Identification of HPLC-Fractionated Tryptic Peptides Using the NanoMate™ 100 System

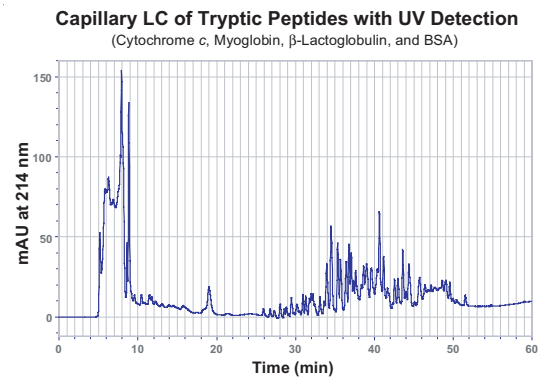
Ultimate "Peak Parking" by Fractionation

Advion BioSciences, Inc. (Ithaca, NY) has demonstrated use of the NanoMate™ 100 system for protein identification from fractionated tryptic digests of a protein mixture by nanoelectrospray mass spectrometry. The NanoMate 100 is an automated sample-handling system coupled with the ESI Chip™ (a 10 x 10 array of nanoelectrospray nozzles etched in silicon).

Proteomic studies currently use various separation techniques followed by on-line, nanoscale, full-scan LC/MS/MS analysis of tryptic digests. While this approach works well, its drawbacks include relatively long runs and special techniques such as "peak parking" to obtain the best data. Using Advion's nanoscale technique, capillary LC fractions from tryptic digests of protein mixtures are collected off-line and the fractions analyzed by automated nanoelectrospray ionization mass spectrometry.

Methods

A standard protein mixture of BSA, myoglobin, β-lactoglobulin, and cytochrome *c* was denatured in 6 M guanidine-HCl and digested by trypsin. The tryptic digests were separated by capillary LC.



HPLC Conditions

Column:	PepMap™ C_{18} (300 µm i.d. by 15 cm, 3-µm particles)
HPLC System:	UltiMate™ capillary nano HPLC
Gradient:	8 minutes, 0% B 68 minutes, 90% B
Mobile Phase A:	5% CH_3CN (v/v) in water with 0.1% TFA
Mobile Phase B:	80% CH_3CN (v/v) in water with 0.1% TFA
Flow Rate:	3 µL/minute
Fractions:	Collected over 1-minute intervals

Nanoelectrospray MS and Database Search

Instruments:	NanoMate, ESI Chip, Thermo Finnigan LCQ™ Deca
Spray Voltage:	1.4 kV
Flow Rate:	100 nL/minute
Acquisition Time:	1.5 minutes
Scan Modes:	MS survey scan and data-dependent MS/MS scan
Searches:	SEQUEST/horse.fasta and bovine.fasta databases

Results

Fractionated samples (3 µL) were collected and analyzed by the NanoMate system over a period of 1.5 minutes. MS/MS analyses of all fractions were complete in 90 minutes. Several peptides were identified within each fraction for each of the proteins digested. An example of the data from the fractions taken from 34 to 40 minutes is shown below.

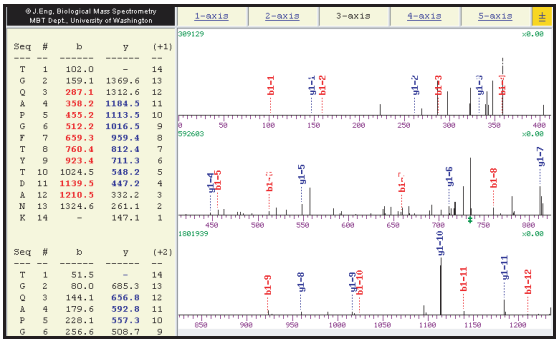
NanoMate ESI — MS/MS Analysis
of the Fractions from 34 to 40 minutes

Time (min)	Fragments (Position in Protein)	Sequences of Peptides
34-35	BSA 257-263	LVTDLTK
	CYT 40-53	TGQAPGFTYTDANK ←
	CYT 9-13	IFVQK
35-36	MYO 32-42	LFTGHPETLEK
	MYO 51-56	TEAEMK
	BSA 35-44	FKDLGEEHFK
	BSA 249-256	AEFVEVTK
	β-LAC 1-8	LIVTQTMK
36-37	CYT 56-60	GITWK
	MYO 32-42	LFTGHPETLEK
	MYO 17-31	VEADIAGHGQEVLIR
37-38	MYO 17-31	VEADIAGHGQEVLIR
	MYO 148-153	ELGFQG
	BSA 402-412	HLVDEPQNLIK
	BSA 437-451	KVPQVSTPTLVEVSR
	β-LAC 108-117	VLVLDTDYKK
38-39	β-LAC 94-99	IPAVFK
	BSA 161-167	YLYEIAR
	BSA 438-451	VPQVSTPTLVEVSR
39-40	CYT 28-38	TGPNLHGLFGR
	β-LAC 108-116	VLVLDTDYK

A representative peptide, identified in the 34 to 35 minute fraction, matched a peptide for cytochrome *c* (peptide TGQAPGFTYTDANK) as shown in the figure below.

SEQUEST Results: Cytochrome *c*

Peptide TGQAPGFTYTDANK in the 34-35 min Fraction



Protein Coverage

100% of the tryptic peptides greater than 500 Da were identified for cytochrome *c* by this method. In addition, 80% of the tryptic peptides greater than 500 Da were identified for myoglobin.

Cytochrome *c* tryptic peptides greater than 500 Da

Peptide Position	Mass	Peptide Sequence	Retention Time of Fraction (min)
1 - 5	589.2	Acetyl-GDVEK	15 - 16
74 - 79	678.4	YIPGTK	31 - 32
40 - 53	1470.7	TGQAPGFTYTDANK	33 - 35
9 - 13	634.4	IFVQK	33 - 35
56 - 60	604.3	GITWK	36 - 37
28 - 38	1168.6	TGPNLHGLFGR	39 - 40
80 - 86	779.4	MIFAGIK	40 - 41
14 - 22	1634.5	Heme-CAQCHTVEK	41 - 42
92 - 99	964.5	EDLIAYLK	43 - 44
61 - 72	1495.7	EETLMEYLENPK	42 - 45

Summary

The NanoMate 100 System has demonstrated utility as a nanoelectrospray platform for rapid analysis of separated samples via 100% coverage found for cytochrome *c*. High-quality MS/MS data were obtained for each fraction with 1.5-minute acquisition times containing as many as five tryptic fragments, and yielding unambiguous identification of the target proteins.

Advantages

- Analysis is not time sensitive.
- Short analysis can be used for screening. Long analysis allows for mining of low-abundance proteins.
- Signal intensity does not change with time.
- Sample fractions can be saved and archived.
- MS time is conserved. Analyze only the peak elution window of interest.
- The decoupling of chromatography from MS/MS acquisition allows for greater flexibility and speed of analysis.

Acknowledgments

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