting (Figure 6A). Notably, many of the quantified proteins are implicated in various disease pathways and are targets in ongoing drug development clinical trials (Figure 6B).

Although lower in throughput, the UltraDeep platform quantified an additional 2,888 proteins in the same sample set, providing a deeper level of insight suitable for smaller-scale studies that demand more detailed investigation (Figure 7).

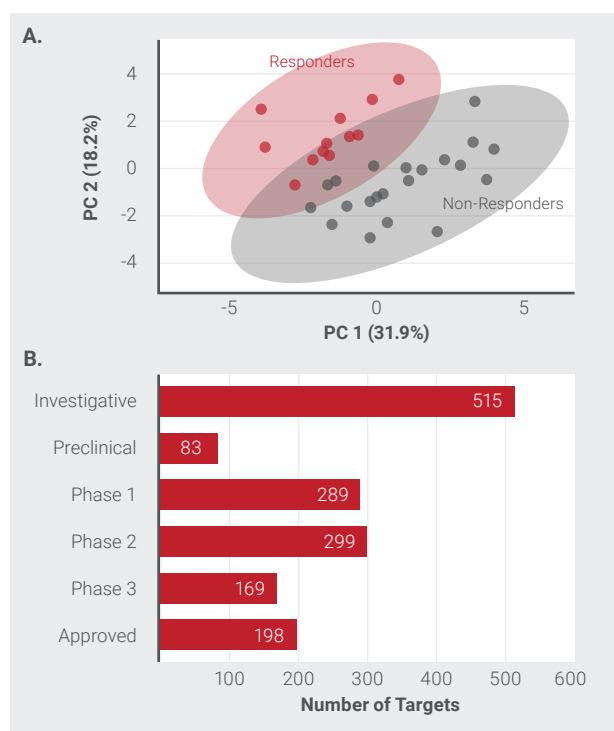


Figure 6. (A) Principal Component Analysis (PCA) shows the power of differentially abundant proteins to stratify patients with different therapeutic responses to therapy. (B) Coverage of therapeutic targets across drug development phases⁷

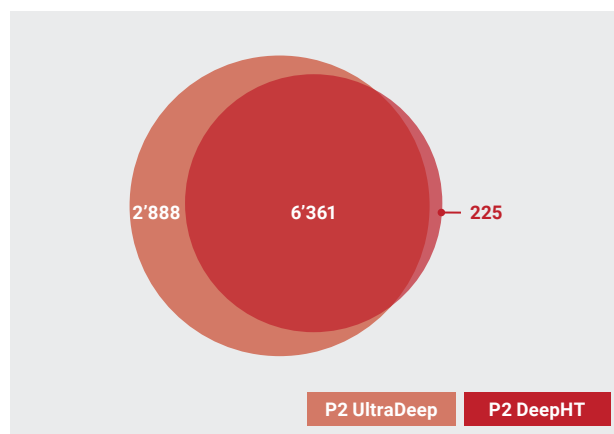


Figure 7. Overlap of the proteins quantified in the High-Throughput (DeepHT) and in UltraDeep offering following P2 enrichment

THE MOST COMPREHENSIVE PLASMA PROTEOMICS SOLUTIONS ON THE MARKET

Get an unbiased view of plasma biomarkers by combining **TrueDiscovery®** with Alamar Bioscience's **NULISaseq™** targeted panels.

Our TrueDiscovery offering is the ideal choice for truly unbiased, hypothesis free analysis, allowing researchers to dig deep into the proteome and uncover novel biomarkers. Aided by the P2 Plasma Enrichment System, the High-Throughput offering is capable of quantifying up to 7,000 proteins, while the UltraDeep option can quantify around 9,000. This makes up a significant portion of the proteome, including thousands of novel proteins that are inaccessible to other techniques.

On the other hand, Alamar Bioscience's NULISaseq multiplex panels excel at targeting a set of well-characterized disease-relevant proteins with high sensitivity and precision. Researchers can now combine the power of these two technologies, diving into the unknown to discover novel biomarkers while validating their findings against known biological signatures (Figure 8).

Both the NULISaseq Inflammation Panel 250 and NULISaseq CNS Panel 120 are available as a research service from Biognosys, providing a complete solution for all your plasma proteomics research needs.

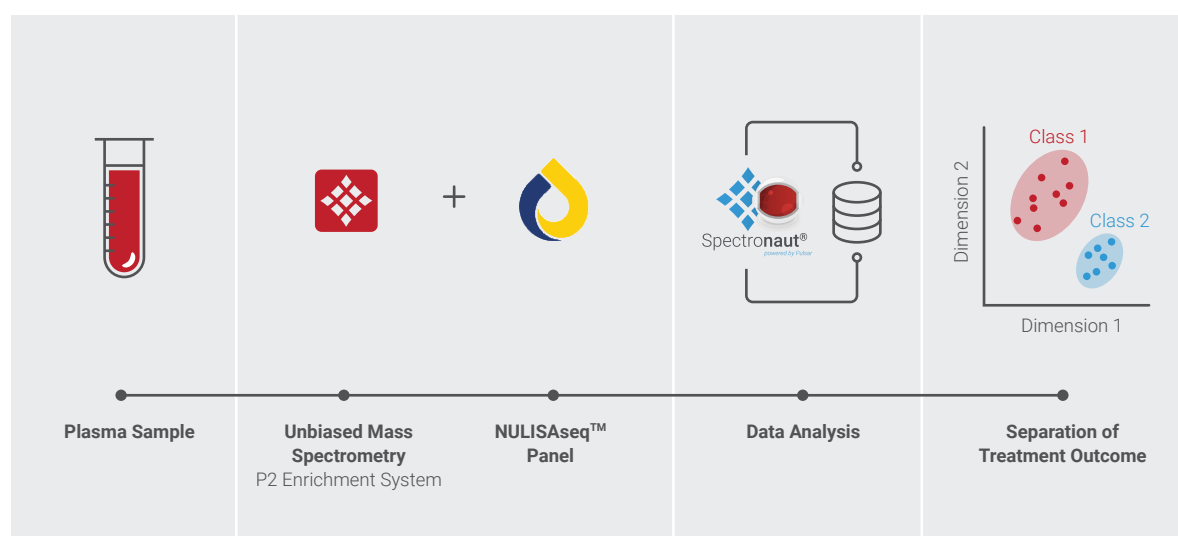


Figure 8. **Combining unbiased mass spectrometry and curated immunoassay panels leads to the most comprehensive proteome coverage for impactful biological insights**

WORKING WITH BIOGNOSYS

From sample preparation to data analysis,
partner with us for **every step of the way.**

Plasma proteomics is incredibly powerful, offering a unique window into disease state and fundamental biological processes. By comprehensively profiling the plasma proteome, we can uncover novel biomarkers, identify therapeutic targets, and gain a deeper understanding of disease mechanisms.

Biognosys is your trusted partner for plasma proteomics analysis. Our TrueDiscovery® platform, combined with the P2 Plasma Enrichment System, delivers unparalleled depth and quantitative precision. With state-of-the-art facilities and expert scientists across Europe and the US, we guarantee the highest quality data and results. At an affordable price point, this offering makes plasma proteomics accessible to a wide range of researchers.

The P2 technology is available as a TrueDiscovery contract research service from our facilities in Schlieren, Zurich, Switzerland and Newton, Massachusetts, US. For end-users of facilities equipped with timsTOF or similar high-performance mass spectrometry platforms, the system is also available under licensing.

Our TrueDiscovery offerings are flexible and cater to a wide range of research needs. For population-based studies, our High-Throughput method enables rapid analysis of thousands of samples, identifying up to 7,000 protein groups with robust quantification. For in-depth investigations, our UltraDeep method provides even more comprehensive proteome coverage, identifying up to 9,000 protein groups. Furthermore, this unbiased discovery approach can be combined with NULISA high-sensitivity, targeted assays panels.

To unlock the full potential of your data, we offer our Proteoverse™ data exploration tool for free alongside TrueDiscovery research services. This intuitive tool empowers researchers to dive deep into their complex proteomic data and extract meaningful biological insights (Figure 9).

Our market-leading solutions, deep expertise and track record of innovation make us the best partner to help you accelerate your research. Our solutions are not just limited to plasma; we offer proteomics services

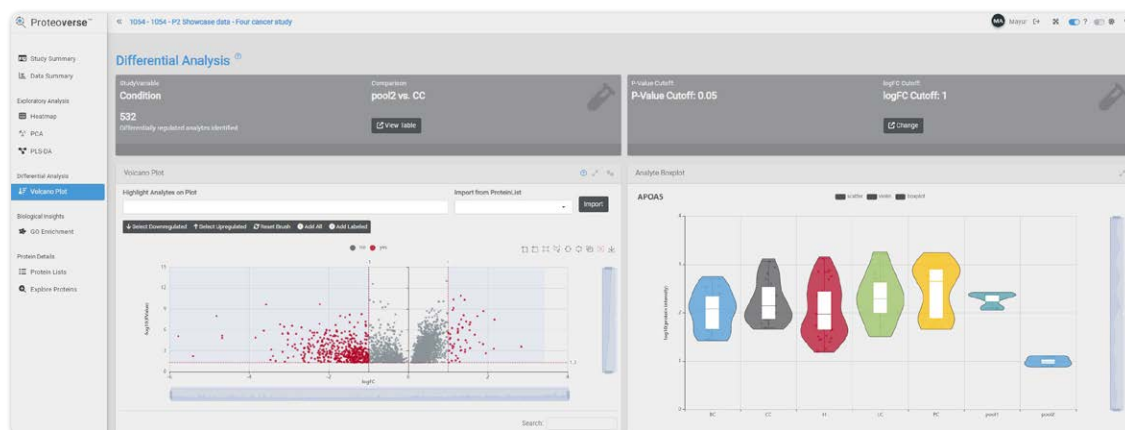


Figure 9. Biognosys' Proteoverse tool interphase for data visualization

for a wide range of tissues and cell lines across early discovery, preclinical studies, and clinical trial assays. Whatever your research needs, we have a solution.

The time is now to harness the power of MS-based proteomics. Explore how Biognosys can help you achieve your research goals by contacting our team today to discuss your specific needs and request a quote. Let's work together to unlock the full potential of your plasma samples.



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Further reading

Biognosys TrueDiscovery Unbiased Insights from Tissues and Biofluids
Available at <https://biognosys.com/services/platforms-applications/#TrueDiscovery>

Biognosys TrueDiscovery Introducing the P2 Plasma Enrichment System
Available at <https://biognosys.com/resources/truediscovery-introducing-the-p2-plasma-enrichment-system/>

Biognosys Unbiased Insights from Tissues and Biofluids
Available at <https://biognosys.com/why-you-should-be-using-rms-based-proteomics-in-hematology/>

Biognosys Proteoverse™ Data Insights
Available at <https://biognosys.com/data-insights-proteoverse/>

• KEY TAKEAWAYS

- Plasma is a rich source of biological information, offering valuable insights into health and disease.
- Advances in mass spectrometry technology have expanded our ability to analyze the plasma proteome. However, the large dynamic range and complexity of plasma make it challenging to study comprehensively.
- Our TrueDiscovery® platform utilizes cutting-edge mass spectrometry to provide analysis of the plasma proteome with unparalleled depth at high throughput.
- The P2 Plasma Enrichment System significantly improves our ability to detect and quantify low-abundance proteins in plasma, with the highest reported proteome enrichment.
- The P2 technology enables robust and reproducible analysis with expanded proteome coverage, identifying up to 9,000 protein groups.
- Our TrueDiscovery® services can be combined with NULISA™ high-sensitivity, targeted panels, offering a complete solution for all your plasma proteomics research needs.
- Our Proteoverse™ tool makes it easy to gain biological insights from proteomics data and is included complimentary with our TrueDiscovery research services.

At Biognosys, we believe that deep proteome insights hold the key to breakthrough discoveries that transform science for better lives. We make the proteome actionable to empower research, drug development, and clinical decision-making with our versatile portfolio of mass spectrometry-based proteomics research services, software, and kits. These solutions provide a multi-dimensional view of protein expression, function, and structure in all biological species and sample types.

Contact us via biognosys.com/contact to discuss your specific needs with one of our scientific consultants.

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