

Providing an analytical platform for state-of-the-art absolute apolipoprotein quantification in human blood plasma using prm-PASEF

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Introduction

With proteomics making a move into precision medicine, there is an increasing pressure on quantitative data quality in studies of blood plasma profiles. Despite the endeavors to develop new data-analysis pipelines, options for improving quantification results early on and prior to the sample preparation are limited. Heavy labelled protein standards such as Quantitative Recombinant Protein Standards (qRePS™) are promising solutions to this challenge with potential to ensure precise quantification that is clinically translatable when combined with targeted mass spectrometry. Apolipoproteins are proteins that have a broad diagnostic importance in cardiovascular risk assessment, identification of lipoprotein abnormalities, monitoring treatment efficacy and genetic disorders.

Methods

ApoEdge™ (ProteomEdge AB, Sweden), consisting of heavy Lys and Arg 13C and 15N quantitative recombinant protein standards (qRePS) targeting all human apolipoproteins, was utilized with prm-PASEF, a powerful targeted mass spectrometry technique, to develop a reference target assay for absolute quantitation of apolipoproteins in human blood plasma (Figure 1). One microliter of blood plasma was diluted and added to the dry panel of ApoEdge followed by reduction, alkylation and digestion. Peptide mixture was analyzed using the timsTOF HT mass spectrometer (Bruker, Germany), operating in prm-PASEF mode. Extracted ion chromatograms were overlapped, and absolute concentration of target proteins calculated.

Results

The final prm-PASEF assay was developed using the dia-PASEF workflow within SpectroDive (Biognosys, Switzerland) and contained 57 peptides. Data was acquired on 8 replicates revealing excellent reproducibility of the assay. All ApoEdge target proteins were absolutely quantified in raw blood plasma with a median coefficient of variation of 7%. The absolute quantitative data revealed concentrations of blood plasma apolipoproteins to range from 49.89 pmol/μl

down to 0.002 pmol/μl covering 5 orders of magnitude in concentrations (Figure 2).

A dilution curve with 12 calibration points (3-fold dilution series) was measured using prm-PASEF in 6 replicates. Calibration curves were generated for 39 peptides covering all 18 human apolipoproteins. Example calibration curves of labelled peptides from APOA1 are shown in Figure 3.

Conclusion and future prospects

The quantitative performance of a multiplex panel of internal standards ApoEdge with prm-PASEF was evaluated. All 18 human apolipoproteins were absolutely quantified with great precision and linearity of response. Combining the simplicity and easily automated sample preparation workflow with high precision in absolute quantification, the mutual synergy has a potential of being translated into the clinical practice to replace the current antibody-based assays that lack the edge of responsibility. Additionally, the high multiplexing capabilities of the timsTOF HT and prm-PASEF workflow allowed to initiate development of a targeted assay for the DiscoveryEdge175, a panel consisting of 177 protein targets including apolipoproteins, complement proteins, coagulation factors, inflammation markers, and other clinically interesting markers. The method consists of 346 peptides to be scheduled within a single targeted MS run. Initial experiments showed good coverage of endogenous and labelled peptides with 312 peptides from 173 protein groups being identified and quantified.

The current work outlines a future potential of internal protein standards and targeted mass spectrometry as direction towards clinical application and diagnostics.

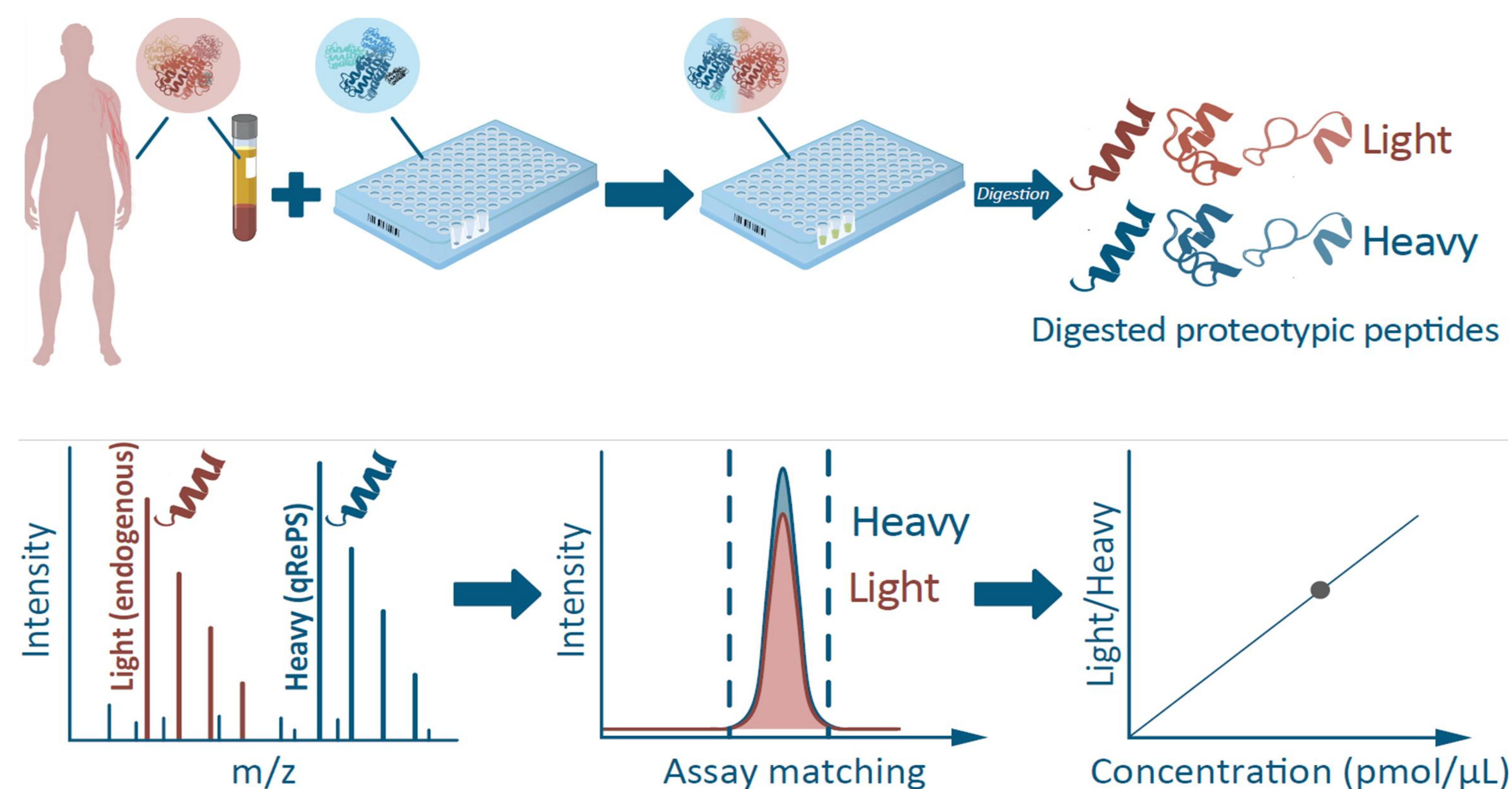


Figure 1: ProteomEdge quantitative workflow starts by addition of blood plasma onto a multiplex panel of qRePS followed by digestion and LC-MS/MS analysis providing targeted absolute quantification.

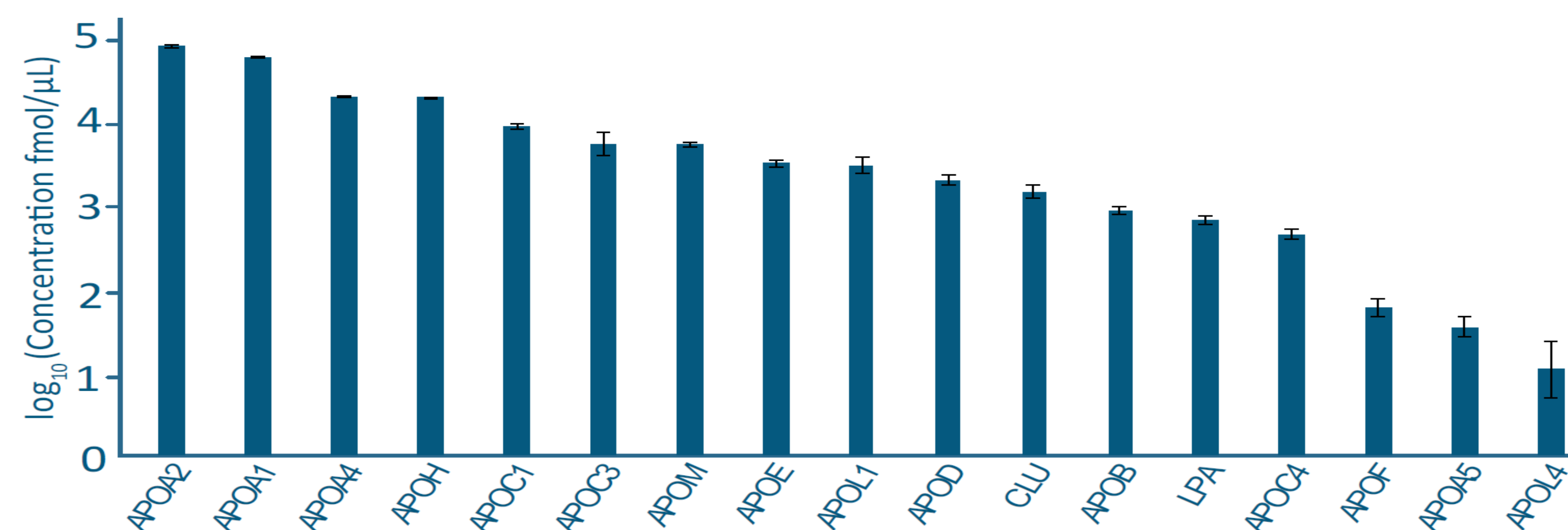


Figure 2: All human apolipoproteins were absolutely quantified in multiplex using ApoEdge and timsTOF HT operating in prm-PASEF mode targeting 57 peptides.

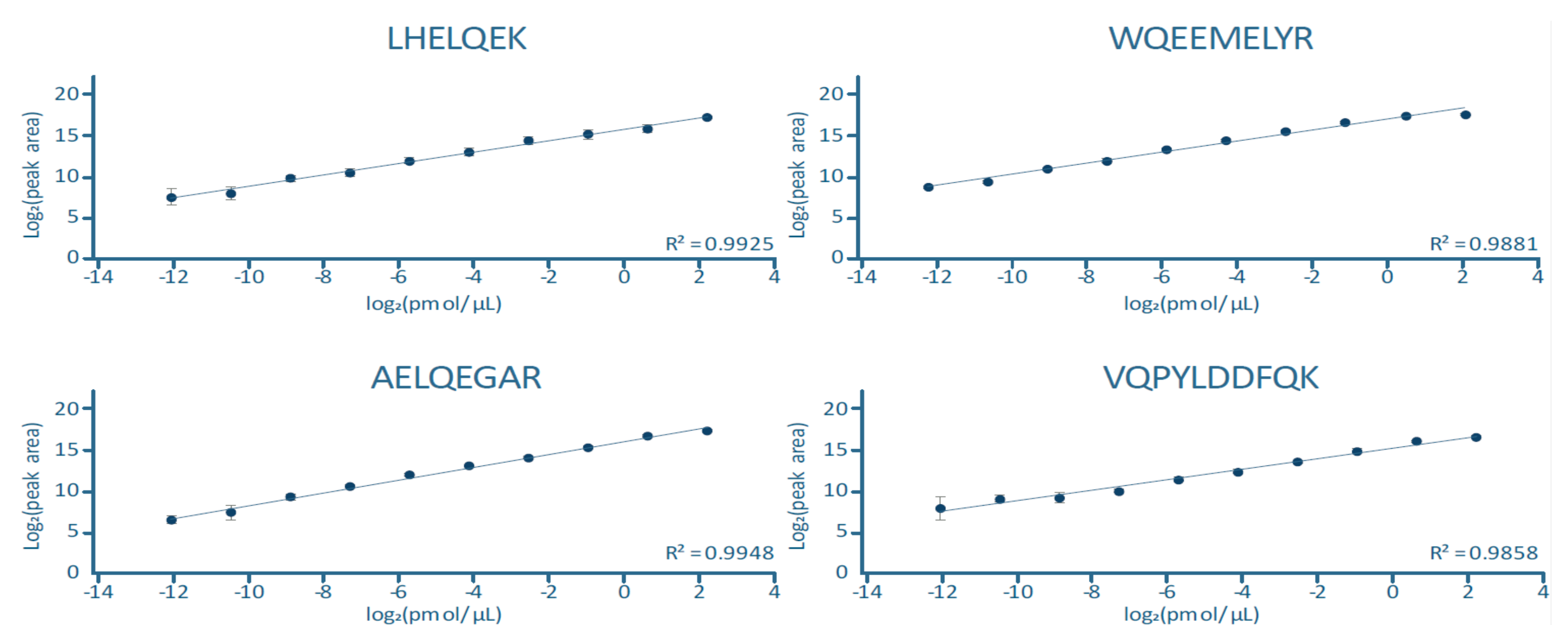


Figure 3: All human apolipoproteins showed good linear range of response providing great synergy of ProteomeEdge internal standards with prm-PASEF workflow.

- prm-PASEF on the timsTOF HT enables accurate and reproducible quantitation of the complete apolipoprotein panel in plasma.
- Initial analysis shows excellent results from a larger panel consisting of 177 proteins, due to the usage of the multiplexing capabilities of prm-PASEF

timsTOF HT