

Unit-6

→ Proteome → coined by Marc Wilkins 1994
→ coined in 1995
→ entire set of proteins

→ Cell (tissue, organism) → proteins produced in certain time & defined conditions.
genome.

→ study of proteome - Proteomics

↓
(all the proteins produced by a cell different conditions)

→ Complexity

→ proteomics helps to understand the character, functions of protein in biological organism.

→ proteomics involves

- Protein purification
- Identification
- Structural analysis
- Interaction
- Function.

→ Technologies such as

- Immunosay
- Mass Spectrometry

are used

Applications

→ Identify proteins involved in pathological processes

→ drug industry include targeted identification and investigations of mechanisms of drug action and its toxicity.

→ Evolutionary studies.

Types of proteomics

- Structural - study of 3D structure
- functional - study of functions.
- Expression Proteomics

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study of qualitative & quantitative expression of proteins in different environments such as diseased & normal individual.

Bioinformatics views

- Data bases of protein sequence
- Data bases of predicted protein structures
- Data bases of protein expression analysis

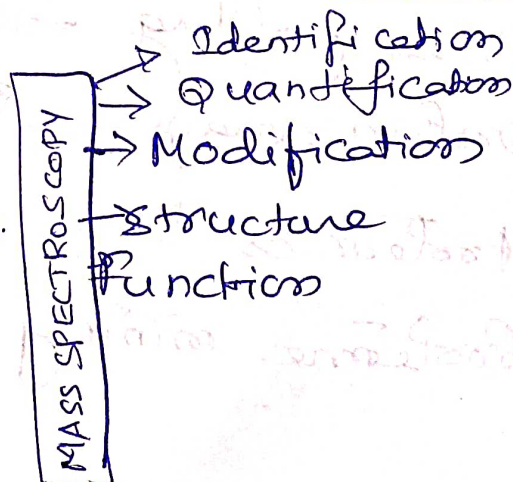
Structural proteomics



Sample → Proteins → peptides.

- studies protein-protein interactions
- Identifies all proteins
- structural analysis of protein.

Functional proteomics



Proteomics - tools

<u>Method</u>	<u>Description</u>	<u>Applications</u>
① Mass Spectrophotometry	Digest protein and fragment peptide to identify proteins	Protein identification, sequence, post-translational modification
② protein chips	Synthesize proteins, peptides, antigens, antibody into a very format and spot onto slides.	Protein interaction with lipid, protein, molecules, drug discovery, post translational modifications
③ Bioinformatics	In silico proteomics	mining database, predicting protein interactions.

Significance of proteomics

- Protein profiling - structure & function
- protein arrays - interactions studies.
- Proteomics to a phosphorylation
- Proteome mining.

Techniques:-

- ① Mass spectrometry (MS2)
- ② NMR spectrometry
- ③ Isoelectric focusing etc.

Proteome Analysis

MS2

Extraction → Pretreatment → Purification → Identification

Ex: Peptidomic
Inactivation
- Heat
- Inhibitors
- Cold acid.

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RP-HPLC Quantification

- ① ELISA
- ② RIA

Proteomics → Tryptic digestion → RP-HPLC → MS2
Capillary reversed.

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Labeled tryptic peptides.

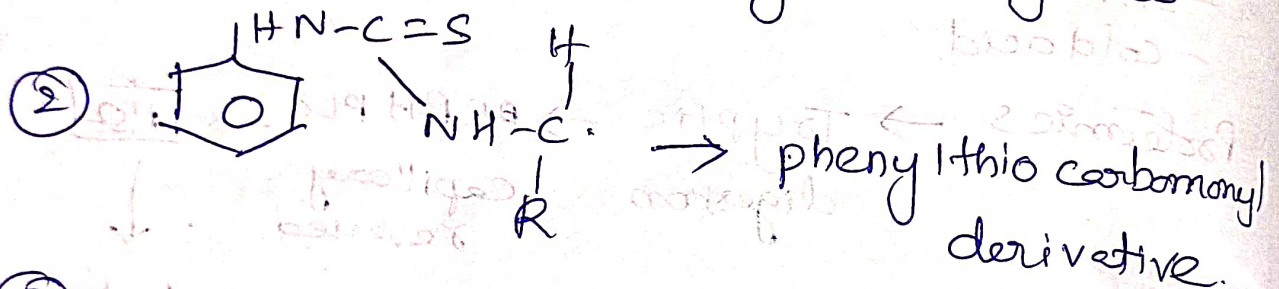
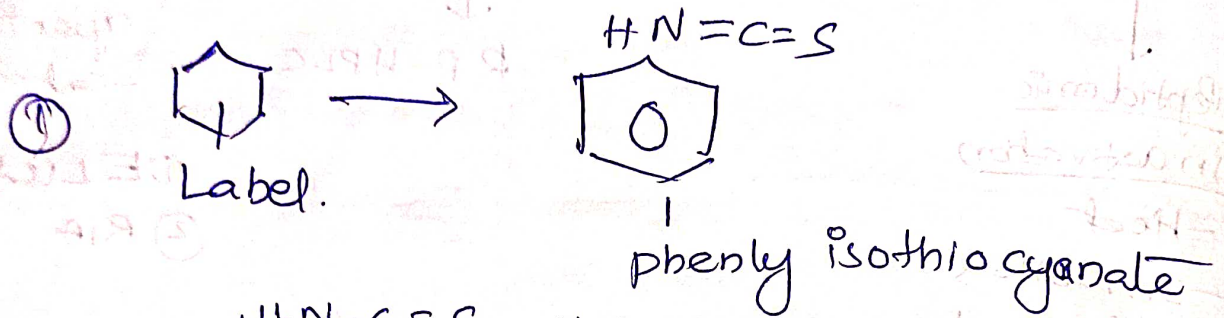
Sequencing techniques of Protein

- ① Edman degradation:- Performed by Edman in 1950
- sequencing of Amino acids in protein

Terminal peptides
N-
C-terminal
Label.
↓
Identify
by chromatography/
electrophoresis.

→ Labelling of N-terminal of protein by phenyl isothiocyanate (Edman's reagent).

→ The reaction b/w Edman's reagent to N-terminal of protein is done in mild Alkaline condition.



③ Phenylthiocarbonyl derivative $\xrightarrow[\text{Condition.}]{\text{Acidic}}$ Extracted by organic solvents & modified

Identified by HPLC $\rightarrow$ Phenylthiohydantoin amino acid derivative

↓
sequence

Identified of N-terminal.

— Maximum Amino acid Identification $\rightarrow 30$.

① Isolate the protein

↓
Purify it

↓
Proteins (3-D)

Breaking of disulfide bridge. $\leftarrow \rightleftharpoons \rightarrow$ β -mercapto ethanol treatment

Poly peptide
(Linear structure)

↓ Trypsin / CNBr treatment
Small peptides

↓
SDS page

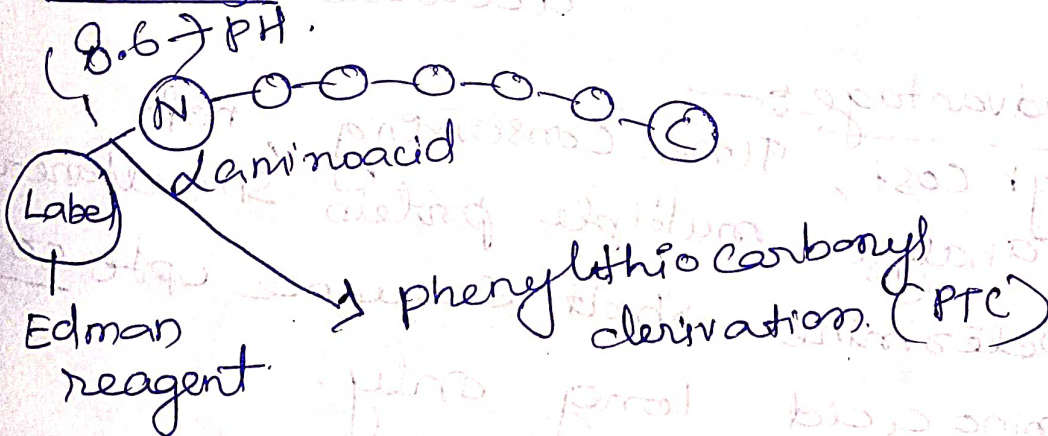
peptide fragments are obtained

Edman sequencing

— 3 steps — chemical method sequencing

① coupling ② cleavage ③ conversion

① coupling



② cleavage

Trifluoroacetic acid

Anhydrous — cut the condition N-terminus peptide bond.

↓
(AZT) Anilinothiazolinone

→ After separation of AZT derivative the remaining peptide is used for another round of reaction with (PITC)

(B) Conversion

→ AZT residue is separated from peptide by extraction in organic solvent (Ethyl acetate)

→ Then AZT residue is converted into more stable form phenylthiohydantoin (PTH derivative)

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Separated and Identified by HPLC
(High performance liquid chromatography)

Advantage

→ Amino acid - Information, sequence accurate

Disadvantages

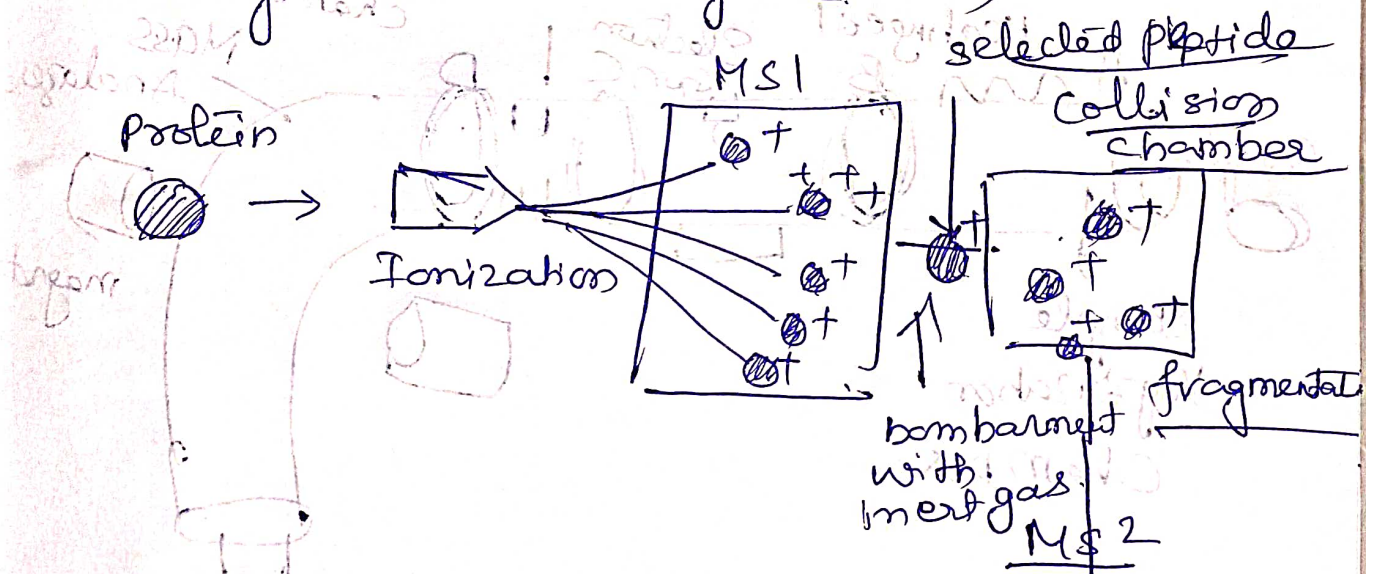
→ High cost, Time consuming, not analyze multiple protein simultaneously
→ Determines short sequence upto 50 amino acid long only.

Mass Spectroscopy — Physical method

- ① production of ions, in gaseous state
- ② Acceleration of Ions in magnetic & electric field
- ③ separation of ions in mass analyzer
- ④ Detection of Ions.

Process

- use to determine primary structure
- molecule sprayed through capillary tube
- forms gas-phase ions
- first instrument sorts ions so that only one emerges (MS1)

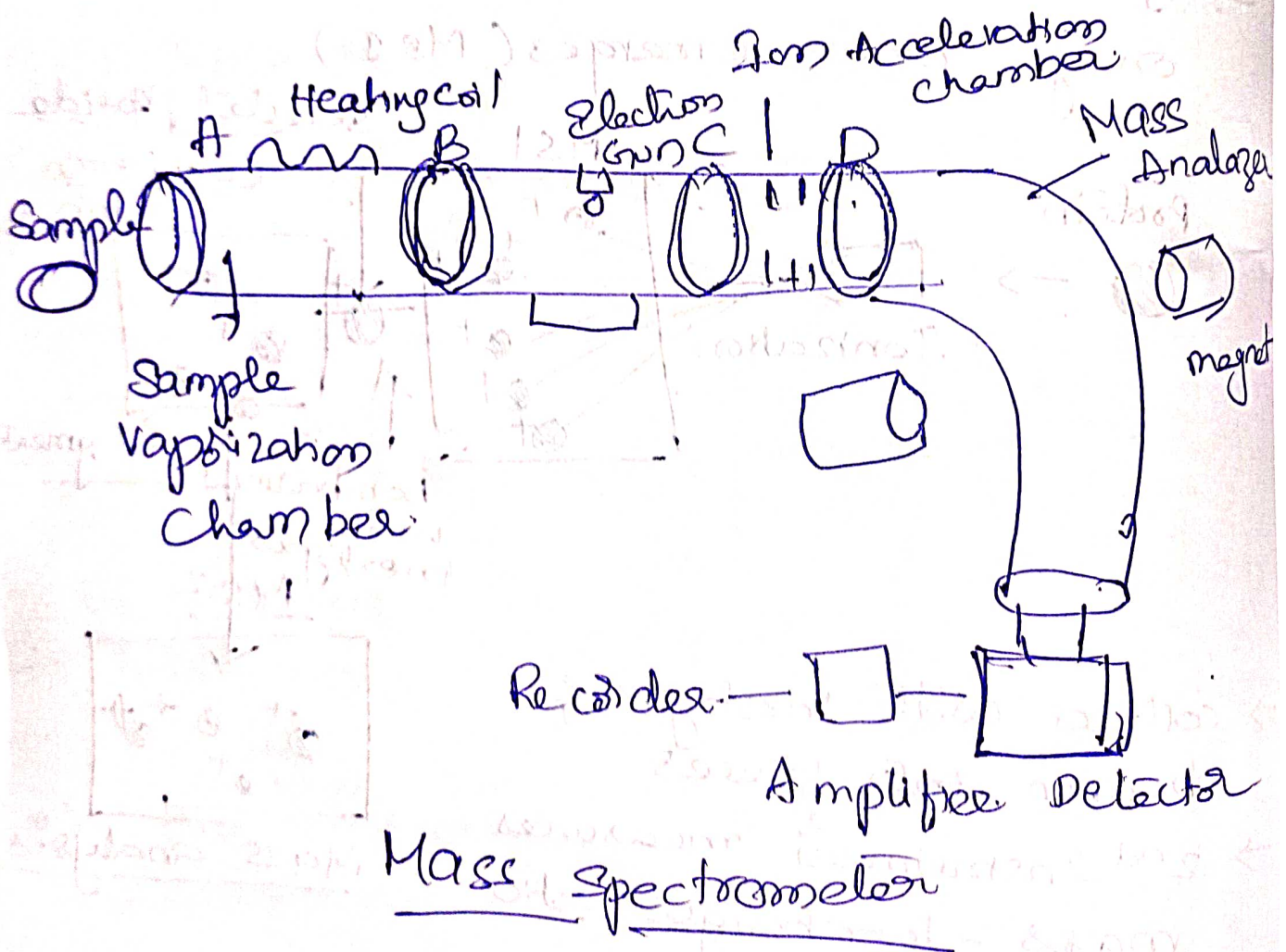
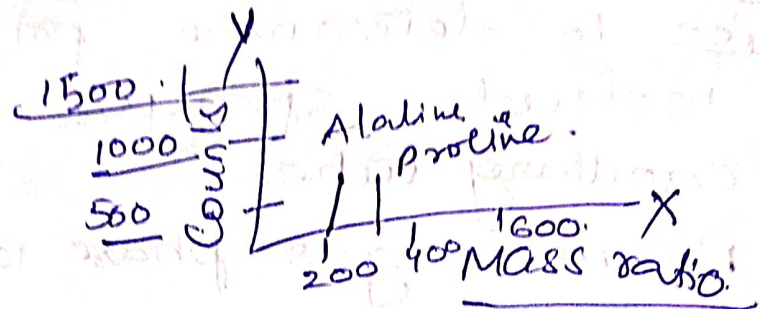


- collides with inert gas & broken into pieces

- 2nd Instrument measures mass-to-charge ratio of pieces (MS2)

- physical breakage of peptide
- can detect any modification.

- Measure charge to mass ratio
- sequence determined by comparing masses of increasingly larger fragments
- Difference in mass of successive peaks identifies each residue.



Protein annotation

— Protein annotations.

— computing protein sequences in the most common way to group and organize protein structures into families.