Ultra-fast Peptide Mass Fingerprinting (UPMF): Online Tryptic Digestion and Automated Fraction Collection for MALDI-TOF Analysis

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Introduction

Reduction

Alkylation

Bottleneck
Digestion
5-18 hours →4-8 minutes

Desalt

MALDI-TOF MS

Database Search

Protein ID

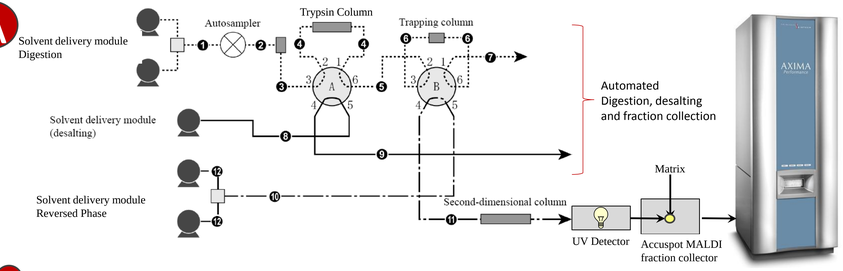
Peptide Mass Fingerprinting (PMF) using MALDI-TOF provides a rapid method of analysis for protein identification. However tryptic digestion is normally performed in-solution or in-gel and digestion times typically range from 4 to 18 hours. In this workflow, the digestion is the rate-limiting step.

Recently, the Perfinity Workstation (Perfinity Biosciences, Inc. W. Lafayette, IN) has been introduced to perform (i) affinity capture of target proteins, (ii) online digestion of the isolated proteins and (iii) trapping/desalting/LC separation of the resulting peptides. The use of immobilized enzyme reaction (IMER) columns, which permit high enzyme-to-substrate ratios while minimizing issues related to autolysis, decreases the digestion time to between one and eight minutes with high reproducibility.

In this presentation, we propose utilizing a modification of this workstation to improve throughput and reproducibility of peptide mass fingerprinting.

Materials and Methods

A



A **Methods:** All materials were purchased from Sigma Aldrich (Saint Louis, MO) unless otherwise specified. Proteins were denatured in denaturing buffer (Perfinity Biosciences W. Lafayette, IN) and reduced for 30 minutes with dithiothreitol (DTT) at 60°C and alkylated with Iodoacetamide for 30 minutes in darkness at room temperature. 10 pmol sample was injected onto a 2 dimensional Prominence Nano HPLC (Shimadzu Kyoto, Japan). Digestion and desalting were performed according to conditions listed to the right. Tryptic peptides were collected for 3 minutes on one spot using the Accuspot (Shimadzu Kyoto, Japan) and 1 µl matrix was spotted before drying. The sample was dried and analyzed by MALDI-TOF MS in reflectron positive mode. Laser power and number of profiles was consistent for each experiment (100 profiles for Albumin, 61 profiles for myoglobin).

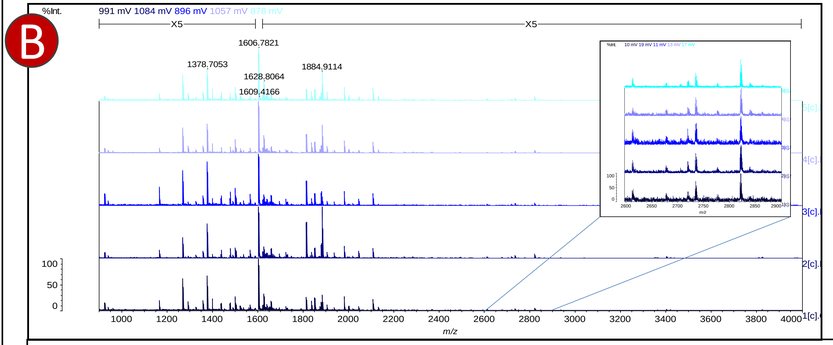
First Dimension:
Column: Perfinity Enhanced Immobilized Trypsin Column
Trap: Piccolo Targa C18 2.5x0.3mm, 5µm
Mobile Phase: Perfinity optimized digest buffer
Flow Rate: 25 µl/min
Temperature: Room Temperature

Second Dimension:
Column: Vydac C18 5cm x 150 µm, 5µm
Mobile Phase A: 0.1% TFA in H_2O
Mobile Phase B: 0.1% TFA in acetonitrile
Flow: 2 µl/min

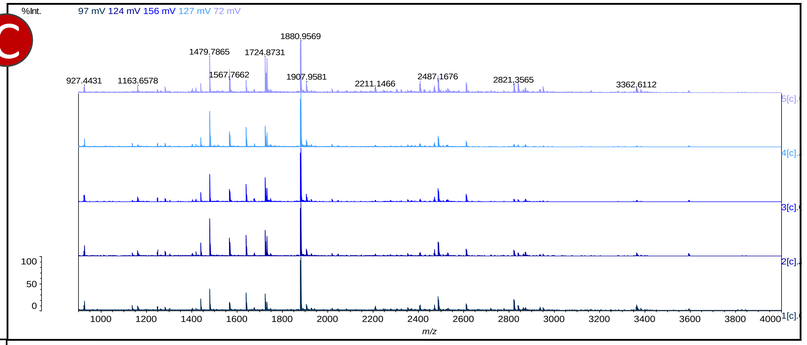
HPLC Conditions		
	Time (minutes)	%B Concentration
Digest time	6 minutes	
Desalting time	2 minutes	
Gradient	0.0	2.0%
	1.0	70.0%
	7.0	70.0%
	7.1	90.0%
	9.0	90.0%
	9.1	2.0%
	11.0	2.0%

Results

B



C



Reproducibility Assessment: Peptide Mass Fingerprints from five injections of myoglobin (B)and albumin (C). Comparison of the features in the spectra indicate that the workflow of the modified Perfinity Workstation using the enhanced immobilized trypsin IMER column provides excellent reproducibility for the peptide mass finger print workflow with a six minute digestion time.

D

Myoglobin					
	Digest 1	Digest 2	Digest 3	Digest 4	Digest 5
Mascot Score	127	141	142	127	129
Sequence Coverage	86%	86%	91%	86%	86%
M.GLSDGEWQQVLNVWGK.V	☑	☑	☑	☑	☑
K.VEADIAGHGQEVLIR.L	☑	☑	☑	☑	☑
R.LFTGHPETLEK.F	☑	☑	☑	☑	☑
R.LFTGHPETLEKFDK.F	☑	☑	☑	☑	☑
R.LFTGHPETLEKFDKFK.H	●	☑	●	●	●
K.FKHLKTEAEMK.A	☑	☑	☑	☑	☑
K.TEAEMKASEDLKK.H	☑	☑	☑	☑	☑
K.HGTVVLTALGGILK.K	☑	☑	☑	☑	☑
K.HGTVVLTALGGILKK.K	☑	☑	☑	☑	☑
K.KKGHHEAELKPLAQSHATK.H	☑	☑	☑	☑	☑
K.KGHHEAELKPLAQSHATK.H	☑	☑	☑	☑	☑
K.GHHEAELKPLAQSHATK.H	☑	☑	☑	☑	☑
K.YLEFISDAIIHVLHSK.H	☑	☑	☑	☑	☑
K.HPGDFGADAQGAMTK.A	☑	☑	☑	☑	☑
K.ALELFR.N	☑	☑	☑	☑	☑
K.YKELGFQG.-	●	●	☑	●	●
	☑	Identified by Mascot			
	●	Not identified by Mascot but present in spectrum			
	☒	Not present			

Bovine Serum Albumin					
	Digest 1	Digest 2	Digest 3	Digest 4	Digest 5
Mascot Score	201	236	250	198	192
Sequence Coverage	45%	44%	47%	44%	41%
-.MKWVTFISLLLFSSAYSR.G + Oxidation (M)	☑	●	●	●	●
R.FKDLGEEHFK.G	☑	☑	☑	☑	☑
K.LVNELTEFAK.T	☑	☑	☑	☑	☑
K.TCVADESHAGCEKSLHTLFGDELCK.V	●	☑	●	●	●
K.SLHTLFGDELCK.V	☑	☑	☑	☑	☑
K.LKPDPNTLCDEFKADEK.K	☑	☑	☑	☑	☑
K.YLYEIAR.R	☑	☑	☑	☑	☑
R.RHPYFYAPELLYYANK.Y	●	●	☑	●	●
K.YNGVFQECCQAEDK.G	☑	☑	☑	☑	☑
K.YNGVFQECCQAEDKGACLLPK.I	☑	☑	☑	☑	☑
K.AWSVARLSQKFPK.A	☒	●	☑	☑	●
K.VHKECCHGDLLECADDRADLAK.Y	☑	☑	☑	☑	☑
K.DAFLGSFLYEYSR.R	☑	☑	☑	☑	☑
R.RHPEYAVSVLLR.L	☑	☑	☑	☑	☑
R.HPEYAVSVLLR.L	☑	☑	☑	☑	☑
K.HLVDEPQNLIK.Q	☑	☑	☑	☑	●
K.HLVDEPQNLIKQNCDQFEK.L	☑	☑	☑	☑	☑
K.QNCDQFEKLGEYGFQNALIVR.Y	●	☑	☑	●	☑
K.QNCDQFEKLGEYGFQNALIVRYTR.K	●	☑	●	●	☑
K.LGEYGFQNALIVR.Y	☑	☑	☑	☑	☑
R.KVPQVSTPTLVEVSR.S	☑	☑	☑	☑	☑
R.CCTKPESERMPCTEDYLSLILNR.L	☑	☑	☑	☑	☑
R.MPCTEDYLSLILNR.L	☑	☑	☑	☑	☑
K.CCTKSLVNR.R	☑	☑	☑	☑	☑
R.RPCFSALTPDETYVPK.A	☑	☑	☑	☑	☑
R.RPCFSALTPDETYVPKAFDEK.L	☑	☑	☑	☑	☑
K.LFTFHADICTLPDTEK.Q	☑	☑	☑	☑	☑
K.CCAADDKEACFAVEGPK.L	☑	☑	☑	☑	●
	☑	Identified by Mascot			
	●	Not identified by Mascot but present in spectrum			
	☒	Not present			

D A comparison of peptides identified by searching the Swissprot database with Mascot using the top 40 peaks. All peptides not appearing in the search results were manually verified as being present in the other replicate injections with one exception.

Summary

The enhanced immobilized trypsin column used with this modified Perfinity Workstation provided significant improvements to throughput for the PMF workflow. Digest times for the PMF workflow were reduced from 4-18 hours to 6 minutes with exceptional reproducibility.