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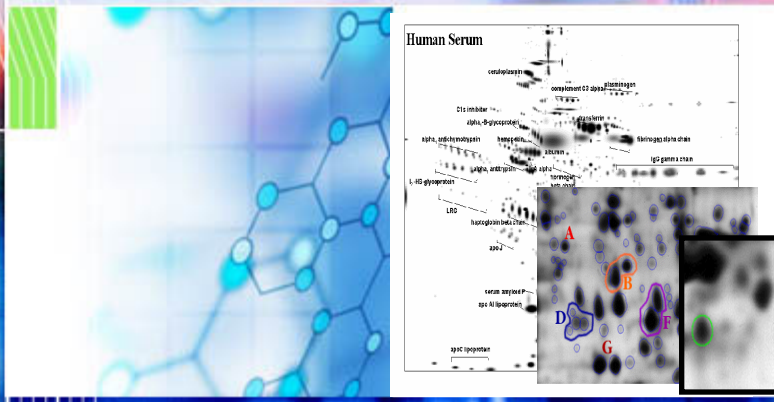
"An LC/MS Interface for Off-line Deposition of Capillary Liquid Chromatography Separations onto MALDI Target Plates"



Jeffrey W. Finch, Daniel B. Wall, Wieben Chen, Scott J. Berger, and Steven A. Cohen

Desoprtion 2002 Conference
September 4, 2002

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Outline

1. Introduction
   a. Peptide Mass Fingerprinting (PMF) by MALDI
   b. Why interface LC to MALDI?
2. The LC-MALDIprep sample deposition module
3. MALDI TOF
4. MALDI (Q-Tof) MS/MS
5. Long-term stability of separations deposited on matrix pre-coated targets
6. Conclusions and future directions

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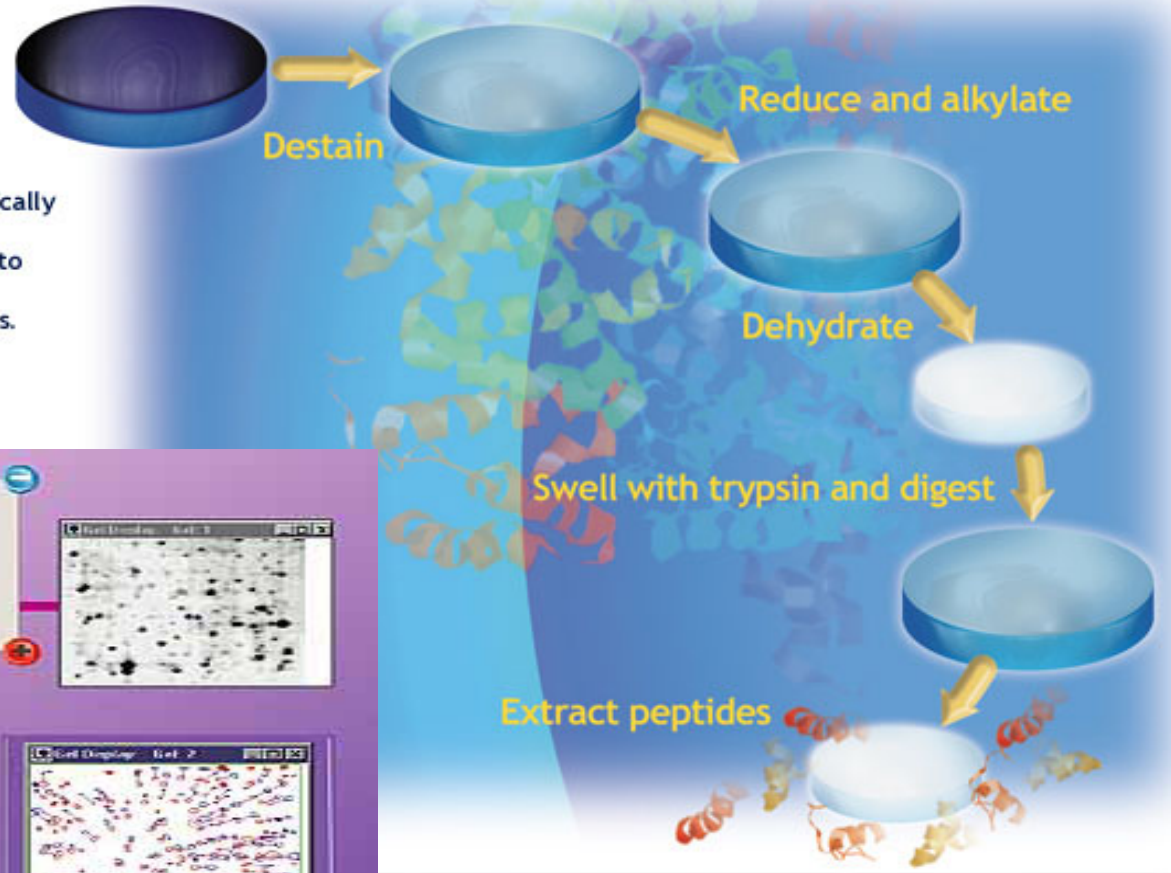
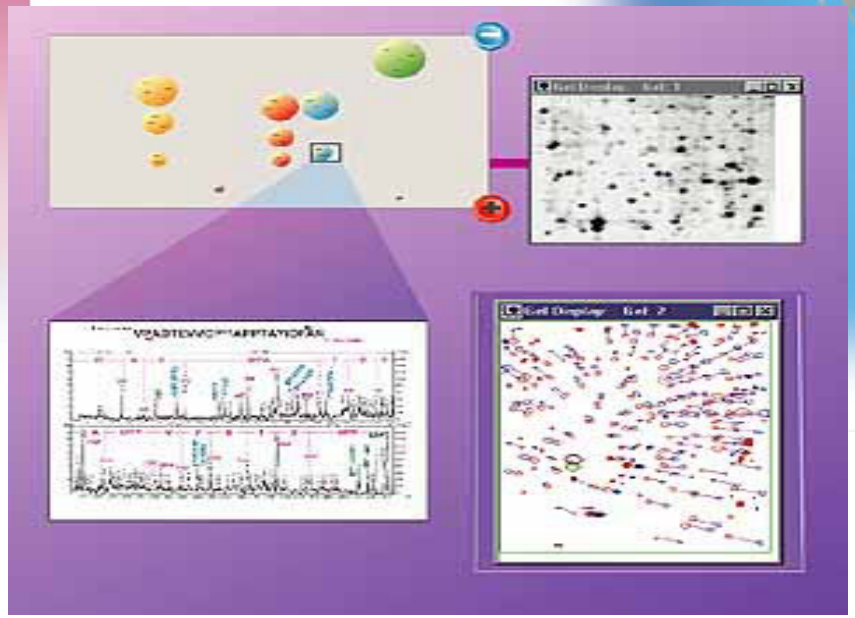
Proteins to Peptides to MS

The excised gel pieces are automatically processed by the MassPREP™ station to deliver the highest recovery of peptides.





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MALDI

- Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry
- Current technology allows measurements on biopolymers (peptides, proteins, antibodies, DNA, enzymes)
- Easy to use
- Sensitive (subfemtomole detection of peptides)
- Peptide mass mapping (or "fingerprinting") for Protein Identification
  - 1D gel spots
  - 2D gel spots
- Major requirement: samples must be relatively "clean"
  - Salts, buffers, detergents, etc. must be removed, or "diluted out", otherwise ion signals of biomolecules are suppressed

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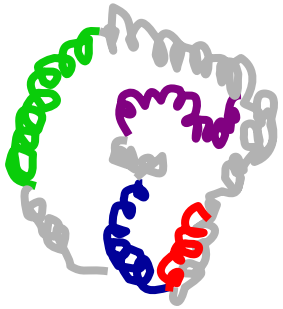
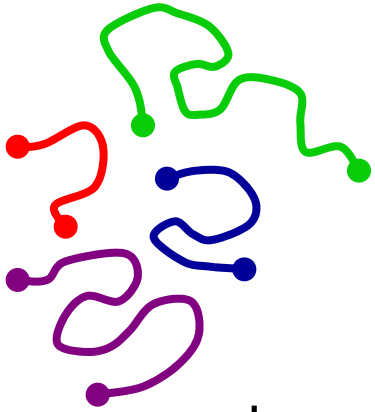
MALDI-TOF Peptide Mass Fingerprinting



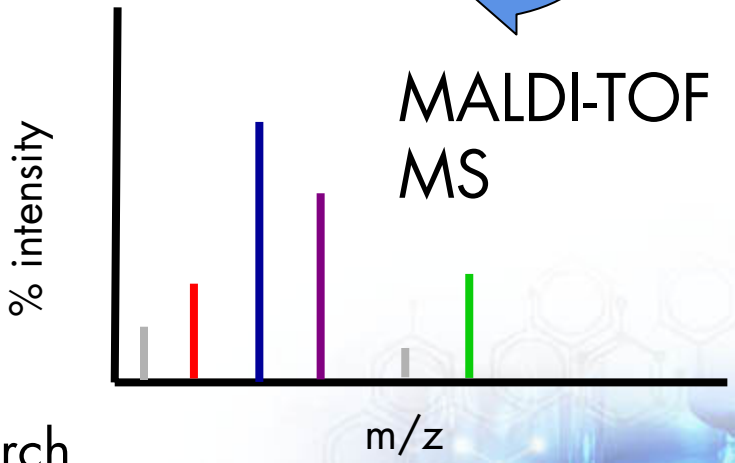
protein(s)

digestion



peptides

MALDI-TOF MS



database search

1) gi|450439 Score: 178 mw 15988 pI 6.75 hemoglobin, beta [Homo sapiens]
sequence coverage: 43%

MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLS

TPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVD

PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH

Protein ID

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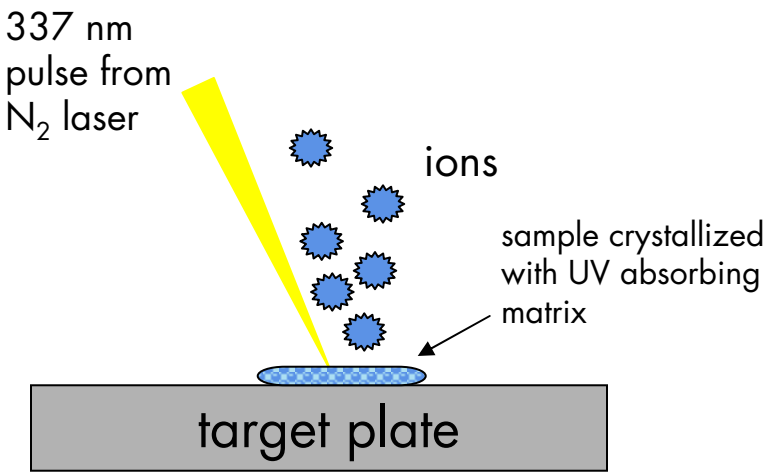
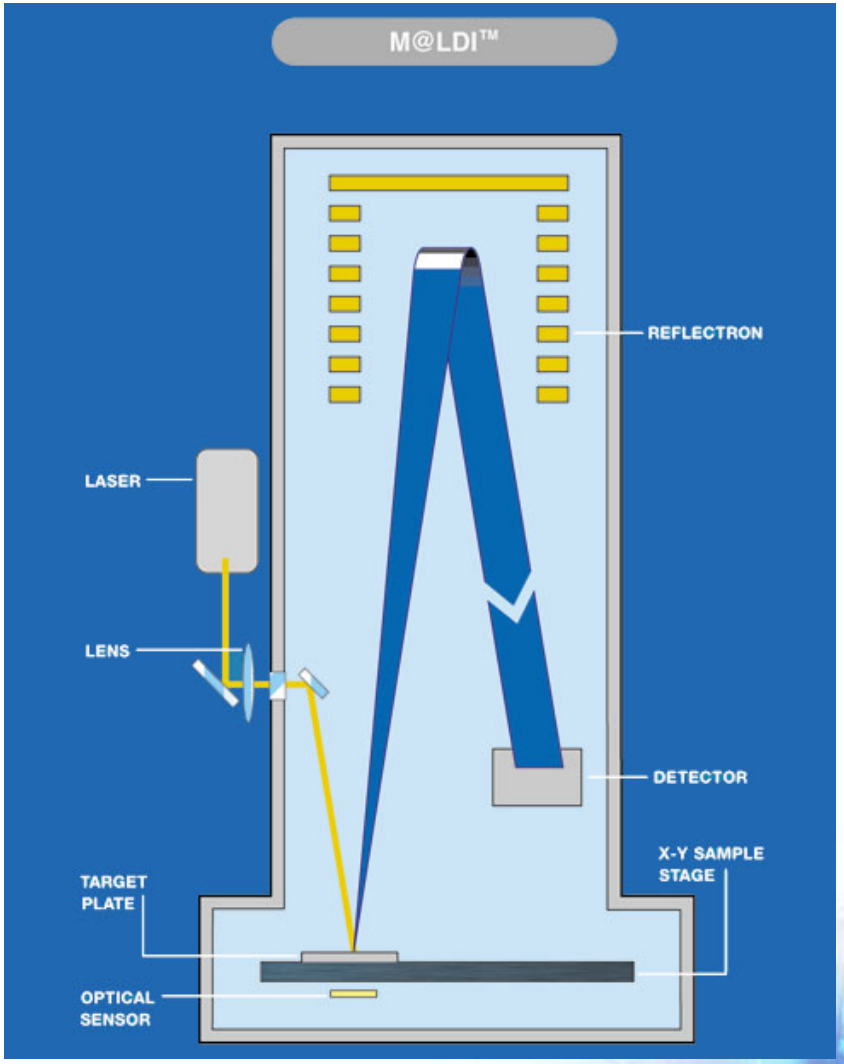
MS Tools: MALDI
Matrix-Assisted Laser Desorption Ionization





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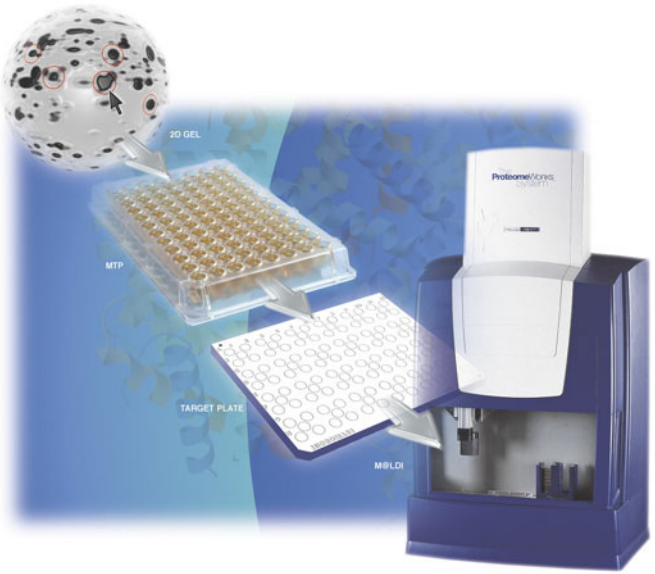
MALDI Sample Preparation

Protein digest samples, analyzed directly or from gel bands or spots typically require sample clean up with a Zip Tip™ or other method to remove salts, buffers, etc. prior to MALDI analysis



ProteinLynx™ Global *SERVER* enterprise edition utilises an advanced sample tracking system to automatically track the fate of a gel spot detected in PDQuest™ through to its identification with ProteinLynx™ Global *SERVER*.

High throughput sample clean-up using 4x Zip-Tip reverse phase disposable tips and precision elution onto a M@LDI™ target plate

Issues with MALDI

- Sample clean-up method, such as a ZipTip™ is typically required to remove salts, buffers, detergents, etc.
  - Limited sample volume and capacity
  - Sample losses (recovery < 50%, etc.)
  - Inability to tolerate high concentrations of salts, buffers, and detergents
- Difficulties in analyzing complex mixtures of proteins
  - Desorption/ionization suppression
  - Resolving peptides which are close in mass
- Shot-to-shot reproducibility

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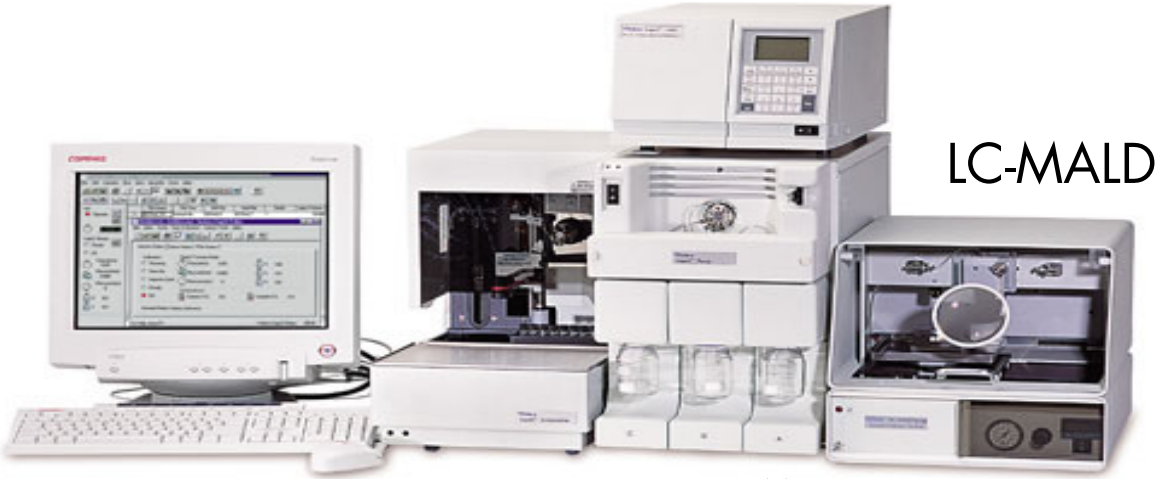
Off-line MALDI LC/MS: LC-MALDIprep Sample Deposition Module



LC-MALDIprep™

Waters CapLC™

Interface between HPLC and MALDI

M@LDI™

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Description of the LC-MALDIprep

- The LC-MALDIprep is a sample deposition module that serves as an interface between reversed-phase HPLC and MALDI mass spectrometry
- Uses a heated capillary nebulizer to desolvate column eluent and spray it onto a MALDI target pre-coated with matrix
- Compatible RP HPLC flow rate range from 5 to 40 μL/min
- Computer control software sets the velocity of the sample stage, the temperature of the nozzle and the collection pattern
- The module can initiate a series of sample collections based on remote inject-start signals
- This device is compatible with a wide range of HPLC and MALDI manufacturers

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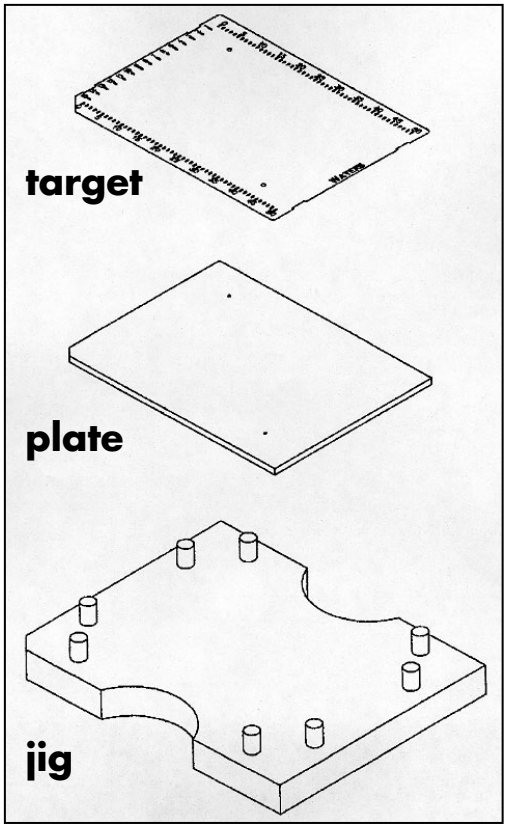
LC-MALDIprep Utilizes an Adhesive Target which is Pre-coated with Matrix

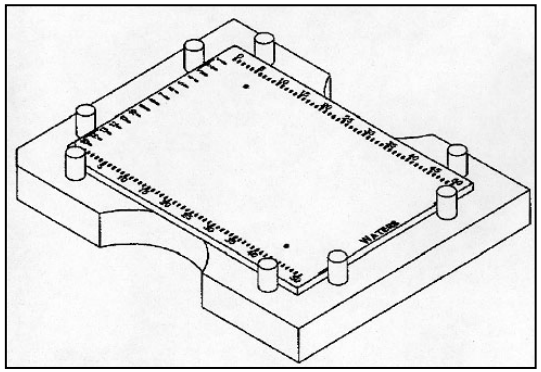
The pre-coated matrix targets are a "one time use" consumable item

Mounting the Target on the MALDI plate

1. Mounting

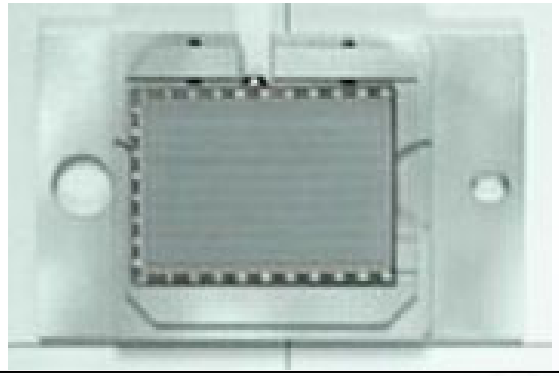


2. Assembled

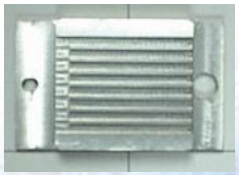


3. LC-MALDIprep Plate Holder

LC-MALDIprep Target Assembly in Plate Holder



… With Lane Shield Mounted



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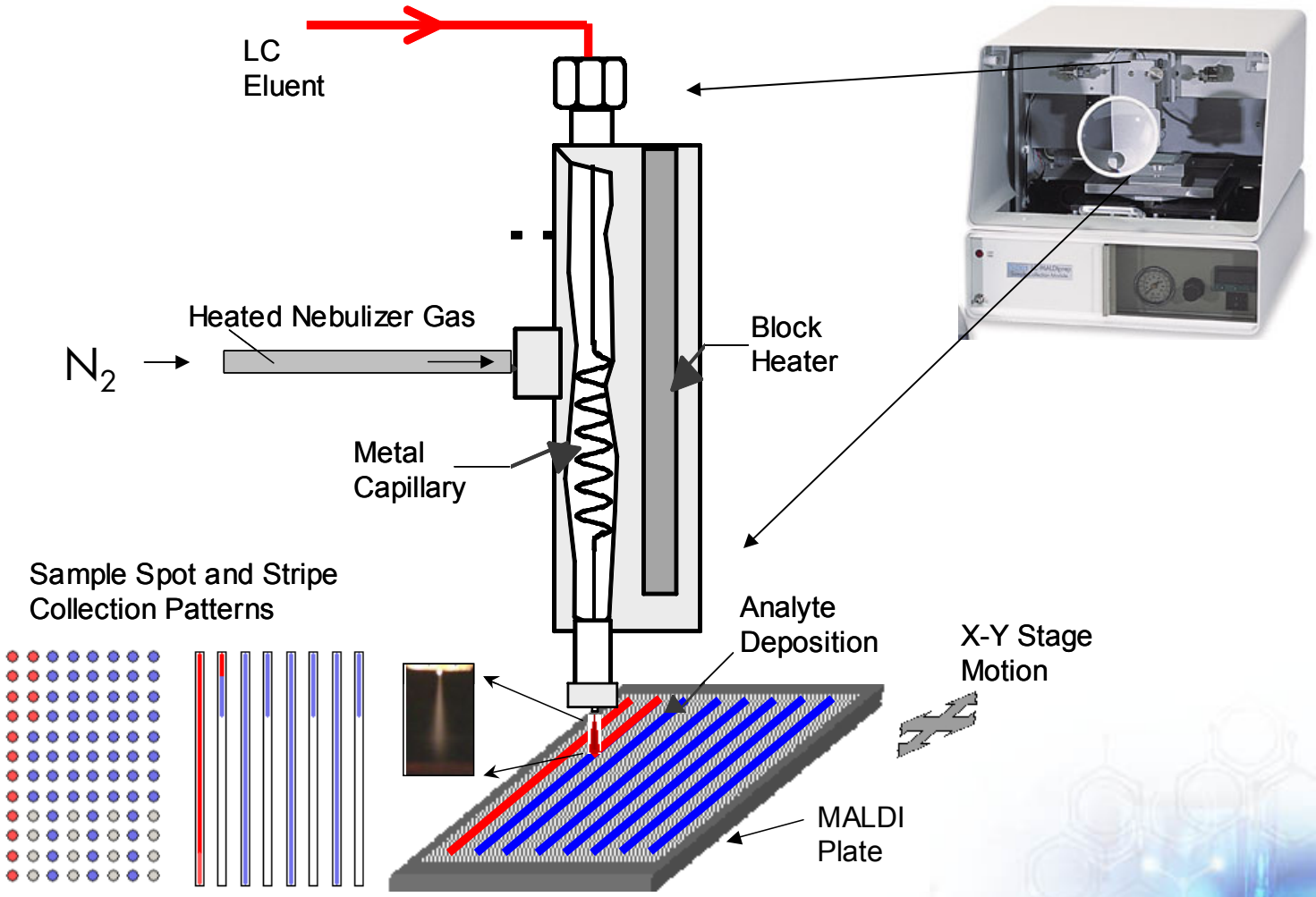
LC-MALDIprep™ Interface:
Heated Capillary Nebulizer with X-Y Stage



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LC-MALDIprep™ Software
Deposition in Tracks

sample deposition in tracks

temperature gradient

Method1.LCMP - LC-MALDIprep Status and Configuration

File Edit View Setup Operation Help

Running Method - Depositing

Current Time:	26.5 mm
Current Temperature:	45.6 °C
Nozzle Position X:	44.75 mm
Nozzle Position Y:	46.20 mm

Position Status

Current Plate: 1

Current Plate:	1
Next Sample Track:	3

Method

Current Method:	Method1.LCMP
Run Time:	30.00 min
Delay Time:	0.10 min
Start Type:	Immediate
Deposition Type:	Tracks
Velocity:	2.00 mm/min

Temperature Offset (°C): 0 Apply

Temperature Status

Current Offset:	0.0 °C
Deviation From Gradient:	0.0 °C

57 54 51 48 45 42 39

0 6 12 18 24 30 36

Ready NUM

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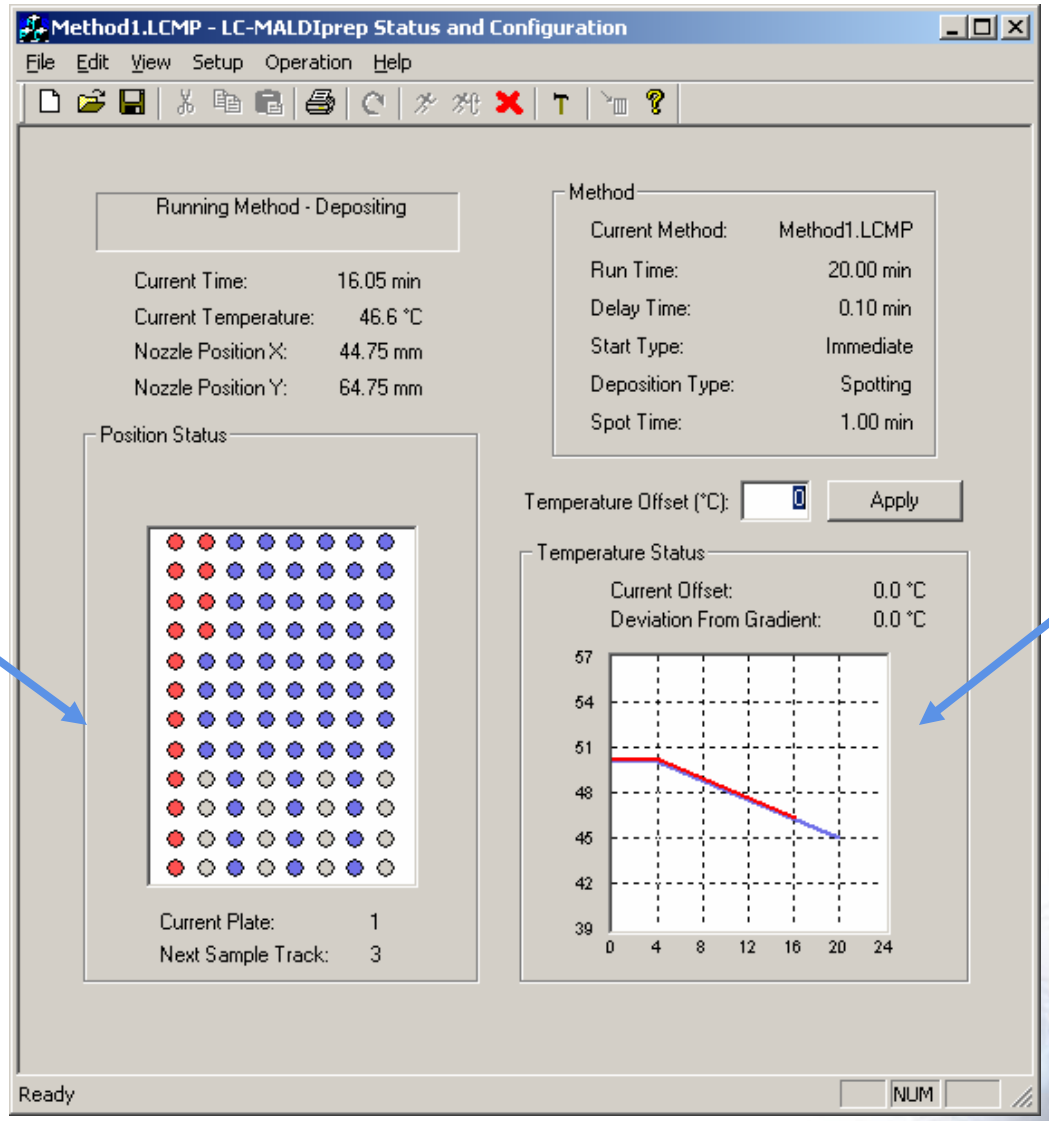
LC-MALDIprep™ Software
Deposition in Spots

sample deposition in spots



temperature gradient

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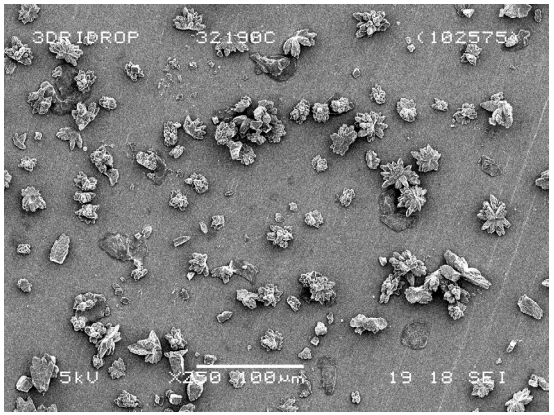
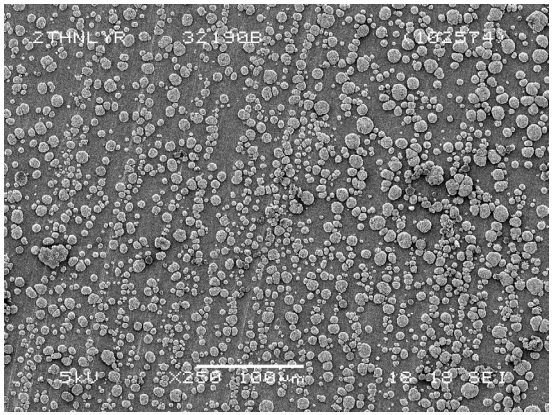
Scanning Electron Microscopy

4HCCA

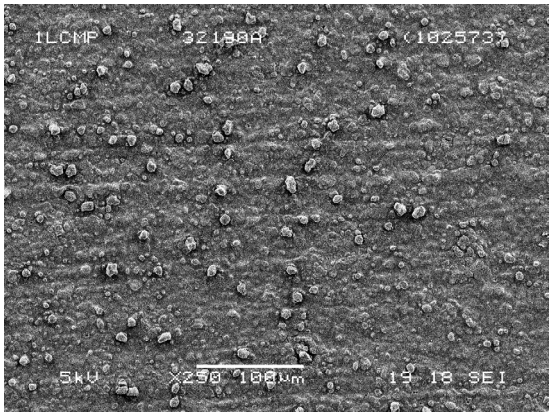
Dried Droplet	Thin Layer	LC-MALDIprep



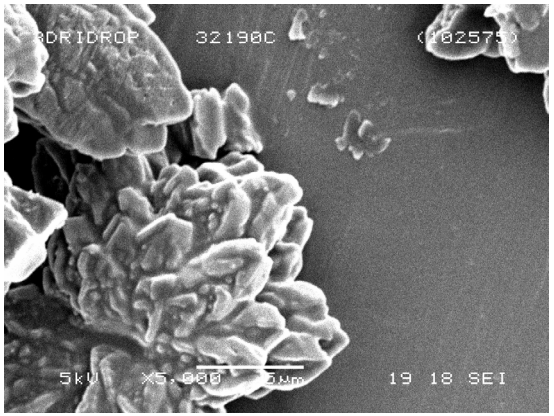
100 µm



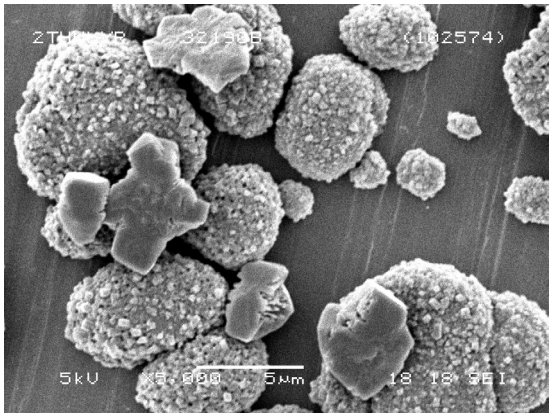
100 µm



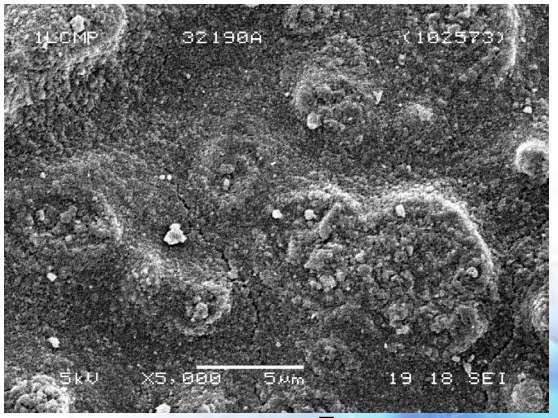
100 µm



5 µm



5 µm



5 µm

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MALDI LC/MS: Typical Experimental Conditions

- Capillary HPLC
  - CapLC with 996 Photodiode array and micro flow cell
  - 0.300 x 150 mm Symmetry C_{18} column (3.5 μm particle size, 100 angstrom pore)
  - 10 μL/min flow rate, Solvent A: H_2O w/ 0.1% TFA, Solvent B: acetonitrile w/ 0.1% TFA, 5% B to 50% B in 26 minutes
- LC-MALDIprep
  - Tracks: 2.5 mm/min stage speed
  - Spots: 0.5 min dwell time
  - Temperature gradient: 60 °C hold: 0-5 min, 60-45 °C 5-26 min
  - Drying gas pressure = 12 psi
- M@LDI R
  - MassLynx 4.0 "Striper" program to control stage and acquire data (tracks)
  - Data reduction program: LC/MS trace ⇨ mass list ⇨ DB search

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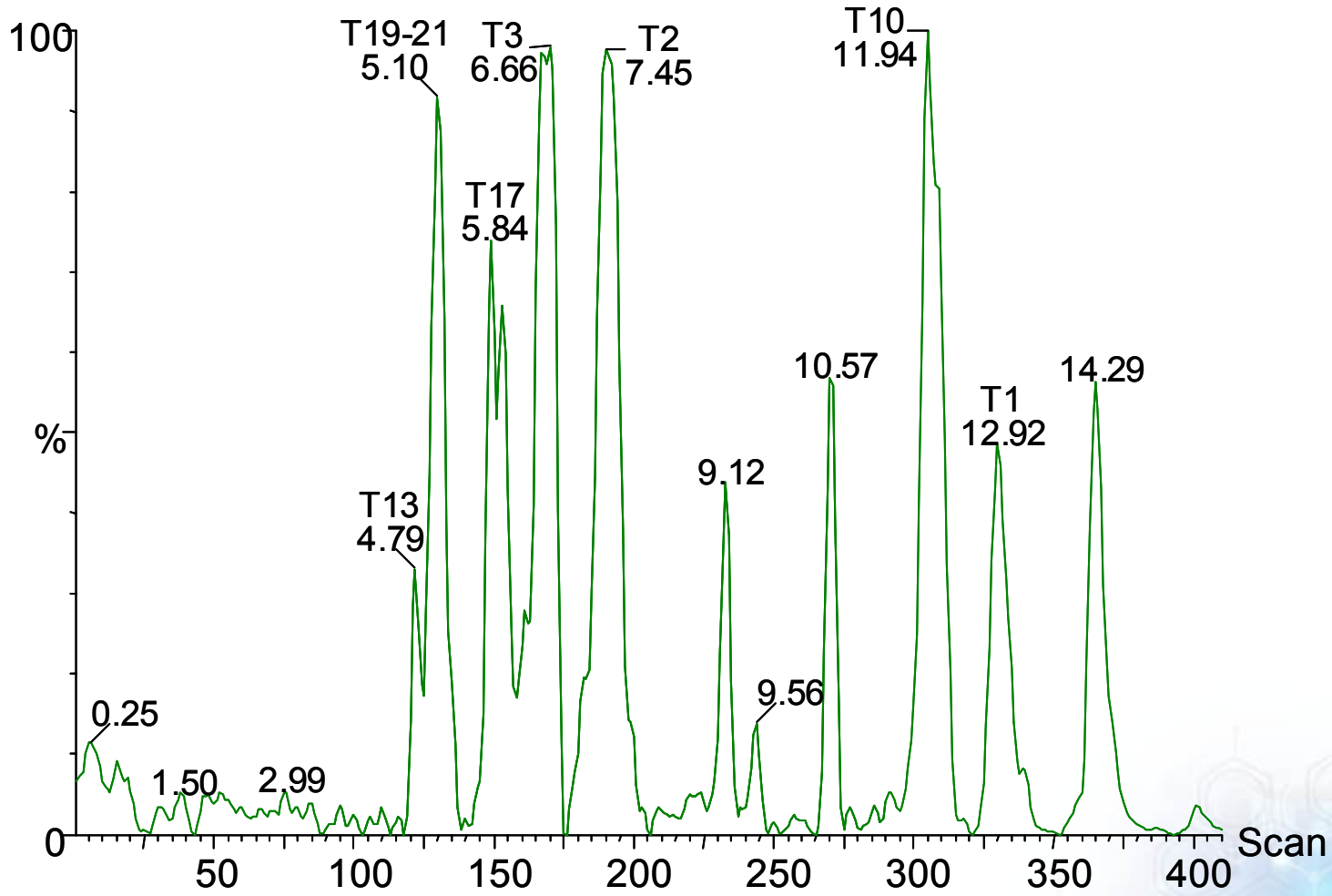
The Base Peak Intensity Chromatogram from LC/MALDI-TOF/MS Analysis of an Apomyoglobin Tryptic Digest Separation



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Sample for Comparing LC-MALDIprep vs. ZipTip & Spotting

- Tryptic digest of high abundance protein in the presence of low abundance proteins
  - Alcohol Dehdrogenase (1250 fmol)
  - Actin (10 fmol)
  - Myoglobin (10 fmol)
  - Cytochrome c (10 fmol)
- Compare detection of tryptic peptides and protein sequence coverage of a capillary LC/MS MALDI separation vs. ZipTip™ & spotting

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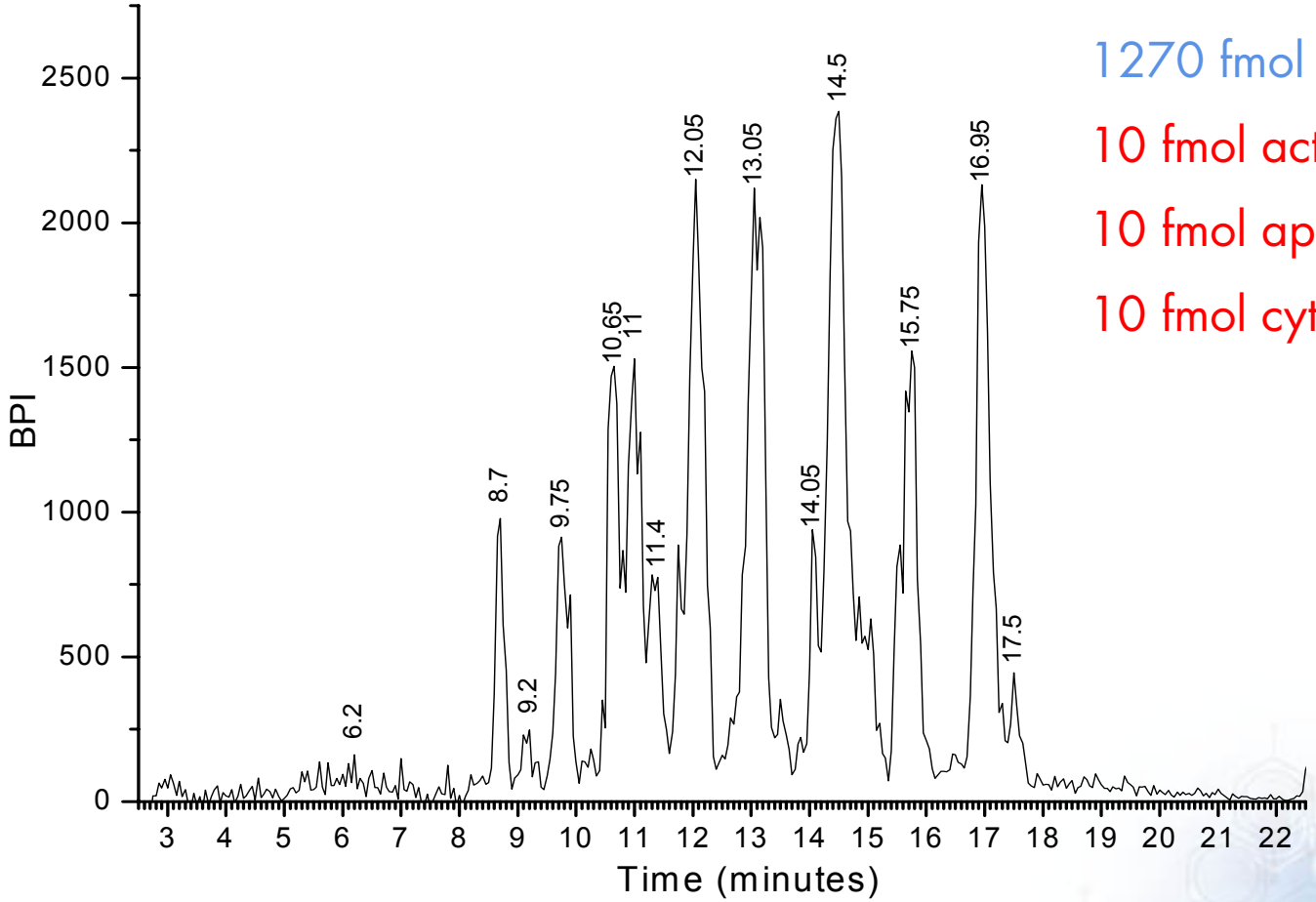
MALDI LC/MS of 4 Protein Tryptic Digest

CapLC with Waters Symmetry C18 .300 x 150 mm column

H_2O/Acetonitrile gradient with 0.1% TFA



1270 fmol ADH

10 fmol actin

10 fmol apomyoglobin

10 fmol cytochrome c

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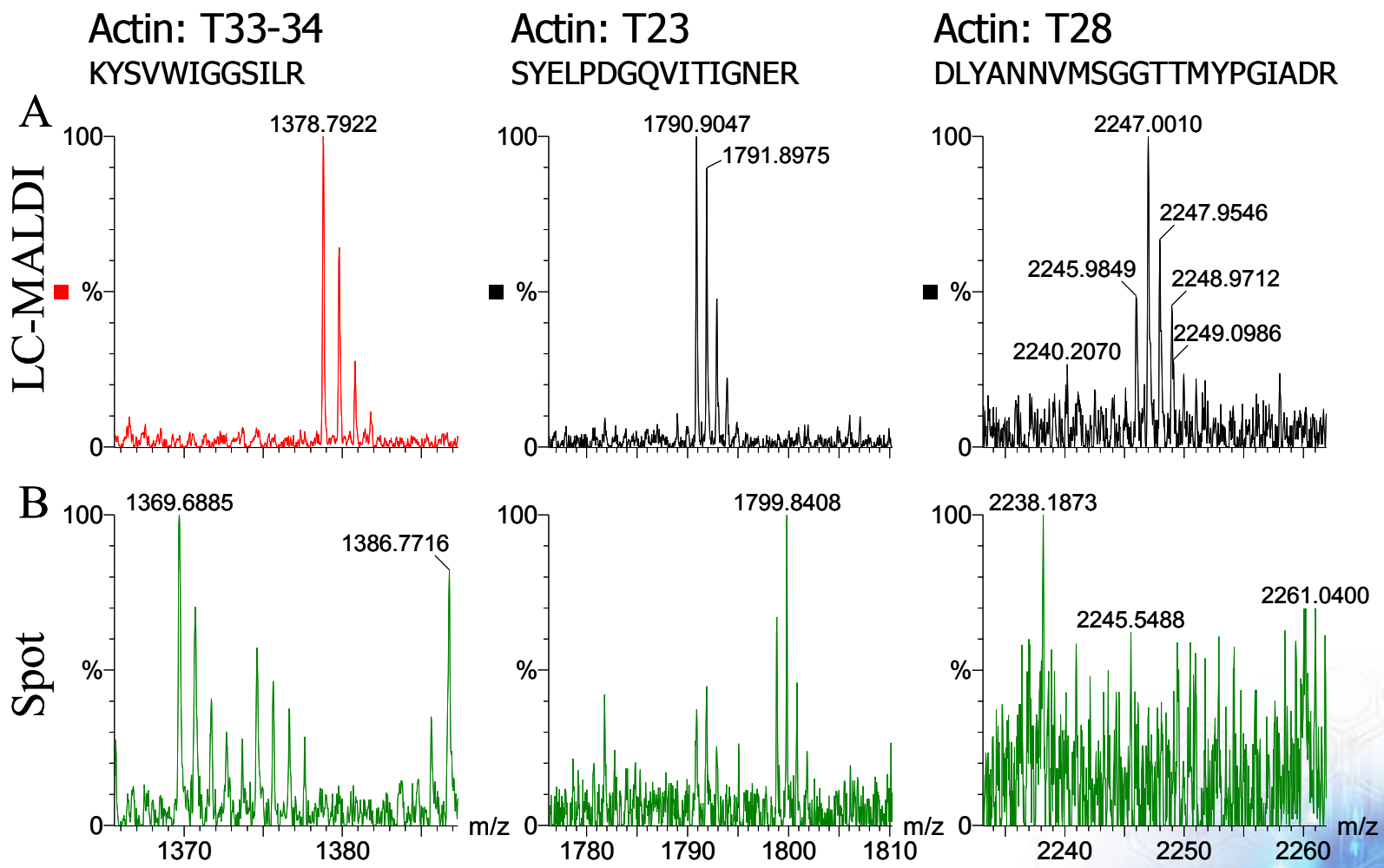
Comparison of Mass Spectral S/N for Tryptic Peptides from Actin (10 fmol) in the Four Protein Digest

LC-MALDIprep versus ZipTip™ and Spot



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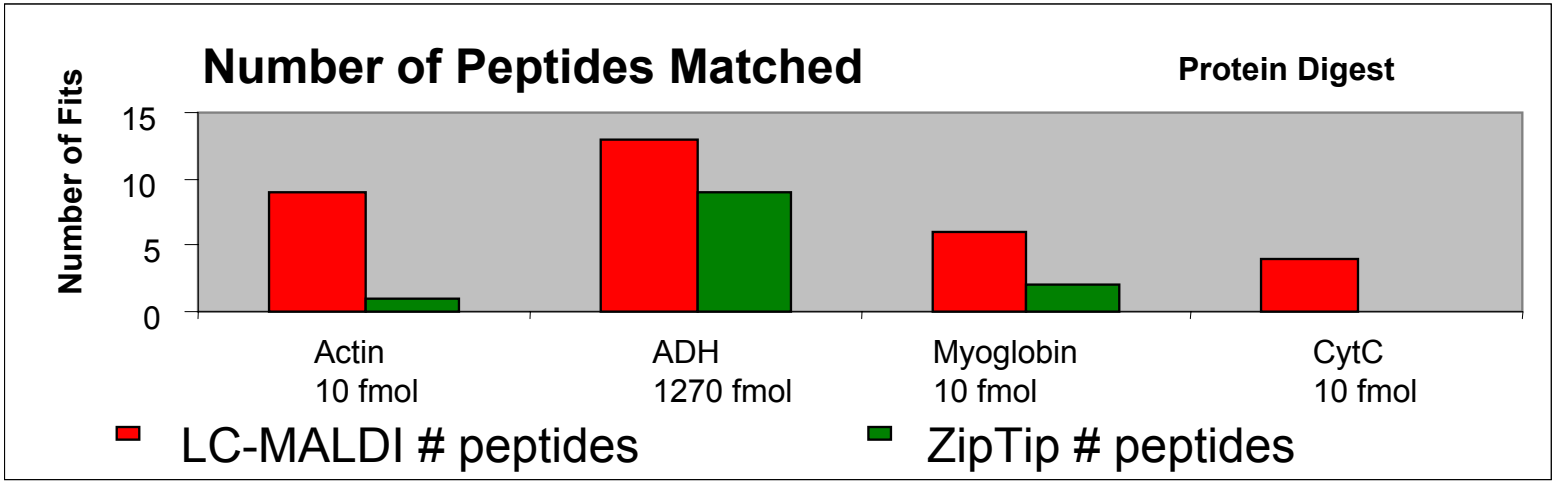
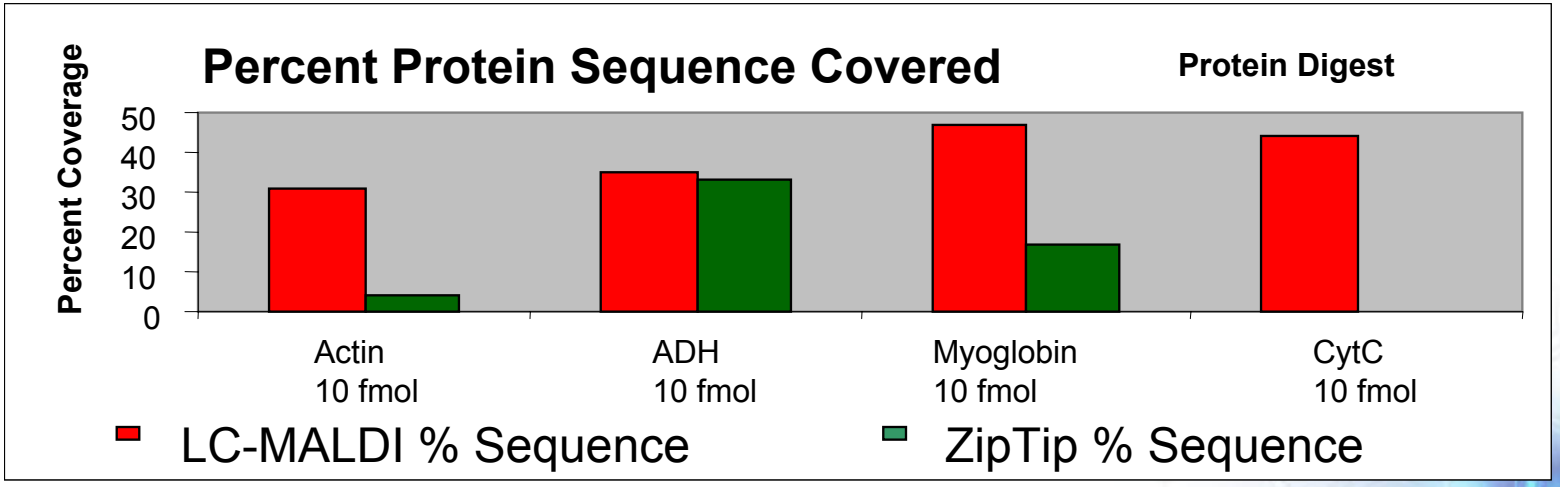
Peptide Mass Mapping of a Four Protein Digest Mixture: LC-MALDI vs. ZipTip ™





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2D in-gel digest of Yeast Cytosolic Proteins

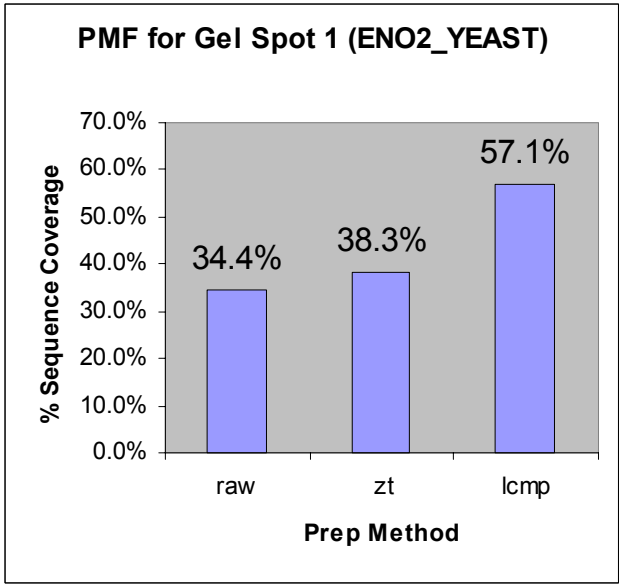
comparison of direct, ZipTip™, and LC-MALDIprep™ analysis

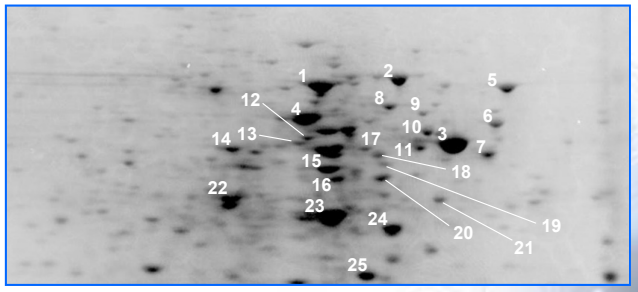
1. Detect and match peptides which are similar in mass

2. Greater number of peptides detected and matched using MALDI LC/MS

ENO2_YEAST

Raw	ZT	LCMP	[M+H]+	Start	End	Sequence	Modifications
	Y	Y	1288.690	346	357	(K)VNQIGTLSESIK(A)	
Y	Y		1373.620	243	254	(K)IGLDCASSEFFK(D)	
Y	Y	Y	1414.788	105	119	(K)LGANAILGVSMAAAR(A)	
		Y	1416.711	15	27	(R)GNPTVEVELTTEK(G)	
Y	Y	Y	1430.760	105	119	(K)LGANAILGVSMAAAR(A)	Oxidation
	Y	Y	1444.656	423	435	(K)AVYAGENFHHGDK(L)	
	Y	Y	1470.664	258	269	(K)YDLDFKNPESDK(S)	
Y	Y		1497.778	126	138	(K)NVPLYQHLADLSK(S)	
Y	Y	Y	1557.724	423	436	(K)AVYAGENFHHGDKL(-)	
Y	Y	Y	1578.770	88	102	(K)AVDDFLLSLDGTANK(S)	
		Y	1600.771	241	254	(K)VKIGLDCASSEFFK(D)	
		Y	1645.900	103	119	(K)SKLGANAILGVSMAAAR(A)	Oxidation
		Y	1669.883	409	422	(K)LNQLLRIEEELGDK(A)	
		Y	1793.905	88	104	(K)AVDDFLLSLDGTANKSK(L)	
Y	Y	Y	1821.922	375	391	(R)SGETEDTFIADLVVGLR(T)	
	Y	Y	1840.893	32	49	(R)SIVPSGASTGVHEALEMR(D)	
Y	Y	Y	1846.848	358	374	(K)AAQDSFAANWGVMVSHR(S)	
Y	Y	Y	1854.967	312	329	(K)TAGIQIVADDLTVTNPAR(I)	
		Y	1856.898	32	49	(R)SIVPSGASTGVHEALEMR(D)	Oxidation
		Y	1925.924	272	287	(K)WLTGVELADMYHSLMK(R)	Oxidation
Y		Y	1960.979	60	78	(K)GVMNAVNNVNNVIAAAFVK(A)	Oxidation
		Y	1972.013	105	125	(K)LGANAILGVSMAAARAAAAEK(N)	Oxidation
		Y	2066.010	272	288	(K)WLTGVELADMYHSLMKR(Y)	Oxidation
		Y	2300.158	28	49	(K)GVFRSIVPSGASTGVHEALEMR(D)	
	Y	Y	2328.098	32	53	(R)SIVPSGASTGVHEALEMRDEDK(S)	
	Y	Y	2344.079	32	53	(R)SIVPSGASTGVHEALEMRDEDK(S)	Oxidation
Y			2828.295	289	311	(R)YPIVSIEDPFAEDDWEAWSHFFK(T)	
Y			2984.432	288	311	(K)RYPIVSIEDPFAEDDWEAWSHFFK(T)	





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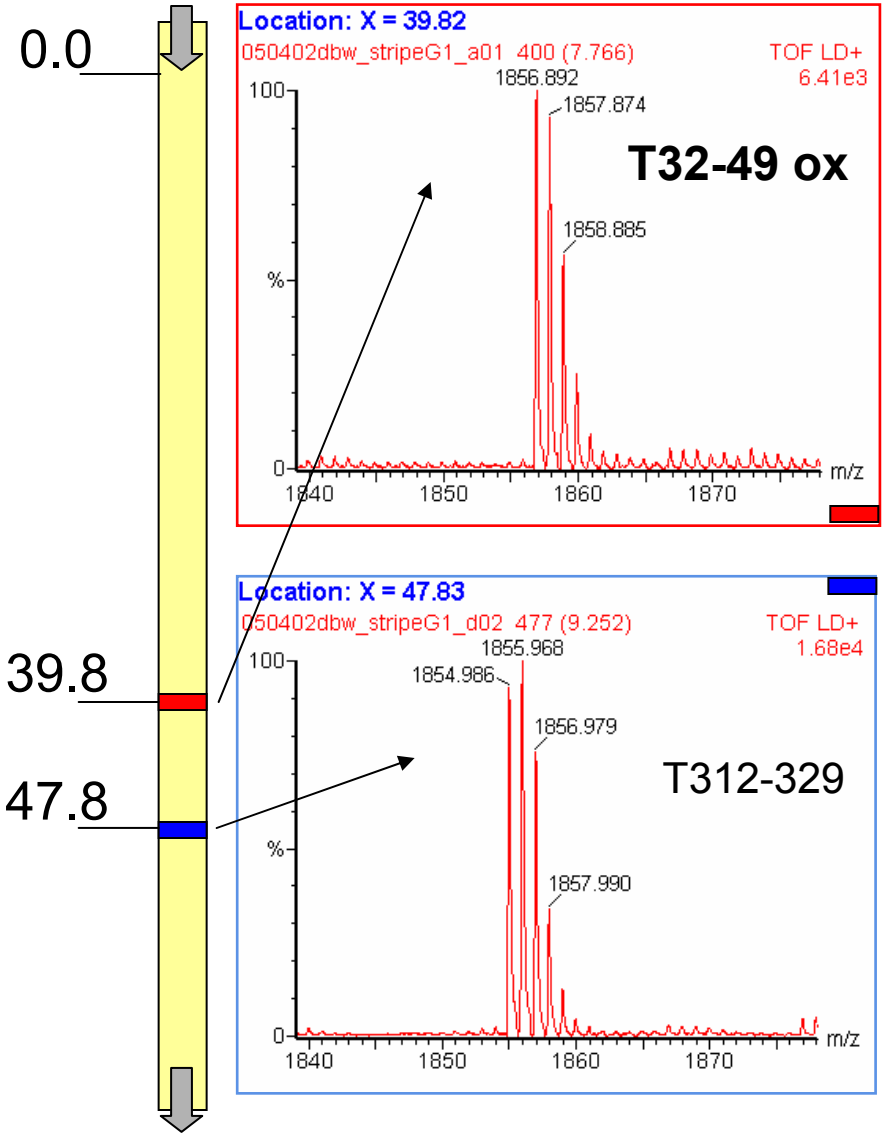
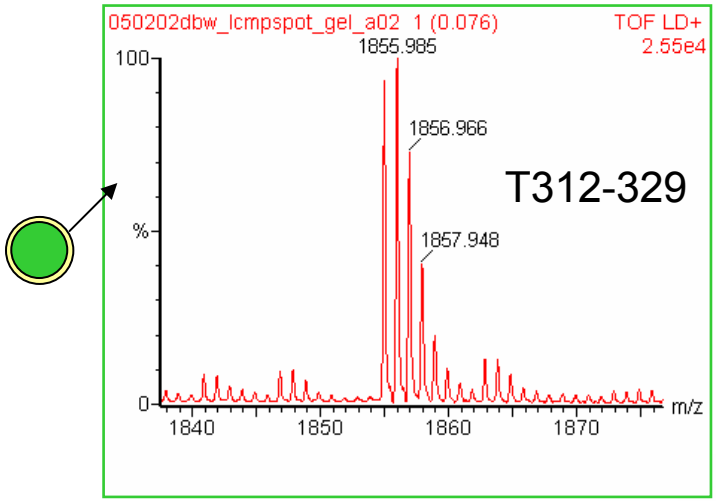
2D in-gel digest of Yeast Cytosolic Proteins

separation & detection of peptides which are similar in mass

Separation: LC-MALDI



Sample Spot: ZipTip



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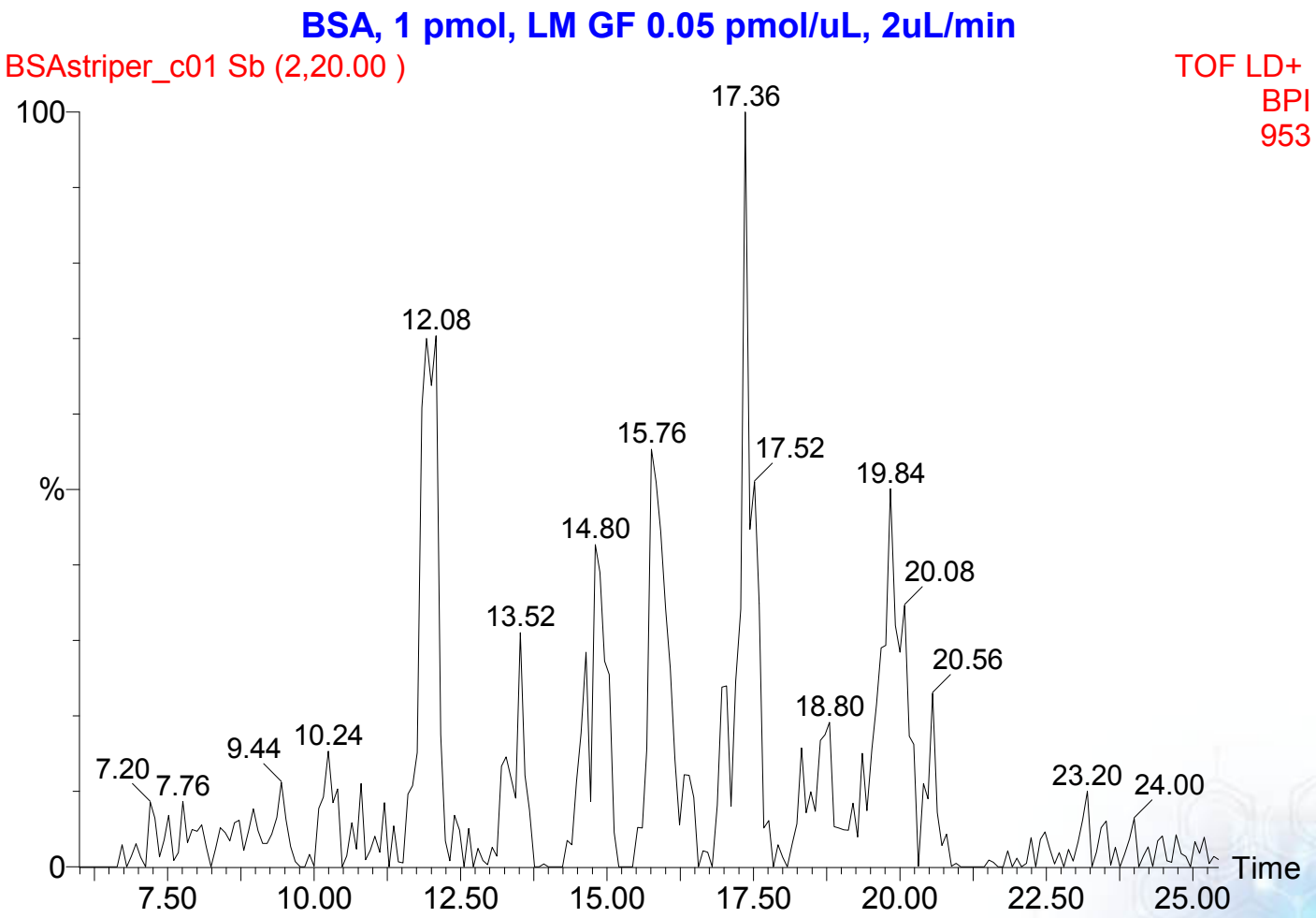
MALDI LC/MS with Internal Lockmass

1 pmol BSA Digest

0.05 pmol/µL glufib lockmass solution co-infused post-column at 2 µL/min



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MALDI LC/MS Results for BSA Digest with Glufib Internal Lockmass

1. 18/34 matches (52%).

Acc. #: P02769 Species: BOVIN Name: Serum albumin precursor (Allergen Bos d 6)

Index: 78059 MW: 69294 Da pI: 5.8

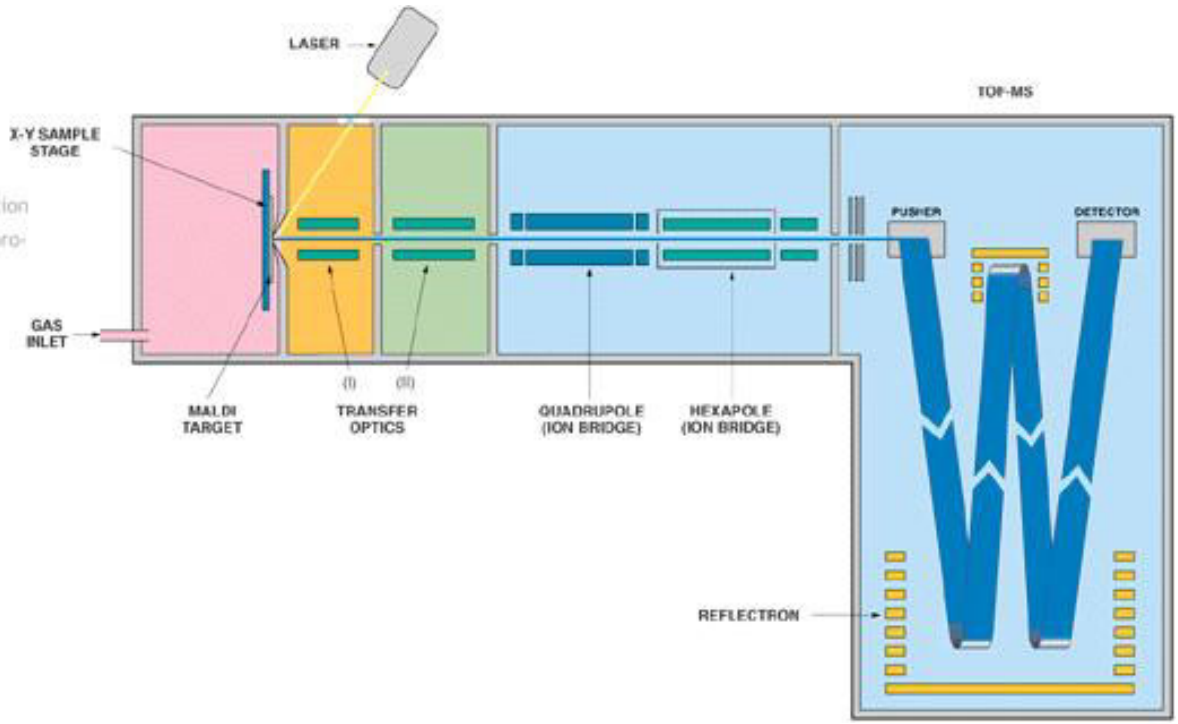
m/z Submitted	MH^+ Matched	Delta ppm	Modifications	Start	End	Missed Cleavages	Database Sequence
1249.6196	1249.6217	-1.7		35	44	1	(R) FKDLGEEHFK (G)
1305.7151	1305.7167	-1.2		402	412	0	(K) HLVDEPQNLIK (Q)
1405.7047	1405.7183	-9.7	1Met-ox	198	209	1	(K)GACLLPKIETMR(E)
1420.6697	1420.6782	-6		89	100	0	(K) SLHTLFGDELCK (V)
1439.8094	1439.8123	-2		360	371	1	(R) RHPEYAVSVLLR (L)
1444.6251	1444.6266	-1		286	297	0	(K) YICDNQDTISSK (L)
1465.5599	1465.5575	1.6		76	88	0	(K) TCVADESHAGCEK (S)
1465.5604	1465.5575	2		76	88	0	(K) TCVADESHAGCEK (S)
1479.7941	1479.7960	-1.3		421	433	0	(K) LGEYGFQNALIVR (Y)
1504.5806	1504.5824	-1.2		375	386	0	(K) EYEATLEECCAK (D)
1534.7512	1534.7497	0.98		298	309	1	(K) LKECCDKPLLEK (S)
1567.7157	1567.7433	-18		347	359	0	(K) DAFLGSFLYEYSR (R)
1639.9407	1639.9383	1.5		437	451	1	(R) KVPQVSTPTLVEVSR (S)
1881.9087	1881.9057	1.6		508	523	0	(R) RPCFSALTPDETYVPK (A)
1908.9009	1908.9053	-2.3		529	544	0	(K) LFTFHADICTLPDTEK (Q)
1930.7540	1930.7509	1.6		581	597	1	(K) CCAADDKEACFAVEGPK (L)
2020.9573	2020.9537	1.8		139	155	1	(K) LKPDPNTLCDEFKADEK (K)
2116.8430	2116.8374	2.6		264	280	1	(K) VHKECCHGDLLECADDR (A)

avg. mass error = 3.2 ppm

Waters

MS/MS with MALDI Q-Tof

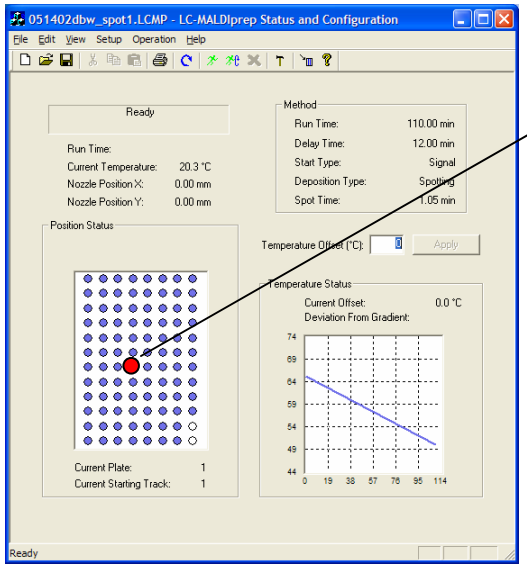
"Q-Tof *Ultima*™ MALDI - the ultimate solution for true MALDI MS-MS in high throughput proteomics."



Waters

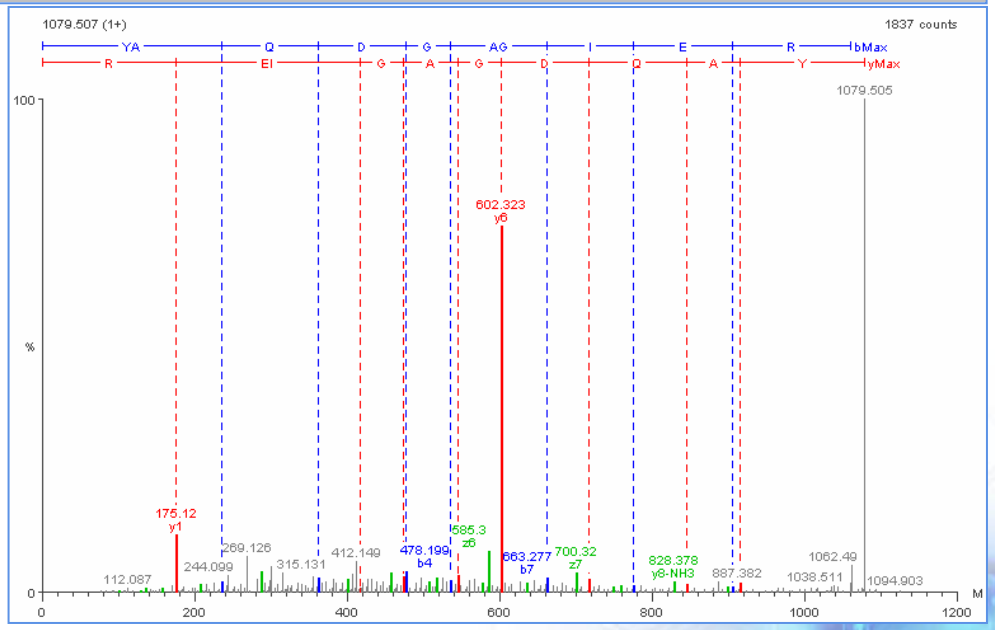
LC-MALDI QTOF Analysis of a Global Digest of Yeast Ribosomal Proteins

Accession number	Score	Match	MW	pI	Description
(P10664)	197.8	1	38942	10.9	60S RIBOSOMAL PROTEIN L4-A (L2A) (RP2)
(P14126)	180.9	1	43608	10.6	60S RIBOSOMAL PROTEIN L3 (YL1) (RP1) (TR



Query	m/z	Charge	Delta	Score	YAQDGAGIER
1	1079.507	1	-0.005	180.9	

Accession number	Score	Match	MW	pI	Description
(P14126)	180.9	1	43608	10.6	60S RIBOSOMAL PROTEIN L3 (YL1) (RP1) (TR



- Analyze complex digest
- Collect separation to 94 fractions
- Consume target protein only

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Stability of Analytes Deposited onto Matrix Precoated Targets
Experiment Conditions

- Mixture of angiotensin I (100 fmol/μL), renin substrate (10 fmol/μL), and ACTH (1 pmol/μL) in 30% acetonitrile with 0.1% TFA
- Infused at 10 μL/min, deposited onto tracks with stage speed =10 mm/min
- Drying gas temperature = 55 °C
- 1.6 mm segment of track analyzed at various time points
- Target stored in dark
- Three different storage condtions
  - 4 °C in desiccator
  - Room temperature in desiccator
  - Room temperature

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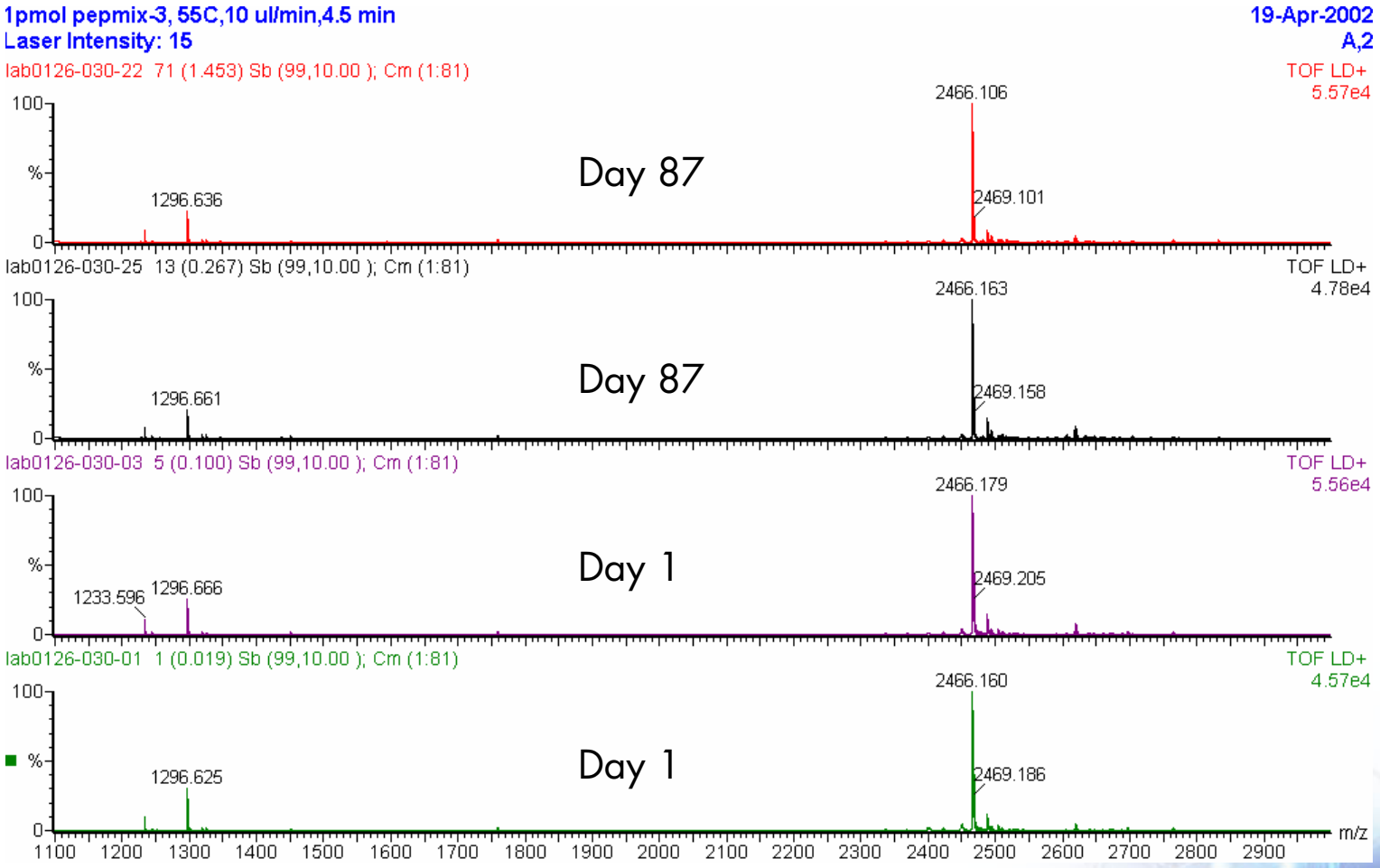
Analyte Stability in Matrix Over Time Acquired at Day 1 and Day 87

Target stored at 4 °C in desiccator



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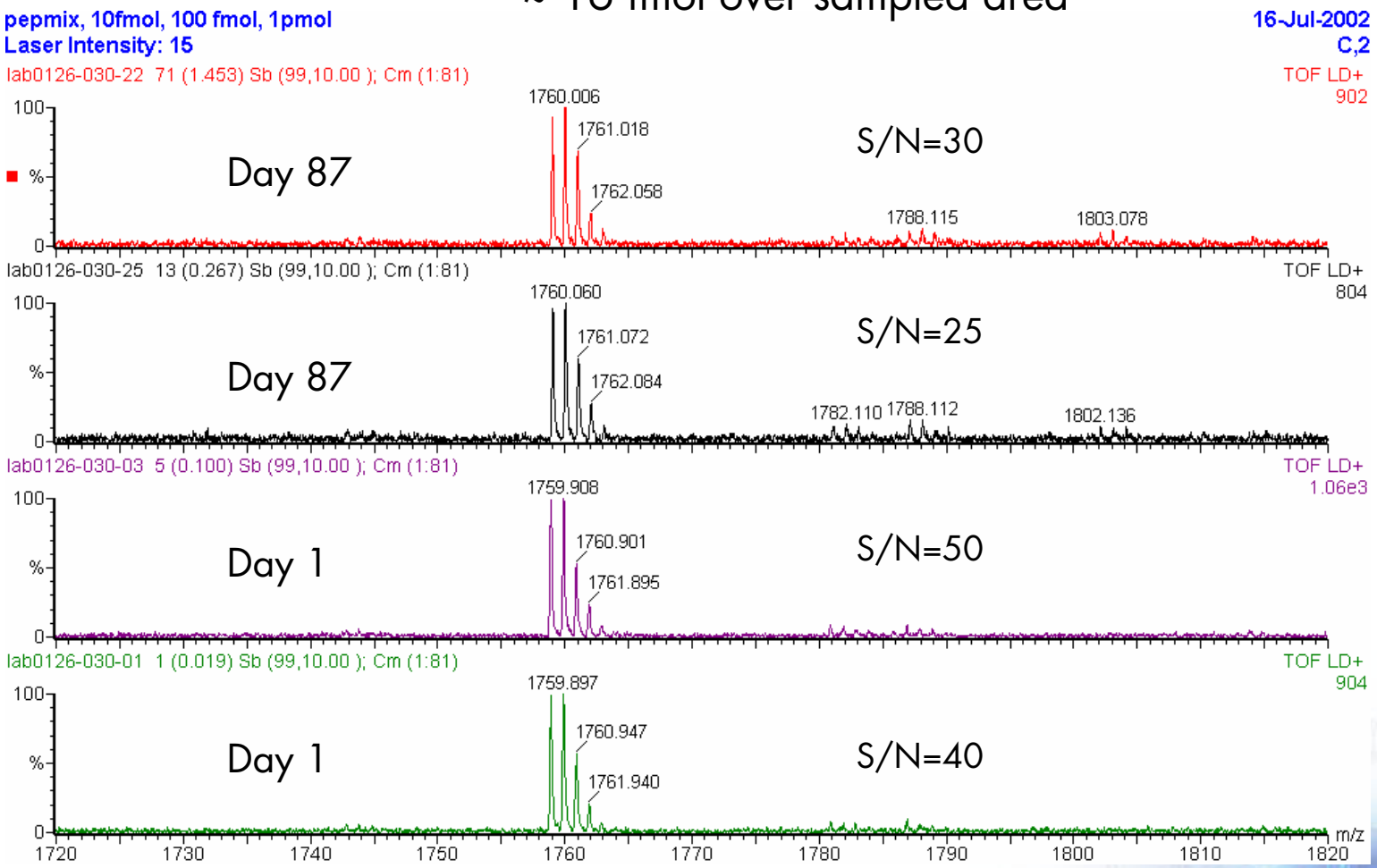
Analyte Stability in Matrix Over Time
Signal-to-noise Ratios for Renin Substrate (10fmol/µL)

Target stored at 4 °C in desiccator

~ 16 fmol over sampled area



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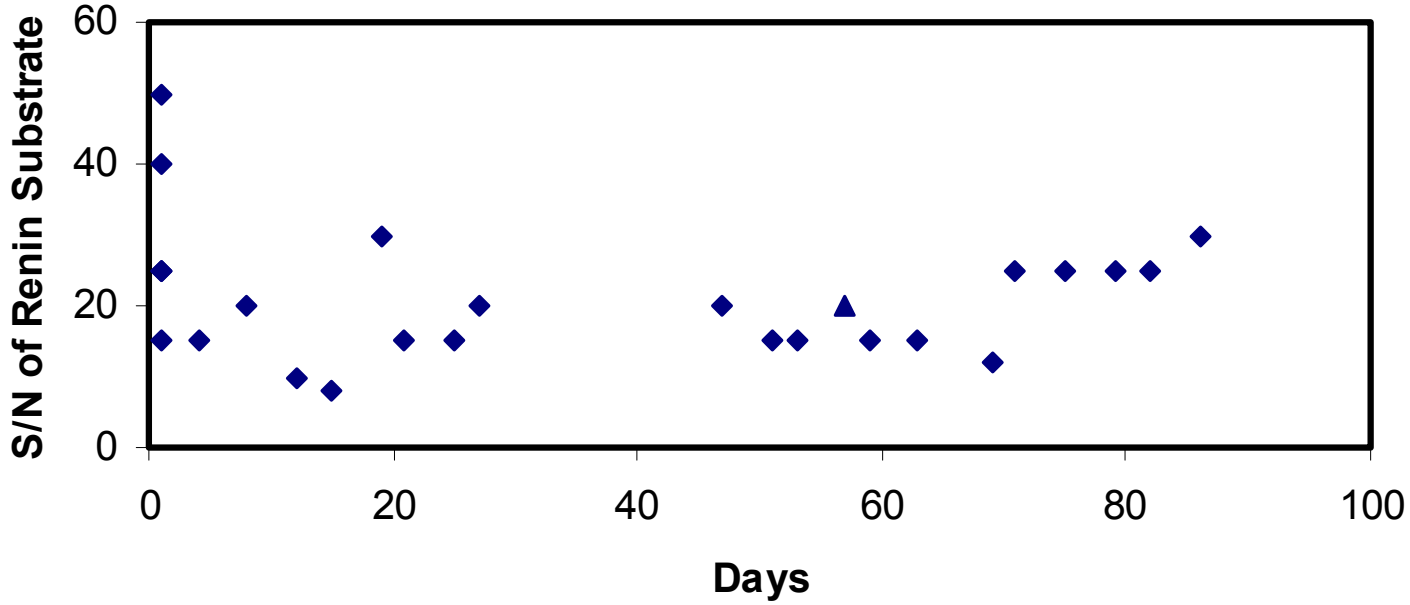
Analyte Stability in Matrix Over Time

S/N of Renin Substrate



Target stored at 4 °C in desiccator

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LC-MALDI Summary

- Collection of analyte from wide range of flow rates: capillary and micro bore (5 to 40 uL/min)
- Robust
- Low femtomole sensitivity
- LC-MALDI reduces ion suppression effects and interference between peptides of similar m/z
- Improved peptide mass fingerprinting results versus ZipTip™ and spot method
- LC separation significantly increases the numbers of peptides sequenced by MALDI MS/MS (QTOF, TOF/TOF, API-MALDI Ion Trap)
- Stable, long-term storage of collected separations that can be analyzed months later
- Work is in progress for interfacing to nanoLC columns

Waters

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- Jeremy Batt

Advance Separations

- Bruce Compton

Chemistry Operations

- Bruce Smith

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Thank you!