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A Fully Automated, Bottom-up Approach for MALDI-TOF MS Based Discovery Workflows

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Novel Aspect

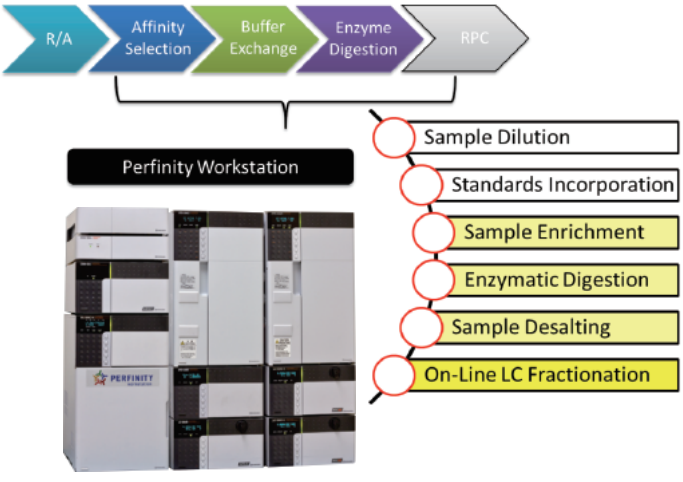
Combining an automated MALDI plate spotter with an automated digestion workstation results in reduced experimental time and improved qualitative results.

Introduction

Bottom-up workflows have been a staple of mass spectrometry based proteomic approaches. Most of these protocols require overnight digestion, sample clean-up and, when involving MALDI, fraction collection, dry down and sample matrix deposition. Such lengthy protocols limit the number of samples that can be analyzed while suffering from great variability in digestion efficiency qualitatively and quantitatively. We present in this work a fully automated solution for MALDI-TOF MS based peptide mapping experiments. Using an online digestion, desalt, reversed phase separation and fraction collection platform, we were able to decrease the experimental time from over 18 hours to less than 30 min (reversed phase separation included – acquisition time not included) while substantially improving sequence coverage.

Methods

An automated sample preparation workstation (Perfinity Workstation (PWS), Perfinity Biosciences) was directly coupled to a MALDI plate spotter (AccuSpot, Shimadzu). Online digestion was performed using an immobilized enzyme column (4 minutes, 50C), followed by reversed phase (Phenomenex Aeris XB-C18, 100 x 2.10 mm, 3.6u) separation (15 min, 2 - 60% acetonitrile in water and 0.1 % formic acid) and fraction plate deposition on a 384 well plate (split ratio, R= 20:1, 3 seconds interval spotting). Benchtop digest was performed overnight using trypsin following standard reduction and alkylation (Trypsin Gold, Promega). Reaction was quenched and solution was dried down, re-suspended and desalted using reversed phase microcolumns (Zip Tip, Millipore). MALDI analysis was performed on a MALDI TOF/TOF (Shimadzu AXIMA Performance MALDI TOF/TOF) in the automated mode, using external calibration. Sequence coverage was assessed using MASCOT Peptide Mass Fingerprinting (PMF) (Swissprot) and was further confirmed by manual review of spectra.



Perfinity Workstation

A unique platform that enables on-line affinity enrichment, enzyme digestion, desalt and reversed phase separation. The workstation can be interfaced with mass spectrometers and used for high-throughput targeted and discovery workflows.

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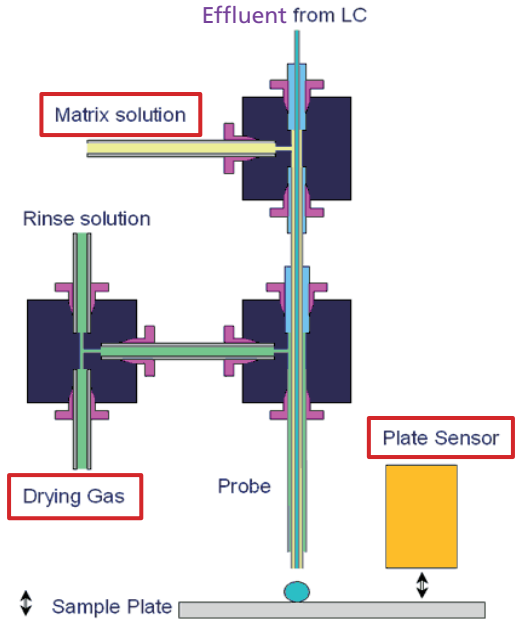
AccuSpot

The AccuSpot is an automated plate spotter instrument that enables mixing LC effluent with MALDI matrices and deposits the mixture on a MALDI plate. The AccuSpot uses concentric flow of effluent and matrix to avoid cross contamination and clogging.



Results

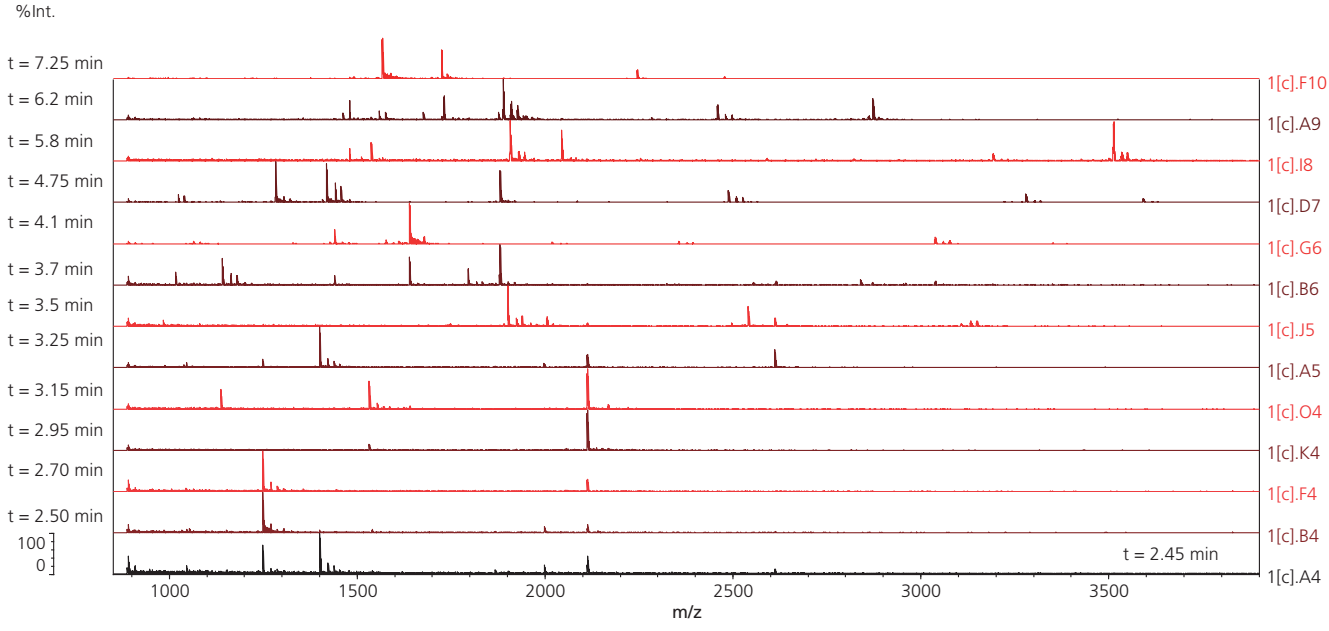


Figure 1. MALDI MS spectra of 13 fractions collected between t = 0-15min.

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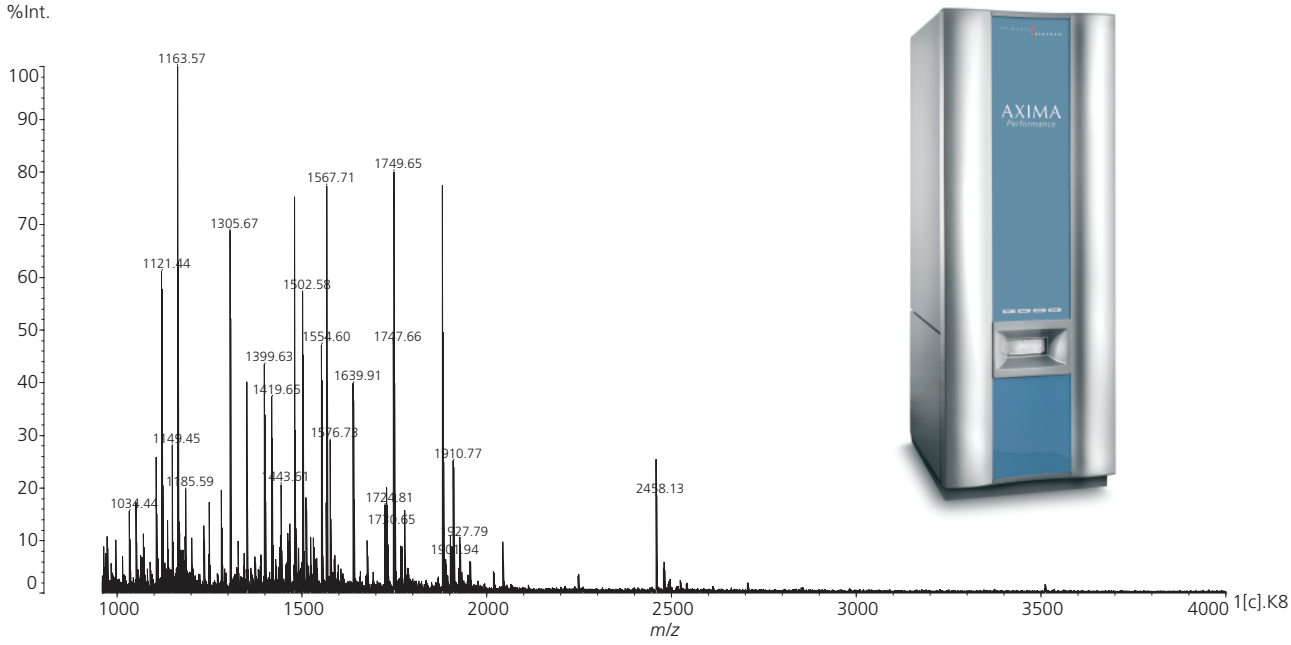

Figure 2. Mass spectrum of BSA digest after micro-column desalting.
Spectra were calibrated externally and submitted to MASCOT PMF search.

(a)

1	MKWVTFISLL	LLFSSAYSRG	VFRRDTHKSE	IAHRFKDLGE	EHFKGLVLIA	
51	FSQYLQQCPF	DEHVKLVNEL	TEFAKTCVAD	ESHAGCEKSL	HTLFGDELCK	
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF	
151	KADEKKFWGK	YLYEIARRHP	YFYAPELLYY	ANKYNGVFQE	CCQAEDKGAC	
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPKAE	
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE	
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAKDAFL	
351	GSFLYEYSRR	HPEYAVSVLL	RLAKEYEATL	EECCAKDDPH	ACYSTVFDKL	
401	KHLVDEPQNL	IKQNCDQFEK	LGEYGFQNAL	IVRYTRKVPQ	VSTPTLVEVS	
451	RSLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC	
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT	
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDKEAC	FAVEGPKLVV	
601	STQTALA					

Manual Digest

(b)

1	MKWVTFISLL	LLFSSAYSRG	VFRRDTHKSE	IAHRFKDLGE	EHFKGLVLIA	
51	FSQYLQQCPF	DEHVKLVNEL	TEFAKTCVAD	ESHAGCEKSL	HTLFGDELCK	
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF	
151	KADEKKFWGK	YLYEIARRHP	YFYAPELLYY	ANKYNGVFQE	CCQAEDKGAC	
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPKAE	
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE	
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAKDAFL	
351	GSFLYEYSRR	HPEYAVSVLL	RLAKEYEATL	EECCAKDDPH	ACYSTVFDKL	
401	KHLVDEPQNL	IKQNCDQFEK	LGEYGFQNAL	IVRYTRKVPQ	VSTPTLVEVS	
451	RSLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC	
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT	
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDKEAC	FAVEGPKLVV	
601	STQTALA					

Automated Digest

Figure 3. Bovine Serum Albumin sequence coverage using benchtop digest (a) and PWS-AccuSpot (b).
Amino acids belonging to the signal peptide (1-18) and pro-peptide (19-25) (underlined) were not included in the sequence coverage calculations. Peptides matched in the sequence are highlighted in bold and light blue.

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Table 1. Comparison of fully automated digestion platform with benchtop digestion.
The PWS-AccuSpot workflow generated a fully spotted plate in 28 min that resulted in sequence coverage of 87%. Overnight digest and sample clean-up, which took over 18 hours, resulted in 68% sequence coverage.

Digestion method	Experimental time (red/alkylation not included)	Matrix used	Sequence Coverage
Automated PWS-AccuSpot	28 min	CHCA	87%
Benchtop In-solution digest	18 hours + desalt	CHCA	68%

Summary

- The PWS-AccuSpot platform was able to improve sequence coverage of the protein standard Bovine Serum Albumin by 19% when compared to the regular benchtop digest at a fraction of the experimental time.
- The platform offers variable parameters that can be optimized: digestion temperature, digestion time, LC gradient and choice of MALDI matrices.
- The PWS-AccuSpot workflow took only 28 min and resulted in a MALDI ready plate. This combination makes for one of the fastest available MALDI-based, bottom-up sample preparation platforms.
- Automation removes user error, improves reproducibility and decreases chances of contamination.
- Optimum coverage required the injection of at least 6 pmol of protein on trypsin column.

Future Directions

More experiments on sequence coverage could be performed. We want to look at the effect of digestion time and temperature on sequence coverage. Sensitivity will also be of interest as we will work on decreasing the split ratio and design a splitless configuration.

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