Targeted mass spectrometric approach coupled with long gradient separation enables highly sensitive, large scale protein quantification in a single analysis

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Overview
- Developed a simple, straightforward approach, long gradient-selected reaction monitoring (LG-SRM), for highly sensitive, large scale protein quantification without the need for sample pre-fractonation.
- Application of LG-SRM enables quantification of low ng/mL levels of proteins in prostate cancer patient sera and IgY14 immunoaffinity depletion further enhances the overall sensitivity for plasma protein quantification.

Introduction
- A major constraint for current targeted proteomics approaches is the limited sensitivity and capacity for simultaneous quantification of hundreds of proteins1.
- Highly sensitive, large scale targeted quantification currently relies on varying degrees of fractionation followed by a number of LC-SRM measurements of individual and/or concatenated fractions2.
- Sample fractionation scheme often requires a relatively large amount of starting material (e.g., >100 µg).
- We present an alternative approach that utilizes a long gradient separation instead of fractionation. The long gradient separation in combination with SRM (LG-SRM) enabled highly sensitive quantification of hundreds of proteins in a single analysis with very small amounts of starting materials (e.g., ~4 µg peptides injected).

Methods
- Human female sera
- Prostate cancer patient sera
- Reduction/alkylation, digestion, SPE cleanup
- Addition of internal standard (heavy-isotope labeled surrogate peptides)

Results

Extract ion chromatograms: DPFIANGER

Calibration curves

Quantification of low ng/mL levels of endogenous proteins in prostate patient sera

Endogenous prostate specific antigen (PSA)

Conclusion
- LG-SRM enables the quantification of low ng/mL levels of proteins in human plasma/serum.
- Further enhancement in the overall SRM sensitivity for plasma protein quantification was achieved by incorporating IgY14 immunoaffinity.
- LG-SRM has ~3 times wider elution time window for a target analyte than conventional LC-SRM, which make it suitable for large-scale highly sensitive quantification of hundreds of proteins in single LC-SRM analysis.

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References

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