ProQuant®: a new precision proteomics platform

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Introduction

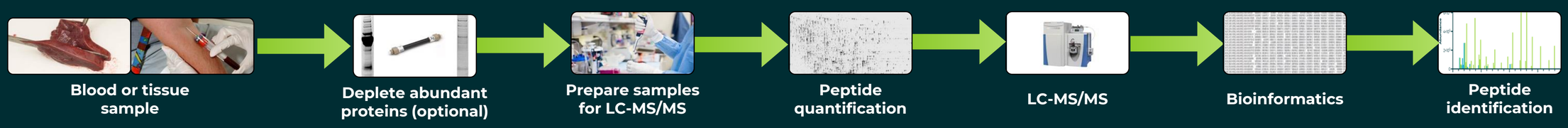
For eight years Methuselah Health UK Ltd worked on developing a label-free proteomics platform that was as precise as possible to enable quantitative analysis of post-translational modifications. Following the acquisition of Methuselah Health by RxCelerate in September 2022 the platform was launched as ProQuant® and is available right now with the power to speed up and de-risk multiple phases of the drug development pipeline.

The ProQuant® platform is based on a standard bottom-up proteomics protocol, with a number of key enhancements that drive the analytical performance. These include optimisations to the sample preparation, changes to the way the mass spectrometer collects data and improved data analytics. We also use machine learning to improve peptide identification and reduce artefacts. Importantly, all these processes are unsupervised, eliminating bias related to the biological question being introduced into the data.

Methods

- Serum samples depleted of 7 of the most abundant proteins using MARS 7 spin cartridge.
- Eluent reduced and alkylated, then digested with trypsin.
- Peptides separated by nanoUPLC on reverse-phase column.
- MS/MS carried out on Thermo QExactive Orbitrap mass spectrometer.
- Peak quantification carried out using a proprietary method.
- Peak identification carried out using Mascot v2.8, combining a narrow robust search and wider error-tolerant search both against SwissProt and the common Repository of Adventitious Proteins databases.
- Resolution of conflicts for identifications of a given peptide was carried out using a proprietary algorithm.
- Protein abundances were calculated by summing all peptide sequences unique to that protein.
- PTM fractions were calculated using a custom script.
- The MS2 fragmentation patterns of key peptides containing modifications of interest were reviewed manually to confirm identity.
- Statistical analyses were carried out in R (v4.2.0) and R studio (v 2022.02.3). FDR correction was carried out using the two-stage step-up method of Benjamini, Krieger and Yekutieli with a desired FDR of 20%.

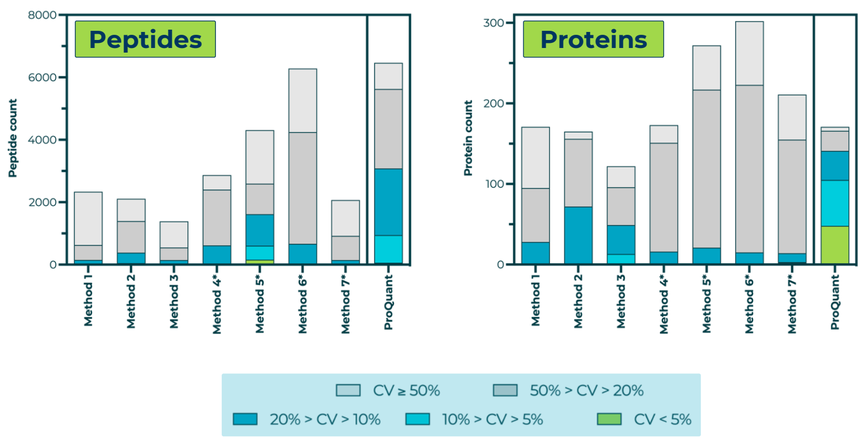
Bottom-up proteomics



Reproducibility

Reproducibility of ProQuant® was compared with alternative approaches by running multiple replicates of multiple serum samples depleted of the most abundant proteins, and then calculating mean coefficients of variation (CV) across the various samples for the peptides and proteins detected.

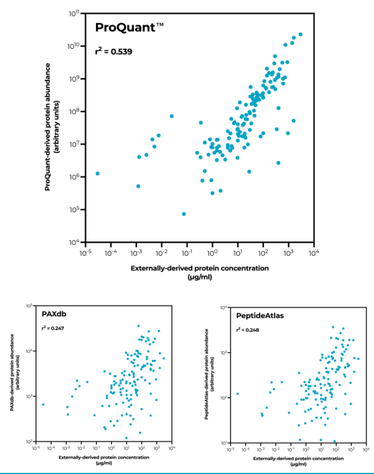
The data shown here demonstrate that the number of peptides and proteins that can be quantified reproducibly is markedly greater using ProQuant® than with alternative methods.



Accuracy

Accuracy of the ProQuant® platform was investigated by comparing proteomics data with an estimate of the concentrations of serum proteins taken from an independent assessment of the serum proteome from the literature.

While an association between proteomics data and literature values is no surprise, data obtained using ProQuant® were much more similar to the independently assessed data than proteomics data from two online repositories of serum proteomics: PAXdb (maintained by the University of Zurich) and PeptideAtlas (published by the Human Proteome Association).



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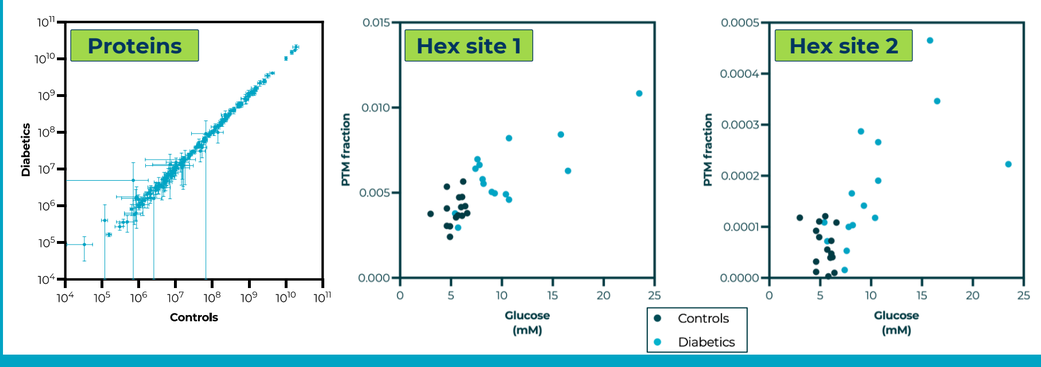
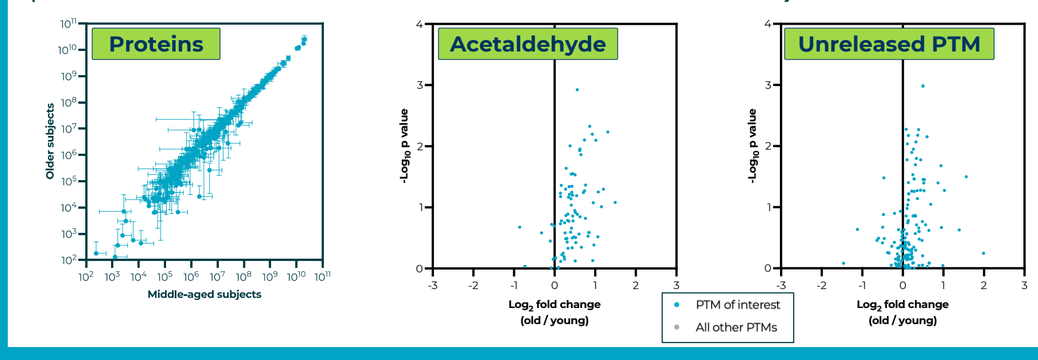
Case study - diabetes

Type II diabetes mellitus (T2DM) is a complex multifactorial disease, with a significant need for new treatments. We used ProQuant™ to investigate new potential biomarkers for T2DM, that may prove to be novel targets for drug discovery. Baseline analysis shows that there are no differences in total protein abundances between patients with T2DM and matched controls that remained significant after adjusting for multiple testing (left panel). By contrast, we identified 7 individual post-translational modifications at specific residues that correlated with fasting glucose at $p < 0.001$, all of which survive FDR correction. All of these were positive correlations with hex modifications of major serum proteins (e.g. mid and right panels). While not biologically novel, we believe that this is the first time that multiple glycation events have been detected in T2DM using a non-hypothesis driven approach, and highlights the power of ProQuant® .



Case study - ageing

Improving our understanding of ageing and longevity may help us to begin unravelling the mechanisms behind age-related diseases, enabling the development of therapies that extend healthy lifespan. We used ProQuant™ to investigate potential differences in the serum proteome between elderly and middle-aged subjects. As with our analysis of the serum proteome of patients with T2DM vs. controls there were no differences in total protein abundances by age. In this study no specific PTMs were different between the two subject groups. However, there were three classes of PTMs which were different in the two subject groups. Acetaldehyde modifications were higher in older subjects, but the effect was due to increased alcohol consumption. There was a trend towards increased deamidation of asparagines which is a novel finding in serum, but analogous to elevated asparagine deamidations seen in eye lens crystallins of older subjects. A third PTM (unreleased identity) was also markedly more abundant in older subjects.



Summary

Multiple optimisations of bottom-up proteomics protocols have led to development of the ProQuant® platform, which has excellent precision and accuracy. We show here that in complex samples we can use ProQuant® to investigate deep into the proteome, using a non-hypothesis driven approach. In one example we identified hex modifications associated with elevated plasma glucose concentrations in patients with diabetes. In another example, we show patterns of changes in three PTMs associated with ageing. In both cases simple proteomic analysis of total protein abundances identify no robust differences.

The ProQuant® platform is now available from RxCelerate and can be used to investigate labelling and cleavage of biologics in vitro and in vivo and identify novel biomarkers in complex biological samples.