



# Sensitive Targeted Quantitative Proteomics Analysis Using Multiple Reaction Monitoring with a Novel ESI Interface

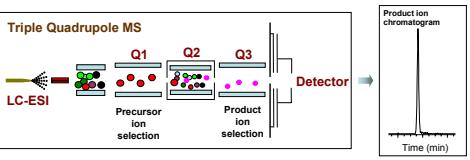
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## Overview

- The research presented here demonstrates significantly improved multiple reaction monitoring (MRM) performance using an in-house modified ThermoFisher TSQ instrument equipped with a novel high efficiency ESI interface incorporating a PNNL-designed dual electrodynamic ion funnel<sup>1</sup>.
- The modified MS/MS instrumental platform has significantly increased our ability to detect low abundant proteins in complex biological matrices.
- Notably, the enhanced MS/MS instrumental platform has achieved the consistent detection of proteins present at low ng/mL levels in non-fractionated blood plasma.

## Introduction

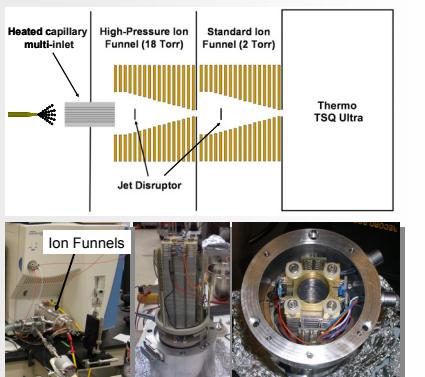
- Identification and accurate quantification of low abundant proteins and peptides in complex biological samples (e.g., plasma and serum) remain challenged by the limited sensitivity, dynamic range, and quantitative precision afforded by routine "global" survey strategies.
- Targeted LC-MS/MS, e.g., MRM, is increasingly being used in hypothesis-driven quantitative proteomics studies, e.g., biomarker verification.
- Sensitive and selective detection of specific peptides is achieved by isolating and monitoring both parent and fragment ions (see below).



Selected reaction monitoring (SRM)

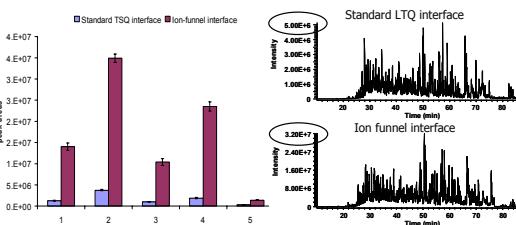
- MRM detection of human plasma proteins at  $\mu\text{g/mL}^2$  and 12 - 40 ng/mL<sup>3</sup> without fractionation has been recently reported.

## Methods

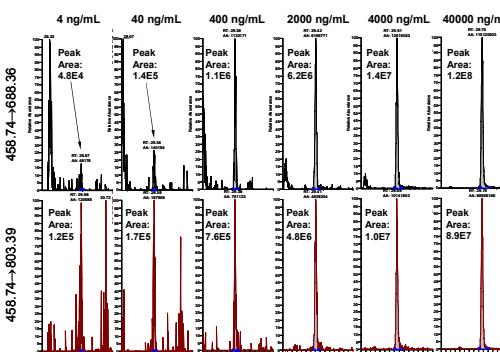


**Fig. 1.** A ThermoFisher TSQ Ultra triple quadrupole mass spectrometer modified in-house with a multi-inlet interface incorporating dual electrodynamic ion funnels.

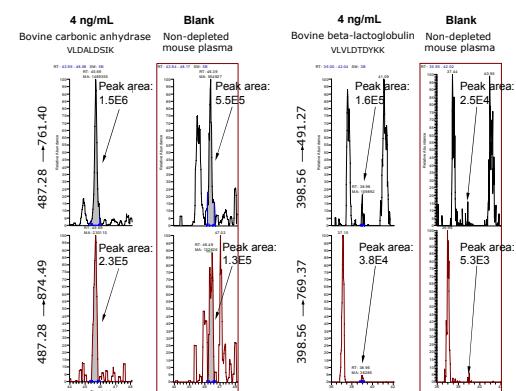
## Results



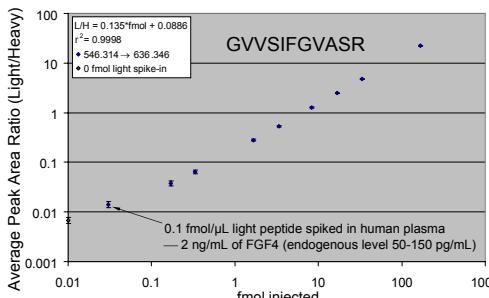
**Fig. 3.** Increased MS intensity using ion funnel: 1) TSQ SRM peak areas for selected standard peptides spiked into mouse plasma (left panel); 2) LC-MS/MS analysis of *Shewanella oneidensis* digest (right panels).



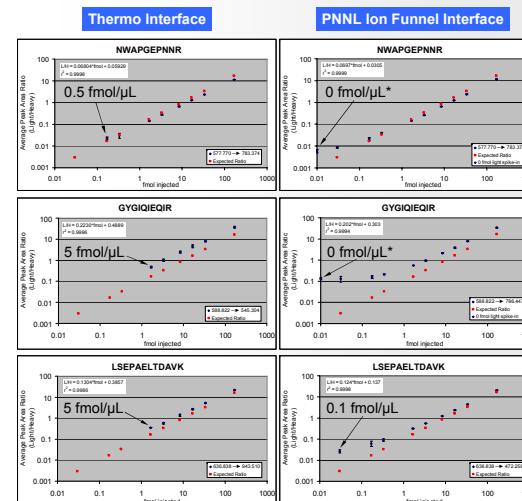
**Fig. 4.** LC-MRM/MS analysis of bovine beta-lactoglobulin (IDALNENK) spiked in non-depleted mouse plasma using the ion funnel interface.



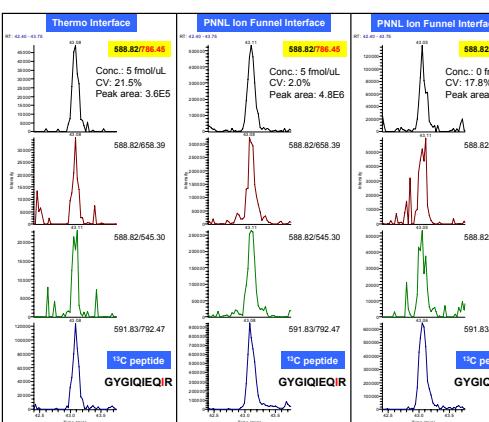
**Fig. 5.** LC-MRM/MS detection of bovine proteins spiked in non-depleted mouse plasma at 4 ng/mL level (with the blank as comparator) using the ion funnel interface.



**Fig. 6.** Calibration curve for human fibroblast growth factor 4 (FGF4; GVVSIFGVASR) using the ion funnel interface. Note, 0 fmol/ $\mu\text{L}$  represents blank without the spiked in peptides (i.e., endogenous level plus residual signal from the  $^{13}\text{C}$  channel).



**Fig. 8.** LC-MRM/MS calibration curves (log scale) for selected peptides, comparing experimental data obtained without (left panels) and with (right panels) ion funnels. Blue squares represent the experimental data; red squares are the calculated  $^{12}\text{C}/^{13}\text{C}$  ratios (as references). The LODs are indicated with arrows. Note, 0 fmol/ $\mu\text{L}$  represents blank without the spiked in peptides (i.e., endogenous level plus residual signal from the  $^{13}\text{C}$  channel).



**Fig. 7.** LC-MRM/MS analysis of GYGQIEQIR (ADAM17) spiked in depleted human plasma. Left panel: limit of detection (LOD) using the Thermo interface; middle panel: detection of the same concentration using the ion funnel interface; right panel: new LOD (\*0 fmol/ $\mu\text{L}$ , representing blank without the spiked in peptide, i.e., endogenous level plus residual signal from the  $^{13}\text{C}$  channel) using the ion funnel interface. See Figure 8 (center panels) for GYGQIEQIR calibration curves.

**Table 1.** Summary of MRM results with the original Thermo and the PNNL ion funnel interfaces.

Peptide	Stats	Thermo Interface	PNNL Ion Funnel Interface
NWAPGEPNNR	Conc. (fmol/ $\mu\text{L}$ ) <sup>*</sup> % CV	0.5 14.3	0 17.1
DSSLLESPVER	Conc. (fmol/ $\mu\text{L}$ ) % CV	0.5 13.3	0.5 16.1
EAAVQSGAGDYLGIK	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 25.4	1 13.9
GYVSIFGVASR	Conc. (fmol/ $\mu\text{L}$ ) % CV	0.5 16.6	0 0
NGELVIHEK	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 13.1	0.5 6.37
LSEPAELTDAVK	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 2.1	0.1 16.4
VDNEELLPK	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 10.3	0.5 8.6
GYGQIEQIR	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 8.4	0 17.8
HGLNSIYVFVR	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 4.4	0.5 4.88
YVSELHLTR	Conc. (fmol/ $\mu\text{L}$ ) % CV	0.1 17.1	0 4.02
TSAFYNFAFK	Conc. (fmol/ $\mu\text{L}$ ) % CV	5 6.7	1 10
GYSIFSAYTK	Conc. (fmol/ $\mu\text{L}$ ) % CV	0 16.6	0 7.25

\* The lowest detectable concentration in the 10-point calibration curve; 0 fmol/ $\mu\text{L}$  represents blank without the spiked in peptides, i.e., endogenous level plus residual signal from the  $^{13}\text{C}$  channel.

## Conclusions

- With the new interface, detection of low ng/mL range of proteins in human and mouse plasma can be achieved without fractionation
- In general, significantly improved limit of detection with the ion funnel interface.
- More reproducible measurements at lower levels with enhanced signal provided by the ion funnel interface

## Acknowledgements

We especially thank Steven Carr's laboratory at the Broad Institute of MIT and Harvard and Amanda Paulovich's laboratory at the Fred Hutchinson Cancer Research Center for their collaborative efforts to portions of this work.

Portions of this research were supported by a grant from the Entertainment Industry Foundation (EIF) and the EIF Women's Cancer Research Fund to the Breast Cancer Biomarker Discovery Consortium, and by the NIH National Center for Research Resources (RR018522).

Proteomics experiments were performed in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the Department of Energy (DOE) and located at Pacific Northwest National Laboratory, which is operated by Battelle for the DOE under Contract DE-AC05-76RL0 1830.

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