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Data-independent acquisition spiked with QconCAT quantification standards (DISQ)

An alternative to targeted methods?

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Introduction

Targeted proteomics by multiple or parallel reaction monitoring (MRM/PRM) is the current gold standard for absolute protein quantification in complex biological samples. Quantification of target proteins is achieved by spiking analyte matrices with heavy-labelled standards in known amounts. However, methods using targeted acquisition require extensive and time-consuming method establishment and the number of target proteins per run is limited.

Recently, data-independent acquisition (DIA) has been established as the new standard for discovery proteomics. Rapid scanning of moving acquisition windows allows for comprehensive analysis of complex biological samples. DIA also enables reproducible and precise protein quantification, due to less missing values compared to standard data-dependent acquisition (DDA) approaches. In contrast to DDA mode, eluting peptides are fragmented several times, allowing for quantification on the MS2 level. Standard DIA approaches are restricted to inter-run relative quantification, but do not allow for intra-run absolute quantification.

We seek to enable absolute quantification of target proteins in DIA approaches by using heavy labeled quantification standards, so-called QconCATs. Here, we present a proof-of-concept study for DIA spiked with quantification standards (DISQ). As an exemplary protein, we spiked the retention time standard RePliCal in a urine background matrix. The presented assay combines the power of exact targeted quantification with the broad discovery depth of DIA. We show that it is possible to quantify down to an attomolar range, if peptides for protein quantification are carefully chosen based on principles also applying for targeted methods.[1]

Results

RePLiCal – a calibrant protein for RT standardization

RePLiCal is an artificial standard protein for RT standardization in proteomics[2]. Here, we used it as a model protein target. RePLiCal gives rise to 27 tryptic peptides, which are dispersed over a broad range of a typical reverse-phase gradient.

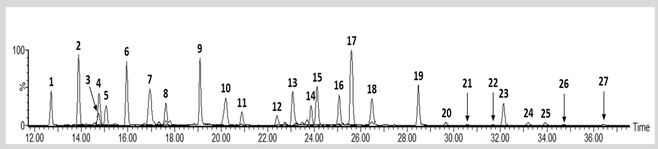
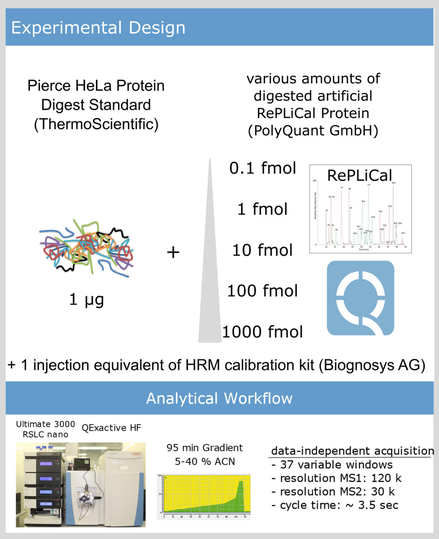


Fig 1: Chromatogram of a 30 min gradient (3-40 % of 0.1 % formic acid in ACN). 5 fmol RePLiCal were spiked into a whole cell yeast lysate tryptic digest (1 µg).

DIA allows for quantitation of RePLiCal down to an attomolar range in a complex sample

To assess the feasibility of protein quantification by DIA, we spiked HeLa whole cell lysate with a dilution series of RePLiCal protein. Each dilution was analyzed in duplicates. In ten DIA runs, we quantified 5,835 proteins with a median CV of 7.0 %.



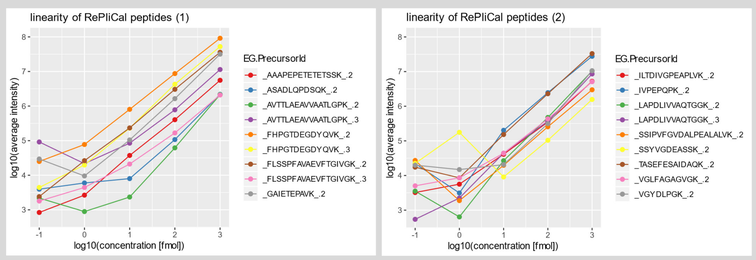


Fig 3: Most tryptic peptides of the RePLiCal protein display linearity in the femtomolar, but not in the low femtomolar to attomolar range.

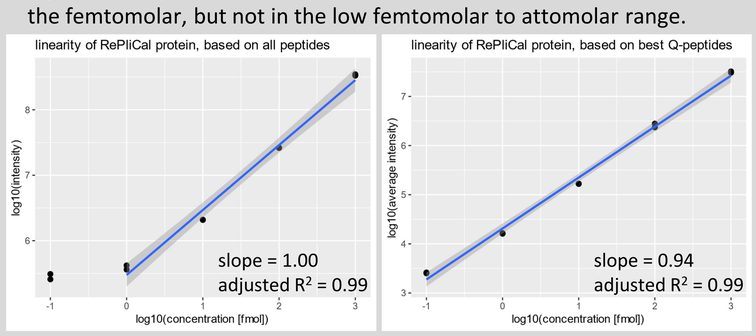
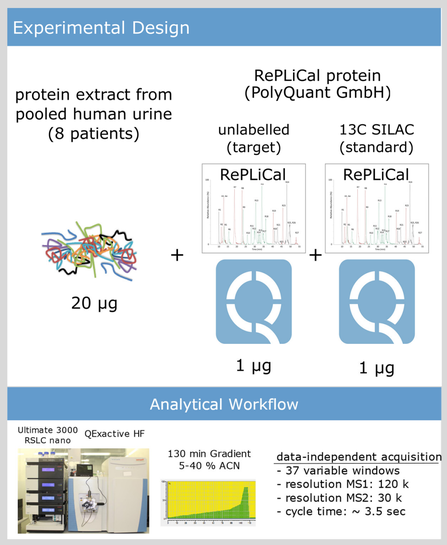


Fig 4: A) Based on all tryptic peptides, the RePLiCal protein can be quantified down to 1 fmol. B) Based only on the best-performing peptides, the RePliCal protein can be quantified down to 0.1 fmol (= 100 amol).

Proteins can be quantified in a targeted manner by spiking heavy labelled standards in DIA mode

To evaluate the possibility of absolute protein quantification in DIA mode, we spiked unlabelled or SILAC-labelled RePLiCal protein into a human urine sample. We measured the samples via DIA and quantified the tryptic RePLiCal peptides on MS2 level. In two DIA runs, we quantified 835 proteins with a median CV of 7.0 %.



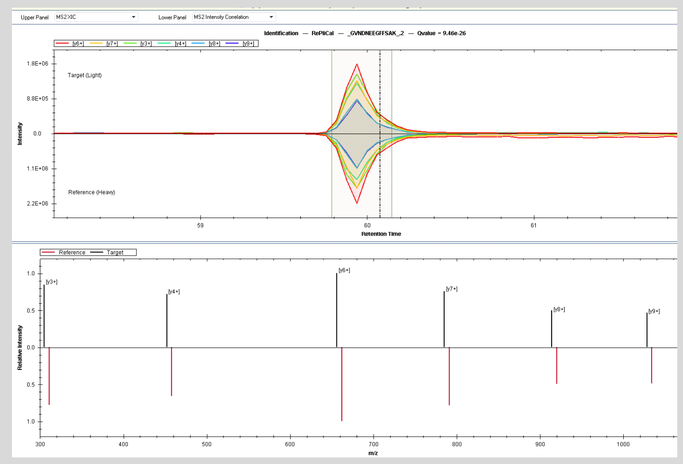


Fig 5: MS2 fragments of a tryptic RePLiCal peptide
A) Elution curve of MS2 fragments. B) Identified y-ions in the light and heavy channel.

QconCATs can provide standard peptides in large scales

QconCATs are labeled synthetic standard proteins, consisting of signature peptides for up to 50 target proteins. QconCATs can be produced cell-free in a highly multiplexed fashion. They may thus be used to generate a high number of standard peptides.

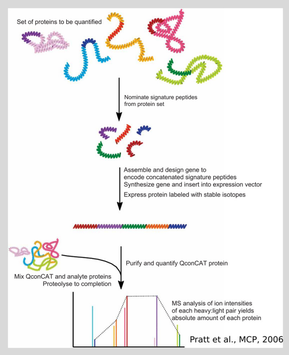


Fig 6: Overview of the QconCAT technique

Conclusion

Our proof of concept study shows the power of protein quantification in DIA mode. We successfully quantified RePLiCal protein in the pico- and femtomolar range in DIA mode in a complex HeLa sample. By carefully choosing optimal peptides for quantification, we were able to extend the linear quantification range even further down to the attomolar range.

Although the larger MS2 windows in DIA mode may reduce the accuracy of the method to some extent, the simple method establishment and almost unlimited number of analytes makes up for this drawback. By the additional untargeted sample analysis, DISQ further allows for the parallel analysis of non-targeted proteins in the same LC-MS/MS run. The DISQ technique investigates the possibility of absolute peptide quantification in DIA mode. It thus combines the benefits of precise target protein quantification with a deep screening of the analytical sample. The DISQ technique also lifts the restrictions of classical targeted proteomics approaches for the number of quantified peptides. If provided with sufficient standards, DISQ allows to quantify hundreds of proteins in an absolute manner in a single LC-MS/MS run. We propose DISQ as an interesting alternative to classical targeted techniques.

References

1) Brownridge, P., Holman, S. W., Gaskell, S. J., Grant, C. M., Harman, V. M., Hubbard, S. J., … Beynon, R. J. (2011). Global absolute quantification of a proteome: Challenges in the deployment of a QconCAT strategy. *PROTEOMICS.*

2) Holman, S. W., McLean, L., & Eyers, C. E. (2016). RePLiCal: A QconCAT Protein for Retention Time Standardization in Proteomics Studies. *Journal of Proteome Research.*

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