

Plasma proteomics: harnessing cutting-edge technologies for unprecedented depth and throughput

Proteomics

Unlocking secrets of the proteome

Mass spectrometry is considered a gold standard technique in proteomics and the most comprehensive approach to truly understanding the proteome. Mass spectrometry, often coupled with liquid chromatography (LC-MS), offers the invaluable benefits of unrivaled sensitivity and specificity for protein identification while also maintaining the versatility needed for detailed information on protein composition and structure. As a result, mass spectrometry-based proteomics can be seen as the universal key uniquely capable of unlocking secrets of the proteome.

While advancements in genomics and transcriptomics have deepened our understanding of biology and ushered in new applications and diagnostic solutions that impact everyday human life, it is widely understood that proteins provide real-time insights for understanding disease onset, progression, and treatment. For example, the potential of the blood plasma proteome as an indicator of disease and health status ushers in new ways in which LC-MS-based proteomics can be utilized to improve overall human health outcomes. However, all of this

Proteograph™ XT Assay Kit



SP100 Automation Instrument

does not come without challenges; the large dynamic range and diversity of protein variants in plasma make it very difficult to achieve the simultaneous depth of coverage and throughput required for large-scale plasma proteome studies, and as a result, much of the human proteome has been inaccessible until recent advancements.

Seer technology

Founded in 2017, Seer Inc. is developing transformative products to facilitate unbiased, deep, rapid, and large-scale proteomics research, with the goal of eliminating obstacles that historically prevented proteomics from keeping pace with other fields. The company's Proteograph™ Product Suite is an integrated solution that includes proprietary engineered nanoparticles, consumables, automation instrumentation, and software enabling nearly any lab to perform proteomic analysis at scale in a matter of hours.

With Seer's Proteograph XT Assay, released in 2023, researchers can now detect novel proteins and protein variants that other methods could potentially miss, resulting in accurate, precise, and reproducible insights with even greater throughput [1]. With this latest iteration of nanotechnology, scalable and unbiased proteomics is finally accessible to the broader scientific community, which may significantly advance understanding of the complexity and nature of biology, health, and diseases. The workflow expands visibility into the proteome without compromise, allowing researchers to gain flexibility, momentum, and capacity in their work (Figure 1).

“Our goal at Seer is to empower the scientific community to achieve exceptional research outcomes with tools that overcome the technological barriers to the proteome.”



Rebecca Rutherford
Sr. Director of Product Management
Seer

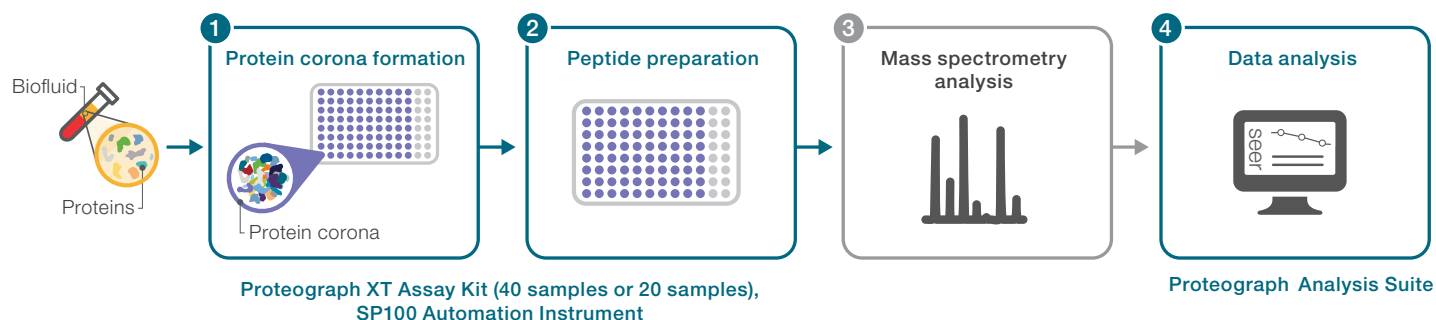


Figure 1. The Proteograph XT workflow. (1) Upon addition of plasma to a nanoparticle (NP) suspension, a stable and reproducible protein corona is formed based on the particles' physicochemical properties. Corona-containing NPs are pulled down and washed, taking advantage of the paramagnetic core. (2) Proteins are then denatured, reduced, alkylated, and digested directly on the nanoparticles using a standard one-pot sample preparation workflow, resulting in release of tryptic peptides into the supernatant. The resulting peptide mixture is then desalted using solid-phase extraction on the SP100 Automation Instrument from Seer. Peptides are then quantified using a fluorescence spectrometer, dried, and resuspended on the SP100 instrument before injection onto a (3) LC-MS system. (4) LC-MS data can be transferred directly to the Proteograph™ Analysis Suite for peptide and protein identification, quantification, and other biological insights.

Partnership toward best performance and throughput

Following the successful launch of the Proteograph XT Assay in 2023, Thermo Fisher Scientific and Seer formed a partnership to harmonize their advancements in proteomics and mass spectrometry technologies. The partnership was made in an effort to accelerate large-cohort studies and unlock new biological insights. Together, Seer's Proteograph XT workflow and Thermo Fisher Scientific's mass spectrometry products offer enhanced speed, sensitivity, and throughput that have been otherwise impossible to achieve without compromising protein depth of coverage and quantification accuracy. Through this partnership Seer has utilized several instruments offered by Thermo Fisher Scientific, with the Thermo Scientific™ Orbitrap™ Astral™ Mass Spectrometer as an integral component and premier mass spectrometry option for its customers at the Seer Technology Access Center (STAC), a premier service offering the broader scientific community access to white glove programs to push their research forward leveraging proteomics (Figure 2).

Rethink what is possible: built for science at scale

The introduction of the Orbitrap Astral Mass Spectrometer by Thermo Fisher Scientific in 2023 marked a significant advancement in mass spectrometry technology and is enabling proteomics at scale without any compromise on depth of coverage. The Orbitrap Astral Mass Spectrometer sets a new standard for performance and discovery with the combination of three mass analyzers: a quadrupole mass analyzer for high selectivity and high ion transmission, an Orbitrap mass analyzer for high dynamic range and high resolution, and a novel Astral™ analyzer for fast and sensitive measurements with complete synchronization of ion transfer and processing throughout the instrument.

This synchronization and parallel handling of five separate ion packets simultaneously enables each mass analyzer to be used at once to optimize performance. The Orbitrap Astral Mass Spectrometer expands the scale and scope of experiments by offering up to four times faster throughput, up to two times deeper proteome coverage, and higher sensitivity with accurate and precise quantification. This is particularly impactful for translational researchers, who can now rapidly and comprehensively capture the dynamic, temporal, and spatial complexity of biology to realize the promise of proteomics.



Figure 2. The Seer Technology Access Center (STAC), with the Proteograph XT workflow for sample processing coupled with the Orbitrap Astral Mass Spectrometers shown here, is located at the Seer headquarters in Redwood City, California, and is enabling demonstration of the entire workflow for large-scale proteomic analysis.

Synergized technology

The combinatory capabilities of Seer's next-generation Proteograph XT Assay and Thermo Fisher Scientific's breakthrough Orbitrap Astral Mass Spectrometer were evaluated to reveal superb discovery power. Combined, these two technologies result in exceptional plasma proteomic depth, detecting >6,000 protein groups and >70,000 unique peptides in a pooled, controlled healthy human plasma sample in less than one hour of LC-MS analysis time. This correlates to over 8 orders of magnitude of proteome coverage reported in the Human Plasma Proteome Project (HPPP) database [2], with a coefficient of variation (CV) of $\leq 12.1\%$. The performance of the Proteograph XT workflow with the Orbitrap Astral Mass Spectrometer enables groundbreaking studies for the translational research community (Figure 3).

STAC with enhanced analytical capabilities

Seer has seen strong demand for STAC projects using the Orbitrap Astral Mass Spectrometer, and currently has the capacity to run thousands of samples per month. As of July 2024, the STAC has served over 48 organizations, including six large pharmaceutical customers. The performance metrics have been impressive, with almost 7,500 protein groups, on average, per study, and a 6.8-fold improvement over neat plasma analysis workflows. These exceptional results achieved through advancement in the technology empower customers to conduct studies with larger numbers of samples, with higher statistical power, to see even smaller changes in the proteome and publish a greater volume of impactful results.

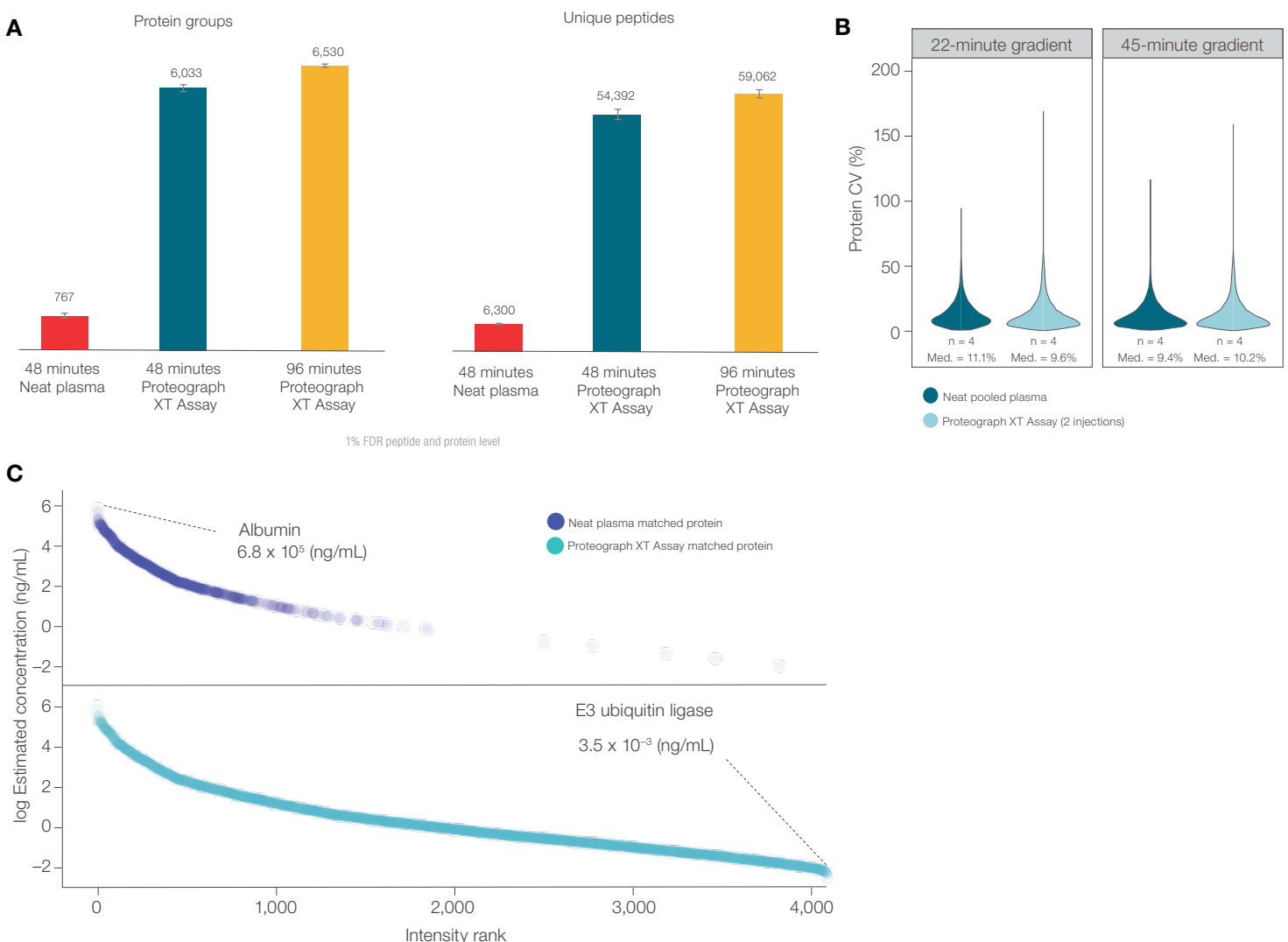


Figure 3. Example results from the Proteograph XT Assay with the Orbitrap Astral Mass Spectrometer. Pooled healthy human plasma was processed for mass spectrometry using a conventional neat plasma digestion workflow and the Proteograph XT workflow. **(A)** The Proteograph XT workflow resulted in more comprehensive identification of protein groups and unique peptides. Results were analyzed using a library-free, data-independent acquisition (DIA) approach. The length of time of each mass spectrometry run is given. **(B)** Distribution of protein CVs for the two workflows. **(C)** The plots depict plasma proteins identified in each workflow. The Proteograph XT workflow demonstrates higher depth of protein coverage and better identification of low-abundance proteins.

Preliminary findings—a glimpse into early results



Study 1: Deep plasma proteomics enables novel biomarker discovery in non-small cell lung cancer

Background

- Lung cancer is the second most diagnosed cancer.
- 80–85% of lung cancers are non-small cell lung cancer (NSCLC).
- Most individuals are diagnosed at an advanced stage and have a poor prognosis.
- Use of noninvasive samples like plasma for biomarker discovery can potentially improve NSCLC identification, treatment, and prognosis with early detection.

Study design

In 2021, Seer conducted a peptide- versus protein-level analysis of 80 healthy samples and 61 early NSCLC samples to demonstrate how differentially expressed proteins can lead to better biomarker discovery and disease characterization.

Study results

The peptide-level resolution and analysis provided by the Proteograph XT Assay and the Thermo Scientific™ Orbitrap Fusion Lumos™ Tribrid™ Mass Spectrometer enabled the identification of many more differences between healthy and lung cancer subjects and revealed important NSCLC-associated protein splice variants [3] (Figure 4).

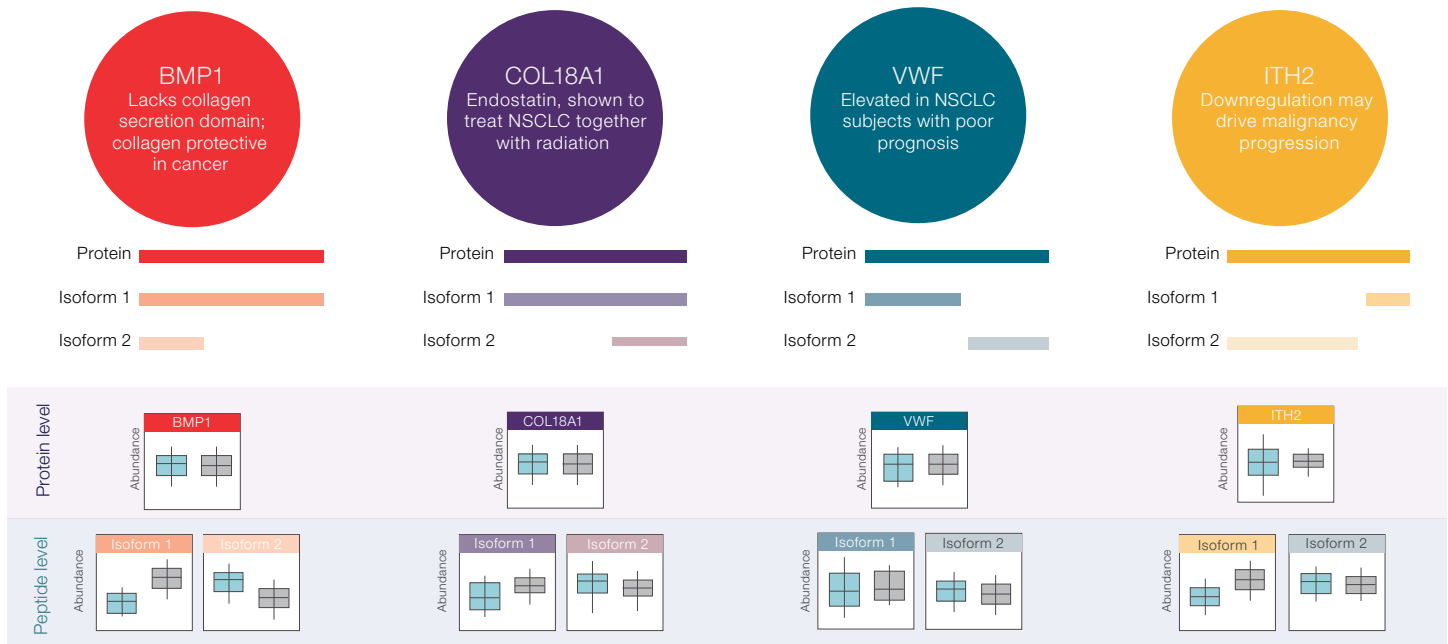


Figure 4. Peptide-level analysis reveals NSCLC-associated protein splice variants. Shown are four examples of proteins that each have at least two isoforms. In each of these four cases, one of the isoforms is more abundant in NSCLC and the other is more abundant in healthy individuals, providing potentially important biomarker information. These differences are not seen at the protein level. Blue box plots: NSCLC; gray box plots: healthy individuals.



Study 2: Deep and rapid large-scale proteomics discovery in Alzheimer's disease

Background

- Alzheimer's disease (AD) affects about 1 in 9 people aged 65 and older.
- 6 million people in the United States are living with the disease, and currently no cure and few treatments are available.
- Studying AD is challenging because it may be progressing for a long time before symptoms appear.
- An accessible biofluid like blood plasma has the potential to be used for detection of predictive biomarkers that could help improve the identification, treatment, and monitoring of AD.

Study design

In 2022, Seer and Massachusetts General Hospital conducted a large-scale AD plasma study on the Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer to expedite proteomics analysis and gain enhanced insight into aging. The end-to-end workflow, with sample processing and LC-MS analysis on one mass spectrometer, was executed within a span of 11 weeks; 1,790 plasma samples were processed, and the resulting peptides were analyzed using the data-independent acquisition (DIA) LC-MS method. Three library search strategies were also investigated [4].

Study results

Over 5,000 plasma proteins were identified with the Proteograph Product Suite, the Proteograph XT Assay, and the Orbitrap Exploris 480 Mass Spectrometer to reveal novel biological insights into complex disease and plasma biomarkers for dementia detection [4] (Figure 5).

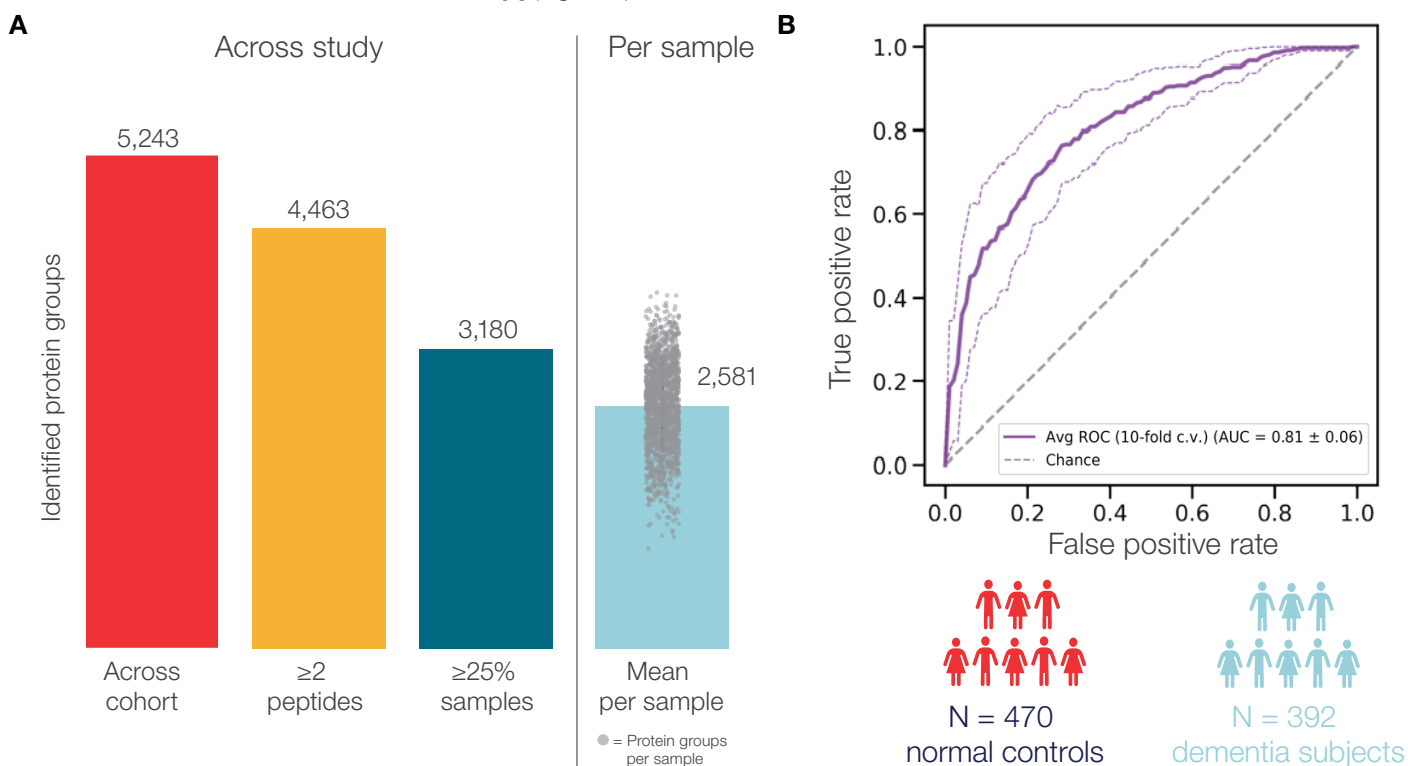


Figure 5. Plasma proteomic analysis for a large AD cohort. (A) Over 5,000 protein groups across the cohort (on average >2,500 per sample) were identified, with the majority being supported by multiple peptides and found in multiple samples. (B) The data were used to set up a classification model for healthy vs. dementia individuals. To evaluate the performance of the model, a receiver operating characteristic (ROC) curve was constructed that illustrates the tradeoff between the true positive rate (sensitivity) and false positive rate (specificity). The ROC curve suggests good discrimination between healthy and dementia individuals, with an area under the curve (AUC) of 0.81.



Study 3: Deep plasma proteome exploration of astronauts

Background

- Weill Cornell Medicine partnered with SpaceX to conduct experiments to discern the molecular basis of changes in the human body during long-term human space travel.
- Biochemical and computational technologies can be used to understand the effects of spaceflight on astronauts by looking at genetic, epigenetic, transcriptional, and proteomic changes.

Study design

Conducted between 2022 and 2023, this study explored changes in the human plasma proteome and underlying biology as a result of spaceflight. Samples were collected from seven astronauts across two missions during pre-flight, post-flight, and the day of landing from space. The combination of Seer technology and the Orbitrap Astral Mass Spectrometer offered increased protein coverage and depth, allowing for additional insights at greater resolution into pathways related to astronaut health and biology.

Study results

With the first-generation Proteograph Assay, 2,998 protein groups across 16 samples were studied, and changes in protein translation and immune and stress response pathways were identified. With the Proteograph XT workflow and the Orbitrap Astral Mass Spectrometer, 7,555 protein groups across 37 samples were identified and evaluated (Figure 6).

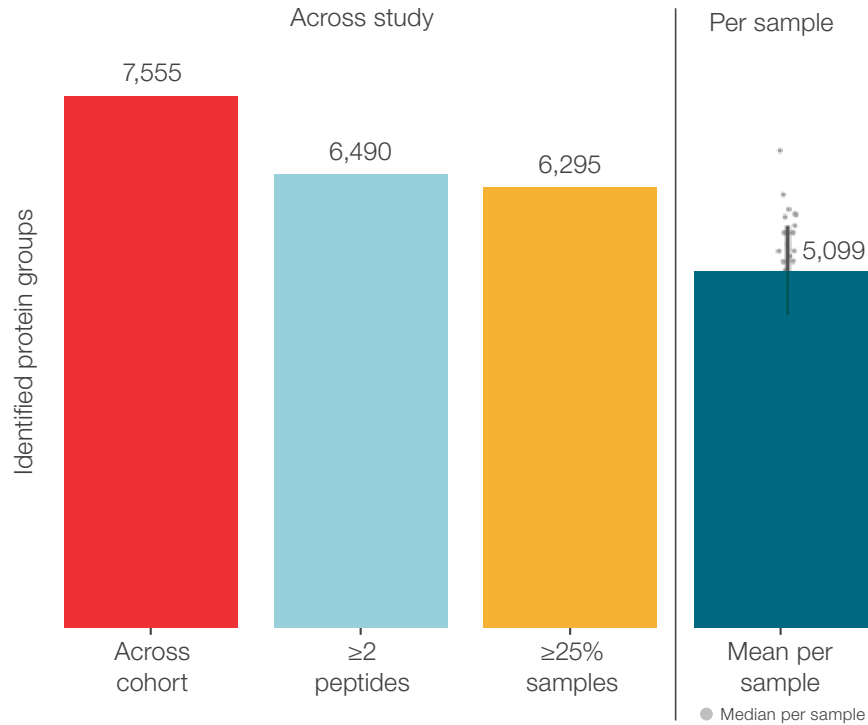


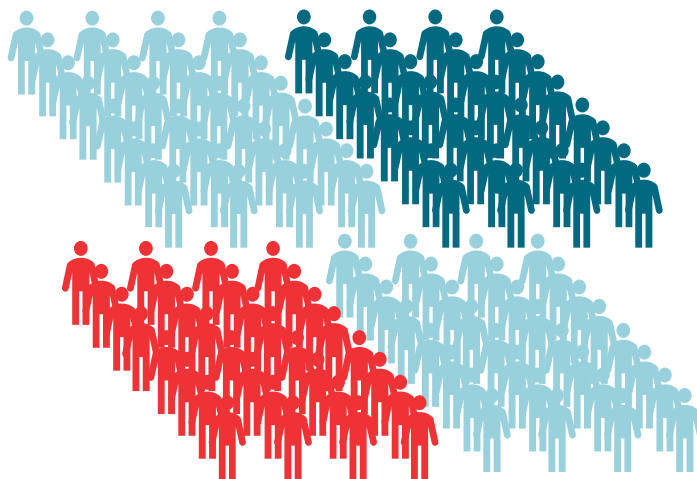
Figure 6. Proteomic analysis of blood plasma from astronauts. Over 7,500 protein groups were identified (average >5,000 per sample), with the majority being supported by multiple peptides and found in multiple samples.

Pioneering a new era of translational research

The recent technological advancements highlighted here are just the beginning of understanding the full potential of plasma proteomics in translational research. With ongoing technological innovations, partnerships, and end-to-end workflows, secrets of the proteome can be revealed, potentially improving our understanding of human health and disease. From studies on NSCLC to Alzheimer's disease, results never thought imaginable are being achieved through the exponentially powerful combination of the Proteograph XT Assay and the Orbitrap Astral Mass Spectrometer.

Proteomics research is now allowing us to discover novel biomarker signatures and deepen our understanding of human health. Together, as the scientific community continues to embrace deep, unbiased proteomics, what we can discover is limitless.

The Proteograph XT Assay with the Orbitrap Astral Mass Spectrometer enables large- and population-scale studies



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