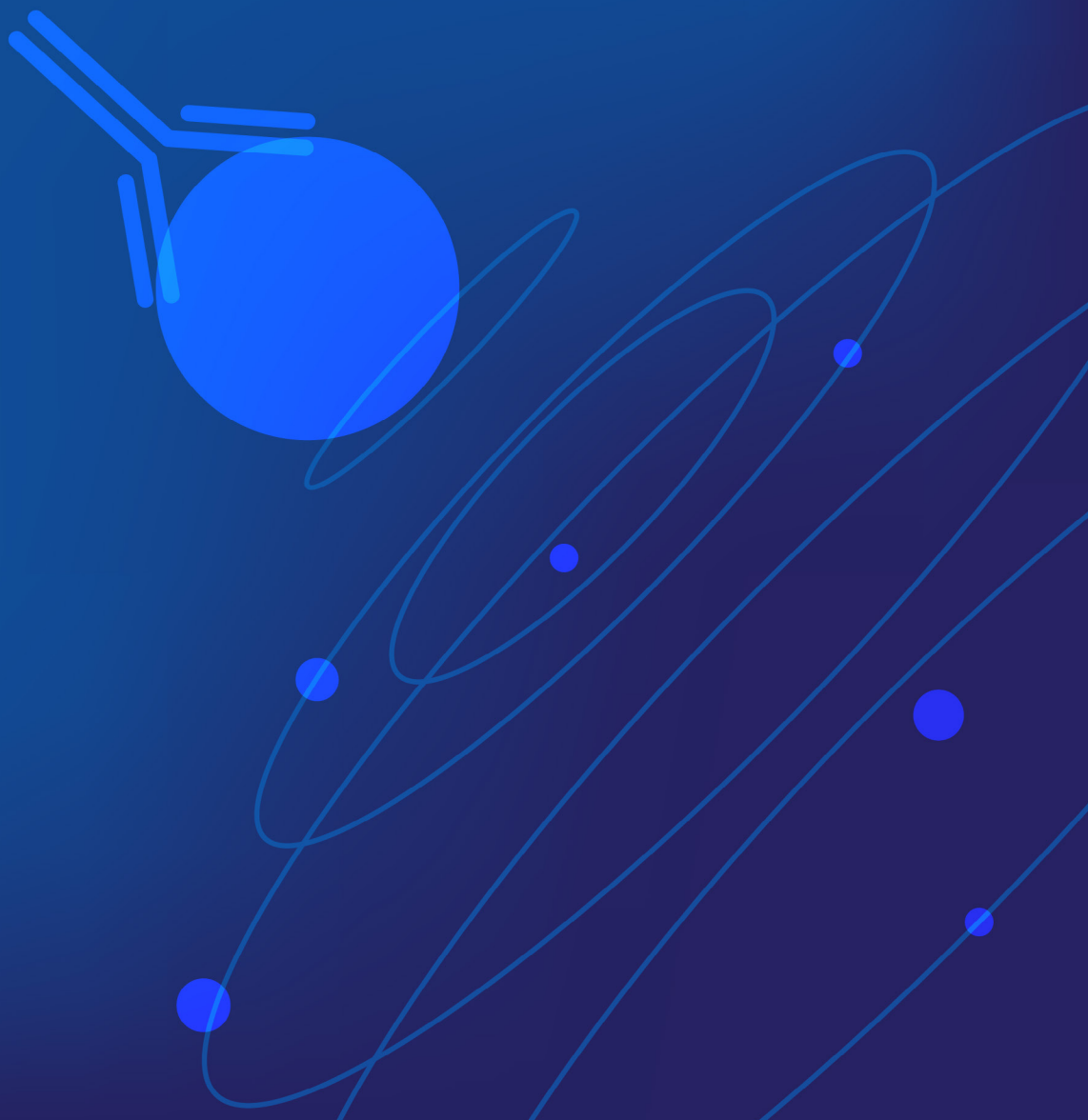
Olink®

Unlocking the power of proteomics: From discovery to application



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Chapter 1

Why proteomics? A new lens for precision medicine

The genomics revolution has transformed our understanding of human biology. It has mapped risk, identified predispositions, and offered hope for targeted therapies.
Yet for many complex diseases, the path from genetic insight to clinical utility remains incomplete. Precision medicine is still a work in progress.
Today, high-throughput proteomics is emerging as the critical next step—providing real-time biological data that genomics alone cannot deliver. This shift opens new frontiers in disease understanding, therapeutic development, and personalized care.

From static code to dynamic biology

DNA is the blueprint, but proteins are the execution. They drive biological processes that define health and disease. While DNA is largely static throughout life, proteins reflect the dynamic state of the body—where disease is active, when it's progressing, and how it responds to therapy.

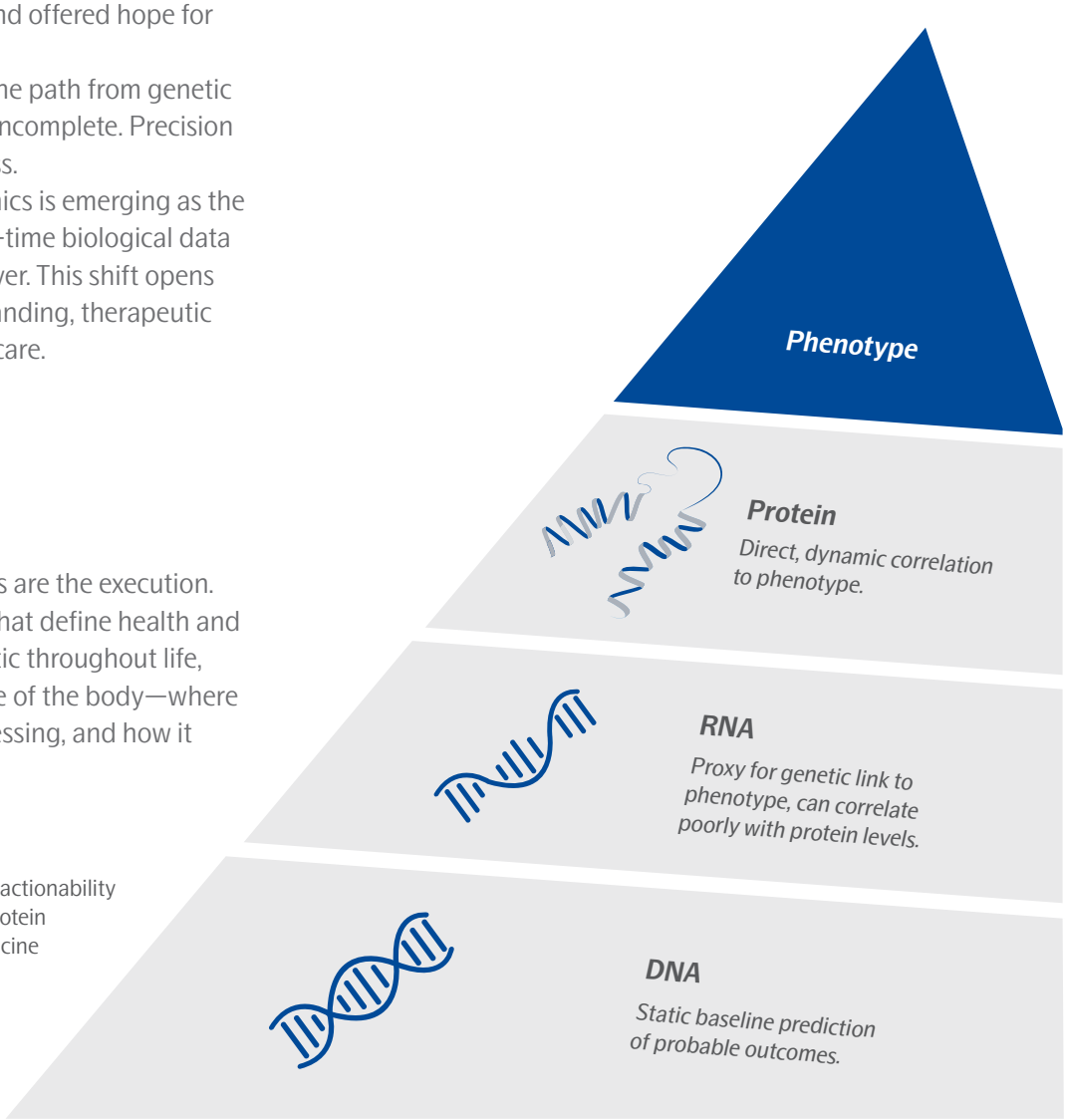


Figure 1.1 Profile and actionability at the DNA, RNA or protein level in precision medicine applications.

The evolution of protein analysis: Building on proven technologies

The field of proteomics has been shaped by decades of technological advancement. Each generation of tools built on the last, bringing us closer to decoding the complexity of protein biology.

- In 1971, ELISA (enzyme-linked immunosorbent assay) introduced a new level of specificity and clinical utility—but as a single-plex assay, it wasn't suited for broader discovery.
- In the late 1970s, Western blotting emerged as another method for protein detection, offering improved resolution.
- By the 2000s, mass spectrometry (MS), especially when combined with liquid chromatography (LC-MS/MS), allowed for deep proteomic profiling, though at the cost of high sample input and technical complexity.
- Multiplex immunoassays, such as those from Luminex and Meso Scale Discovery, provided the ability to analyze tens of proteins simultaneously, increasing throughput and sensitivity.
- More recent MS advancements—SRM, PRM, and SWATH-MS—offer peptide-level quantification and analysis of post-translational modifications with greater speed, with limitations related to workflow complexity, throughput and cost.

In the last decade, a new wave of affinity-based multiplex proteomics has emerged. Platforms like Olink have introduced sensitive and simplified high-plex solutions using NGS and qPCR.

Olink's NGS-based proteomics marks a true step change—combining the specificity of immunoassays with the scalability and precision of sequencing, backed by a rigorous 3-step, 15-parameter verification process. The antibody-based detection minimizes nonspecific binding and reduces dependency on the shape or availability of a single epitope, delivering insights you can trust. This positions the Olink platform not only as a powerful research tool but also as a clinical enabler.

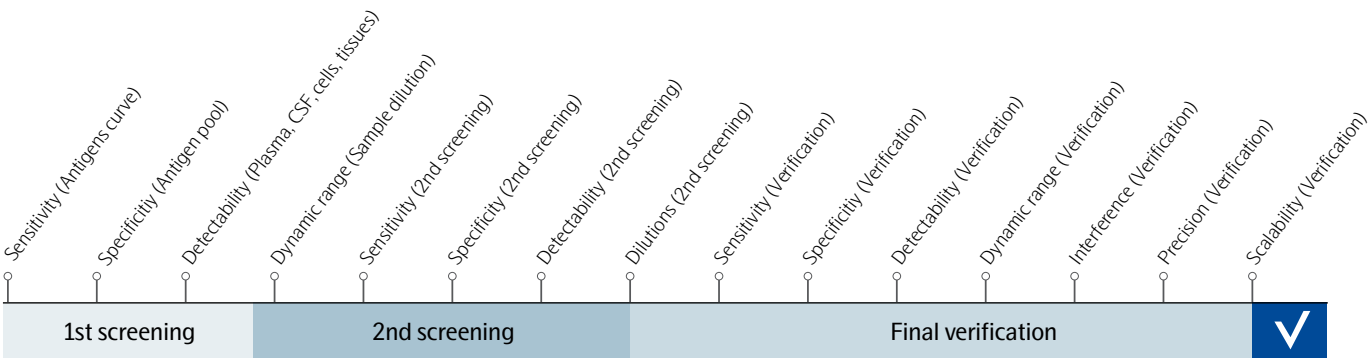
In contrast, aptamer-based technologies are engineered to bind a single epitope and rely heavily on specific protein conformations, which might increase the risk of nonspecific binding and false positives.

These innovations now make it possible to explore the proteome at population scale—delivering the specificity of immunoassays, the breadth of mass spectrometry, and the scalability of sequencing technologies.

The caveats of aptamers

Read more

Table 1.1 Olink's rigorous 3-factor 15-step analytical validation



Why proteomics matters

Proteins aren't just passive reflections of biology. Nearly all drugs act on proteins, not genes. While DNA reveals inherited risk, protein levels change in real time, signaling when disease is active, how it's progressing, and whether a treatment is working. This is why proteins like APOB, PSA, and CRP are already widely used as clinical biomarkers, and why scalable proteomic tools are key to the future of precision medicine.

Precision medicine needs more than DNA

Despite the massive scale of genomic data available today, three fundamental questions remain challenging:

Precision goal	Genomics	Proteomics
Find the right drug	Genetic clues often indirect	Proteins are the direct drug targets
Find the right patient	Strong for rare conditions	Enables broad segmentation across diseases
Find the right time	Genetic sequence is stable	Protein levels are dynamic and time-sensitive

Proteomics bridges these gaps—bringing us closer to actionable precision medicine that guides effective treatment.

Chapter 2

Population-scale proteomics in action: New insights from the UK Biobank

"(The UK Biobank is) the single most impactful dataset that has ever been examined in the history of genetics research."

Dr. Cristen Willer
Professor of Internal Medicine,
University of Michigan

The UK Biobank Pharma Proteomics Project (UKB-PPP) marks a transformative moment in biomedical science. It's the largest study of its kind and a blueprint for what happens when genomics and proteomics meet at scale. This large-scale initiative integrates high-throughput proteomics into one of the world's most extensively phenotyped cohorts—demonstrating the true potential of proteogenomics to uncover novel biology, validate therapeutic targets, and create predictive tools that advance human health. With over 54,000 participants, 13 pharma partners, and Olink® Explore at its core, the first phase of UKB-PPP demonstrates the power of population-scale proteomics to unlock new insights into human biology (**Sun et al., Nature, 2023**).
UKB Announcement Blog, Jan 2025

This collaboration brought together academic researchers and pharmaceutical companies, generating over 17 million protein measurements and mapping more than 14,000 significant protein quantitative trait loci (pQTLs).
Sun et al., Nature 2025
Publication Highlights Blog, March 2025

Proving the power of proteogenomics

UKB-PPP validated what the field has long hypothesized: integrating genomics and proteomics reveals deeper biological insights than either alone. By linking genetic variants to protein levels and downstream disease risk, researchers can now distinguish causal from correlative biomarkers and accelerate target validation.

Olink article – Realizing the Potential of Population-Scale Proteogenomics
This is proteogenomics in action—connecting genotype, proteotype, and phenotype to drive discovery.

The UKB-PPP: Scope, scale, and significance

UKB-PPP is the largest proteogenomic study to date, with over 54,000 plasma samples profiled using the Olink® Explore platform **UK Biobank resource**
These samples were analyzed across more than 2,900 proteins, generating over 150 million data points in the pilot phase alone. The data—collected from deeply phenotyped individuals with genetic, lifestyle, imaging, and health records—has been made available to the research community through UK Biobank's open-access infrastructure.

This resource has already delivered groundbreaking insights across disease biology, risk prediction, and drug discovery. Key findings from the pilot phase include proteomic markers of biological aging and a multi-disease risk model based on protein signatures.

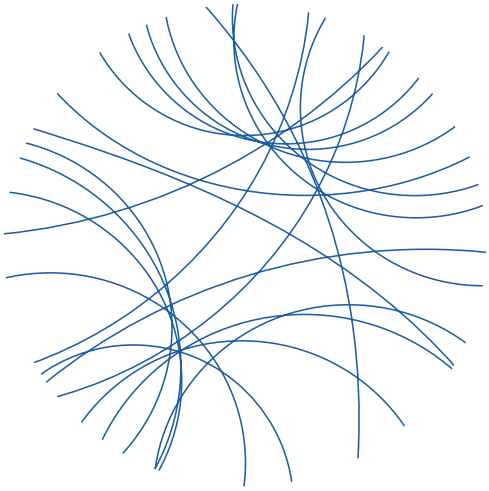
Podcast with Dr. Whelan: Interview on the UK Biobank

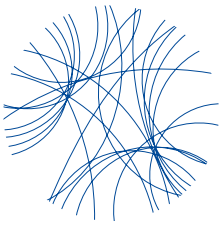
Watch podcast

Breakthrough applications

Findings from UKB-PPP span multiple domains:

- Aging clock: Researchers identified a composite proteomic signature that tracks biological aging more accurately than chronological age. This "aging clock" may support preventive health strategies in the future.
- Proteomics risk scores: Researchers introduced ProRisk—proteomics risk score prediction models that outperform traditional polygenic scores in key diseases. These scores integrate genetic and protein-level information for better segmentation and clinical decision-making.
- Causal Target Identification: Using Mendelian randomization and pQTL mapping, the project linked genetic variation to disease-relevant proteins, helping validate drug targets with functional evidence (**Sun et al., Nature, 2025**).





Proteomic aging clock: Measuring biological age from a single blood sample

In one of the first major analyses, researchers developed a data-driven model of biological aging using Olink® Explore data. A set of 204 proteins was identified as highly predictive of chronological age in over 45,000 participants, with correlation coefficients exceeding 0.94. The model generalized across diverse populations, including validation in both Chinese and Finnish cohorts.

Importantly, deviation from predicted age—so-called "age acceleration"—was strongly associated with future risk of chronic disease and all-cause mortality, even after adjusting for traditional risk factors. This proteomic aging clock offers a robust, accessible metric for assessing health span and disease susceptibility at scale.

Watch the webinar: Leverage UK Biobank insight to unlock proteomic discoveries

Presented by
Austin Argentieri PhD, Research Fellow, Analytic and Translational Genetics Unit, Harvard Medical School; Affiliate Member, Broad Institute of MIT and Harvard

Roberta Benedetto, PhD, Field Application Scientist, Olink, part of Thermo Fisher Scientific
Klev Diamanti, PhD, Senior Data Scientist, Olink, part of Thermo Fisher Scientific

Watch webinar

ProRisk: A single score for predicting multiple diseases

Another major finding from UKB-PPP is the development of ProRisk™, a multi-disease proteomic risk score built using neural network modeling. Based on protein profiles from over 52,000 individuals, the model integrates signals from more than 1,400 proteins to stratify individual risk for 45 common diseases.

The results are compelling. ProRisk demonstrated strong predictive performance (C-index > 0.80) across a range of endpoints, including cardiovascular disease, type 2 diabetes, neurodegenerative conditions, and certain cancers. In many cases, the model outperformed traditional biomarkers or clinical predictors—despite being derived from a single baseline blood sample.

Building a genetic atlas of the plasma proteome

In addition to phenotypic modeling, UKB-PPP has made major contributions to the understanding of genetic regulation of proteins. Using genome-wide association studies (GWAS), the pilot study identified over 14,000 significant protein quantitative trait loci (pQTLs), 67% of which were cis-acting, providing genetic validation of assay specificity.

These results provide critical resources for causal inference studies. By linking protein levels to disease outcomes, researchers can prioritize high-confidence drug targets and identify proteins with therapeutic potential—accelerating target discovery and validation.

Empowering global research through open data

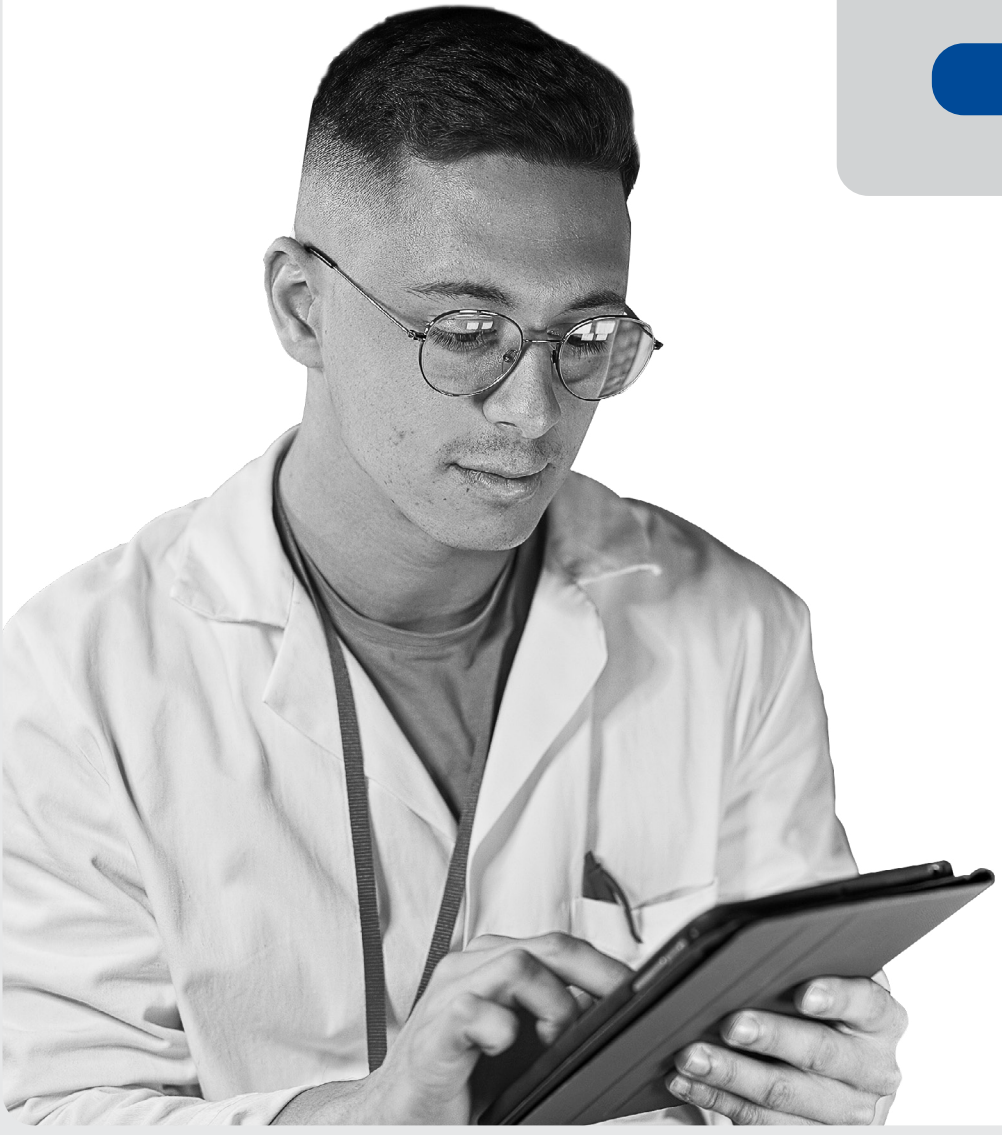
The data generated in UKB-PPP is already driving discovery. In 2024 alone, the pilot dataset supported over 100 peer-reviewed publications across a range of disciplines. These studies have identified new therapeutic targets, deepened understanding of disease mechanisms, and validated proteomic tools for use in clinical and research settings.

Ongoing expansion of the project will scale this impact further. In the next phase, over 600,000 additional samples will be analyzed with Olink PEA™ technology—creating an unprecedented proteomic resource for population health research.

Publications

Landmark articles based on the UKB-PPP data were recently published in Nature, as summarized in our blog.

Read summary



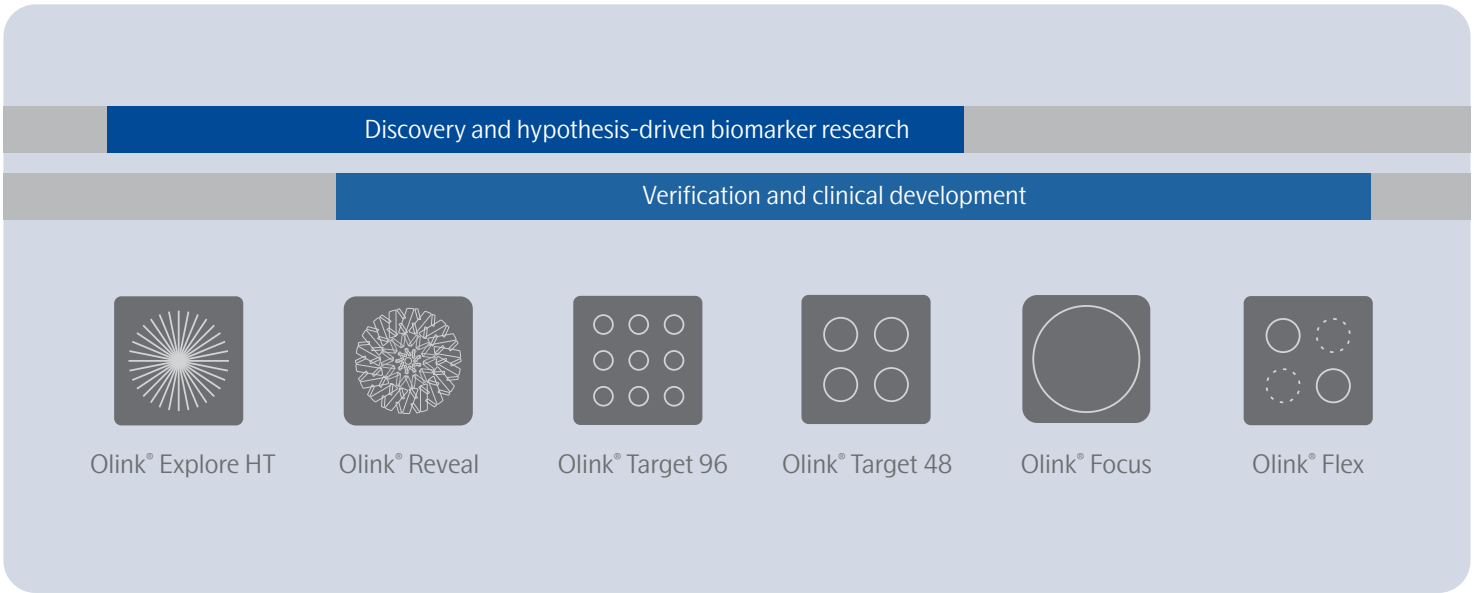
Chapter 3

From discovery to impact – translational applications of high-throughput proteomics

The true potential of proteomics lies in how it drives actionable insights across every stage of human health research. Olink's high-throughput platform enables researchers to move fluidly from exploratory discovery to clinical translation, all while maintaining scientific rigor and reproducibility and turning protein signals into meaningful advances in precision medicine. By using the same Proximity Extension Assay (PEA™) technology across Olink® Explore, Reveal, Target, Flex, and Focus, findings can be confidently carried from hypothesis to clinical reality.

This chapter explores key translational use cases across therapeutic areas, highlighting how leading researchers are using Olink solutions to discover biomarkers, guide therapeutic development, segment patient samples, and support clinical research and diagnostic development.

Figure 3.1 Integrated proteomics workflow.



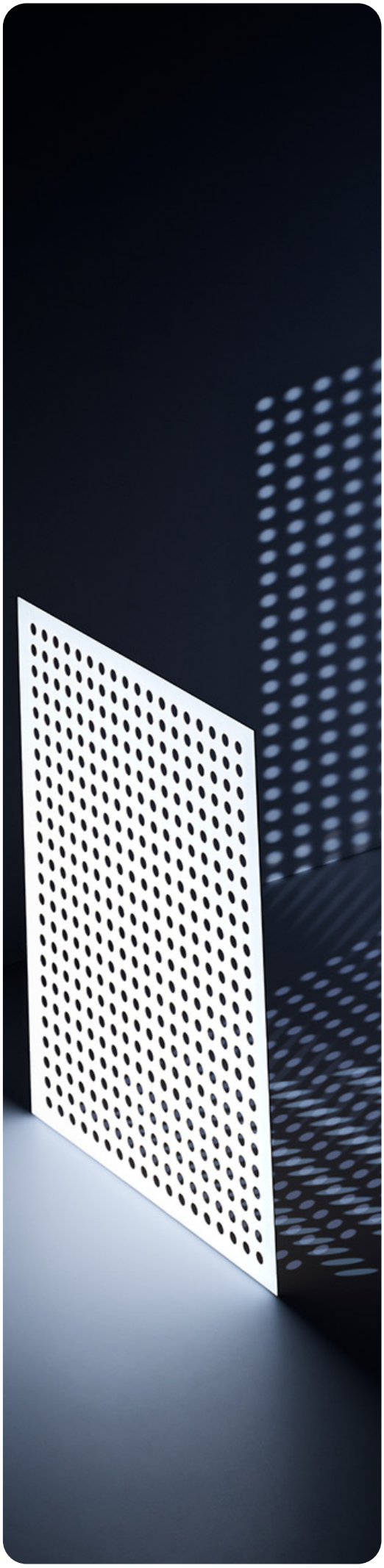
Early detection of disease

Oncology: Multi-cancer early detection with plasma proteomics

In a population-level study, researchers profiled plasma proteins using Olink® Explore 3072 in treatment-naïve patients diagnosed with 18 different types of solid tumors, as well as healthy individuals. The study revealed sex-specific differences in cancer-associated proteins, with approximately 80% of significant proteins in men showing no significant changes in women, and vice versa. Despite these differences, both male and female models achieved outstanding performance with just 10 proteins each (AUCs > 0.98) for pan-cancer detection. The models also accurately identified the tissue of origin for most cancers in over 80% of cases, surpassing the sensitivity of circulating tumor DNA (ctDNA)-based approaches. These findings support the potential of high-throughput plasma proteomics for scalable, non-invasive multi-cancer early detection (**Budnik et al., BMJ Oncology, 2024**).

Cardiopulmonary disease: Biomarker of right ventricular dysfunction in PAH

Right ventricular (RV) dysfunction is a critical determinant of prognosis in pulmonary arterial hypertension (PAH), yet the molecular underpinnings of RV remodeling are not fully understood. In a study led by Université Laval, researchers used a combined approach of transcriptomic analysis of RV tissue and proteomic profiling of plasma samples with Olink® Explore 384. They identified latent transforming growth factor beta binding protein 2 (LTBP-2) as a plasma biomarker significantly associated with RV function. Elevated LTBP-2 levels correlated with increased cardiac fibrosis and adverse remodeling, offering mechanistic insight into RV pathophysiology. Importantly, LTBP-2 levels added predictive power to existing risk segmentation models for long-term survival in PAH, supporting its potential as a clinically useful prognostic biomarker (**Boucherat et al., Nature Cardiovascular Research, 2022**).

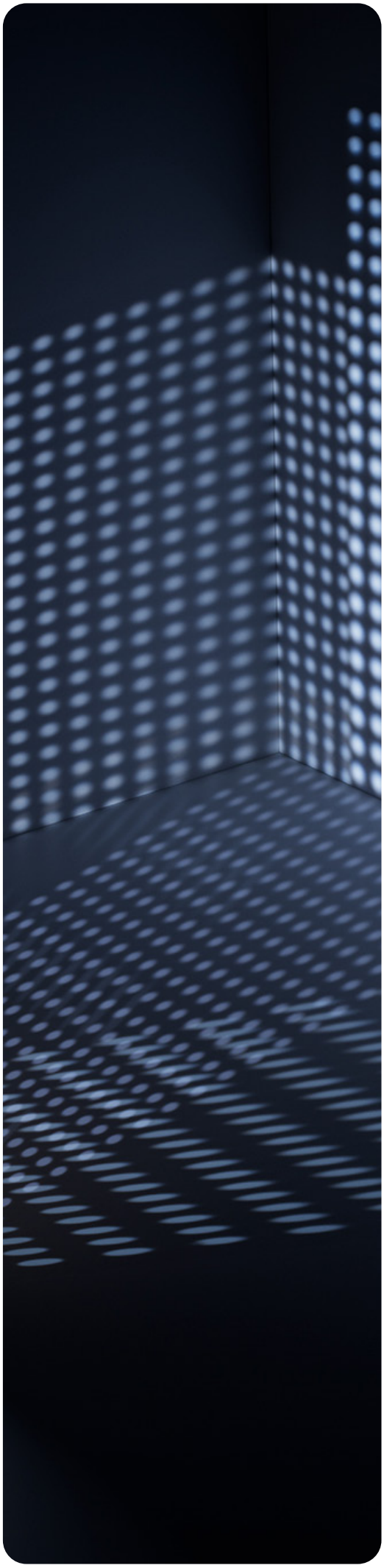


Immunology and inflammation: preclinical detection in Crohn's disease

In a prospective, nested case-control study conducted at Mount Sinai Hospital in Toronto, researchers profiled serum samples from healthy first-degree relatives of Crohn's disease (CD) patients using five Olink® Target 96 panels. Among the 25 proteins found to be differentially expressed in individuals who later developed CD, key markers such as MMP-9, CLEC4D, CXCL9, and MEPE stood out. These proteins were linked to immune activation, microbial response, and epithelial barrier dysfunction—hallmarks of CD pathogenesis. The findings highlight the potential for proteomics to reveal predictive biomarkers for preclinical disease, enabling earlier diagnosis and preventive strategies (**Leibovitzh et al., Gut, 2023**).

Neurology: Blood-based biomarkers for Alzheimer's disease

Researchers at the Hong Kong University of Science and Technology conducted large-scale plasma proteomic profiling to identify protein biomarkers for Alzheimer's disease (AD) screening and staging using Olink® Target 96 panels. From over 400 dysregulated proteins, a refined 19-protein panel was developed that accurately classified clinical AD with an AUC of 0.97–0.98. Several of the proteins were also strongly associated with specific stages of the disease, enabling potential applications in disease monitoring. Key Olink-based findings—including CASP-3 and CD8A—were independently validated using singleplex ELISAs. These results support the development of a high-performance, blood-based test for early AD detection and therapeutic segmentation (**Jiang et al., Alzheimer's & Dementia, 2021**).



Mechanistic insight

Neurology: Proteomic insights into refractory MS relapse treatment

In a study exploring therapeutic alternatives for relapsing multiple sclerosis (MS) patients unresponsive to standard methylprednisolone (MPS) therapy, researchers compared the immunological effects of a second high-dose MPS treatment versus immunoadsorption (IA), a novel apheresis-based therapy. Utilizing the Olink® Target 48 panel, the team found that IA significantly outperformed MPS, inducing a marked shift in the cytokine repertoire. Specifically, IA reduced circulating levels of B cell maturation cytokines and B cell-derived mediators implicated in neuroinflammation. These proteomic findings suggest that the immunomodulatory effects of IA are largely driven by B cell suppression and underscore its potential as a targeted treatment strategy for steroid-refractory MS relapses (**Pfeuffer et al., Journal of Neuroinflammation, 2022**).

Oncology: NSCLC and immunotherapy response

Olink® Explore identified distinct protein signatures in non-small cell lung cancer (NSCLC) patients responding to PD-1 inhibition. Immune activation markers, including cytokines and checkpoint proteins, differentiated responders from non-responders, providing a foundation for improved segmentation and response prediction in immunotherapy (**Loriot et al, Annals of Oncology, 2021**).

Immunology and inflammation: Biomarker-guided therapy in hidradenitis suppurativa

Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disorder with complex pathophysiology and inconsistent therapeutic outcomes. In a phase 2 clinical trial, researchers used Olink® Target 96 panels to profile serum proteomic changes in HS patients treated with fostamatinib, a spleen tyrosine kinase (SYK) inhibitor. Baseline proteomic profiles revealed differentially expressed proteins (DEPs) correlated with disease stage. Importantly, distinct protein signatures were observed in responders versus non-responders by week 12, with early indicators traceable to baseline. These findings highlight the potential for proteomics-based biomarkers to stratify patients for targeted treatment and optimize therapeutic outcomes in HS (**Flora et al., The Journal of Investigative Dermatology, 2023**).

Disease prediction

Oncology: Early detection of lung cancer

In a study leveraging the Liverpool Lung Project (LLP) and UK Biobank Pharma Proteomics Project, researchers used Olink® Explore 3072 to identify plasma protein biomarkers predictive of lung cancer years before clinical diagnosis. The analysis included 131 pre-diagnosis cases (spanning 1–10 years before diagnosis), 90 newly diagnosed patients, and 237 healthy controls. Using machine learning models, the team achieved median AUCs of 0.76–0.90 for samples collected 1–3 years pre-diagnosis and 0.73–0.83 for those taken 1–5 years pre-diagnosis. External validation using UK Biobank data confirmed robust predictive performance (AUCs up to 0.7 a full 12 years before diagnosis), with biomarker performance independent of confounding factors such as smoking status. These findings demonstrate the potential for plasma proteomics in developing early detection tools for lung cancer (**Davies et al., EBioMedicine, 2023**).

Neurology: Inflammatory signatures predicting stroke outcome

In a prospective study of 534 patients with ischemic stroke, researchers at the University of Gothenburg used Olink® Target 96 panels to identify 20 plasma proteins associated with functional outcomes at 3-month follow-up. A 9-protein model accurately predicted favorable versus unfavorable recovery with an AUC of 0.81, which increased to 0.92 when combined with stroke severity measures. Pathway analysis revealed enrichment of NLRP3 inflammasome signaling and immune regulatory networks, implicating inflammation in post-stroke recovery. These findings offer novel biomarkers for outcome prediction and potential therapeutic targeting (**Angerfors et al., Journal of Neuroinflammation, 2023**).

Immunology and inflammation: Predicting progressive fibrosing interstitial lung disease (PF-ILD)

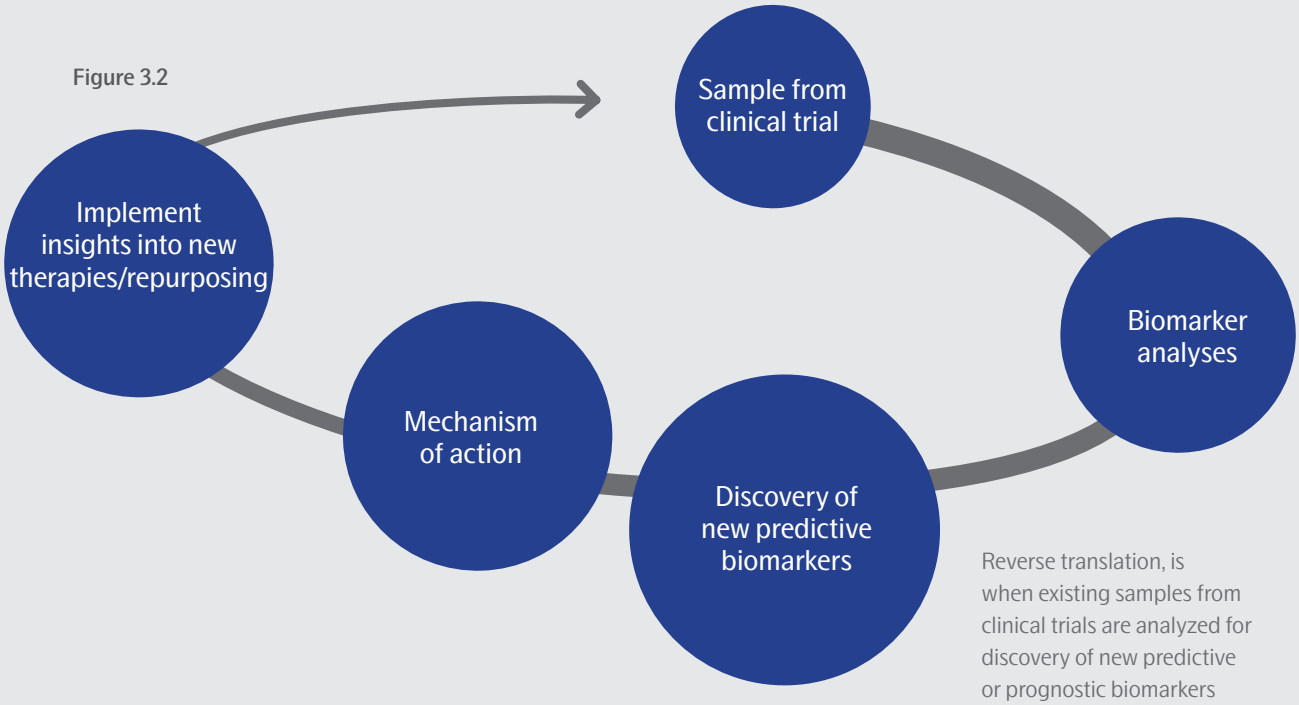
In an effort to improve prognostic accuracy for patients with fibrosing interstitial lung disease (ILD), researchers applied the Olink® Explore 384 Inflammation panel to derive a 12-protein biomarker signature predictive of disease progression. Using machine learning across a multicenter cohort, the team demonstrated that this signature had a negative predictive value of 0.91—indicating that fewer than 10% of patients without the high-risk proteomic profile would progress within one year. Notably, integrating this signature into clinical trial design was projected to reduce sample size requirements by over 80%, dramatically increasing trial efficiency. This work addresses a critical gap in PF-ILD management, providing a robust tool for risk segmentation and therapeutic targeting (**Bowman et al., The Lancet Respiratory Medicine, 2022**).

Drug repurposing and reverse translation

Immunology and inflammation: Genetic drivers of inflammation and therapeutic targeting

In a large-scale proteogenomics effort led by the SCALLOP consortium, researchers integrated genome-wide association studies (GWAS) with Olink® Target 96 data from over 14,800 individuals across 11 cohorts to investigate the genetics of circulating inflammatory proteins. Through protein quantitative trait loci (pQTL) analysis and Mendelian Randomization (MR), the study identified 180 significant associations between 108 genomic regions and 70 proteins. Findings were validated using the ARISTOTLE study, with strong concordance (r = 0.97) for both cis- and trans-pQTLs. Notably, MR analysis uncovered up to 22 significant causal protein-disease associations, including a pQTL for IL-12B linked to inflammatory bowel disease (IBD)—supporting the clinical efficacy of IL-12/IL-23-targeting therapies like ustekinumab. This work demonstrates how integrated proteogenomics can reveal causal pathways and guide therapeutic development for immune-mediated diseases (**Zhao et al., Nature Immunology, 2023**).

Figure 3.2



Reverse translation, is when existing samples from clinical trials are analyzed for discovery of new predictive or prognostic biomarkers and potentially provide insight into the mechanism of action for new therapeutic development or repurposing existing on-market therapies.

Neurology: Proteogenomic mapping of neurological disease risk

In a large-scale proteogenomics study, researchers used two Olink® Target 96 panels to analyze serum samples from over 3,000 individuals across two independent cohorts, aiming to uncover protein quantitative trait loci (pQTLs) linked to neurological diseases. Genome-wide association studies (GWAS) integrated with proteomic data identified 214 independent genetic variants associated with 107 proteins—114 of which were novel. Among the findings, strong evidence of causal relationships emerged for key protein-disease pairs: CD33 and Alzheimer's disease, GPNMB and Parkinson's disease, and MSR1 and schizophrenia. These pQTLs present opportunities for therapeutic repurposing and deeper understanding of disease pathways. Validation with SomaScan for overlapping proteins revealed moderate to strong correlations, further supporting the robustness of the Olink-based results (**Png et al., Nature Communications, 2021**).

Oncology: Mechanistic insight into cancer cachexia

In the TRACERx clinical trial, researchers utilized Olink® Explore 3072 to investigate the biology underlying cancer-associated cachexia (CAC) in patients with non-small cell lung cancer (NSCLC). CAC, a multifactorial metabolic syndrome, is linked to poor prognosis and increased treatment toxicity. The proteomic analysis revealed that circulating levels of GDF15 at the time of relapse were significantly associated with reductions in body weight, skeletal muscle mass, and adipose tissue—hallmarks of cachexia. These findings deepen the mechanistic understanding of GDF15's role in CAC and suggest its potential as a therapeutic target to mitigate adverse effects in NSCLC treatment (**Al-Sawaf et al., Nature Medicine, 2023**).

Treatment response

Oncology: Immune checkpoint response in lung cancer

In the Phase III Lung-MAP S1400I trial, a multi-omics approach—including Olink® Target 96 Immuno-Oncology Panel—was applied to uncover immune and molecular correlates of treatment response in patients with squamous non-small cell lung cancer (SqNSCLC). While the combination of ipilimumab and nivolumab did not demonstrate overall benefit compared to monotherapy, deeper analysis revealed immunological and proteomic differences in patients who did respond. Olink proteomics identified circulating proteins associated with improved progression-free and overall survival, supporting a mechanistic rationale for patient segmentation and offering candidate biomarkers for clinical translation (**Parra et al., Clinical Cancer Research, 2024**).

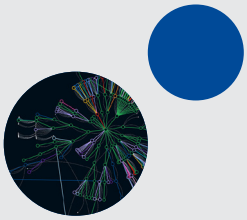
Patient segmentation

Oncology: Predicting immunotherapy response in NSCLC brain metastases

In a prospective clinical study, researchers investigated the predictive value of cerebrospinal fluid (CSF) cytokine profiles in non-small cell lung cancer (NSCLC) patients with brain metastases undergoing immune checkpoint inhibitor therapy. Using the Olink® Target 96 Immuno-oncology Panel they analyzed both plasma and CSF samples from 28 patients and revealed that baseline levels of multiple cytokines were significantly lower in CSF than in plasma. However, in patients who experienced an intracranial response to therapy, higher baseline CSF levels of LAMP3 and lower levels of CXCL10, IL-12, CXCL11, IL-18, TIE2, HGF, and PDCD1 were significantly associated with treatment response and progression-free survival. The resulting 7-protein CSF signature achieved high predictive accuracy (ROC = 0.91), outperforming PD-L1 expression (AUC = 0.72). These findings highlight the clinical potential of CSF-based proteomic biomarkers for immunotherapy segmentation in NSCLC brain metastases (**Li et al., OncoImmunoloy, 2023**).

Neurology: Biomarker discovery in Niemann-Pick Type C1

Niemann-Pick disease type C1 (NPC1) is a rare, fatal pediatric-onset neurodegenerative disorder with no approved therapies and highly variable clinical presentation. Researchers at the National Institutes of Health used Olink® Explore 1536 to analyze cerebrospinal fluid (CSF) from 28 NPC1 patients and 30 healthy controls, identifying ten novel protein biomarkers significantly altered in disease. These proteins—validated by ELISA—may offer critical insight into disease mechanisms and serve as markers for monitoring therapeutic response in future clinical trials (**Campbell et al., Biomarker Research, 2023**).



Clinical translation: From panels to practice

Neurology: Serum-Based Monitoring of MS Disease Activity

The lack of validated clinical tests to track disease activity or progression in multiple sclerosis (MS) presents a significant challenge for clinical management. Octave Bioscience (Menlo Park, CA) conducted a biomarker discovery study using Olink panels (1104 proteins) and Luminex bead-based assays (215 proteins) to identify markers associated with clinical and radiographic endpoints in MS. The study's remarkable findings, presented as a poster at the 2019 European Committee for the Treatment and Research in Multiple Sclerosis (ECTRIMS) conference, unveiled a multivariate protein signature comprised of 21 proteins (**Chitnis et al., Nature Communications, 2024**). This signature demonstrated exceptional performance (AUC = 0.93) in classifying clinical disease activity status, surpassing that of the previously identified single biomarker, neurofilament light (NfL), which had an AUC of 0.717. Notably, the protein biomarker model they derived effectively classified clinical disease activity, with the involved proteins representing significant pathways in MS pathophysiology. These promising preliminary results were verified in additional cohorts and laid a foundation for subsequent analytical and clinical validation. Building on the biomarker discovery study, scientists at Octave Bioscience embarked on the development of a Laboratory Developed Test (LDT) with clinical utility. They designed a custom panel using Olink® Focus, consisting of the 21 protein biomarkers identified in the discovery phase. The Multiple Sclerosis Disease Activity (MSDA) panel underwent rigorous technical validation, with fifty-one plates processed using two different production lots over a year (**Qureshi et al., Proteomics - Clinical Applications, 2023**). The evaluation criteria included accuracy, precision, sensitivity, assay interference, sample stability, and sample reanalysis across different equipment, reagents, location, and personnel. Of the 21 evaluated biomarkers, 18 met the acceptability criteria and were included in the final algorithm (specifically the proteins APLP1, CCL20, CD6, CDCP1, CNTN2, CXCL13, CXCL9, FLRT2, GFAP, IL-12β, MOG, NfL, OPG, OPN, PRTG, SERPINA9, TNFRSF10A, and TNFSF13B). The assay's accuracy was confirmed by mixing samples from four affected patients at different dilutions, and the percent recovery for each biomarker was determined to be within established limits. The expected Disease Activity Scores were calculated and compared to the observed scores, with a measured r^2 correlation of 0.997, well above the established acceptability criterion of >0.85. Octave Bioscience developed this multiplexed assay to measure each patient's MS disease biology, revealing underlying pathways and disease mechanisms through the quantitative analysis of serum proteins. This assay forms a quantitative dimension of their comprehensive management system, termed the "Octave MS Precision Care Solution" for overall patient management. After the successful analytical validation of the panel, the crucial next phase was the clinical validation of the MSDA protein signature in extended cohort studies. In an article published in clinical immunology (**Chitnis et al., Clinical Immunology, 2023**), the 18-protein MSDA test was validated based on associations between algorithm scores and clinical/radiographic assessments using serum samples from 614 MS patients. The multi-protein model was trained based on presence/absence of gadoliniumpositive (Gd+) lesions and was also strongly associated with new/enlarging T2 lesions, and active versus stable disease. The data obtained showed that the test was clinically validated, significantly outperforming the top-performing single-protein model, and providing a quantitative tool to enhance the care of MS patients.

Oncology: Early detection in ovarian cancer

Olink® Target 96 panels were used to perform high-throughput proteomic profiling of 593 plasma proteins across three cohorts of patients with ovarian cancer and benign tumors. The researchers identified and validated an 11-protein signature that demonstrated high diagnostic performance (AUC = 0.94, PPV = 0.92, sensitivity = 0.85, specificity = 0.93) for detecting ovarian cancer across stages I–IV. The final signature was converted into a custom low-plex PEA™ panel reporting in absolute concentrations and evaluated in a fourth independent cohort. This study highlights the translational potential of Olink technology to move from discovery through validation to clinical utility, with the proposed panel offering a promising tool for early detection, triage, and referral of patients with adnexal ovarian masses. (**Enroth et al., *Communications Biology*, 2019**).

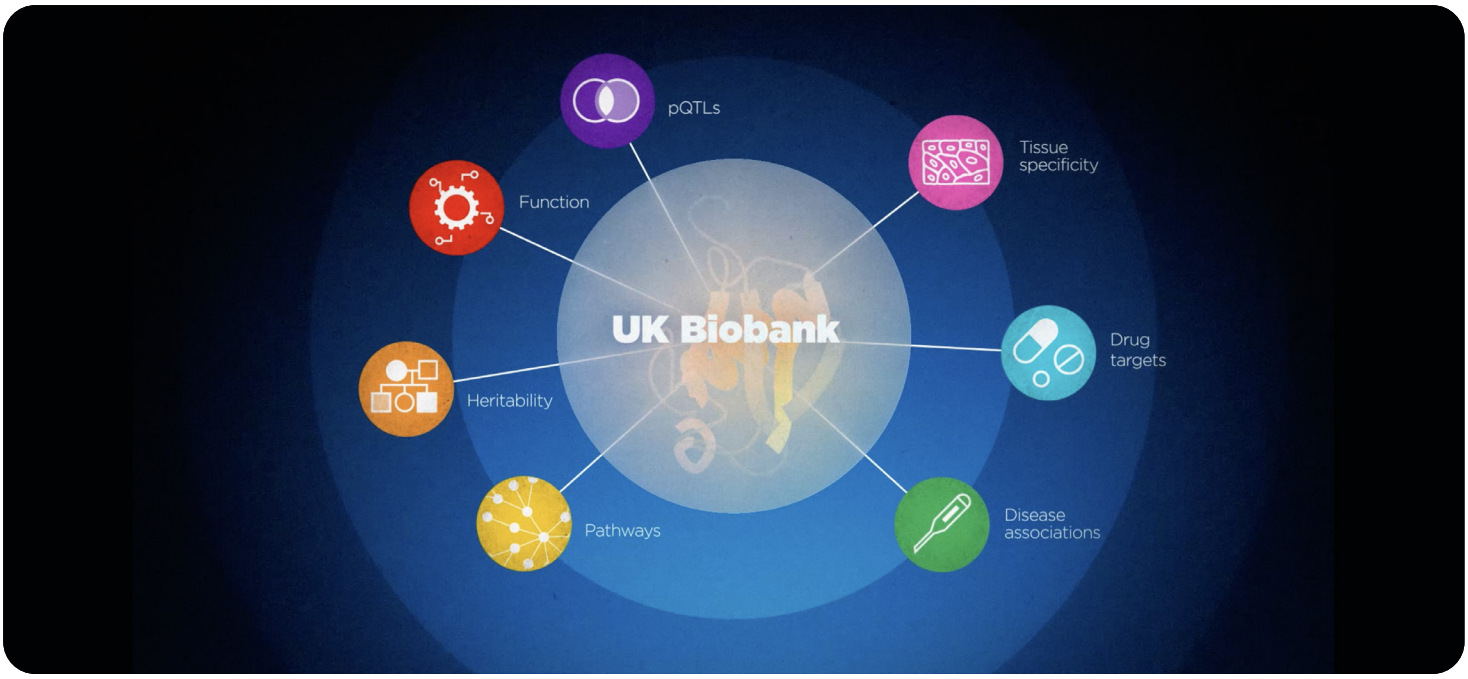
Through large-scale discovery, deep mechanistic insight, and integration with genetic data, Olink's proteomics platforms are enabling a shift from reactive to proactive precision medicine. By delivering specific, scalable, and reproducible data, Olink empowers researchers and clinicians to transform protein signatures into precision action.

Chapter 4

From sample to answer: The role of data science in proteomics

High-throughput proteomics opens new doors for understanding human biology—capturing thousands of proteins across diverse samples, timepoints, and conditions. But discovery is just the starting point. The real power of proteomics lies in transforming vast data sets into actionable insight. Olink's integrated solutions are designed to streamline this process. Whether the goal is mapping disease mechanisms, prioritizing therapeutic targets, or building predictive models, the platform provides the tools, workflows, and services required to accelerate progress from data generation to biological discovery. With standardized technology across discovery, validation, and translation, Olink supports researchers in confidently moving from raw data to real-world impact.

Figure 4.1

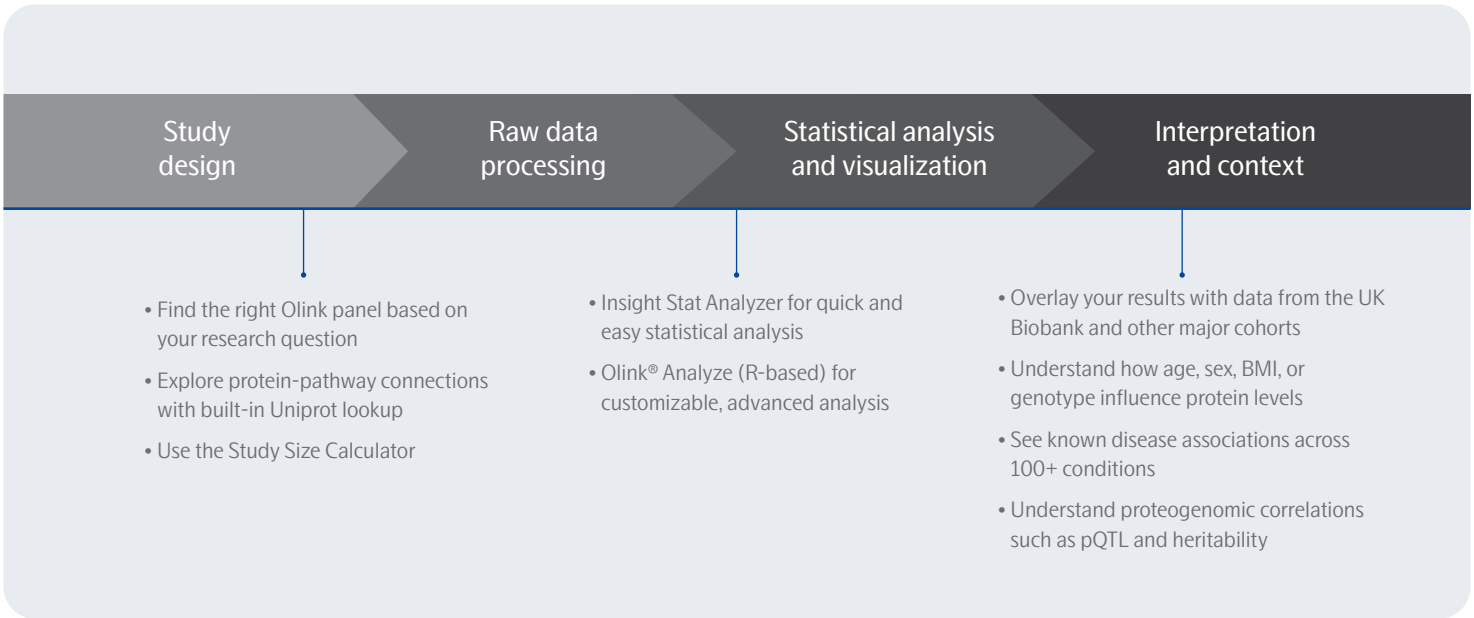


From raw data to biological answers

Olink's data workflow is designed to support researchers from experimental design through to biological interpretation. Whether the goal is discovery, validation, or clinical application, the platform provides integrated tools and services that transform proteomic data into actionable insight.
Key elements of the Olink data journey include:

- Experimental design resources to support optimal study planning, maximizing statistical power while minimizing bias.
- Automated software for raw data processing, performing quality control (QC) and generating normalized protein expression (NPX) values.
- Visualization and statistical analysis tools that reveal key patterns and biological signals. These include volcano plots for identifying group differences in NPX values, heatmaps of significant proteins, scatterplots, and principal component analysis (PCA) to examine sample clustering. For longitudinal or multi-study designs, normalization and bridging reports are available to harmonize NPX data across multiple batches or time points.
- Olink® Insight: an interactive, online bioinformatics platform that integrates experimental data with reference datasets from landmark studies such as the UK Biobank Pharma Proteomics Project (UKB-PPP)—the largest population-scale proteomic resource to date. Olink® Insight enables overlay of protein results with disease associations, normal population ranges, and genetic variant correlations (pQTLs). A built-in Publication Explorer links findings to relevant literature, providing additional context and supporting further hypothesis generation.

Figure 4.2



Unlock a bioinformatic treasure trove

Olink® Insight is an interactive data analysis platform developed by Olink's dedicated Data Science team, with extensive expertise in managing and interpreting high-throughput proteomic data. The platform integrates findings with curated reference datasets from some of the world's leading population and clinical studies, including:

- The UK Biobank Pharma Proteomics Project (UKB-PPP), the largest proteomic study to date
- The China Kadoorie Biobank
- The Arivale wellness cohort
- A pan-cancer cohort of over 1,500 patients from the Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN)

By combining experimental data with these large-scale resources, Olink® Insight supports exploration of disease biology, novel biomarker identification, and translational research.
Built-in annotation layers provide biological context to proteomic results—linking proteins to disease associations, genetic variants, pathways, and population-level reference ranges. This enables researchers to better interpret their findings and generate hypotheses for future studies.

Table 4.1 How Olink® Insight can provide answers.

Application	Biological questions	How Olink® Insight can provide answers
Pathway analysis	What molecular pathways are perturbed in disease or drug response?	Olink® Insight connects protein IDs to UniProt, Reactome pathways
Heritability and pQTL	Which proteins are genetically regulated?	Overlay your proteins of interest with pQTL and heritability coefficient data from UK Biobank
Tissue specificity	Where are these proteins expressed, and in which tissue contexts are they relevant?	Insight offers expression profiles across tissues (e.g., GTEx), enabling rapid filtering by biological relevance.
Disease association	Are your protein biomarkers associated with disease?	Overlay your proteins of interest with disease association data from UK Biobank

High-throughput proteomics generates vast, complex data—but meaningful conclusions require more than measurements. Olink combines high-quality data generation with advanced bioinformatic tools and expert analytical support to streamline interpretation and accelerate discovery.

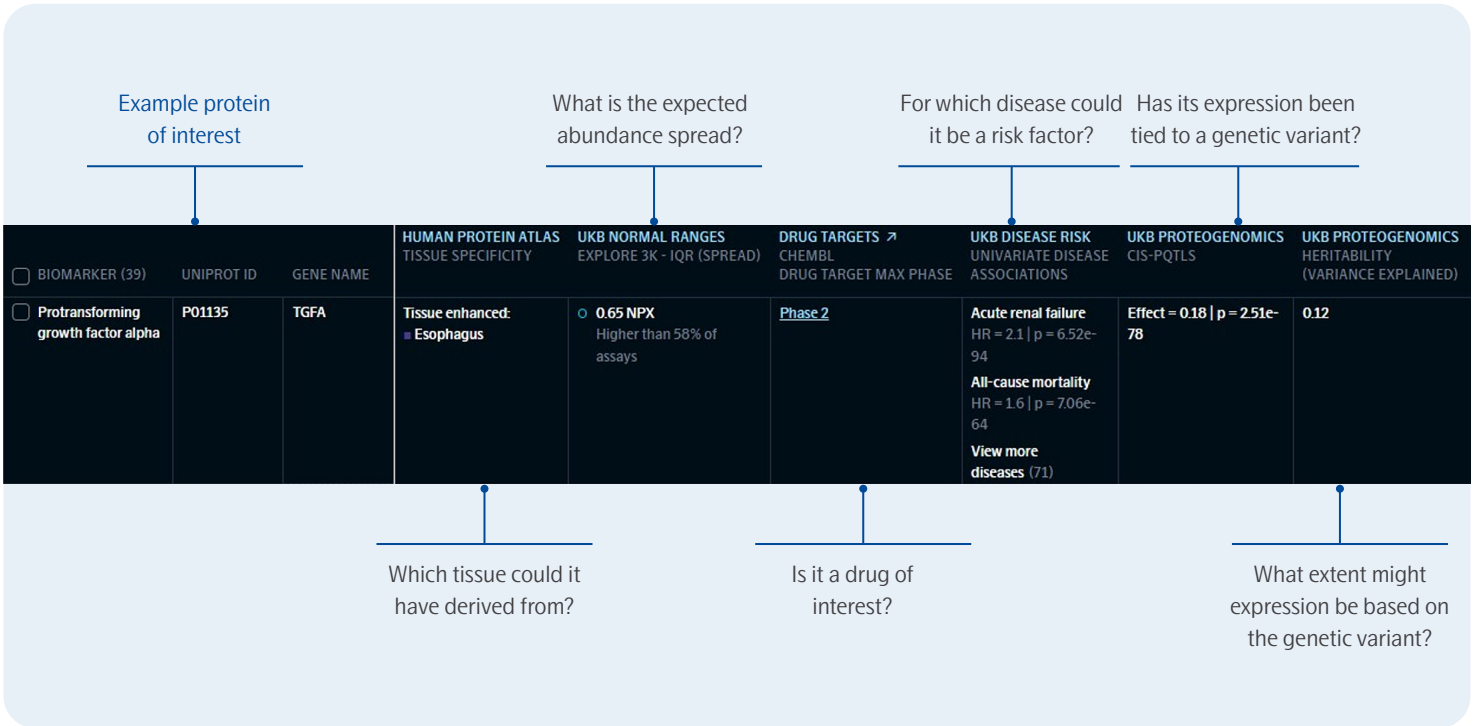
By connecting large-scale protein expression to real-world biology, Olink enables researchers to uncover new mechanisms, validate biomarkers, and accelerate biological conclusions.

· Sun, B., et al., Nature Communications Medicine, (2025).

· Olink. (2025). Realizing the Potential of Population-Scale Proteogenomics.

· Olink. (2025). Blog: Publication Highlights – January 2025.

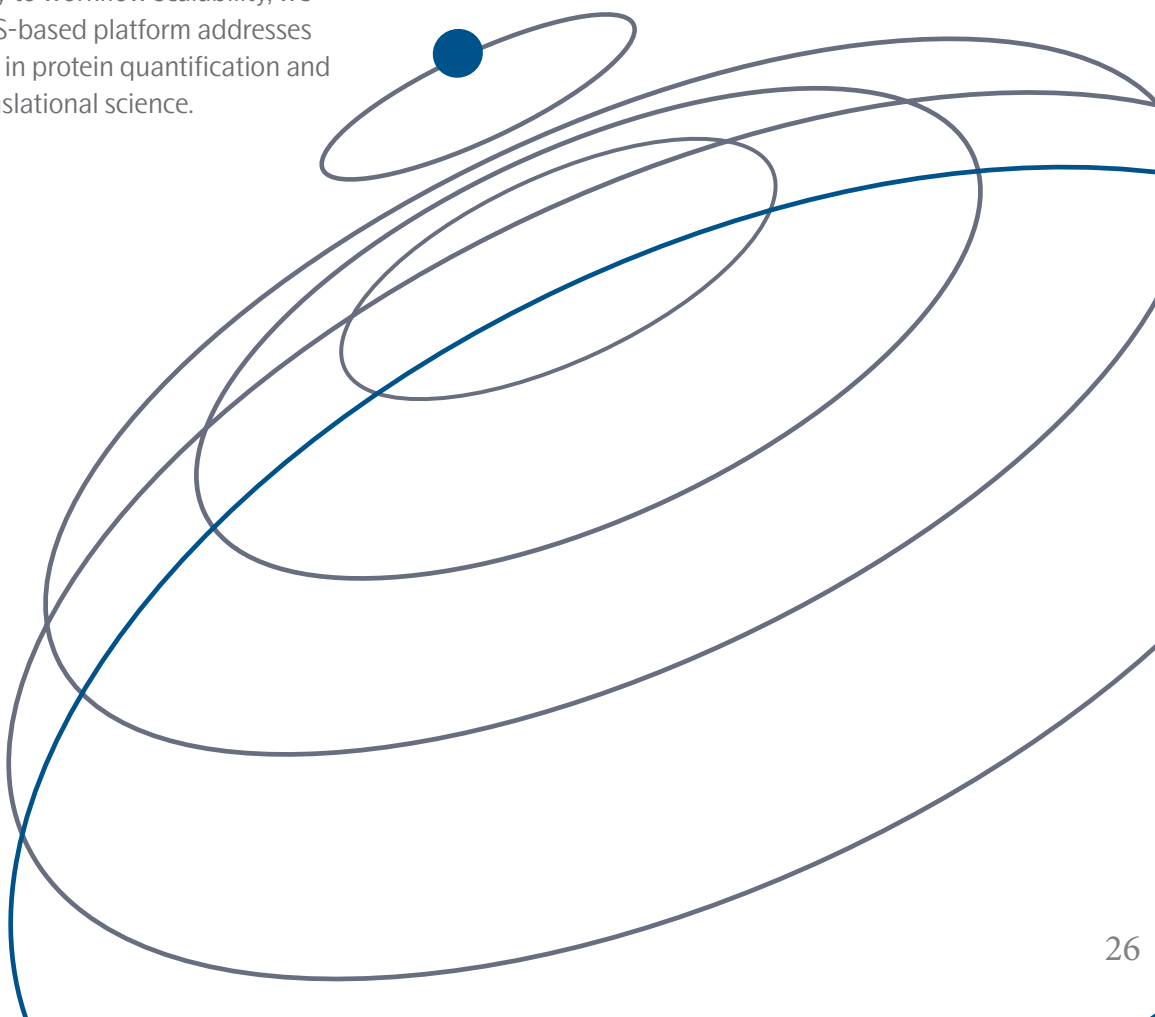
Figure 4.3



Chapter 5

The proteomic landscape – Technology, specificity, and scale

As the need for actionable, high-resolution biological insights grows, so does the demand for proteomic technologies that can deliver them. This chapter explores how the Olink platform compares to legacy tools like ELISA and mass spectrometry, and how high-throughput solutions are shaping the future of proteomics.
From analytical specificity to workflow scalability, we highlight how Olink's NGS-based platform addresses long-standing limitations in protein quantification and expands the reach of translational science.



The evolution of protein analysis

Protein analysis has evolved rapidly since the development of immunoassays in the 1970s. The enzyme-linked immunosorbent assay (ELISA), first introduced in 1971, set the foundation for quantitative protein detection and is still used widely as a gold-standard single-plex technique.

Mass spectrometry (MS), particularly when combined with liquid chromatography (LC-MS/MS), emerged in the 2000s as a powerful tool for proteomics. More recently, advanced MS-based techniques like Selected Reaction Monitoring (SRM), Parallel Reaction Monitoring (PRM), and SWATH-MS have enabled sensitive quantification of peptides and post-translational modifications.

Multiplexed immunoassay platforms such as Luminex (bead-based) and Meso Scale Discovery (planar electrochemiluminescence) have allowed researchers to quantify tens of proteins simultaneously, albeit with trade-offs in specificity, cross-reactivity, and scalability.

Affinity-based proteomic platforms—such as those from Olink and SomaLogic—represent the latest generation, with Olink's Proximity Extension Assay (PEA™) offering exceptional specificity, sensitivity, scalability and diverse sample types (especially complex biological samples like plasma and serum).

Olink vs. mass spectrometry: Complementary strengths

While mass spectrometry remains unmatched for untargeted proteome discovery, it often requires extensive sample preparation, large input volumes, and longer workflows. In contrast, Olink PEA™ technology enables high-throughput, multiplexed measurement of thousands of proteins with as little as 2 µL of plasma or serum.

Figure 5.1. Comparison of major protein analysis platforms across key features.

Feature	Mass spectrometry	Olink® Explore HT
Sample preparation	Complex, multi-step	Minimal, streamlined
Throughput (1-2 weeks, one instrument, one operator)	~1,000–2,000 samples (optimized LC-MS workflows)	10,000–20,000+ samples (standard HT setup with NovaSeq)
Sensitivity	High for abundant proteins	High across 10-log dynamic range (from fg/ml to mg/ml)
Specificity	High, but may detect isoforms	Very high, dual-antibody recognition + NGS readout
Sample volume	50–200 µL	Just 2 µL
Workflow	Complex, variable	Automated, standardized
Best use	Discovery, post-translational modification analysis	Scalable profiling, longitudinal studies

Olink technology and mass spectrometry platforms are often complementary. Mass spectrometry enables deep proteome exploration, including the ability to study post-translational modifications. In contrast, Olink excels in high-throughput profiling of the human proteome, including low-abundance proteins, with the added flexible scalability to conduct more focused biomarker studies for translational research—all on the same platform. This is particularly advantageous in settings where standardization and minimal sample input are critical.

Specificity at scale: The power of dual-antibody recognition

Olink's PEA™ technology is the next generation of proteomics and uniquely combines dual-antibody recognition with NGS or qPCR readout, minimizing cross-reactivity and false positives common in high-plex immunoassays.

Key features:

- Each protein is recognized by two antibodies tagged with complementary oligonucleotides
- Only when both bind in proximity on the same target is a DNA barcode formed
- Barcodes are quantified using NGS and qPCR, ensuring a highly specific signal

Olink PEA™ shows no measurable cross-reactivity—even between closely related proteins. This is confirmed by Olink's rigorous three step, 15-factor analytical verification process, recombinant protein testing, orthogonal assay comparison, and cis-pQTL validation across cohorts.

Publications
See the data, link to white paper on specificity

Download paper

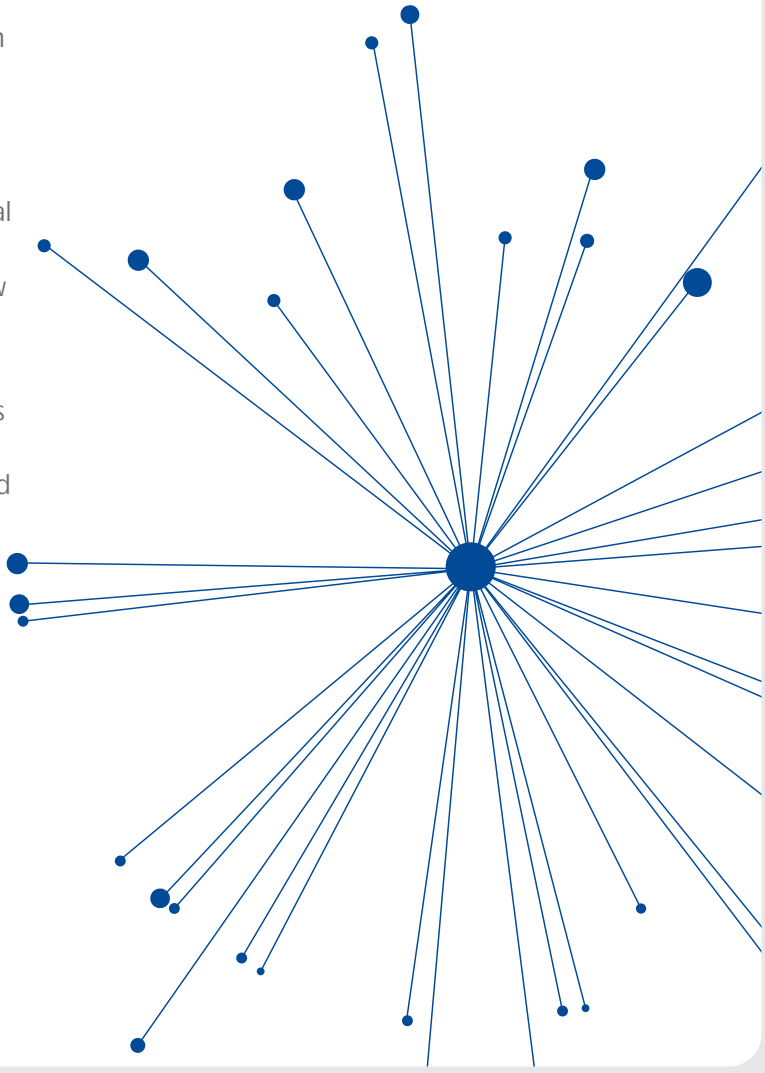
Figure 5.2. Illustration of Proximity Extension Assay (PEA™) mechanism.

Consequences of poor specificity

There are two types of cross-reactivity. The first type is biological cross-reactivity, where the proteins are highly homologous and an antibody may bind to the same epitope on both, in vivo or in vitro. The second type is technical cross-reactivity from non-specific binding due to the method used.

Without sufficient specificity, there is likely to be inaccurate quantification and misidentification of the target proteins, which can have serious implications. In studies aimed at identifying protein biomarkers that could provide actionable biological insights, improve disease diagnosis, treatment, and monitoring, and help develop and optimize new therapies, the consequences of poor specificity in the assay method used can be disastrous. Possible consequences of poor specificity:

- Valuable time, money and precious samples wasted on studies that provide incorrect and misleading information
- Misidentification of proteins leading to erroneous conclusions and misdirection of subsequent research
- Misplacement of future resources focused on irrelevant proteins and incorrectly implicated biological pathways
- Even if stable signatures are identified for a biological state, they may not be attributed to the correct proteins, and incorrect identification of potential new therapeutic targets can lead to costly failures in drug development projects
- Unnecessary delays in gaining key biological insights that could expediate better understanding of disease, development of more effective therapies and ultimately, better outcomes for patients



Detectability: A biologically meaningful readout

With the Olink's PEA™ technology, detectability reflects true biological presence and concentration of a protein in each sample. It is not just a technical attribute, but a measure of biological relevance.

Olink® Explore HT delivers robust, validated detectability across diverse cohorts, capturing genuine biological variation rather than technical noise.

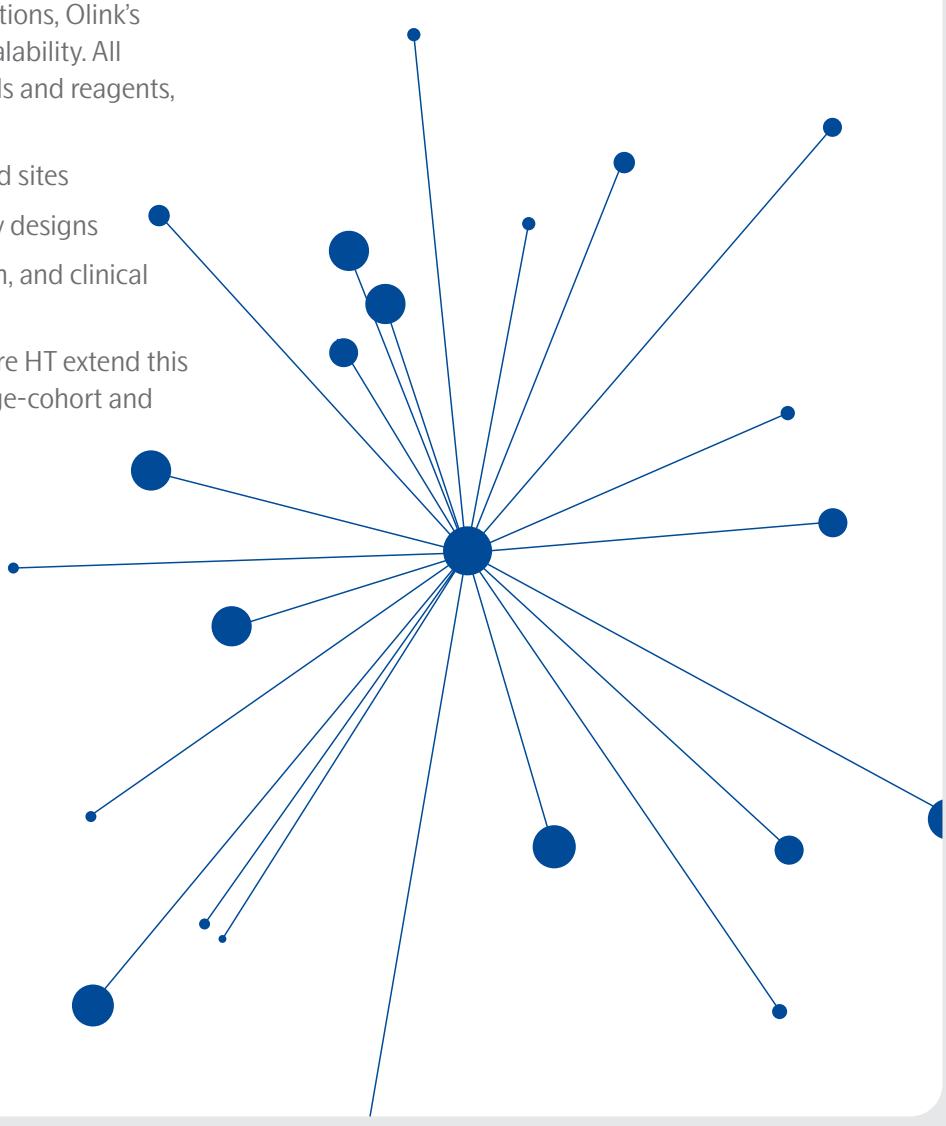
- NPX values show near-perfect correlation between panel sizes (96-plex vs. 3000-plex), confirming consistent assay performance
- Assays retain specificity and dynamic range, regardless of study scale or complexity

Scalable and standardized

From single studies to global collaborations, Olink's proteomics platform is designed for scalability. All Olink panels use standardized protocols and reagents, ensuring:

- Reproducibility across timepoints and sites
- Compatibility with longitudinal study designs
- Efficiency across discovery, validation, and clinical translation

Platforms like Olink® Reveal and Explore HT extend this capability even further, supporting large-cohort and multi-arm study harmonization.



High discovery power with minimal sample input

Olink® Explore HT allows the measurement of up to 5,400+ proteins from just 2 µL of plasma, serum, and other sample types—enabling large-scale population studies without compromising sensitivity and specificity. Explore HT leverages a 10-log dynamic range and uses next-generation sequencing (NGS) for precise quantification. Minimal sample volume is critical in clinical research because it enables testing across multiple assays or timepoints from limited biospecimens—essential for longitudinal, and biobank studies, or where sample availability is constrained.

Looking ahead

As the field of proteomics continues to mature, the need for standardized, sensitive, and scalable platforms will only grow. With Explore® HT, Reveal, Target, Flex and Focus, Olink is advancing a new era of translational proteomics—where biological insights are not only discovered but applied, tested, and translated with confidence.

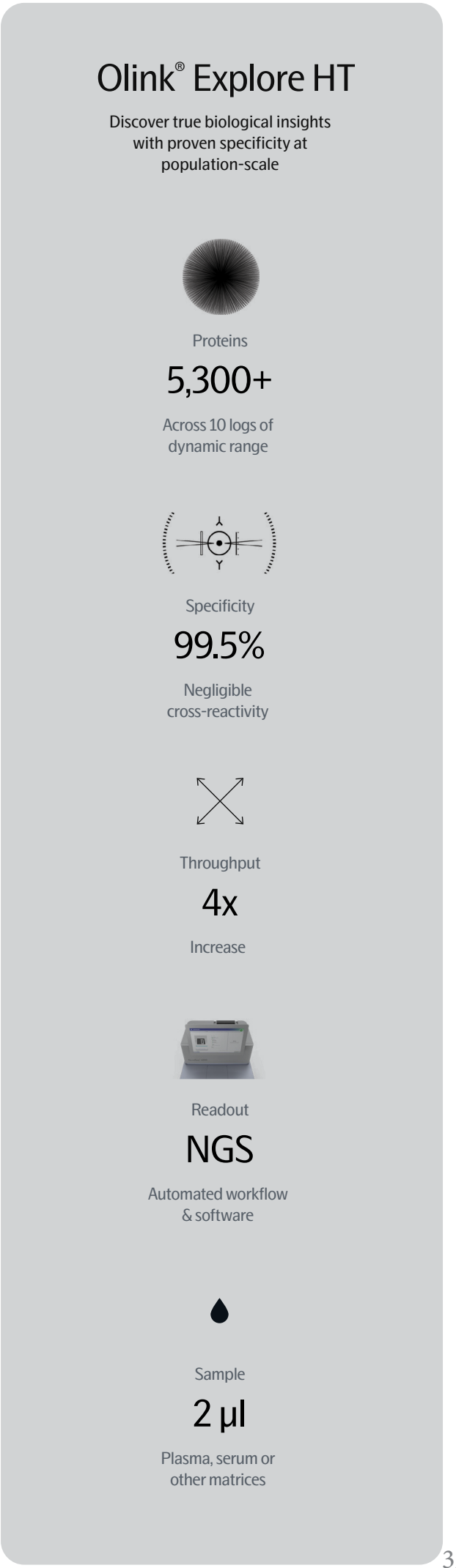


Figure 5.3

Conclusion: Unlocking the power of proteomics

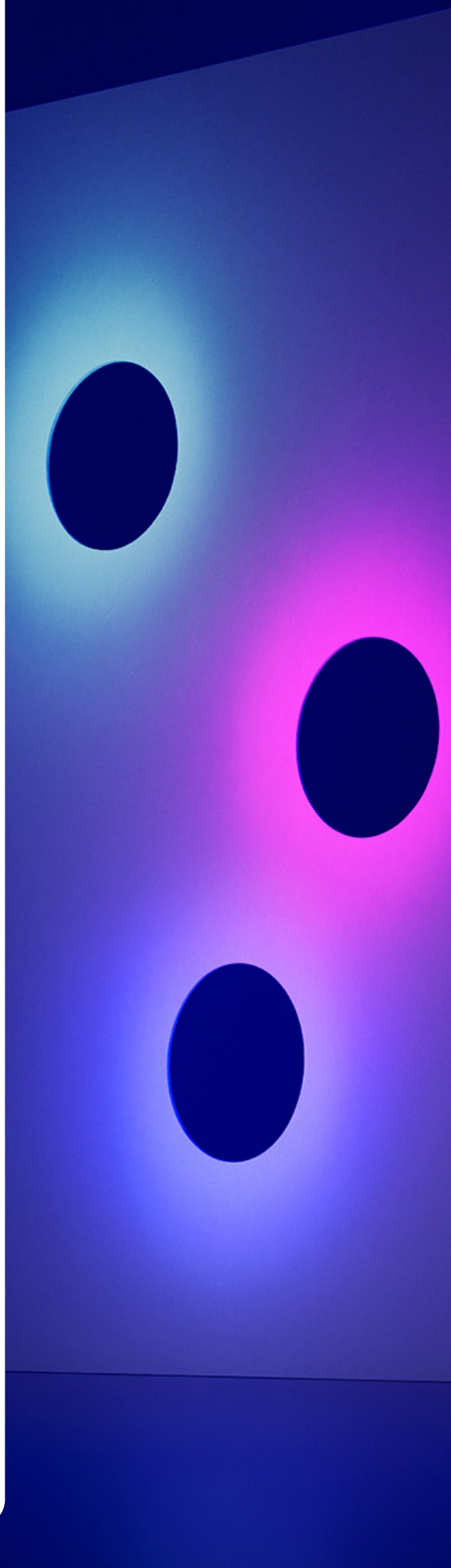
High-throughput proteomics ushers in a new era of biological exploration—one that builds on decades of scientific progress to offer unprecedented insight into the molecular basis of health and disease. By enabling the simultaneous analysis of thousands of proteins from minute sample volumes, this approach allows researchers to interrogate complex biological systems with greater depth, scale, and precision than ever before.

Proteins are the functional drivers of biology and the direct targets of nearly all therapeutic interventions. Unlike genomic data, which provides a static view of inherited risk, proteomic data reflects real-time biological activity—capturing changes in disease state, therapeutic response, and environmental influence. This makes proteomics an indispensable tool for advancing translational science and precision medicine.

The strength of Olink's platform lies in its unique ability to combine high specificity with scalability. Built on Proximity Extension Assay (PEA™) technology and powered by next-generation sequencing (NGS), Olink's solutions deliver reproducible, high-throughput data across thousands of proteins, ensuring that researchers can detect low-abundance biomarkers and explore subtle biological variation across large cohorts. Whether supporting biomarker discovery, patient segmentation, or drug development, Olink provides a consistent and reliable foundation for clinical translation.

Through integrated platforms like Olink® Explore, Olink® Reveal and Olink® Insight, researchers can efficiently generate and interpret proteomic data—linking proteins to pathways, genetic variation, tissue specificity, and disease outcomes. This combination of robust assay performance and intuitive analysis tools accelerates the path from raw data to actionable insight.

As demonstrated by landmark initiatives such as the UK Biobank Pharma Proteomics Project, the integration of proteomics with genomics is transforming population-scale research. This proteogenomic approach enables the identification of causal biomarkers, the validation of therapeutic targets, and the development of more predictive disease models—advancing the field of medicine from inherited risk toward real-time, individualized care.



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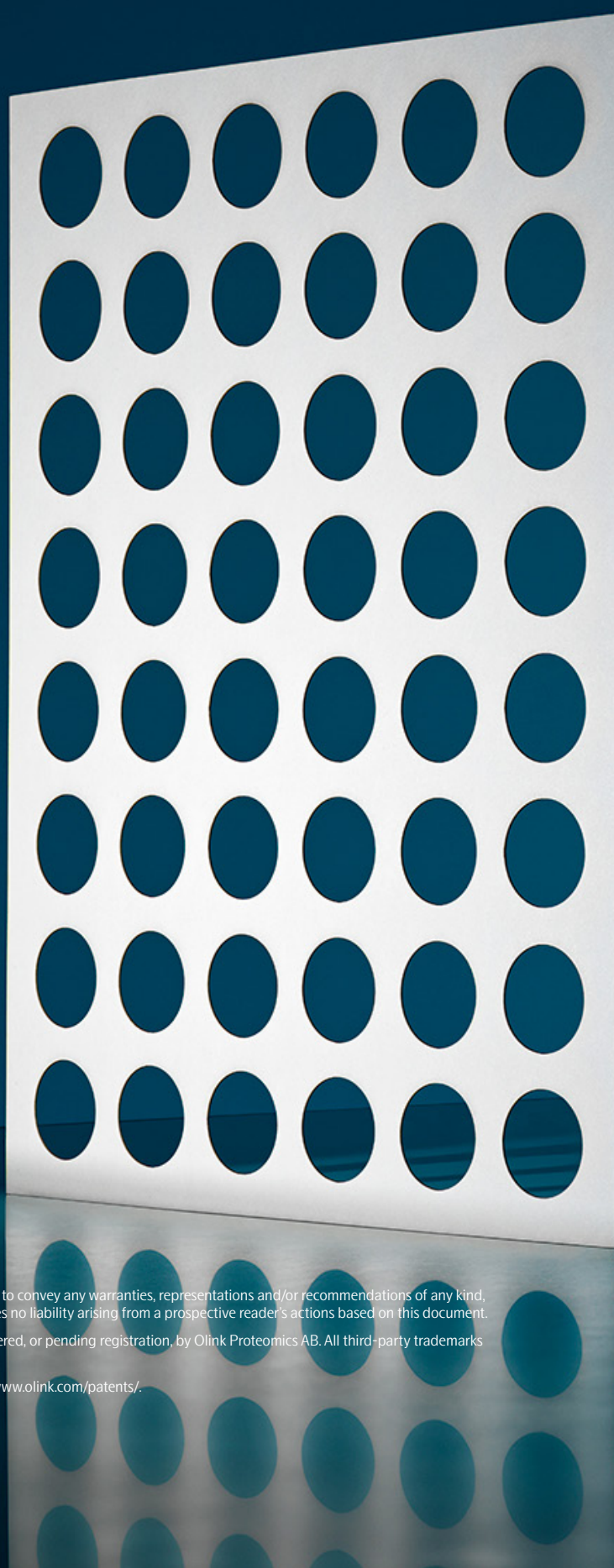

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