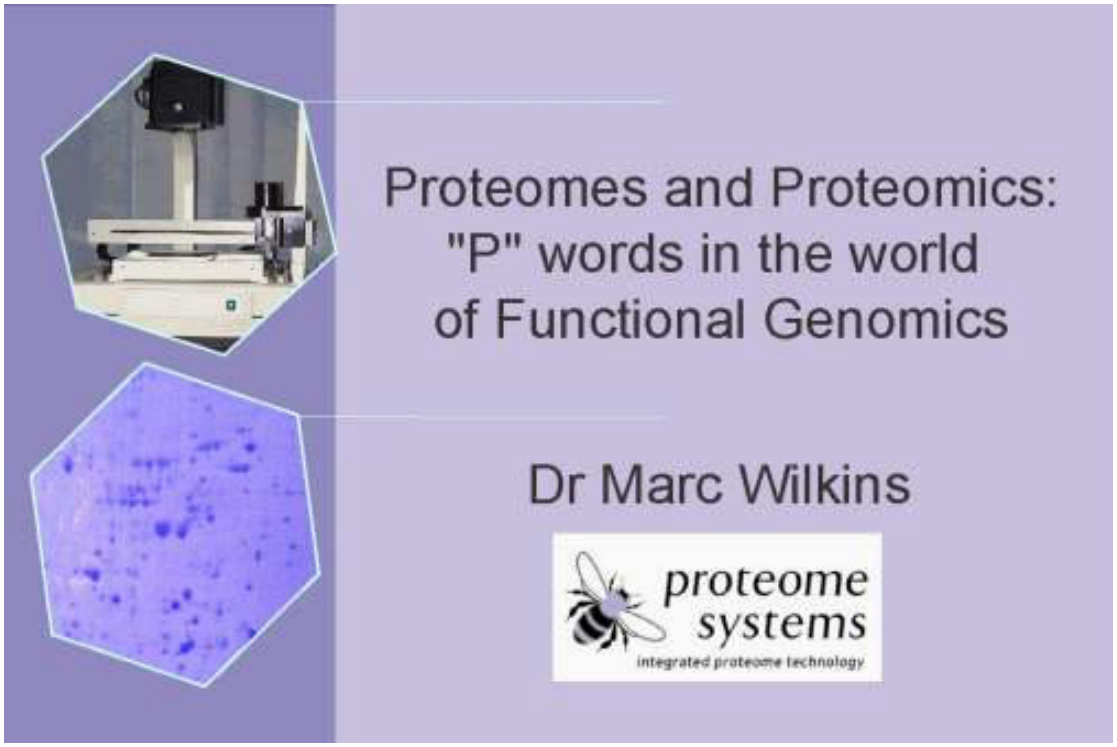
slide01.jpg (504x336x24b jpeg)

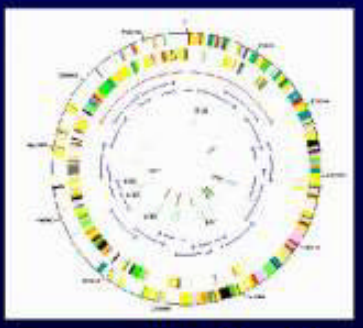


slide02.jpg (504x336x24b jpeg)

Genes, Genomes and Gene Products

- availability of genomic sequences has changed biology forever

- genomes now available for many dozens of organisms
  e.g. *Escherichia coli*
  Bacillus subtilis
  Saccharomyces cerevisiae
  Drosophila melanogaster
  Homo sapiens



Mycoplasma genitalium genome map
Fraser *et al.* 1995

- genomes define the informational content of an organism

- however, a genome sequence does not tell you how an organism works

- study of proteins, the functional molecules, is essential

proteome systems

slide03.jpg (504x336x24b jpeg)

What is a Proteome?

- proteome = PROTEin complement expressed by a genOME or tissue
  (Wilkins *et al.* 1995 Biotechnology and Genetic Engineering Reviews 13, 19-50)

- proteomes are dynamic

- proteomes change as a function of:
  - time
  - development
  - extracellular conditions
  - intracellular conditions

Dictyostelium discoideum development

proteome systems

slide04.jpg (504x336x24b jpeg)

What is Proteomics?

- proteomics is the study of proteomes

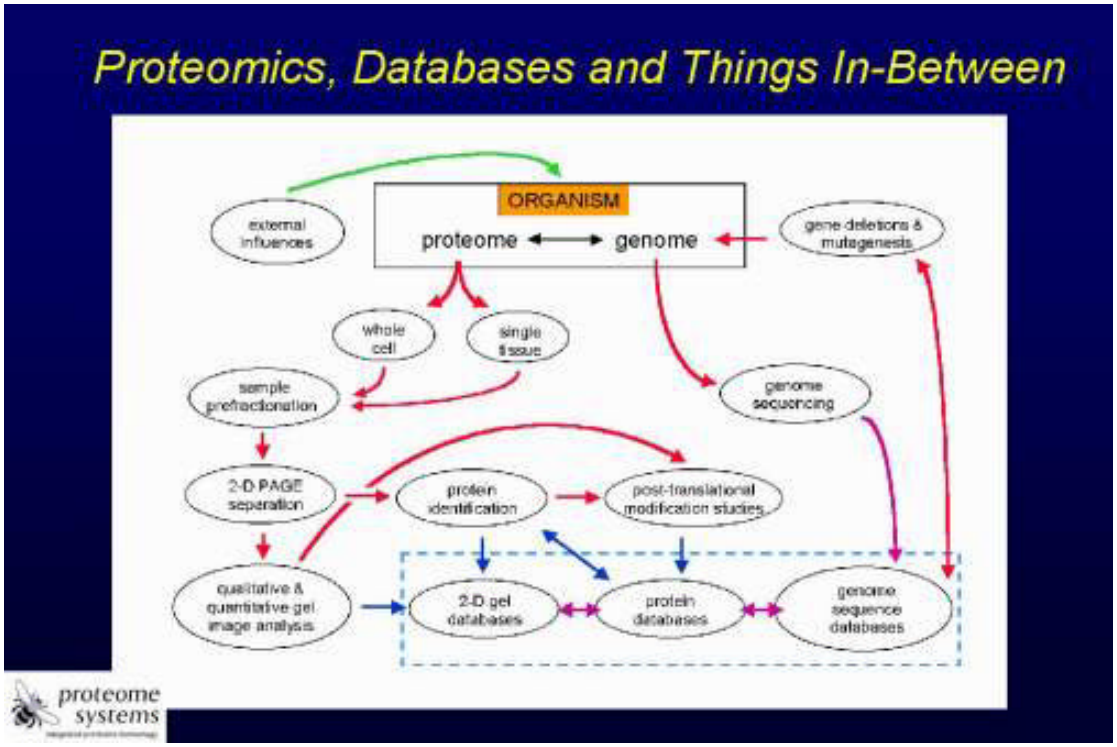
- proteomics aims to:
  - separate, identify and characterise proteins on a large scale
  - define levels of proteins in cells / tissues and how these change
  - investigate protein complexes
  - elucidate protein functions, pathways, and interrelationships

proteome systems

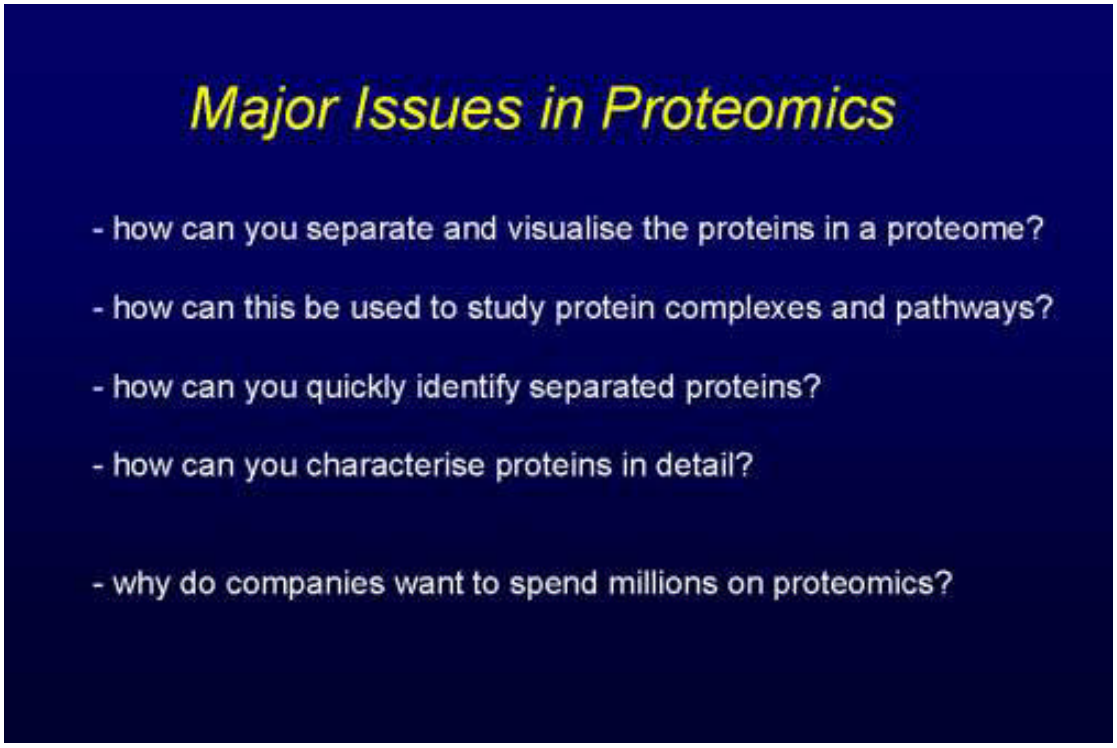
Dictyostelium discoideum development

D. discoideum slugs after 2-D PAGE

slide05.jpg (504x336x24b jpeg)



slide06.jpg (504x336x24b jpeg)



slide07.jpg (504x336x24b jpeg)

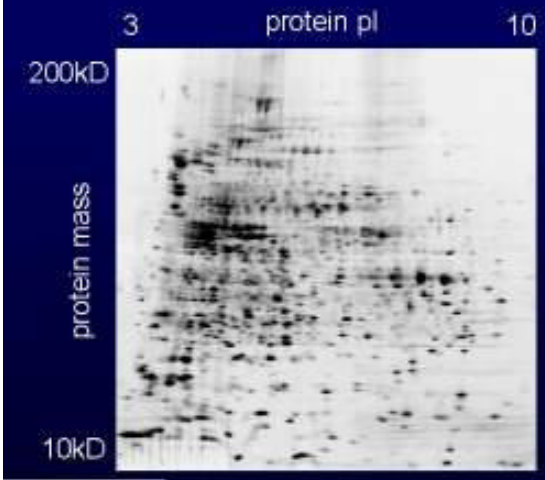
Traditional Protein Chemistry

- column chromatography
  (e.g. ion exchange or reversed phase)
- one protein at a time
- purification may take months

proteome systems

slide08.jpg (504x336x24b jpeg)

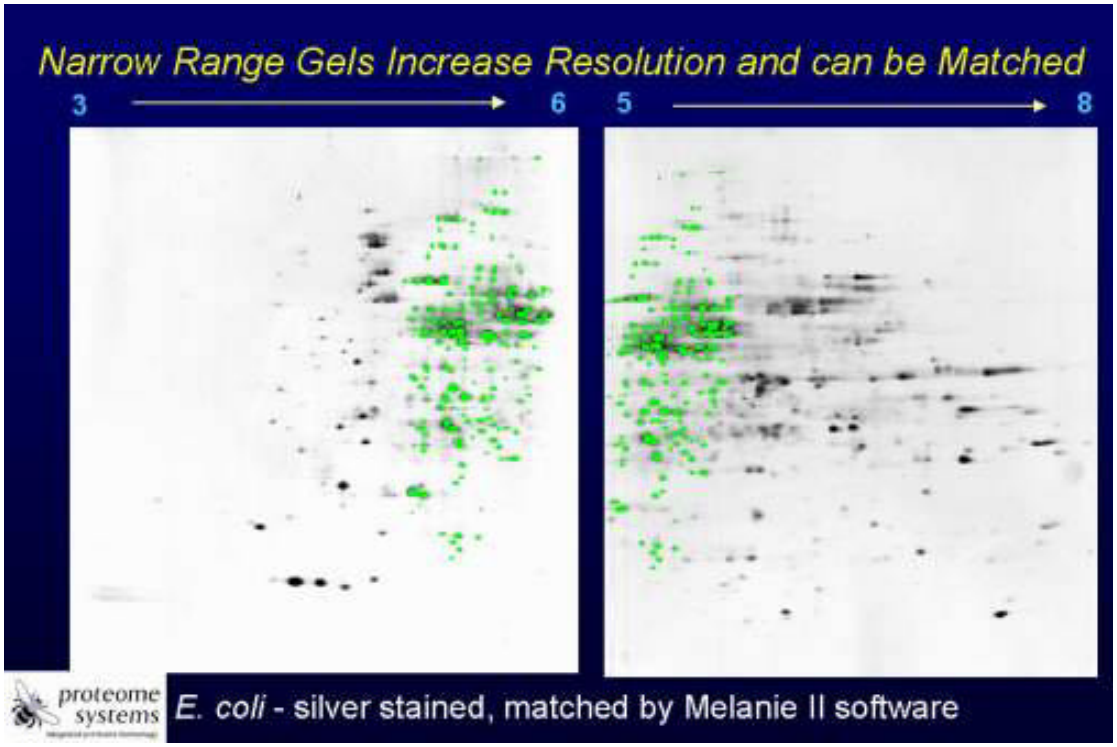
Proteomics Array: two dimensional gel electrophoresis



- first dimension charge-based separation
- second dimension mass-based separation
- up to thousands of proteins purified at once (e.g. 6000)
- proteins purified in parallel in 1-3 days
- image is reference map for cell, tissue or protein complex

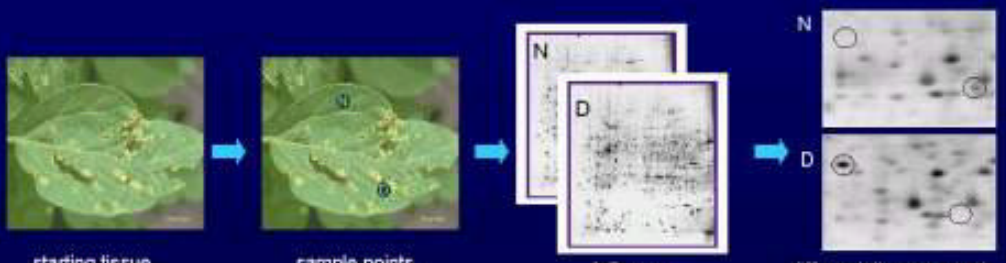
proteome systems

slide09.jpg (504x336x24b jpeg)



slide10.jpg (504x336x24b jpeg)

Discovery of Differentially Expressed Proteins



Differential display can reveal:

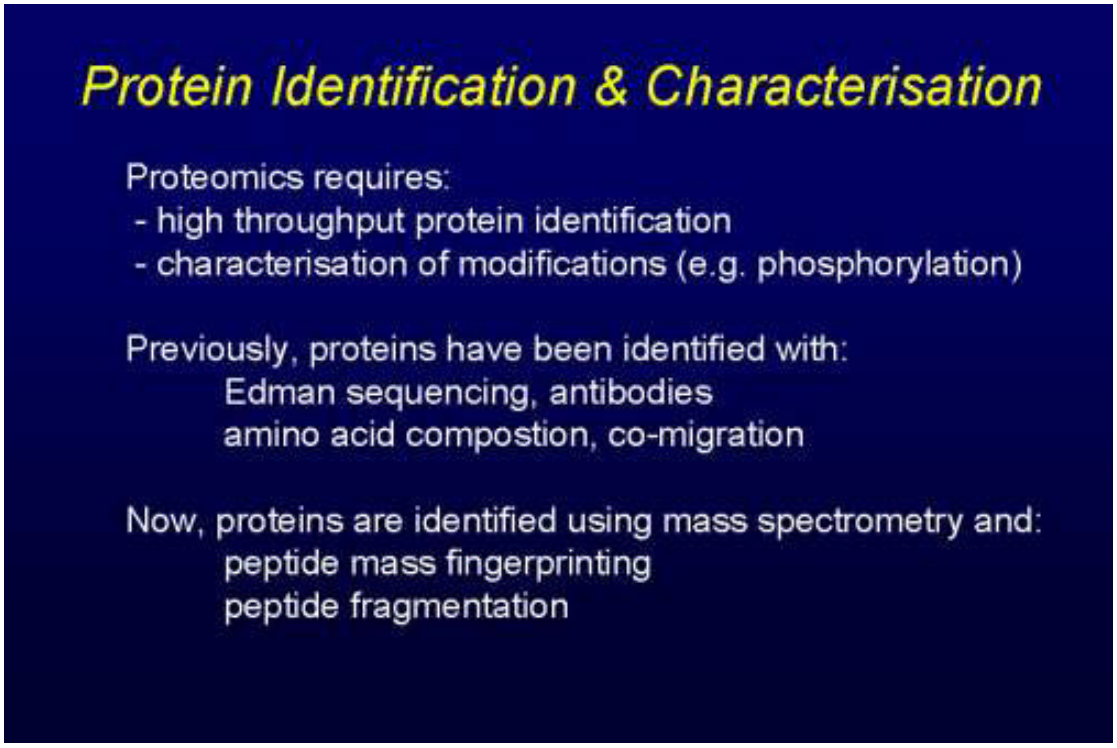
- changes in protein expression
- altered processing or modification of proteins

These changes may be responsible for a phenotype.

Co-regulation or co-modification of proteins can provide clues to pathways and function

proteome systems

slide11.jpg (504x336x24b jpeg)



slide12.jpg (504x336x24b jpeg)

Mass Spectrometry of Proteins

Mass spectrometers precisely measure the mass of molecules

Mass spectrometers have two parts:

1) ion source
2) measuring apparatus

Common ion sources for protein analysis are:

- electrospray ionisation (ESI)
- matrix assisted laser desorption/ionisation (MALDI)

Measuring apparatus these are teamed with are:

- quadrupoles
- time of flight (TOF) detectors
- ion traps
- combinations of the above

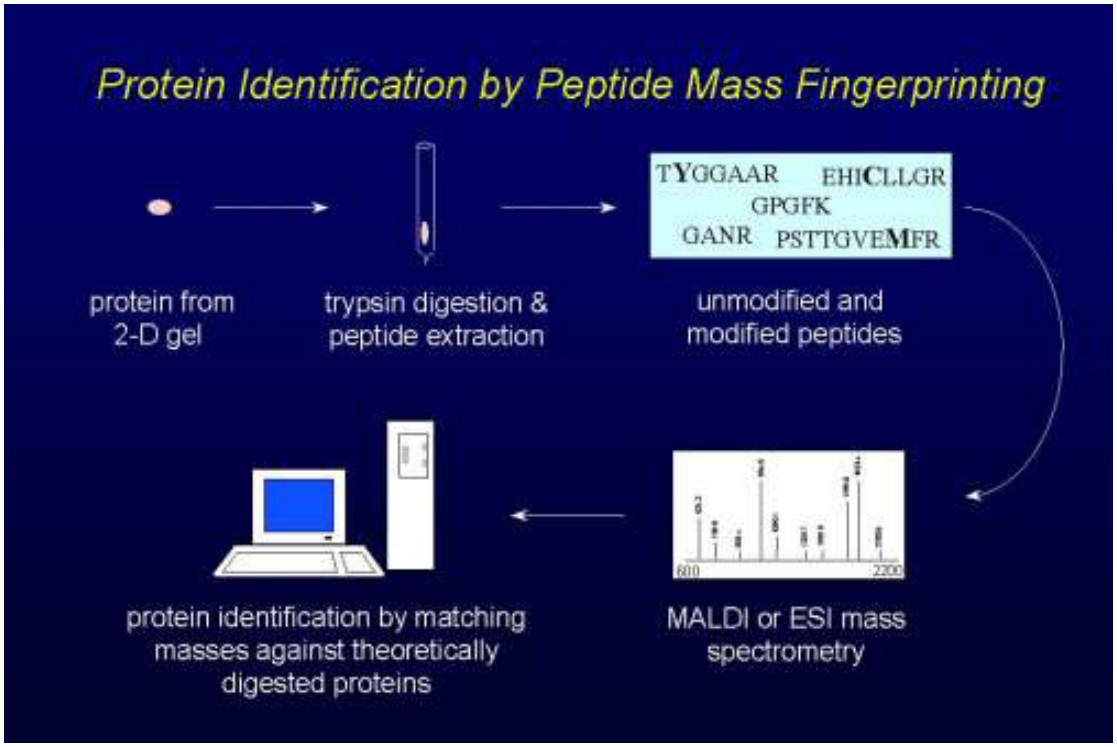
Bruker Biflex III

Common platforms:

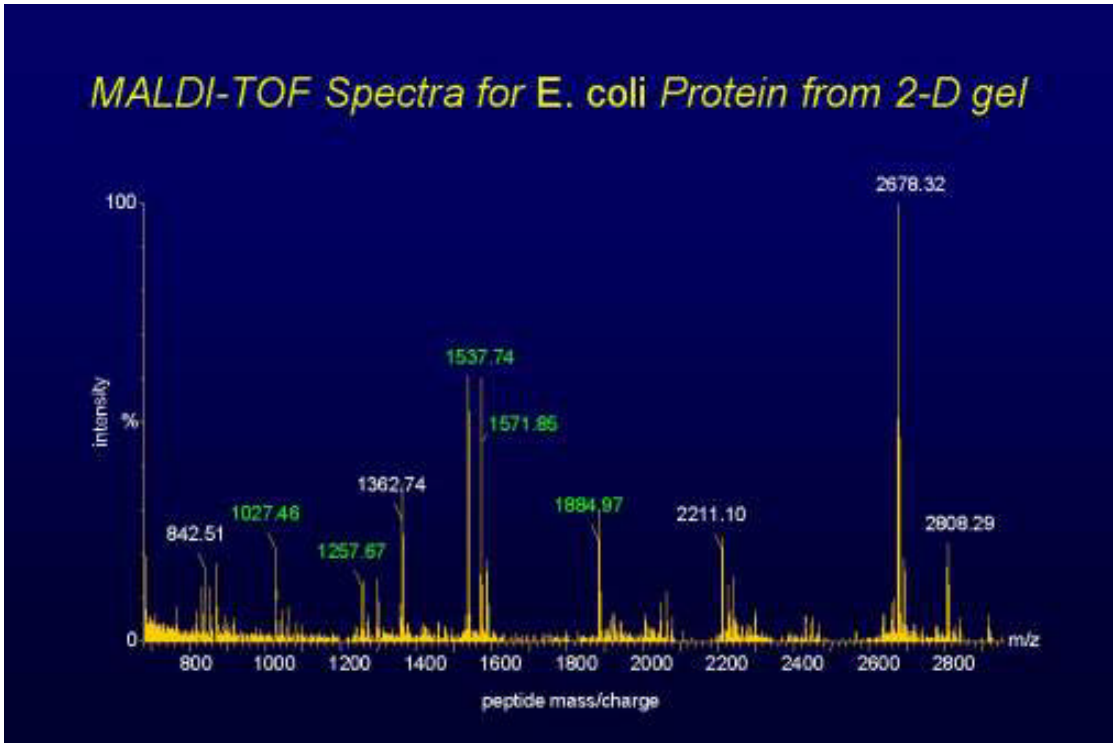
- MALDI-TOF, ESI-TOF, ESI / ion trap, (triple) quadrupole TOF

proteome systems

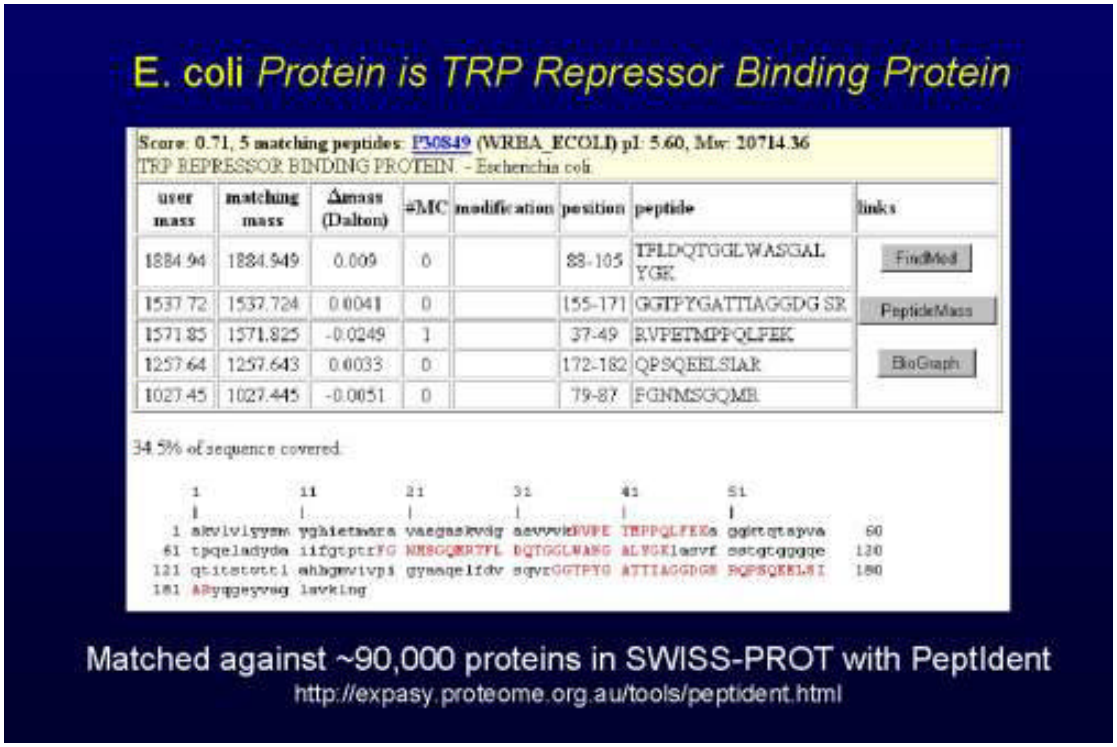
slide13.jpg (504x336x24b jpeg)



slide14.jpg (504x336x24b jpeg)



slide15.jpg (504x336x24b jpeg)



slide16.jpg (504x336x24b jpeg)

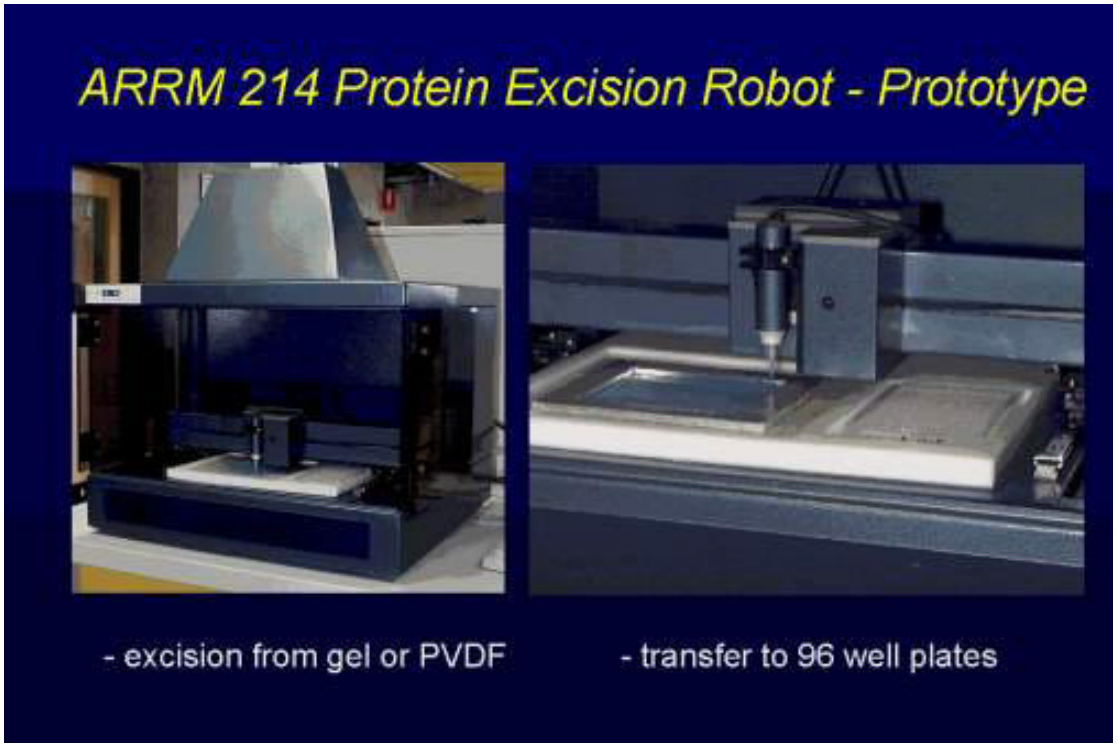
Automation of Protein Identification

384 well plates

High-throughput labs now use automated:

- spot excision
- trypsin digestion
- MALDI target loading
- spectra acquisition
- calibration
- peak picking
- database matching

slide17.jpg (504x336x24b jpeg)



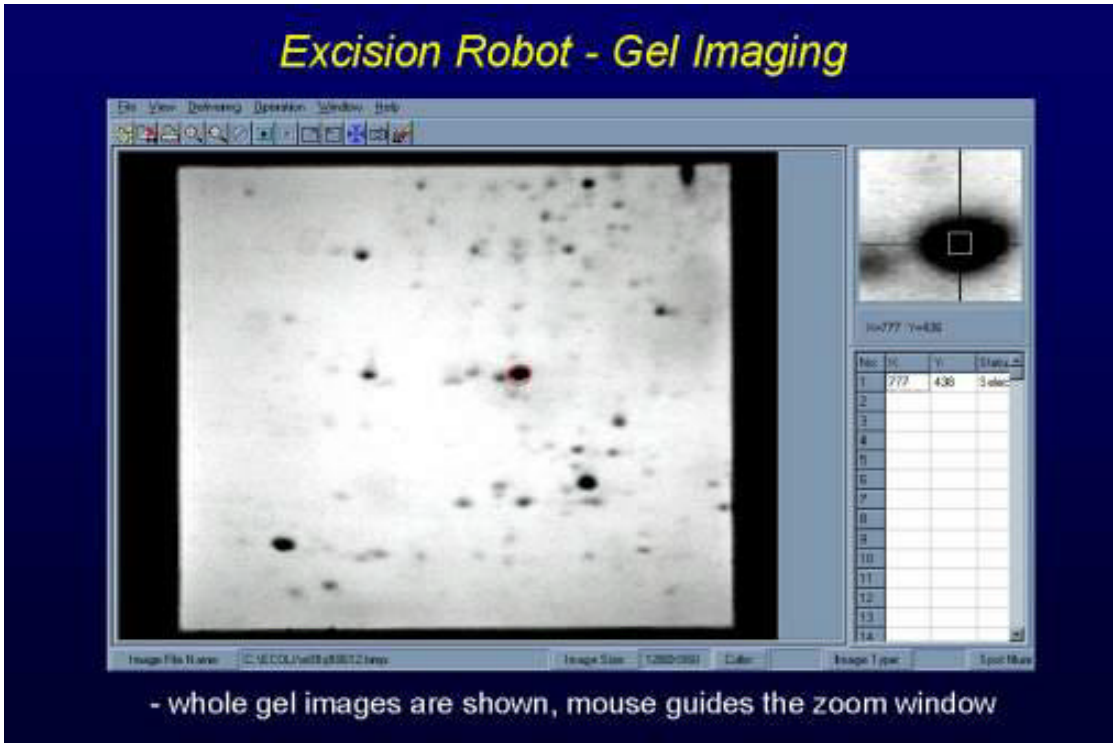
slide18.jpg (504x336x24b jpeg)

Protein Excision Robot - Production Model

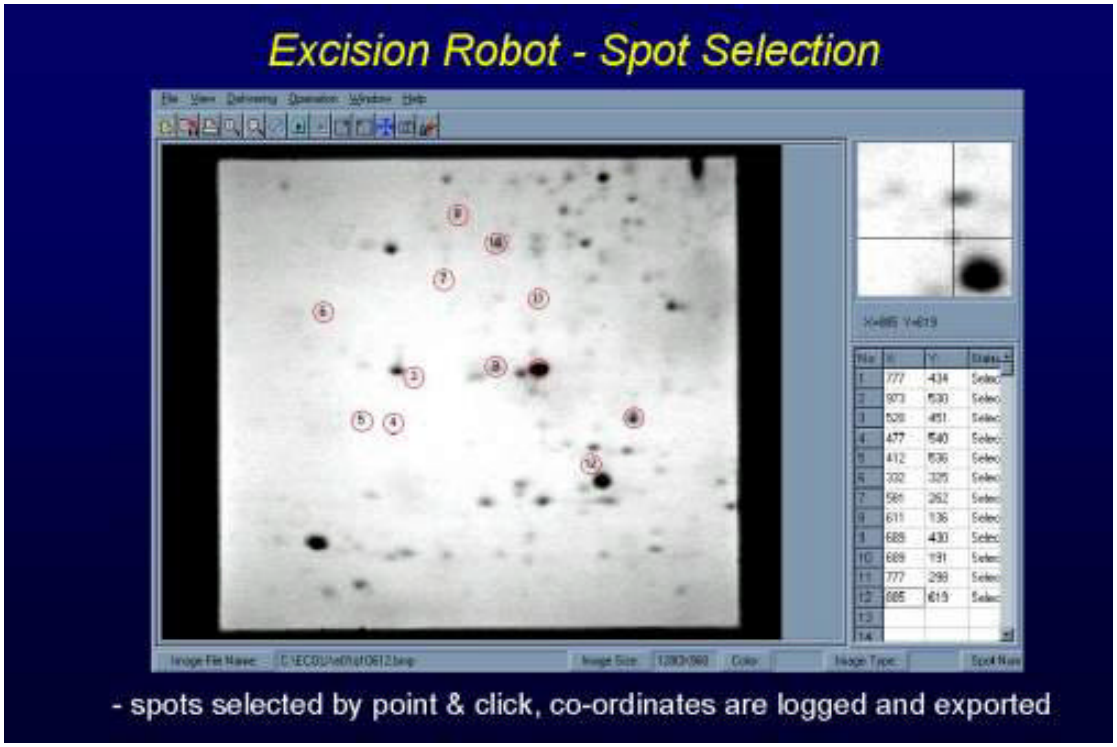
- developed by Proteome Systems, APAF and ARRM Biotech
- marketed internationally by Bio-Rad

proteome systems

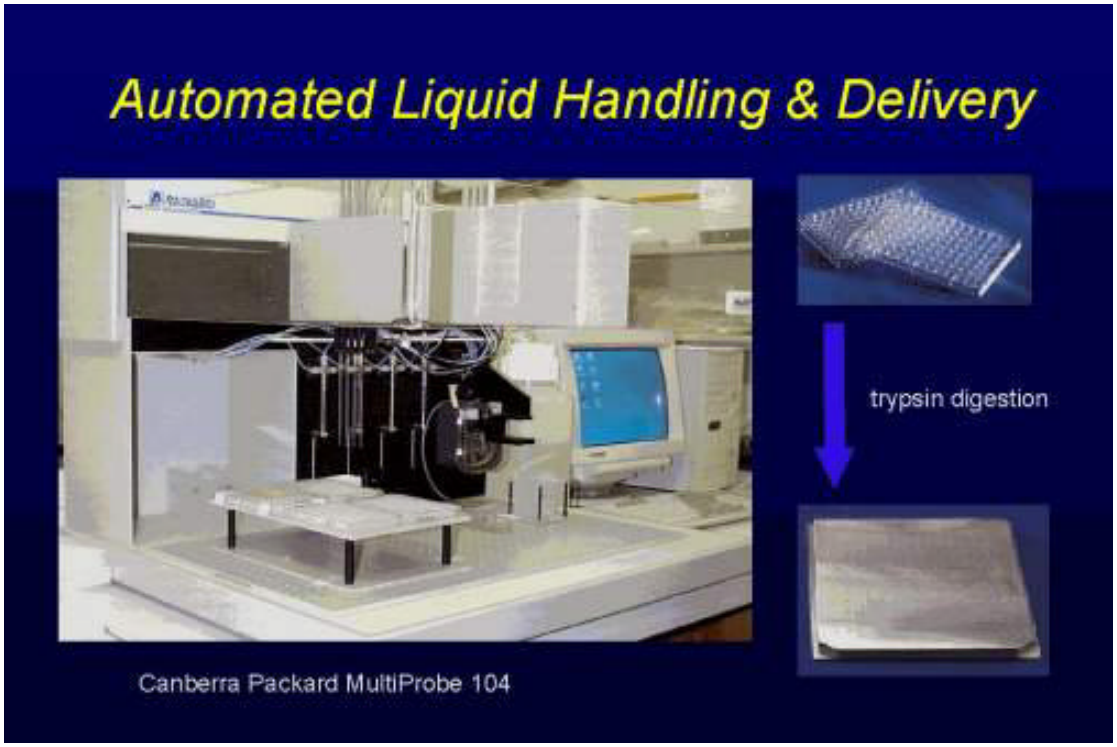
slide19.jpg (504x336x24b jpeg)



slide20.jpg (504x336x24b jpeg)



slide21.jpg (504x336x24b jpeg)



slide22.jpg (504x336x24b jpeg)

Automated Mass Spectrometry

Proteomics requires automated

- spectra acquisition
- calibration
- (deconvolution)
- peak picking
- data export

Voyager DE-STR

MALDI-TOF MS offers

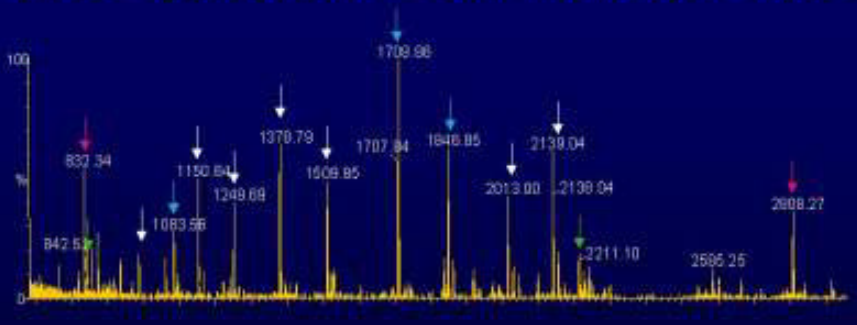
- high throughput (500+ per day)
- high degree of automation

Micromass LCT

ESI-TOF machines currently slower & less automated

slide23.jpg (504x336x24b jpeg)

Mass Spectra Require Interpretation



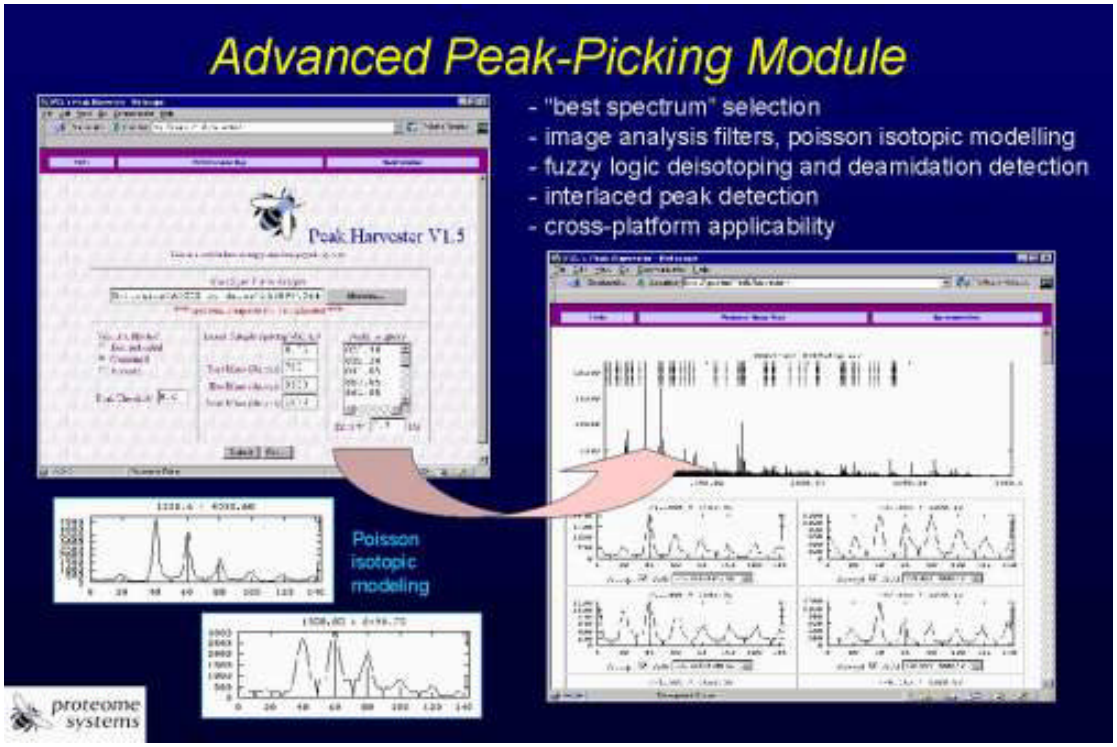
2 calibration peaks, > 2 junk peaks

7 hits with P02339, 3 large unmatched peaks - modifications

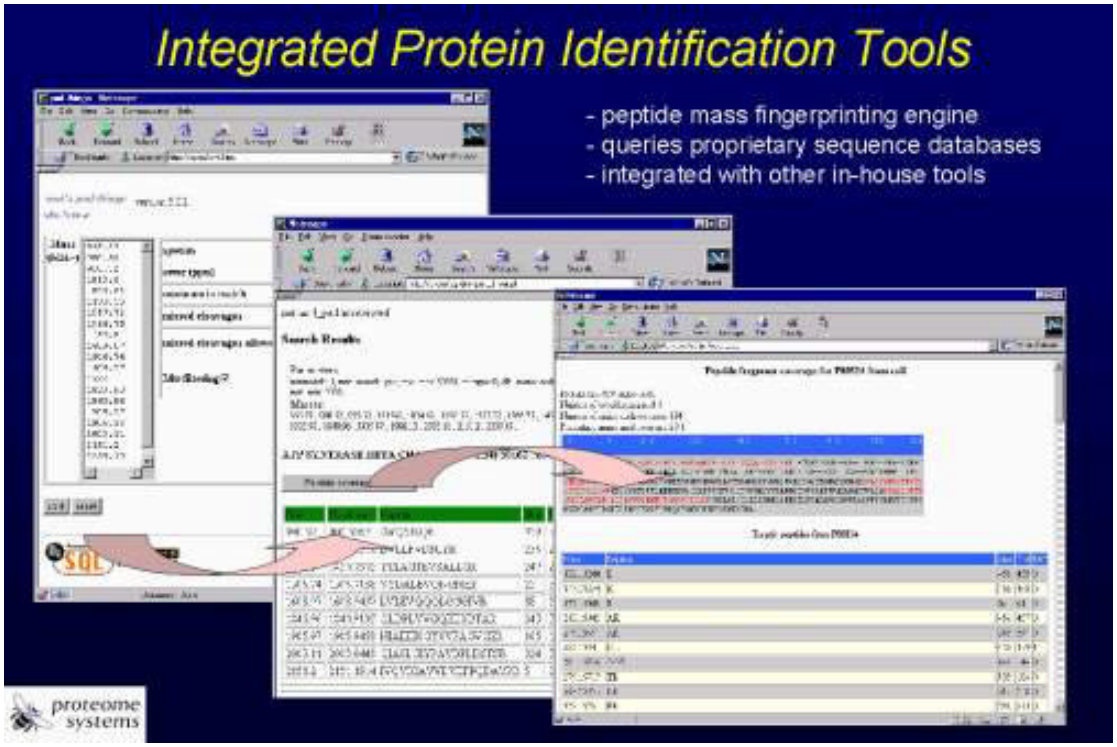
lots of noise

Can interpretation and database matching be done automatically?

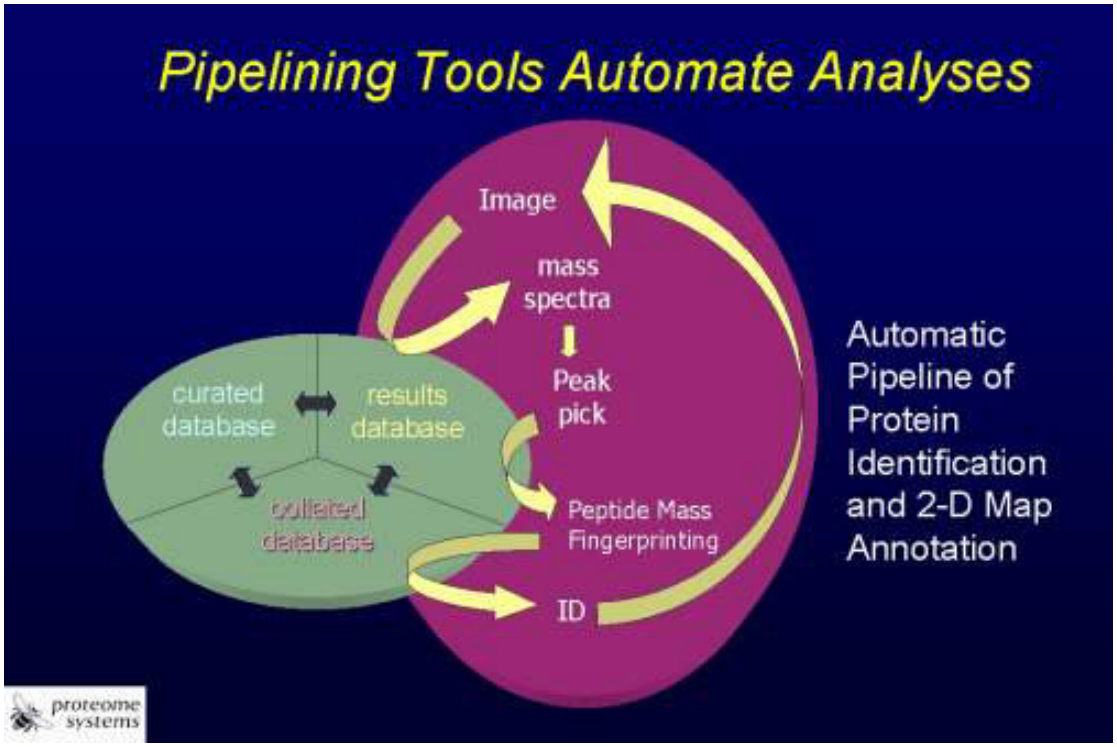
slide24.jpg (504x336x24b jpeg)



slide25.jpg (504x336x24b jpeg)

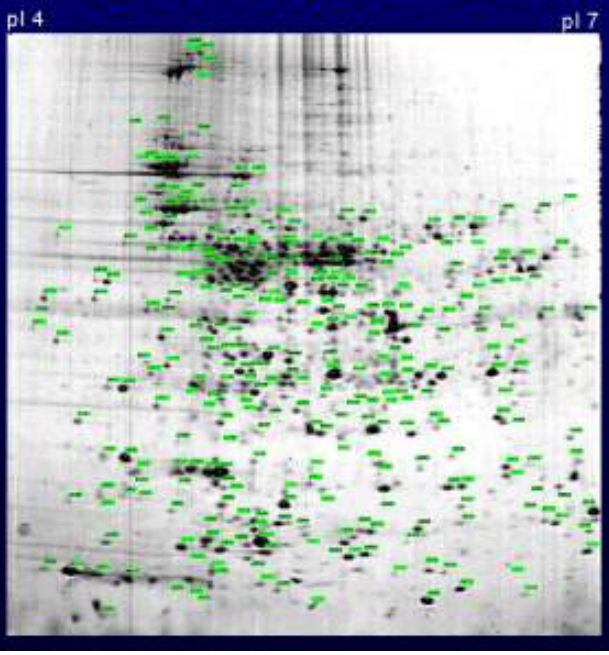


slide26.jpg (504x336x24b jpeg)



slide27.jpg (504x336x24b jpeg)

2-D Array of E. coli *Whole Cell Lysate*



- 1069 analyses from 5 gels

- 587 (55%) spots identified
- 55 (5%) putatively identified

- 324 spots ID on 3mg master

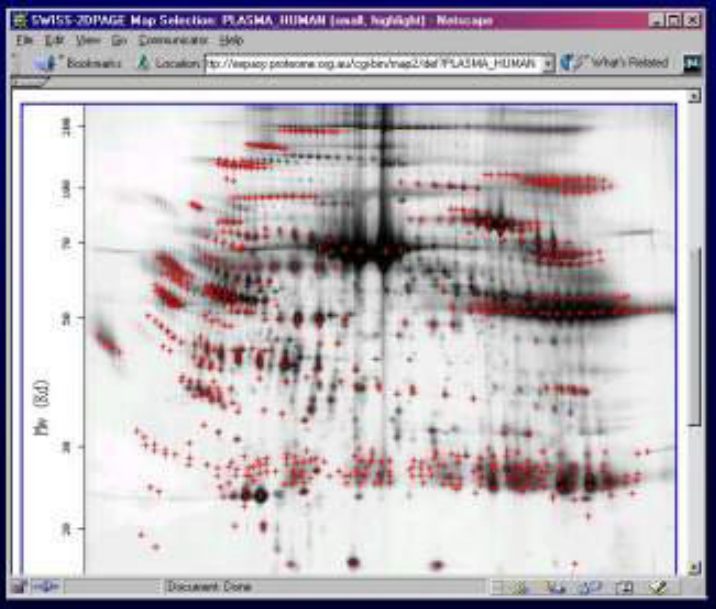
- 192 gene products

- All steps automated but for reference image annotation

- This project completed in 8 weeks with 1.5 people (including development time)

slide28.jpg (504x336x24b jpeg)

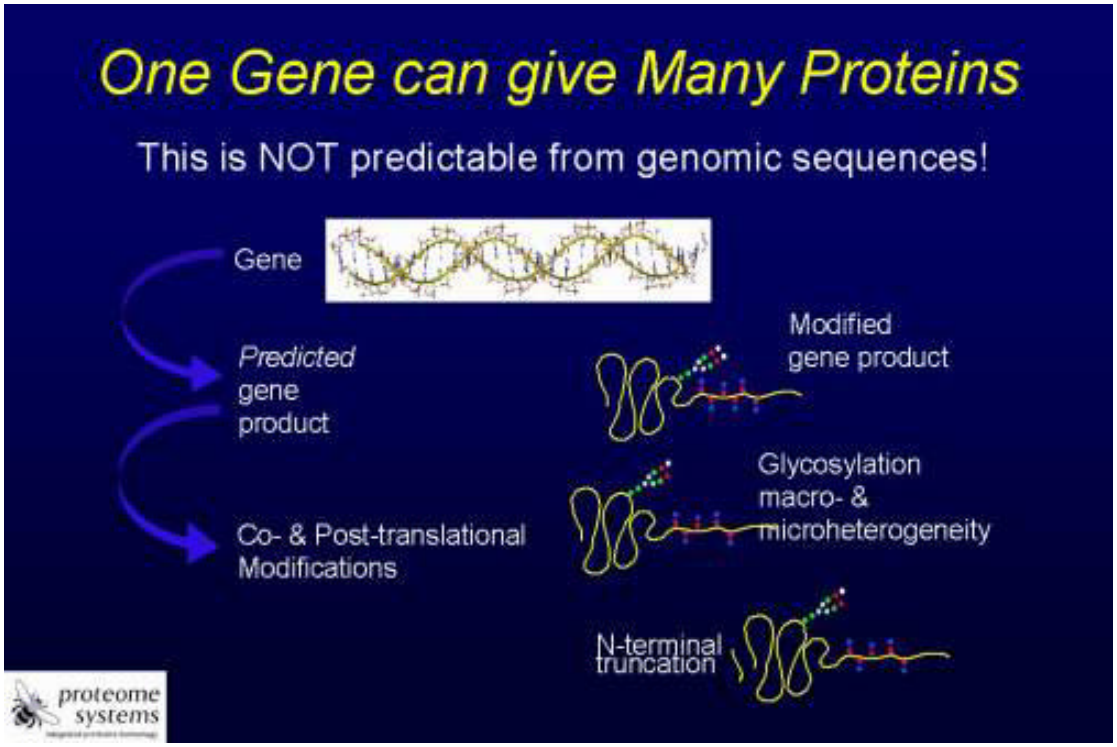
Annotated Reference Maps on the Web



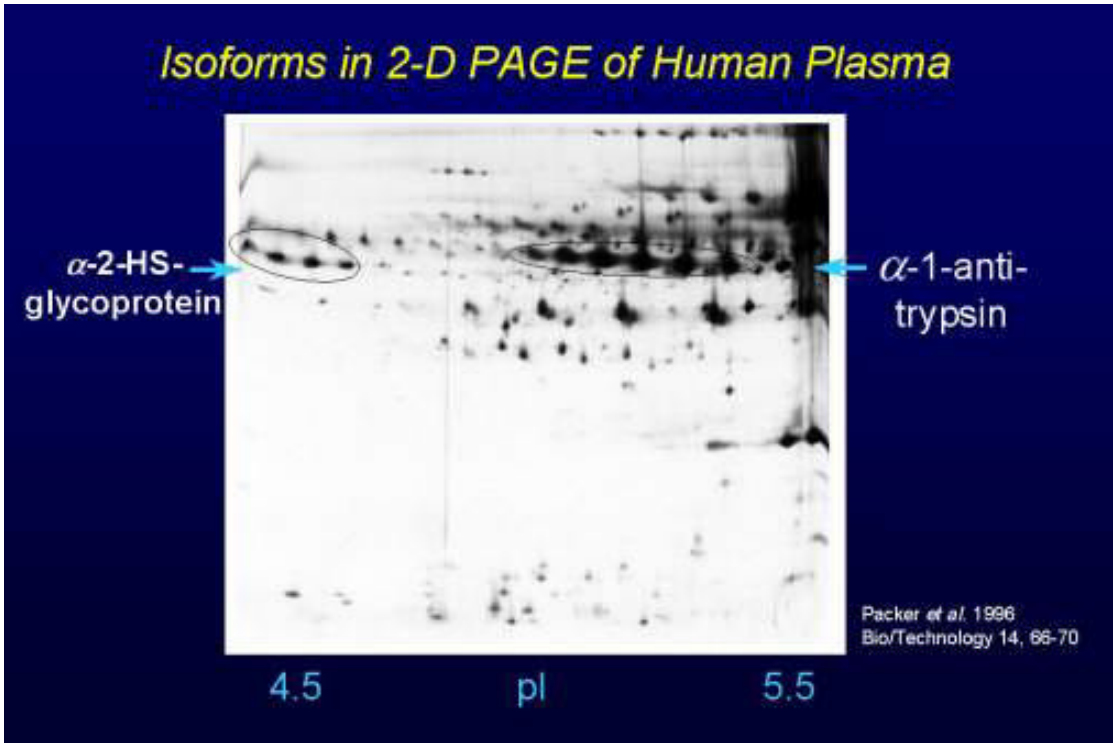
E.g. human plasma
from SWISS-2DPAGE

www.expasy.ch

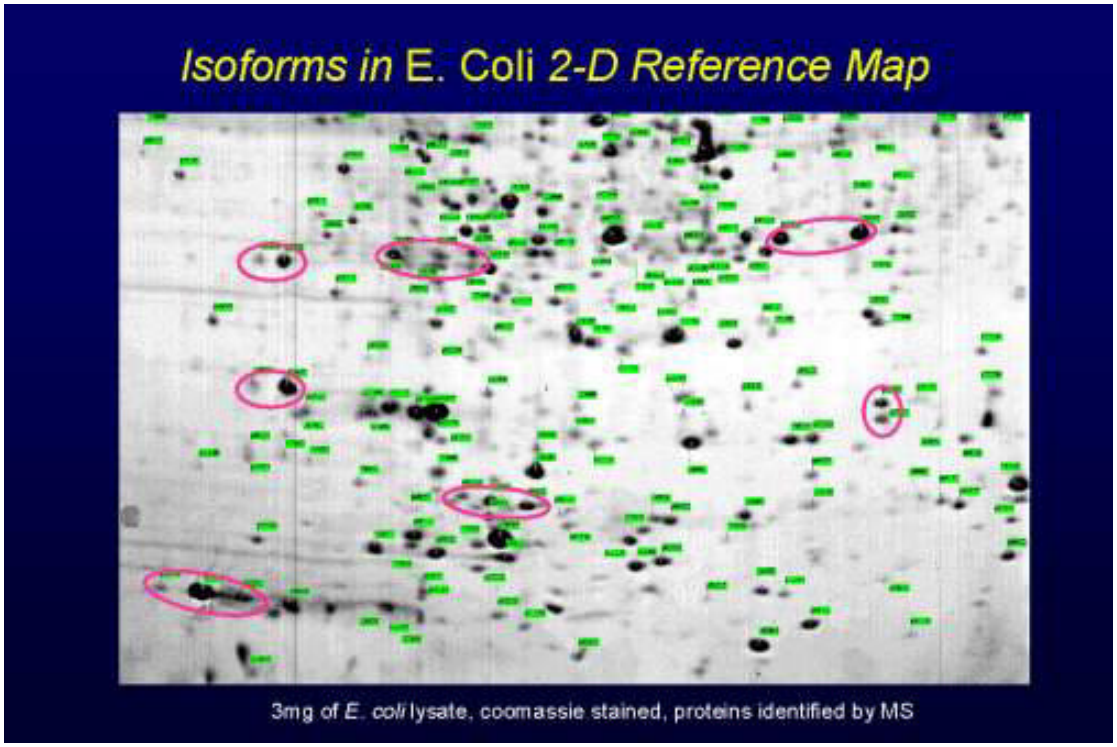
slide29.jpg (504x336x24b jpeg)



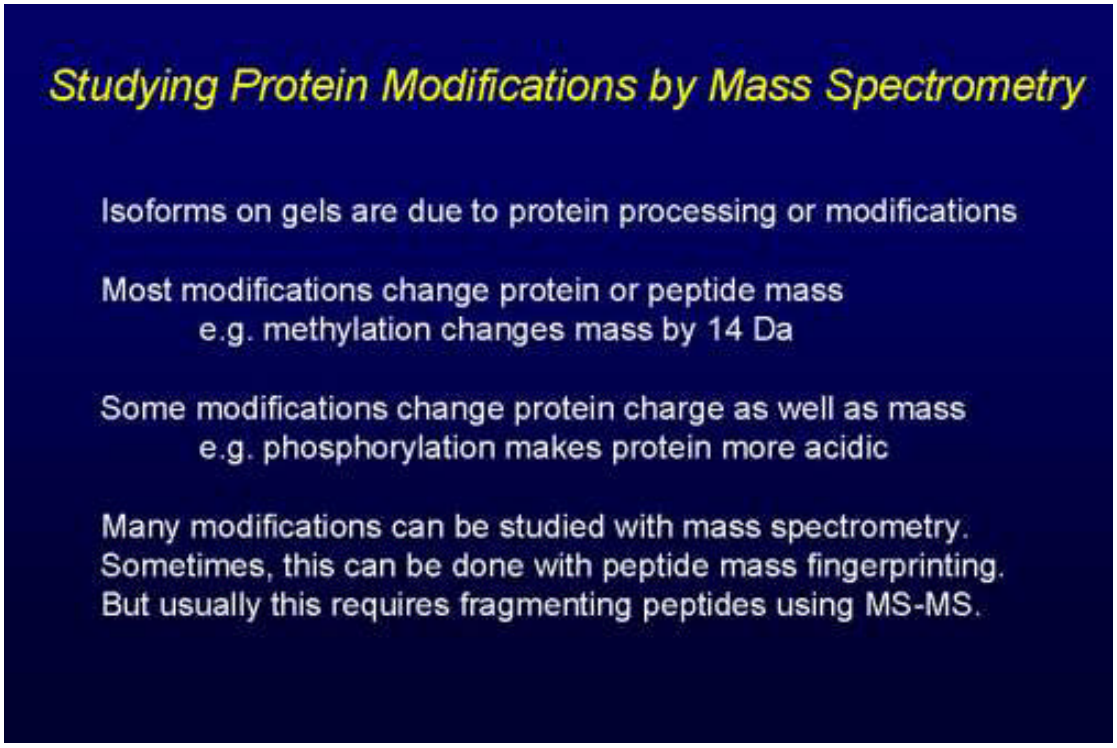
slide30.jpg (504x336x24b jpeg)



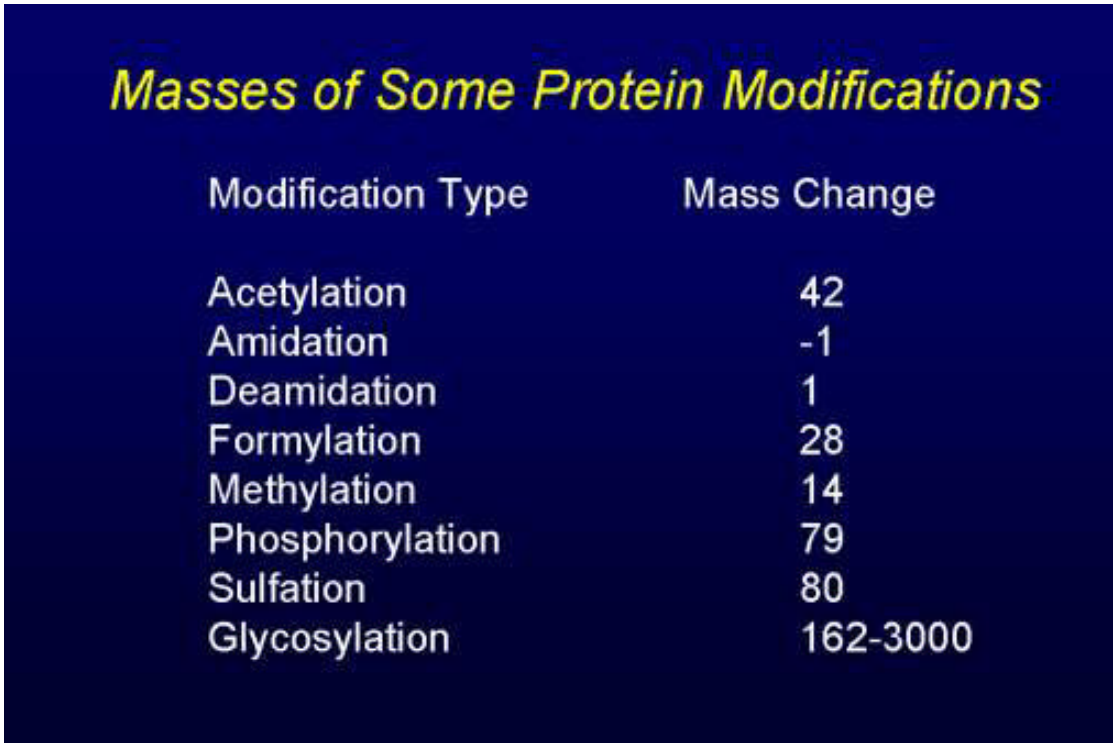
slide31.jpg (504x336x24b jpeg)



slide32.jpg (504x336x24b jpeg)



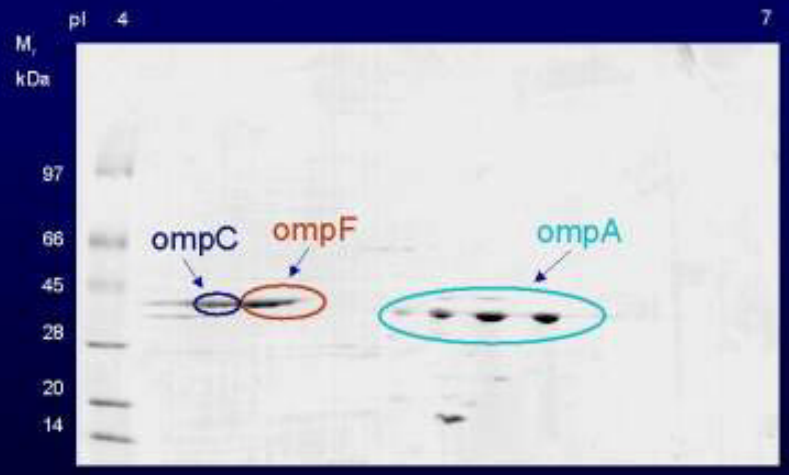
slide33.jpg (504x336x24b jpeg)



slide34.jpg (504x336x24b jpeg)

E. coli *Membrane Proteins on Mini 2-D PAGE*

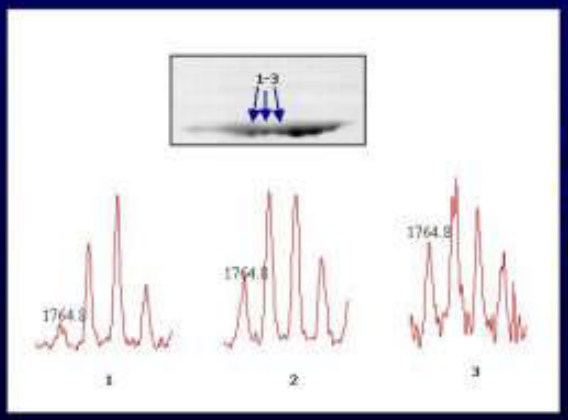
Why are there Isoforms?



solubilisation solution = 7M urea, 2M thiourea, 1% ASB 14, 2mM TBP, 40mM Tris base, 0.5% CA
coomassie stained gel

slide35.jpg (504x336x24b jpeg)

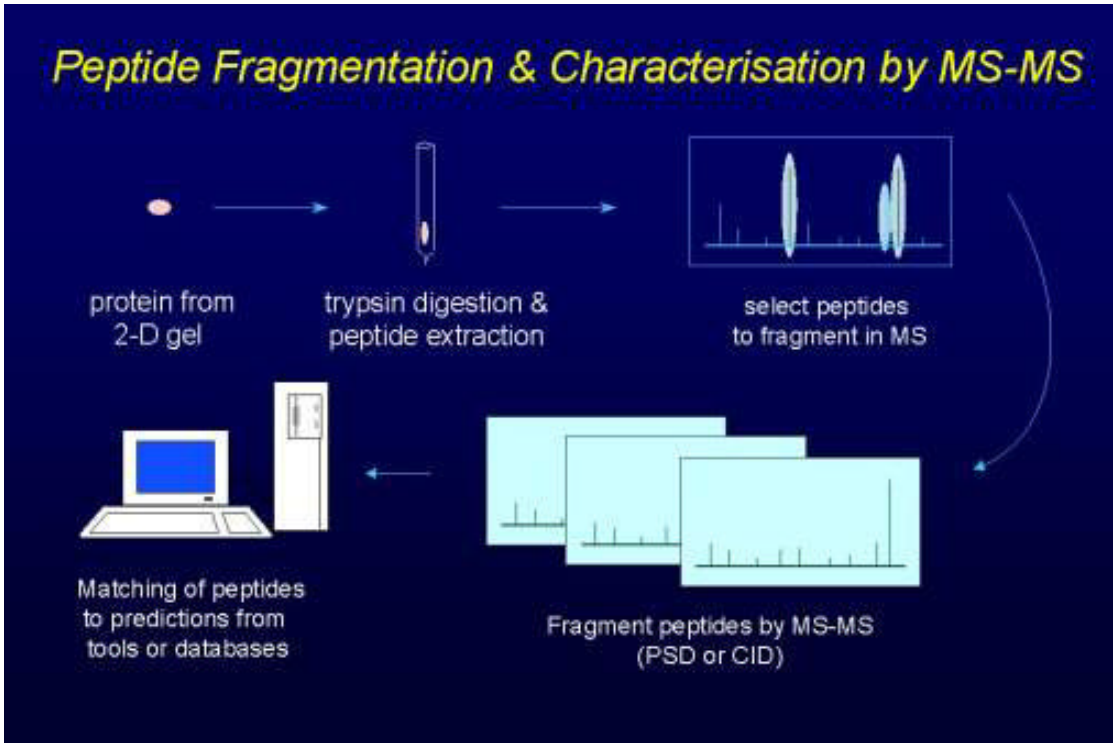
Deamidation of 1764.8 in ompC Causes Isoforms



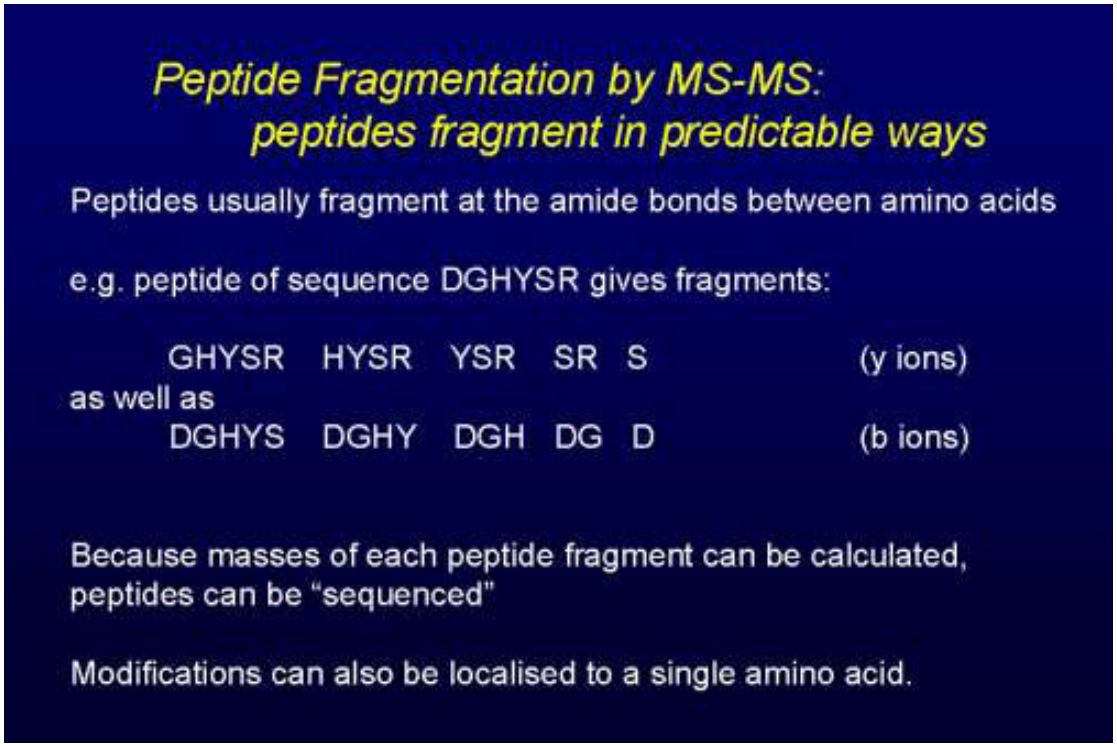
peptide sequence	protein pI
NGNPSGEGFTSGVTNGR	4.48
DGNPSGEGFTSGVTNGR or NGNPSGEGFTSGVTDGR	4.45 (-0.03)
DGNPSGEGFTSGVTDGR	4.4 (-0.08)

N to D deamidation causes 1 Da incease in peptide mass

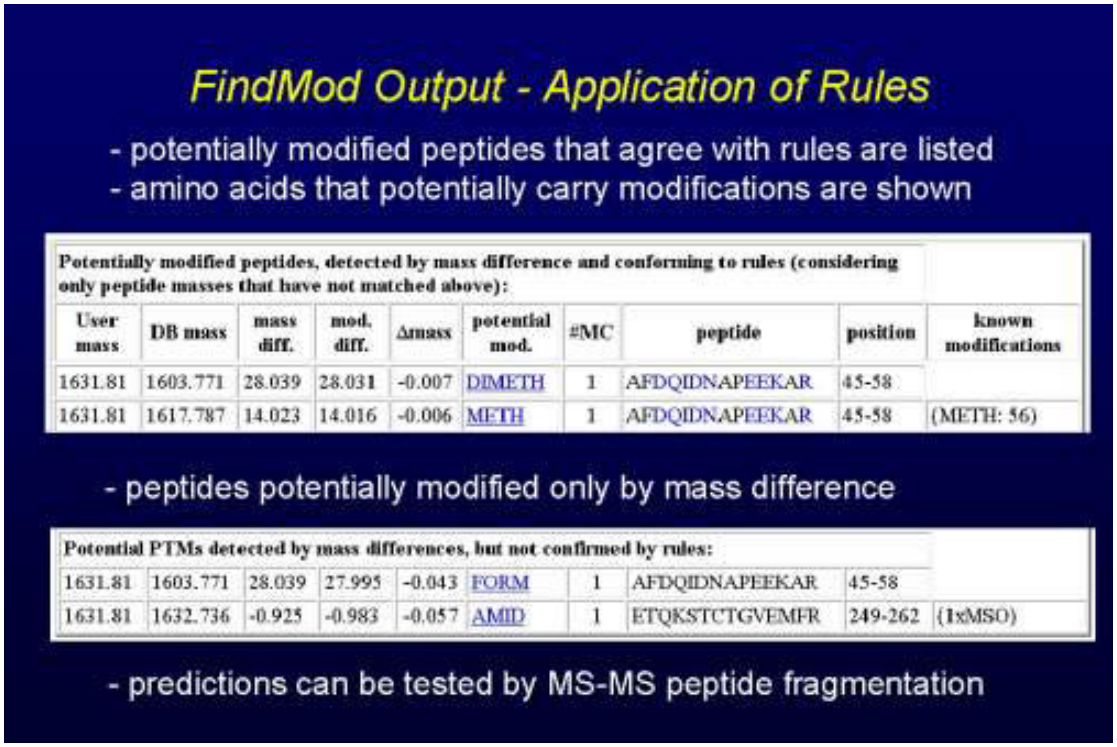
slide36.jpg (504x336x24b jpeg)



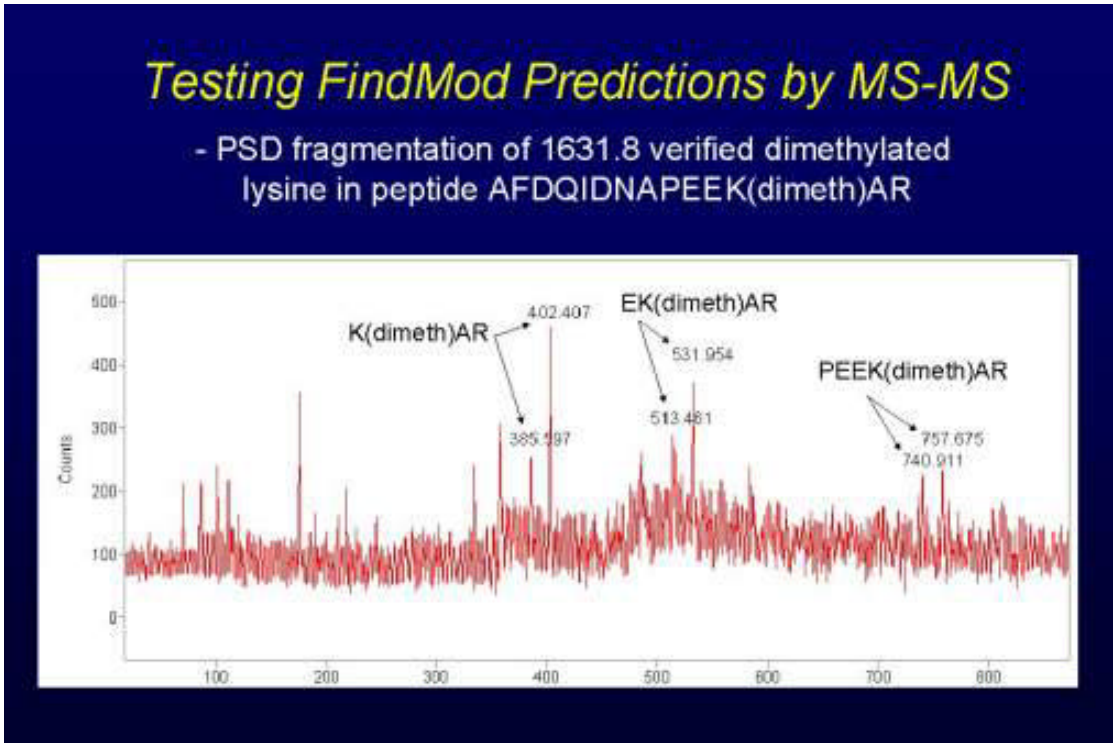
slide37.jpg (504x336x24b jpeg)



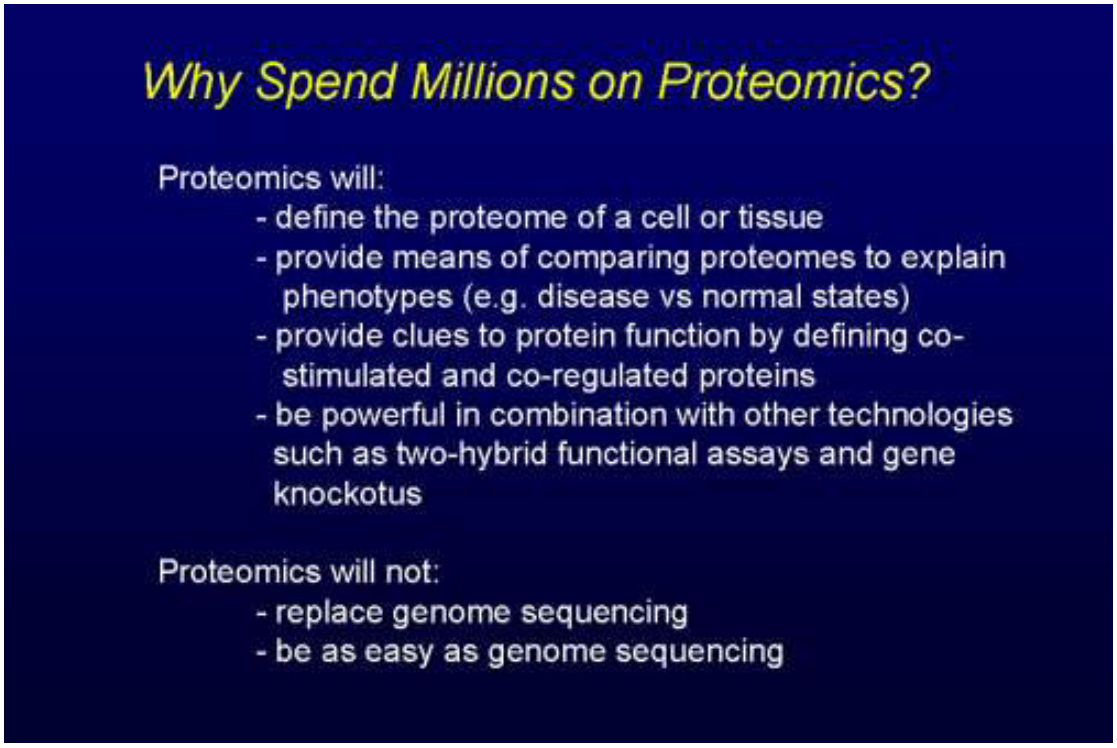
slide38.jpg (504x336x24b jpeg)



slide39.jpg (504x336x24b jpeg)



slide40.jpg (504x336x24b jpeg)



slide41.jpg (504x336x24b jpeg)

References and Further Reading

General references on proteomics:

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proteome systems