

RAPID QUANTITATIVE ANALYSIS OF SELECTED SERUM GLYCOPROTEINS USING ISOTOPICALLY LABELED PEPTIDES BY LC-MALDI MS USING A 2-KHz LASER

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SUMMARY

- High throughput quantitation using isotopically labeled synthetic peptides
- Parallel 4-column reversed phase LC separation coupled to MALDI MS
- Chromatographic resolution preserved using 6-sec deposition rate
- Rapid MS acquisition using a lab-built MALDI MS instrument operating at 2-kHz
- Preliminary results: six selected serum glycoproteins were quantitated in 10 samples
- Classification of cancer vs. normal samples based on quantitative data

INTRODUCTION

Targeted proteomics is well-suited to high throughput analysis because only a relatively low percentage of sample components need to be identified and quantitated. LC-MALDI MS is a suitable technique for such analysis since the LC analysis is decoupled from the MS data acquisition facilitating analysis of only specific pre-selected positions on the MALDI plate. In addition, multiplex chromatography can be performed in parallel, which further increases throughput.

Standard MALDI MS instruments require at least several seconds to acquire a single MS spectrum, which limits throughput. We have developed a novel MALDI MS instrument with a 2-kHz repetition rate laser to provide a platform for high throughput analysis.

We have applied a previously-developed strategy for selective capture of peptides that are N-linked glycosylated in the intact proteins, for quantitative analysis of human sera. Peptides isolated from serum samples were spiked with synthetic, stable isotope labeled reference peptides that were selected based on prior experiments in which the peptides were sequenced and identified.

EXPERIMENTAL

SAMPLES

- Serum samples: 4 cancer and 6 normal samples
- Peptides glycosylated in original proteins were enriched similarly as described in Ref. 1
- Six synthetic reference peptides, see Table 1, were spiked into the samples
- Isotopically labeled peptides: +7 Da (Leu with 7 x ¹³C), +6 Da (Val with 6 x ¹³C)

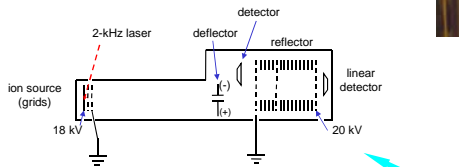
4-COLUMN MULTIPLEX LC ANALYSIS

- Four 100 µm i.d., 15 cm long columns packed with 3 µm Magic C18 particles
- Nanomate LC system was used for gradient delivery (total flow rate 4 µl/min) split onto 4 columns
- Separation using 40-min linear gradient
- Post-column MALDI matrix addition to individual LC eluents
- MALDI matrix: 7 mg/ml CHCA in 50/50 ACN/water in 0.1% TFA

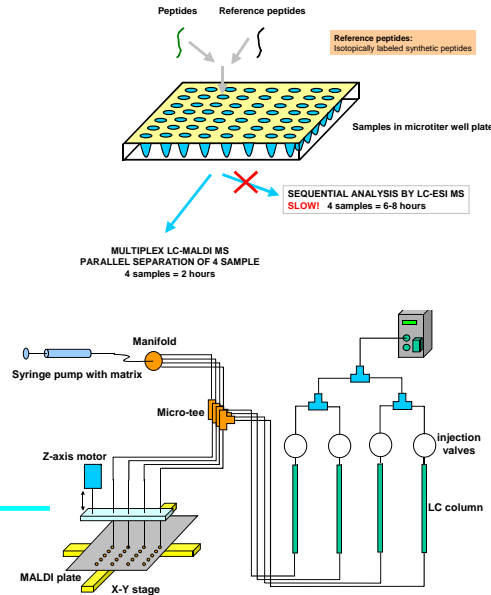
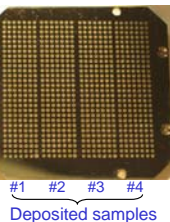
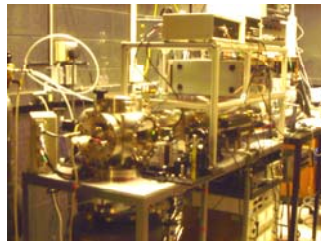
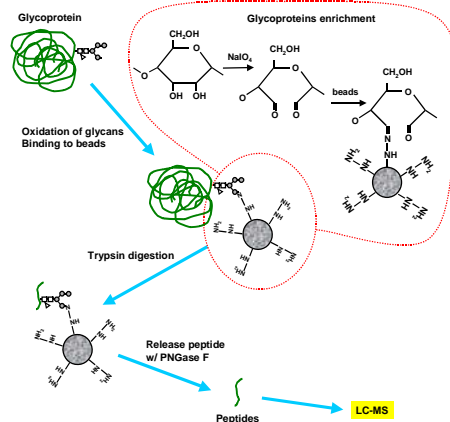
2-kHz MALDI MS ANALYSIS

- High repetition laser 2-kHz Nd:YAG 355 nm (Laser Compac Corp.)
- High voltage power sources
 - Low voltage drift (~10 ppm/°C)
 - High power (higher frequency = higher load)
- Data acquisition system 20 samples/sec + averager (Aquiris Corp.)
- Compatible with AB4700 plates
- For more information see poster WP27

2-kHz MALDI MS INSTRUMENT



- Speed: up to 5 MS spectra/sec
- Sensitivity: amole
- Mass accuracy: ~10 ppm (internal calibration)
- Resolution: ~8,000



DATA ACQUISITION SPEED

HPLC - MALDI MS: 40-min LC gradient using 6-sec spot → 400 spots

Laser rep. rate	Shots/spot	1 spectrum	Total acq. time
20 Hz	1000 shots	50 sec	6 hours
200 Hz	1000 shots	5 sec	40 min
2000 Hz	1000 shots	<1 sec	8 min

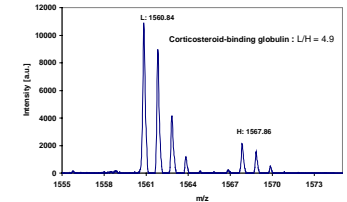
kHz laser NOT ONLY high number of samples/time

- Sensitivity, mass accuracy – averaging of more laser shots
- Suppression effects – separation to more fractions
- Dynamic range – separation to more fractions
- Sample utilization – more sample can be used
- Time for re-analysis of the same sample

Other applications of the 2-kHz MALDI MS instrument

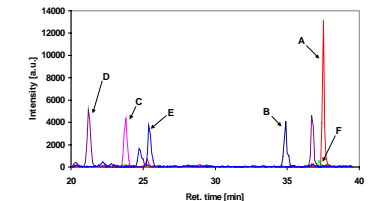
- MALDI imaging
- LC-MALDI MS based proteomics

EXAMPLE OF QUANTITATION USING PEAK INTENSITIES



Sample: Cancer 4
MS spectrum: 2-kHz MALDI MS instrument, baseline subtracted

EXTRACTED ION CHROMATOGRAMS FOR 6 REFERENCE PEPTIDES



BIC = m/z ± 0.1; m/z values in Table 1.
Sample: Cancer 1

RESULTS

Table 1. List of quantitated proteins and corresponding peptides

Label	Protein annotation	Peptide sequences	Reference peptide	[M+H] ⁺ (L)	[M+H] ⁺ (H)
A	Leuveninrich alpha-2-glycoprotein	LPFGLLADYTLR		1425.95	1432.97
B	Corticosteroid-binding globulin	AGLLGGLGFDLTER	AGLLGGLGFDLTER	1560.84	1567.86
C	Clustrocin	LAKLGGGQDYR	LAKLGGGQDYR	1688.82	1695.84
D	Methoprosthemase inhibitor 1	FVGTPEVNTLTYR	FVGTPEVNTLTYR	1753.88	1760.90
E	Neogenin	TLSDVPSAAPQDLSEVR	TLSDVPSAAPQDLSEVR	1897.90	1904.92
F	Lumican	LGDFEGLVNLTRHGVNR	LGDFEGLVNLTRHGVNR	2196.16	2203.18

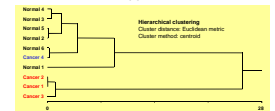
* previously glycosylated Aen
Bold red isotopically labeled amino acid, peptides synthesis Cyt Signaling Technologies

Table 2. Summary of calculated ratios

	A	B	C	D	E	F
Cancer 1	3.7	2.2	4.2	1/9.1	1/55.7	1/7.1
Cancer 2	4.1	1.5	5.0	1/9.8	1/45.2	1/2.6
Cancer 3	7.2	1.9	6.7	1/6.2	1/28.3	ND
Cancer 4	3.1	4.9	13.5	1/16.7	1/15.6	1/2
Normal 1	3.5	7.5	26.3	1/24.3	1/55.6	ND
Normal 2	5.3	8.9	17.4	1/31.9	1/30.1	ND
Normal 3	5.6	10.6	12.0	1/28.9	1/29.3	ND
Normal 4	6.5	12.1	15.3	1/18.4	1/20.1	ND
Normal 5	4.4	10.5	18.0	1/14.8	1/24.8	ND
Normal 6	3.1	5.6	15.5	1/24.3	1/21.3	1/1.2

Value represents ratio of peak intensities L:H at chromatographic apex (MALDI signal RSD < 30%, n=3)
* Reference peptide overlaps with another peak
ND not determined due to low intensity

PRELIMINARY DATA ANALYSIS



- Analysis of 10 samples (4 cancer and 6 normal)
- Each sample analyzed only once (performed sample amount)
- MALDI MS data obtained 3-times for each sample
- Variation in MALDI MS replicates negligible compared to the variation between samples
- Clustering using reference peptides A-E

CONCLUSIONS

- Parallel LC-MALDI MS allows high throughput relative or absolute quantitation of selected proteins
- The technique is applicable for quantitation of samples with multiple standards
- Reference peptides are characterized by LC-MS prior to the analysis
- Retention time, exact molecular weight and presence of a peptide pair provides sufficient information for peptide identification – MS/MS is not required
- Quantitation can be carried out using either MS intensity of the peptides or by integrating of the MS intensity over the elution of the chromatographic peak

REFERENCE

1. Zhang, H. L. X.; Martin, D. B.; Aebersold, R. Nature Biotechnology 2003, 21(6), 660-666.
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3. Chen, H.-S.; Rejtar, T.; Andreev, V.; Moskovets, E.; Karger, B. L.; Anal. Chem. 2005 77(8): 2323-2331.