Mass Spectrometry Based Proteomics Professor Sanjeeva Srivastava Department of Biosciences and Bioengineering Indian Institute of Technology, Bombay Mod 02 Lecture Number 10

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Topics to be Discussed Today:

- # Basics of MALDI-TOF MS
- # An overview of typical Proteomics experiments
- # MALDI Experiment Sample Preparation
- # MALDI-TOF Instrumentation
- # Data Analysis for Protein Identification

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Welcome to the proteomics course, today we will talk about ...

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Matrix Assisted Laser Desorption Ionization Time Of Flight MALDI TOF.

In previous lecture, we talked about basics of mass spectrometry, the various combinations of mass analyzers and ionization sources. Now it is time for us to combine those and start discussing these in more detail.

So, today let us focus on the MALDI TOF which is one of the very widely used techniques in proteomics. This provides a high throughput platform for several applications, including molecular weight determination, protein identification as well as post translational modification studies.

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Let us first start about basics of MALDI-TOF. So, MALDI is an efficient process for generating gas phase ions of peptides and proteins for mass spectrometric detection. MALDI is one of the most widely used ionization technique, currently applicable in the proteomics area.

This ionization method was independently developed by two scientist Koichi Tanaka and Hillenkamp. Tanaka also received the Nobel Prize for his novel contribution into soft ionization technique such as MALDI.

So, let us go through the some of the basic concepts involved in the MALDI TOF. We can split that in two parts one is MALDI which is ionization source another is TOF which is a mass analyzer

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Let's first talk about Matrix Assisted Laser Desorption Ionization or MALDI. So analyte or the proteins of interest are mixed with matrix which is usually an aromatic compound.

There are various types of matrices available, which we talk about in more detail when we come to the sample preparation and matrix selection. But just for your reference we can use 2-5-dihydroxy benzoic acid, we can use sinipinic acid and there are several other choice.



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Once you have selected a matrix for the experiment then analyte and matrix can be dissolved in an organic solvent after which then it can be placed on a metallic target. As you can see in the slide, the first left section shows you how to place the analyte and matrix together on the sample plate. Now, once you have placed the matrix and the analyte on the target plate you can put that in the vacuum chamber and apply high voltage.

Now these crystals are targeted with short laser beams, as you can see in the slide, then rapid sublimation can convert analyte into gas phase ions.

Now these ions once generated, they can accelerate away from the target plate through the mass analyzer which is Time of Flight TOF tube and they can reach towards the detector.

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Section II

An overview of typical Proteomics experiments

Involving 2DE Gel & MALDI-TOF MS

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Sample preparation

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... and spotting

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The protein sample must be prepared suitably before it can be analyzed by mass the spectrometer.

If you have run the 2D Gel, first of all, the purified protein of interest need to be excised from the gel on which it has been electrophoresed and dissolved in a suitable buffer.

Depending upon the application, if you have purified a protein, you can separate that on the gel and cut that band. Or if you have a mixture of the proteins in 2D gel, you can just excise that particular spot.

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Spot can be dissolved ...

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... in the suitable buffer

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Trypsin is then added to this mixture in order to carry out...

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...digestion of the protein

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Trypsin cleaves the protein at the C-terminal of its arginine and lysine residues. But that is not always universal. If you have a proline present immediately after ...



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... then it will hinder that.

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But overall, the protein is

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... digested into smaller fragments ...

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... of manageable size.

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Once the protein sample has been digested, all the salt, buffers and any detergents must be removed from the sample. After doing in-gel digestion...



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...and before proceeding for the mass spectrometry analysis, in between an efficient step is to use some filters...

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... or ZipTip which can eliminate some of these contaminants and salts. It offers several advantages such as quick verification, sample enrichment and ensures that there is no contamination.

So there are multiple advantages of using ZipTips. However it can purify only limited volume of the sample and also it adsorbs some amount of protein sample thereby leading to losses.



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The purified protein sample can be mixed with an aromatic matrix compound such as ...



... alpha-cyano-4-Hydroxycinnamic acid or Sinapinic acid in the presence of an organic solvent.

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The components are mixed thoroughly.

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And then the solution containing the organic matrix with the embedded analyte of interest can be spotted on to a metallic MALDI sample plate.

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MALDI gives you an opportunity to analyze large number of samples ...



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... in a high-throughput fashion.

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The target plate containing the spotted matrix and analyte can be further placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy ...

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... gets absorbed by the matrix and is transferred to the analyte molecules ...



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... which undergo rapid sublimation resulting in gas phase ions

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The gas phase ions generated are accelerated and travel through the flight tube at different rates.

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The lighter ion moves rapidly ...

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... and reaches the detector first while the heavier ions ...

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...migrate slowly. These ions are resolved and detected ...

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...on the basis of their mass to charge ratio and a mass spectrum is generated.

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Parameters such as geometric design....

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... power supply quality, calibration method, sample morphology, ion beam velocity etc. all of these factors affect the accuracy of mass detection.

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So now you know how to perform the cleaning step by using ZipTips. Now you have the sample ready and you have selected the matrix. So now let me show you these various steps involved before you can actually start the MALDI experiment

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So, you need to select the matrix, you need to prepare the matrix. You have already done the sample purification.

Now sample need to be deposited on the MALDI plate. Either you can mix it with matrix or you can do this separately. There are various combinations one can try and then once both sample and matrix are deposited on the MALDI target plate then you are ready to do the drying and then plate can be used for MALDI-TOF uh instrument for further analysis.

Let's first talk about matrix selection. So the important step in MALDI-TOF analysis is the selection of appropriate matrix for the sample. The matrix selection mostly depends on the molecular weight of the target to be analyzed and often the type of application which you intend to do by using these instruments.

So these matrices are low molecular weight organic compounds with low vapor pressure and volatile nature. Most of the matrices are acidic in nature so it can easily excite the photon and ionize analyte for the analysis.

However there are few basic matrices are also available.

In the slides I am giving you an overview of few matrices and some of their properties. But there are many more properties which is not mentioned here. But just to give you certain major features of these matrices commonly used for various applications.



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So, the one is alpha-cyano-4- hydroycinnamic acid. When you have peptides less than 5000 Daltons or lipids and nucleic acids one can use this matrix. One can also use Sinapinic acid if peptides and proteins are having more than 5000 Daltons and it can also be used sometimes for the lipids.

Then you have options such as 2-5-dihydroxybenzoic acid also known as DHB ...

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...small molecules and peptides which are not ionized by the other molecules can be analyzed by using this matrix. TriHydroxyAcetoPhenone, THAP, this is used for small nucleotides and also used for phosphorylation and specialized applications.

Then we have picolinic acid which is generally used for the nucleotides. So these are only few representative matrices. As you can see there are many options available for selecting the matrix depending on the molecular weight and the type of applications.

But regardless of this, these matrices absorb energy from the laser source and converts both matrix and analyte into the gaseous phase. Matrix can also analyze analyte molecule by providing energy which comes from the laser bombardment.

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Now once you have selected the matrix, matrix can be prepared by mixing it into a suitable solvent and vortex it for few minutes so that it can dissolve properly.

Now you are ready with both, your analyte, the protein which you want to analyze as well as the matrix which we have selected for your application.

Now, one needs to think how to deposit sample on the MALDI target plate. So there are many ways of deposition of sample and matrix onto the MALDI plate.

Mostly sample and matrix are mixed in an eppendorf tube and then mixture is directly deposited by using a micropipette onto the MALDI plate.

But one can also try various combinations. In one approach the sample is first deposited to the MALDI plate followed by the matrices deposited above it and then it is properly mixed before drying process can happen.

Other way of doing it is to apply that with the sandwich-based method. In which a small amount of matrix is deposited on the plate, then you add the protein sample and again the matrix is spotted on top of it so that you have enough matrix in the below and above of the analyte.

So one can try different combinations of placing the matrix and the analyte. And then once you have placed all of this sample of interest on MALDI plate then you are ready to dry the target plate.

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After spotting is done and the MALDI plate is dried almost 30 minutes, then the instrument can be turned on and MS analysis can be performed.

Now there are various types of configurations of these instruments available as well as there are various types of commercial software which help to operate the hardware.

It's not possible to go into individual detail but I am going to show you the generic steps in the following video of MALDI-TOF instrumentation

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How to perform analysis of in-gel digested samples using MALDI?

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MALDI-TOF Instrument Operation (Refer Slide Time 16:33)

After in-gel proteolytic digestion and Zip Tipping, the sample is further subjected to mass spectrometric analysis. Here we are depicting the MALDI-TOF analysis of tryptic digested proteins

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MALDI is performed in two steps. In first step, the compound for the analysis should be dissolved in a solvent containing small organic molecules, known as matrix.

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This mixture is dried before analysis and liquid solvent used in the preparation of the solution is removed.

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So in this video by depicting the matrix preparation as well as instrumentation I will try to give you the overview of MALDI-TOF instrumentation.

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Spot the mixture on the MALDI plate. How uniformly you can plate these mixtures on the MALDI plate ensures your good spectra and data quality later on

Completed on the MALDI plate

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the samples are allowed to dry for 30 minutes, after which the instrument is switched on and the MS analysis can be performed.

While these steps are happening you need to ensure

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So click on the software and ...
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... open the acquisition window and then click on "open door".

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Insert MALDI target plate ...

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... face up with the cut-off corner to the front.

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Insert MALDI plate		

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... peptide spectrum is generated.

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Now we have shown here ...

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...a spectra for the Pepmix

So now you are clear with how to perform the MALDI TOF experiment. Now let us add one more mass analyzer. So now we have a configuration of MALDI TOF-TOF.



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So MALDI can be coupled to the tandem Time of Flight in combination with another Time of Flight, so TOF-TOF or with hybrid Quadrapole Time of Flight analyzers which are separated by the collision cells.

Now for proteomic application, it is recommended to use the TOF-TOF or Q-TOF. The peptide ions are accelerated through the first Time of Flight tube as you can see in this slide and then they are dissociated by introducing an inert gas into the Collision cell.

This process allows collision induced dissociation spectra from the MALDI produced from the precursor ions. Now these hybrid configurations are more sensitive than the triple quad and the single Time of Flight.

So the combination of TOF-TOF allows the protein identification through the peptide mass fingerprinting and high throughput analysis of the protein or proteome is possible with the hybrid TOF analyzers.

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Open the Matrix Science browser window to carry out online data analysis.

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... to a particular species or a group of species to which the sample belongs. When you are not sure of organism select all entries.

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(Refer Slide Time 23:19)

Depending upon user needs the parameters can be changed. Protein mass is the mass in that protein and is optional.

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Henry Hand State			Click on 10
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Report top 10 hits.

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(Refer Slide Time 23:51)

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... and paste in the selected box for Mascot search.

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Click on search.

(Refer Slide Time 24:02)

Data Output	

Data output

(Refer Slide Time 24:06)



The output can be seen in 3 sections. In section 1 the summary of spec parameters defined by user

(Refer Slide Time 24:14)

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Section 2 Mascot's co-histogram the number of proteins with score is plotted along the graph

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Section 3 summary report in which the matched protein from the database with the details of important parameters are displayed either in concise format protein format and the data can be exported too.

(Refer Slide Time 24:38)



Data Analysis

(Refer Slide Time 24:43)



If the search parameters are not the best fit, the software generates the error message. Depending on the error message the user needs to change the parameter setting and do the search again

(Refer Slide Time 24:54)



In section 2 the Mascot score histogram the number of protein hits and their score is displayed along the graph

(Refer Slide Time 25:03)

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Protein view section displays matching of the query peptide to the protein sequence in the database. The sequence type, the matched region, what is the expected and the calculated value of the query peptide and the sequence details

(Refer Slide Time 25:17)

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Click on Address Bar		

For better protein identification and to increase protein score CID of each peak generated ...

(Refer Slide Time 25:23)



... is carried out to generate MS/MS data

(Refer Slide Time 25:27)

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For such data analysis MS ion search option is selected from the Matrix Science browser window

(Refer Slide Time 25:42)

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In MS/MS search tool more input parameters like quantitation, MS/MS tolerance, peptide charge instrument etc in addition to fields for PMF and rest other parameters are similar to that of the peptide mass fingerprint

Depending upon the process carried out for data generation a selection in the quantitation must be made. In case of label--free quantitation ...

MASCOT	MS/MS lons Sear	ch	
Your name	pater parker	Ernal	vipformula1@gmail.com
Search title			
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...select none in quantitation tab

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MASCOT	MS/MS Ions Search			
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Remaining parameters remain same as PMF. Browse the MS/MS raw data file and ...

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...search the results using Mascot

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...that has been used to generate the raw data. When we don't know the name of the instrument select default.

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The result output generated is almost similar to PMF output.

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The accession ID indicates the protein information obtained from the database marked as green

(Refer Slide Time 26:37)



The protein hit outside the green box indicates the p value less than 0.05 which is statistically significant....

(Refer Slide Time 26:45)



... where as hits inside the green box indicates random matching

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Mascot search Results

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The score the peptide score is the sum of highest ion scores for each sequence with duplicate matches being excluded.
(Refer Slide Time 27:03)

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Calculated pI value, predicted isoelectric point of the protein

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ensi Aan (Wr) 1884); (atculated pi value: A.BF
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A A A A A A A A A A A A A A A A A A A
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VERSION PRODUCT PROTEMES ALCORPOL AND THE
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Sequence coverage indicates the percent of matching peptide

(Refer Slide Time 27:24)

Mascot search results	
Probably show white the p12/9304 have 105 bismess currents for install (1990) (plane support) France is served at 1.0 bismediatent/dataget(12) (1, 4) 40 pers surgers hav note hits- and france free and	
Nonmart nam (Mrs. 1994). Lainument provins 4 Alf Mille Kull North of <u>a 1971/2017</u> appret at Web-social <u>assumption (1970)</u> for participation about applications.	
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Food madifications: Londonialmenthy ICL Younder matifications: Automation (R) Clansraph by Property Automatic Linear allow of AR weblan, space matification in P Engineers: Converge 1-10.	
All peptides are deployed with matching peptides because of ref. or 05/25/2011 F1020742 California or 05/25/2012 F1020742 California	

All the peptides are displayed with matching peptides indicated in red

(Refer Slide Time 27:32)

Mascot	search res	sults				
Pristan	~					
Show	preticing pe	utides also				
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Sect.P	epiides By	* tesidar	Number 1	t tecres	and a	an E Decreasing Mass
Start - End	Observed	Hereset)	Mitala	Deita		Sequence
111-119		111.174	1918.9797	11		K. WANGGLOCVPPELLAR N Deidetion (N) (1000 Scott 113)
147 154	491.7200	141.4254	182.4913	47		A FEGAVATE & CONSIGNER AL
147 - 154	492.7765	961,446.4	982,8913	-44	0	R. FGEAVWER A CIRCLICOLE ADD
147 154	497.2348	962,4551	982,4913	37	- 21	K.FGEAVWER.A (host score 11)
241 238	1005.4435	2008.8955	2007,6133	100	6	R. LANFINFORTUGAVIOR & Deletion (M) Clark score 14
141. 114	474 2984	2023 6743	2025.0104	427	6	R. LAMPENFOFFVCAR/TON C 2 Oxidation (N) class score 27

The protein view obtained on selecting the particular protein link is very similar to protein view observed in PMF. It provides detailed information

(Refer Slide Time 27:41)

Pre	Relis vi	earch rei ew	unts				
1	90 m p	redicted pe	ptides also				
Inde	ales De	qianing & o	nd of each				
pept	-	_	_	under 1			un o betreened were
(Ing) IN	Observed	Hr(expl)	Writelia)	Delta	#155	Sequence
111	14	-	1918.8748	1918,9797	-11	1	R. MANEGALGEVPPELLAR N. Oxidation (N) (Lans scate 112)
142	141	482.2200	1918.9029	1918,9797	42		E. WAMEGALGCYPPELLAS. III. OxMARIAN (W) (1993 NERVE 41)
147 -	154	492.2305	982.4464	982.6913	-4	0	K.FGEAVWER.A CLOSE SCACE AD
107	154	492.2348	102.4551	982.4913	17		K.FGEAVWER.A (http://www.31)
241	438	670.6393	2018.8755	2009.0155	11	-	R. LANFINFOFFYGANTOR, C. Deldation (R) (1918 SLOTE 12) 8 LANFINFOFFYGANTOR, C. Deldation (R) (1919 SLOTE 13)
	114	474 2484	2023.8743	2075.0104	422		R. CAMPENFORTWOATVTOK C 2 Outdation (N) Consistence 22)

... about each of the matched peptide displayed The start ...

(Refer Slide Time 27:46)

Masco	ot search re	sults				
0	ow predicted pe	optides also				
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٠.	Otherved males	ny neity	rber	n morea	ting a	ass @ Decreasing Mass
Star a	and the second	Mrieupti	Wiald	Delta	-	Sequence
194.1	-	1918.8746	1918.9797	35		R. WANLGALGCUPPELLAR.W. Oxidation (W) times access 1120
126 1	12 960.4587	1914,9029	1918.9797		- 81	E. WANEGALGENTPELLAR. N. Oxidation (M) (inclusion All)
147 1	4 492.2200	982, 4234	982.4913	1		R. FGLAVNER, A COMMENCE MAD
10.1	497.1148	887.4551	982.4912	17	1	E.F.C.LAVWER.A. Cont. State 101
241-2	470.4795	2008.8354	2009.0155	.19	0	R.LAMPINFOFFVOAIVIGE.C Oxidation (M) (Iors score 42)
241 2	18 1009 46.15	2068,9124	2009.0155	-81	9	E LANFENFEFFVQARVTER C Oxidation (M) (into actors 35)
241 . 21	E 676.2985	2025.8741	2015.0104	427	0	R. LAMFLMFCFFVCA/VICK. G 2 Oxidation (M) Contributing 22

... and end position of amino acids ...

(Refer Slide Time 27:50)

Protein view										
She	predicted pe	ptides also								
Sort Peptides By		***	ruisted mole	e ular se	+(491)	D Decreasing Mass				
			~							
Start - Er	d Observed	Mr(expt)	MICIO	Delta	Miss	Sequence				
126 . 142	960.4445	1918.8746	1912/0797	-55	2	R. WANLGALGCVFPELLAR.N Oxidation (M) (Lana acure 113)				
126 - 142	960.4587	987,4254	982,4913	-00		R. WARLGALGCYPPELLAR. R. Oxidation (M) (1011 Score 44)				
147 - 154	492.2305	982.4464	982.4913	-44	0	K.FGEAVWER.A CONSIGNER HOP				
147 154	492.2348	982,4551	982,4913	-17	2	K.FGEAVWER.A (1001 SCORE 32)				
241 - 234	670.6393	2008.8955	2009.0133	37		R.LANFONFOFFYGANTOK C Oxidation (M) (Jons score 12) 8.1ANFONFOFFYGANTOK C Oxidation (M) (Jons score 15)				
241 - 234	474.2986	2025.4741	2023.0104	427	0	R. LAMPIMPOPPULAIVIGE. C 2 Oxidation (R) Itons score 22				

... calculated and ...

(Refer Slide Time 27:51)

		search res	sults				
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1	how	predicted pe	utides also	1			
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		Cop.	erimental m	olecular well	(M. 14	sing m	ass © Decreasing Mass
		_			_		
				Manhaman Sarah 1	Bulks	Mitt	Laborate
Start	End	Observed	Mr(expl)	WY(CBIC)	PELLA		Preprinte
Start 126 -	End 142	Cheerved 950-1445	Metenpt3	MY(CASC)	-55	0	R. WAMLGALGCVFPELLAR.N. Oxidation (M) (jump peors 112)
Start 126 - 126 -	End 142 142	Cheerved 967-005 960-0587	Mr(expl) 1918.8746 1918.9029	MY(CASC) 1918,9797 1918,9797	-55	0	R. WANEGALGCVFPFLLAR.N. Oxidation (N) (1003 access 112) B. WANEGALGCVFPFLLAR.N. Oxidation (N) (1003 access 112)
Start 126 - 126 -	End 142 142 154	Cheerved 960.4445 960.4587 492.2200	Meteopeta 1918.8746 1918.9029 982.4254	M/(CASC) 1918.9797 1918.9797 982.4913	-55 -40 -47	000	R. WANI, GALGCVPP(LLAR.N. Oxidation (N) (<u>Loss access 112</u>) R. WANI, GALGCVPP(LLAR.N. Oxidation (N) (<u>Loss access 112</u>) R. FGEAVWFR, A. (<u>Loss access 64</u>)
Start 124 - 126 - 147 -	End 142 142 154	060-4446 960-4465 960-4587 492-2200 492-2305	Mr(expl) 1918.8746 1918.9029 987.4254 982.4464 982.4464	M/(CAIC) 1918.9797 1918.9797 982.4913 982.4913	-55 -40 -47 -46	00000	R WARLOAGCVIPPELLAR N. Oxidation (N) (<u>Intersect 111</u>) R WARLOALCVIPPELLAR N. Oxidation (N) (<u>Intersect 111</u>) R JGLAVNTRA (<u>Intersect</u> 651 R JGLAVNTRA (<u>Intersect</u> 651 R JGLAVNTRA (<u>Intersect</u> 651
Start 126- 147 147 147	End 142 142 154	Observed 960:4446 960:4587 492.2200 492.2305 492.2348	Mr(expt) 1918.8746 1918.9029 987.4254 982.4464 982.4551 7008.551	MY(CBIC) 1918,4797 1918,4797 982,4913 982,4913 982,4913	-55 -40 -47 -46 -17	000000	REWARD GALECYPPELLAR IN Oxidation (M) (JUNE JUNE 112) START GALECYPPELLAR IN Oxidation (M) (JUNE JUNE 45) R.FGLAVWER A (JUNE 35) R.FGLAVWER A (JUNE 35) R.FGLAVER A (J
Start 126- 147- 147- 147- 147- 147- 147- 147-	End 142 142 154 154 154	000000000 960-4646 960-4587 492.2200 492.2305 492.2345 870.6395	Mrtexpt) 1918.8746 1918.9029 987.4254 982.4854 982.4551 2008.8966 2008.8966	MY(CBIC) 1918.0797 1918.0797 982.4913 982.4913 982.4913 2009.0155	-55 -40 -47 -46 -17 -59	0000000	AVANI, GALGOVIPPI, LAR. N. Oxidation (N) (Inter score 110) 8. WARI, GALGOVIPPI, LAR. N. Oxidation (N) (Inter score 110) 8. WARI, GALGOVIPPI, LAR. N. Oxidation (N) (Inter score 110) 8. VARIANTIA - Origin score (S) 8. VARIANTIA -

... experimental molecular weights...

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Protein	WH .					
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Start - End	Garned	Wriespij		Delta	6	leasence
176 . 147		1111.4744	1918,9797	-15	~	B. WARLGALOCVEPTLAN. IN Oxidation (N) Cana Marr. 1127
147 - 154	492.2200	162.4254	182,4913	42		K. WARE CALCE VIPTLAN W CONDITION (W) HIPL ROPE AL)
147 - 154	417.7305	887.0464	982,4913	-11	-	K. TGERVWYK, A COMMANNER, SDA
10-258	470.4295	2008.8966	2009.0135	-39		R. LANFENFOFFYGANVICE. C. Deidetten (M) (Jans BLace, 42)
241 258	5005.4435	2068.9134	2009.0155	-11	8	R. LAWFUNFOFFVOARVIOR G. 2 Oxfation (N) (Inter score 3)

... number of missed tryptic cleavages,

(Refer Slide Time 27:56)

Pret	gin vi	earch ret e#	ults				
12	ton p	redicted pe	ptides also				
2	ort Pr	utides By	* tesidue	Number 1	0 Increa	ting pe	an © Decreasing Mass
Start	End	Observed	Hr(expt)	#rialo	Detta	Miss	(and the second s
126	142	100.4445	1918.8748	1918,9797	-33	1	K. WAR GALGEVFFELLAR N. Oxidation (N) flore score 113) 8. WARGALGEVFFELLAR N. Oxidation (N) flore score 431
147	154	492.2200	942,4254	982,4913	-87	0	K.FGEAVWER.A COME MARY BAD
147 -	134	497.7305	982.4464	982,4913		- 1	K.FGLAVWER.A CONLACKE.SD
101 -	154	670.6395	2008.8964	2009.0135	-59		R. LAMPENFORTYQUIVIGE.C. Oxidation (M) (See acars (D)
244	14.8	1005 4435	2068.9134	2009.0135	-55	Ψ.	R. LANFINFOFFVQARVTOR. G. Oxidation (M) (Inco score 35)

... sequence of each peptide segment and their corresponding ion scores are shown

(Refer Slide Time 28:02)

ħ	otein vi	lew.					
	Show (predicted pe	ptides also	1			
	Sort P	eptides By					
- Mertpattin			• trudae	Numper	ncrea	and w	use for calculation of the protein ware.
Star	t - End	Observed	Mr(expt)	Mr(calc)	Delta	Hiss	Sequence
126	- 147	960.4445	1918.8746	1918.9797	-55	0	R. MANLGALGEVEPELLAR.N. Oxidation (M) ELECTROPILLA
126	142	960.4587	1918,9029	1918.9797	-40	8	R. WANEGALGEVYPELLAR.N. Oxidation (M) (1201 hccl 41)
147	- 154	492.2305	982.4464	982.4913	-44	0	K.FGEAVWER.A LINEL SCOTE 400
147	154	492.2348	982.4551	982.4913	.17	0	E.FGEAVWFEA (1011 SCORE 32)
241	- 238	670.6395	2008.8964	2009.0155	-59	0	R. LAMPEMPGPPVQAIVTGR. G. Oxidation (M) (Jona score. (2)
	1.1.10	1003,4633	10004.4174	10010.0133		×.	IL CYNLOL AND CLARKEN C. C. CHARLENN (M) (CALFTON TO)

The highest ion scores are used for computing the final protein score

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Mascel search results Peptide view #Lins frageweitation of Fedanistic
Match is Query 41 M22 Trible: Sum of 11 acces Trible: Sum of 11 acces Trible: Sum of 11 acces Trible: Although and State State Trible: Sum of 11 acces Trible: Sum of
Data file Crubers/harini/Deshtopix8/3/CLC-KG-HS data analysis Raw data file- mgf files/Data file1.mgf Click mouse within plot area to zoom in by factor of two about that point
Or, Plat fram 100 to 900 Da Full range
Label all possible matches @ Label matches used for scoring #

Each peptide in Tandem MS/MS undergoes through second round of fragmentation when it passes through the second mass analyzer before it reaches the detector

(Refer Slide Time 28:16)

Mascot search results Peptide view MS/35 Fragmentation of PGERVMPK Found in (1)(17)3114, Webnew (portain for IRAGE/S1943)66 (Homo sagiens)
Match to Query 4: 982-4356 Bange values for the x-balls that can be Tritler Sward 6: Is kaos in /s 17.Jacq004.FRODexision Data Tile Cupershaming: and the graduat representation representation. Plas Tile Cupershaming:
Click mouse within plot area to a
Or, Plot from 100 to 900 Da Full range
Label all possible matches @ Label matches used for scoring #

This provides significantly larger amount of information regarding each peptide segment which can be viewed by clicking on the peptide link provided in the summary report. The fragmentation pattern is displayed graphically ...

(Refer Slide Time 28:35)

Ma	Mascot search results										
Mail Fine Inter Mail	d modificat Score: 66 Ches 1 23/7	tions: Carbo Expect: 0.0 8 fragment	ral peptide amidomethy 00036 ions using	Micalco ((C) (appl 16 most int	y to specifi	ed re	sidues or t (p)	ermini only	n		
	Immon		1	6	v	Seq	y	Y'	y ⁴	,	
1	120.0604	120.0808		148.0757		r				1	
2	30.0338	177.1022		205.0972		6	434.4301	819.4036	818.4196	1	
3	102.0550	306.1448	288.1343	334.1397	316.1292	t	779.4087	762.3821	761.2981	1	
1	44.0495	377.1819	359.1714	405.1769	387.1663	A	450.3661	433.3395		5	
5	72.0808	476.2504	450 2798	504.2453	484.2347	٧	579.3289	542.3024		4	
6	159.0917	642.3297	644.3191	690.3246	672.3140	w	480.2605	463.2340		3	
7	120.0608	809.3981	791.3875	837.3930	819.3824	+	294.1812	277.1547		1	
1	101.1073					ĸ	147.1128	130.0843		ī	

...which can be zoomed into as per the requirement

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(Refer Slide Time 29:01)

Summary

- # Basics of MALDI-TOF MS
- # An overview of proteomic experiment using 2DE gel & MALDI-TOF was demonstrated
- # MALDI Experiment:
 - Sample preparation discussed
 - MALDI-TOF instrument demonstrated
 - PMF and MS/MS analysis was discussed

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