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, **l**ong **g**radient-**s**elected **r**eaction **m**onitoring (LG-SRM), for highly sensitive, large scale protein quantification without the need for sample pre-fractionation.
- 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 proteins[1].
- 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 fractions[2].
- The 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)

↓

LG-SRM MS

Sample preparation

Human serum samples were processed as previously described[2].

LG-SRM analysis

~4 μg of tryptic digested peptides were loaded onto two types of C18 columns (75 μm i.d. × 100 cm, packed with 3 μm Jupiter C18 bonded particles; 75 μm i.d. × 40 cm, packed with 1.7 μm BEH particles) and a 300 min gradient was used for peptide separation with a flow rate of 100 nL/min.

The Waters nanoACQUITY UPLC system was directly coupled on-line to the Thermo Scientific TSQ Vantage instrument for LG-SRM measurements.

Data analysis

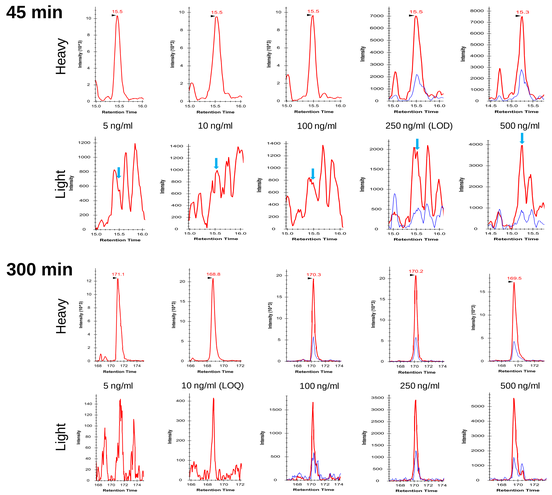
Surrogate peptide SRM signals from LG-SRM were compared with those from conventional LC-SRM to assess SRM sensitivity based on limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values were defined as the lowest concentration point of target proteins at which the S/N of surrogate peptides is at least 3 and 10, respectively.

The RAW data from TSQ Vantage were loaded into Skyline software to create high resolution figures of extracted ion chromatograms of multiple transitions monitored for target proteins.

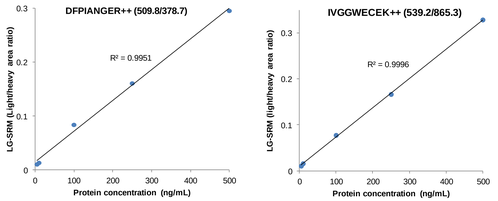
Results

Proof of concept

Extract ion chromatograms: DFPIANGER



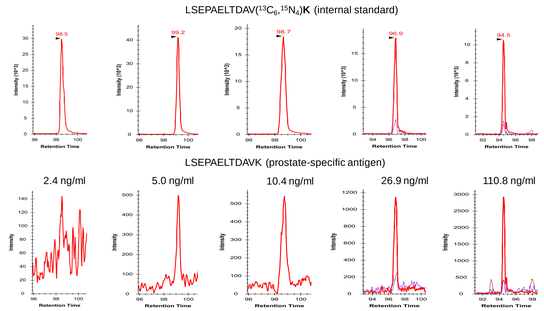
Calibration curves



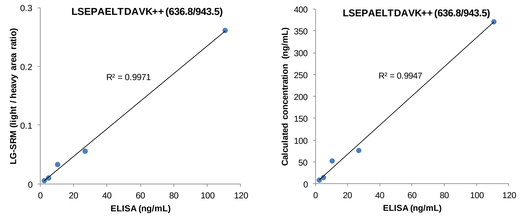
Target protein	Surrogate peptide	LOD (ng/mL)		LOQ (ng/mL)	
		LC-SRM	LG-SRM	LC-SRM	LG-SRM
Bovine carbonic anhydrase	DGPLTGTYR	500	100	2000	250
	DFPIANGER	250	5	1000	10
Prostate-specific antigen	IVGGWEC$_{cam}$EK	100	5	1000	10
	LSEPAELTDAVK	250	5	1000	10

Quantification of low ng/mL levels of endogenous proteins in prostate patient sera[3]

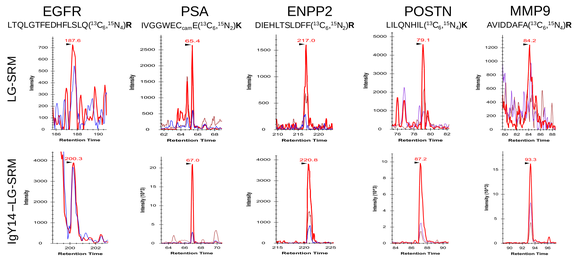
Endogenous prostate specific antigen (PSA)



Correlation curves[2]



Further enhanced LG-SRM sensitivity for quantifying endogenous plasma proteins by coupling with IgY14 depletion



Conclusions

- 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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