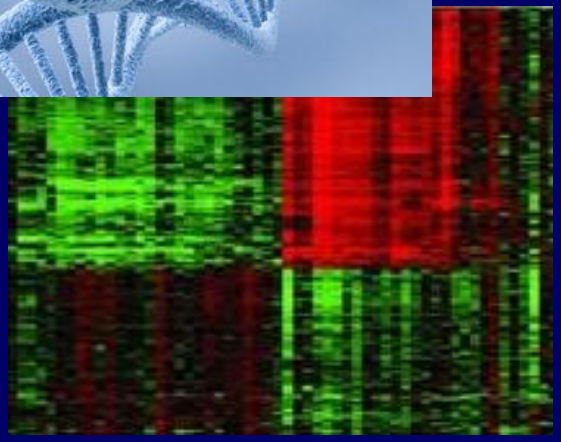
Proteomics in Infectious Disease



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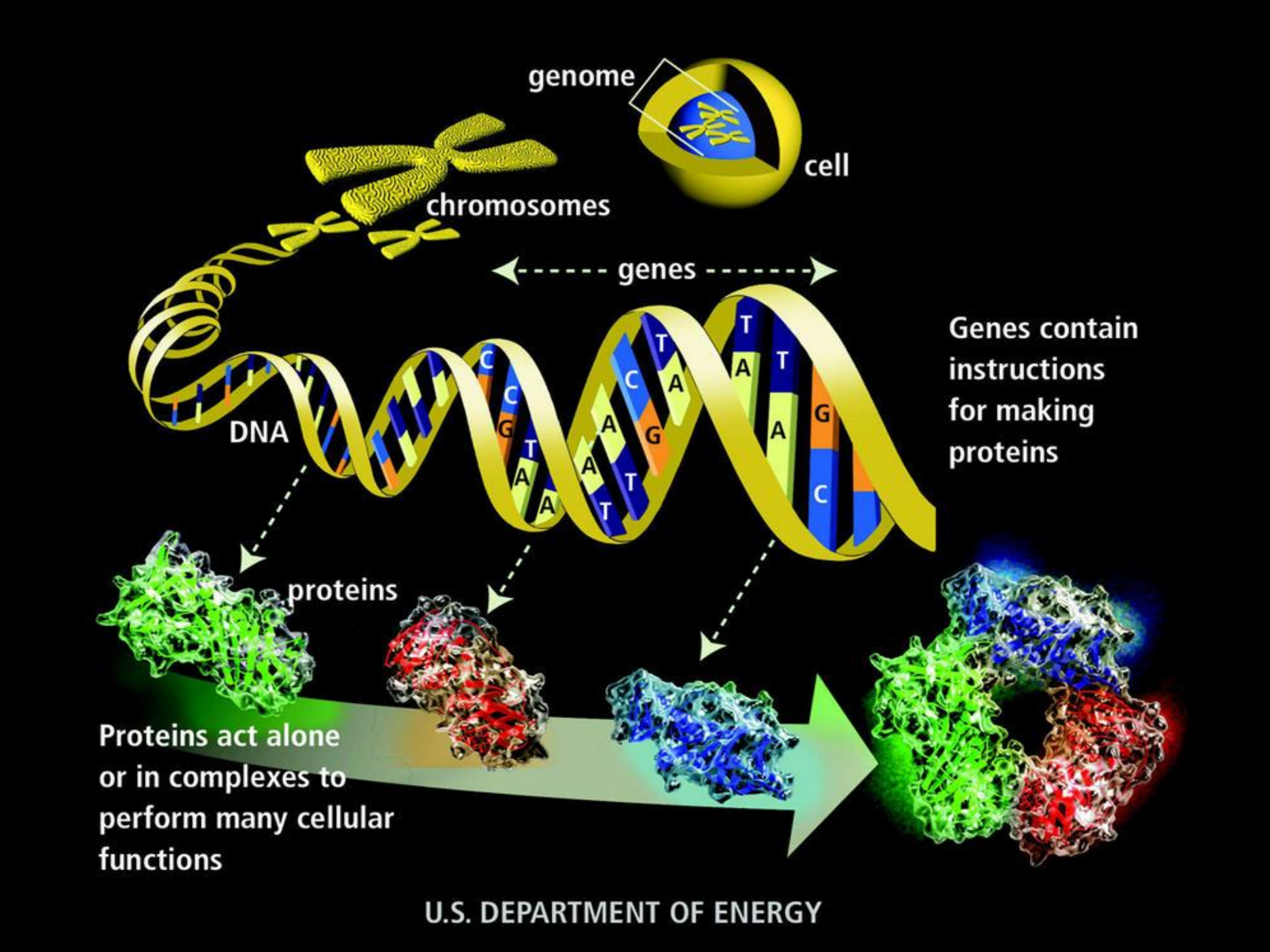
Imperial College London
Jan 2012


genome
cell
chromosomes
genes
DNA
Genes contain instructions for making proteins
proteins
Proteins act alone or in complexes to perform many cellular functions
U.S. DEPARTMENT OF ENERGY

Proteome

- Proteome
  = PROTEins expressed by a genOME

- The proteome is
  - **Time-** and **cell**- specific
  - Includes isoforms and post-translational modifications
  - Very dynamic with time and in response to environment
  - Different between cell types

Proteomics

- Classical proteomics

  - total proteomes (e.g. from two differently treated cell lines)

- Functional proteomics

  - more limited protein sets

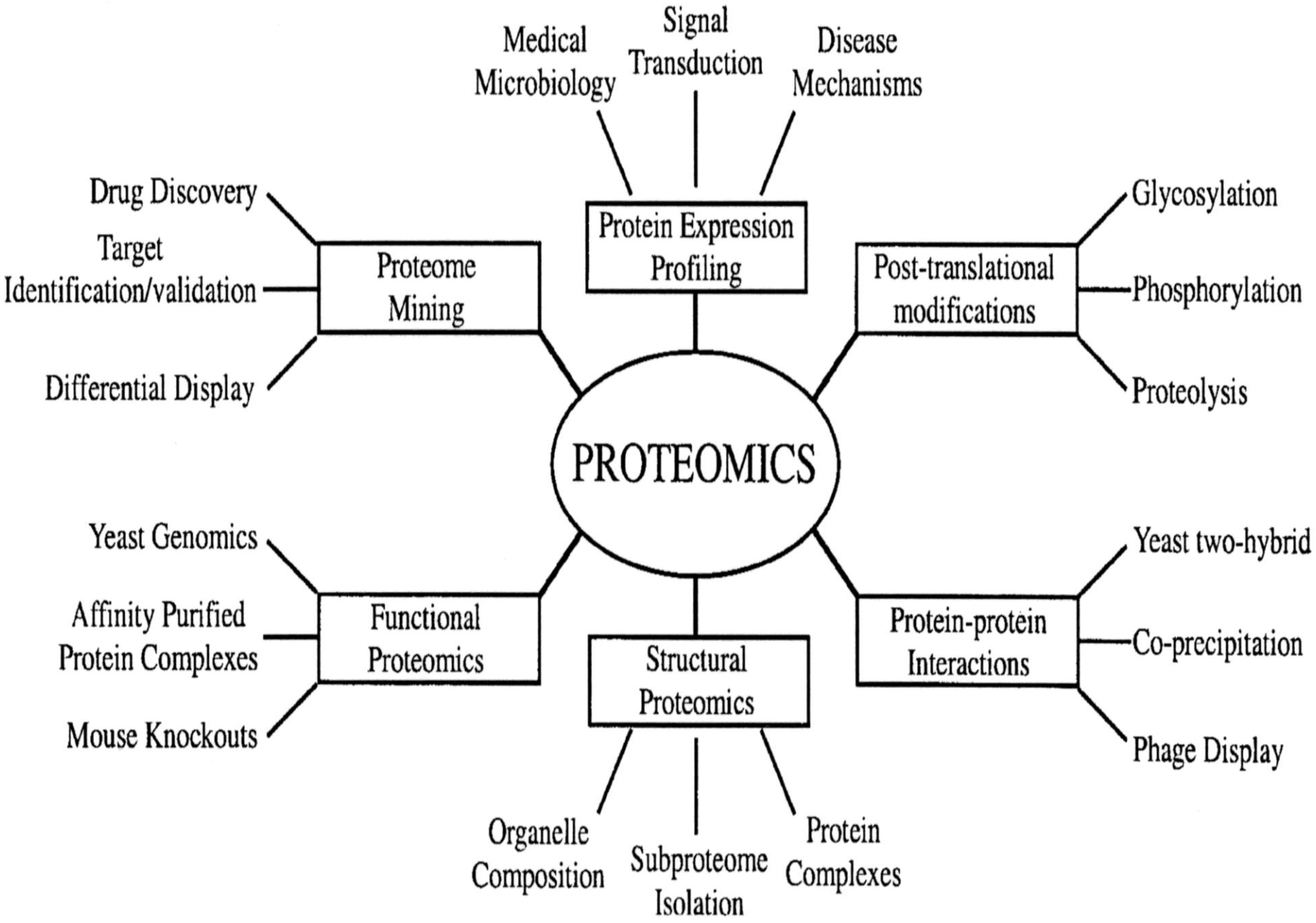


FIG. 1. Types of proteomics and their applications to biology.

Why is proteomics important?

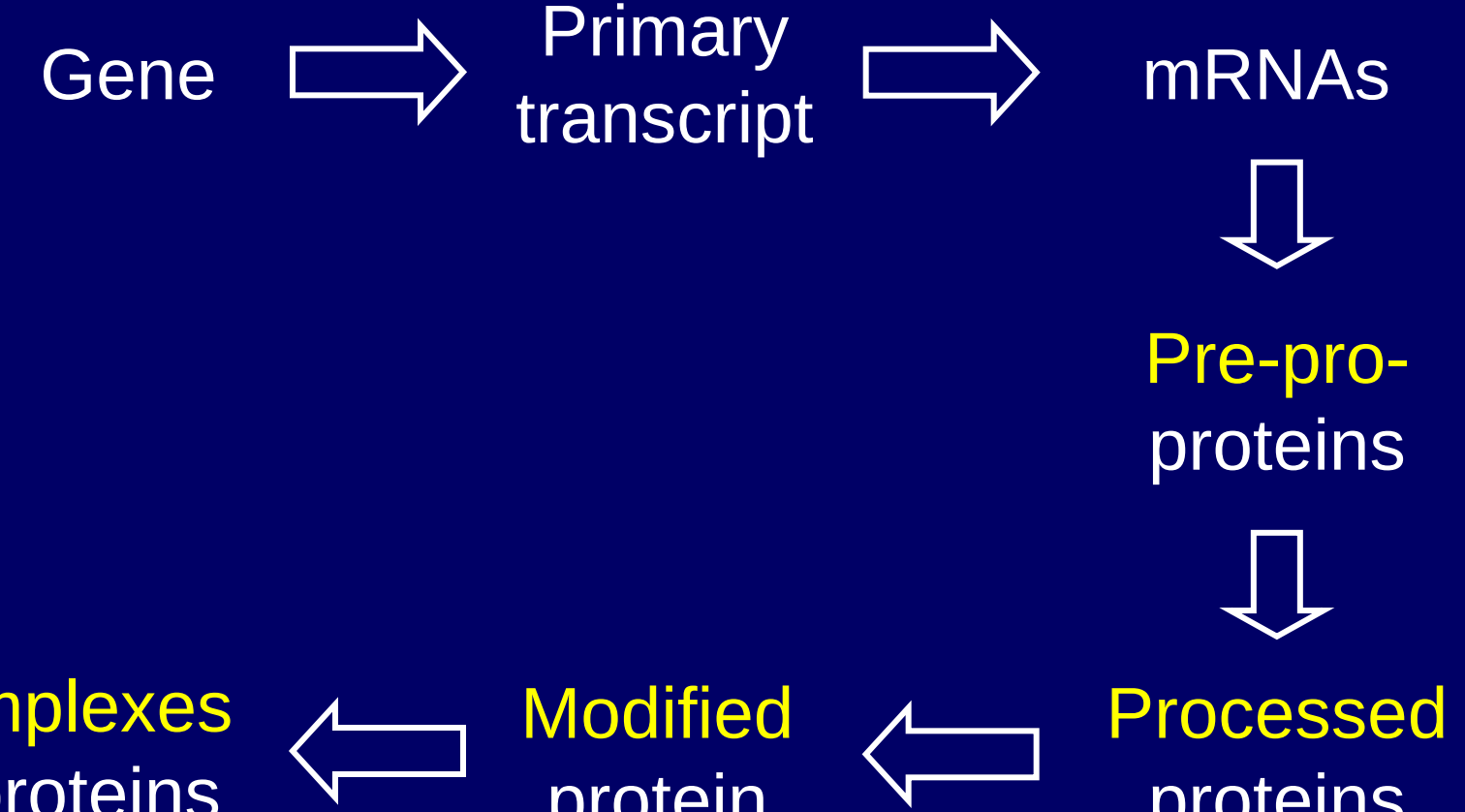
- Proteins are active agents in cell – they execute the biological functions encoded by genes
- Gene sequences (or genomes) and transcriptome analyses NOT sufficient to elucidate biological functions

"DNA makes RNA, RNA makes protein, and proteins make us."
Francis Crick

Four main questions

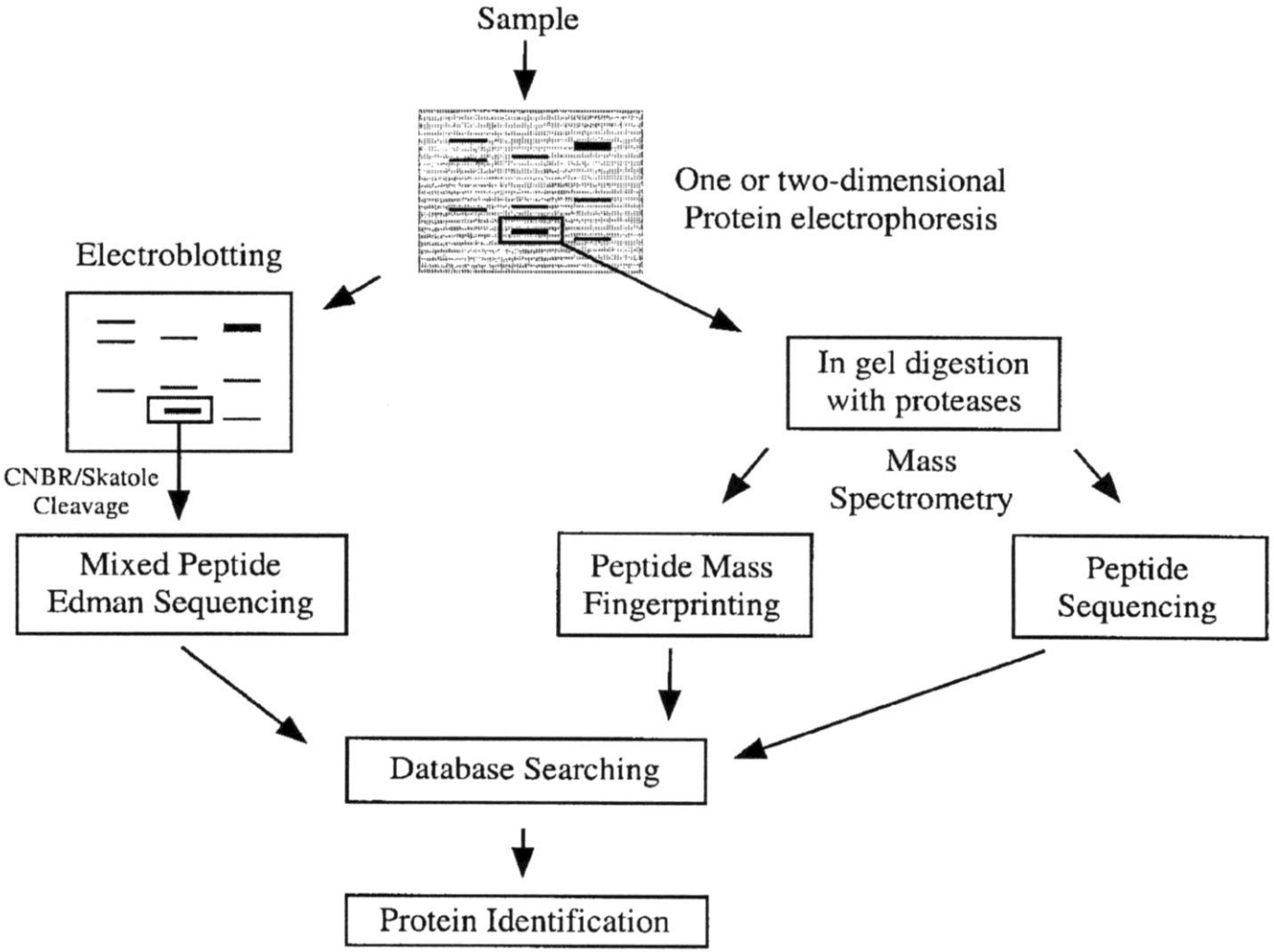
(1) What proteins are present?
(2) What other proteins does a particular protein interact with? (networks)
(3) What does a particular protein look like? (structure)
(4) What is the function of that protein? (context)

Proteomes are complex

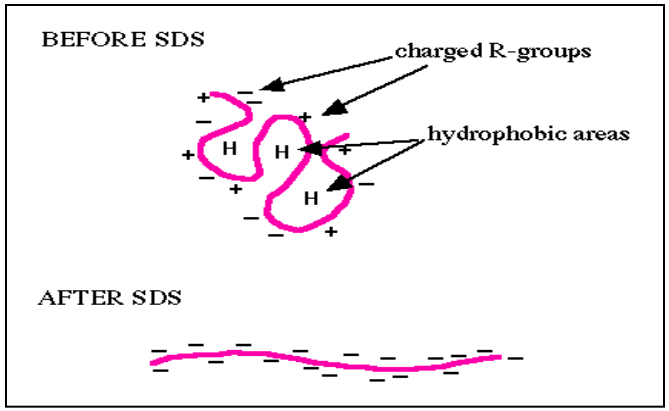


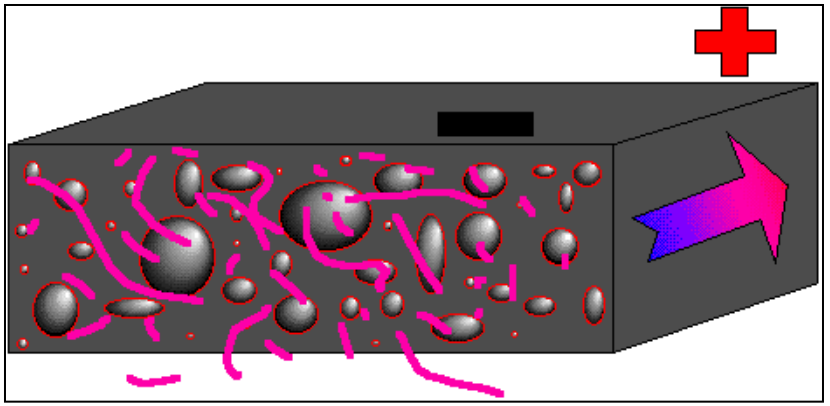
- Differential display of proteins (2DE)
  - mutants, knockouts, different stimuli….
- Protein identification
  - affinity purification (1DE, 2DE)
  - proteinchips (proteins or Abs)
- Protein modification
  - phosphorylation, glycosylation….
- Protein-protein interaction
- Protein ID by mass spectrometry

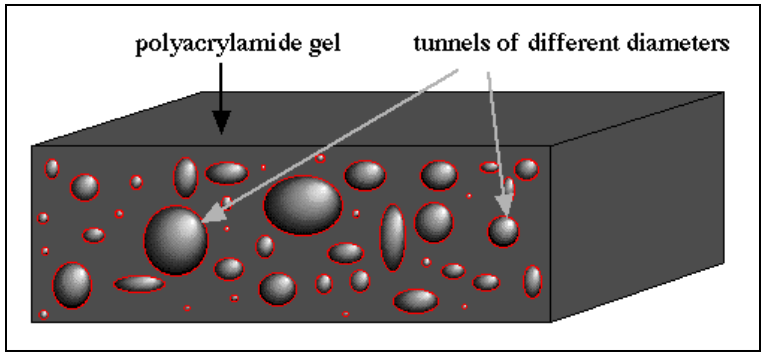
Overview of protein identification

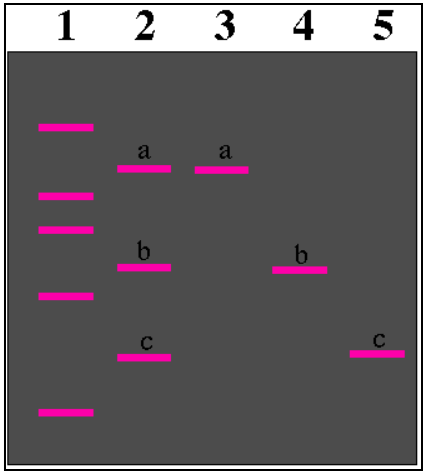


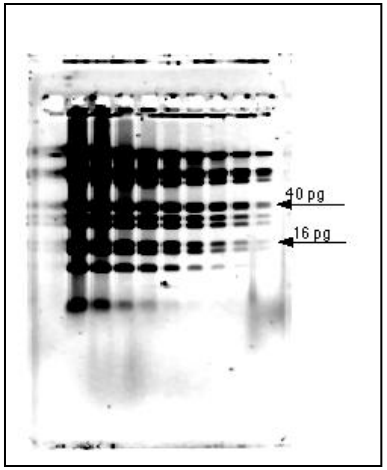
1-D SDS-PAGE


BEFORE SDS
charged R-groups
hydrophobic areas
H
H
H
AFTER SDS




polyacrylamide gel
tunnels of different diameters


1
2
3
4
5
a
a
b
b
c
c


40 pg
16 pg

Two dimensional electrophoresis (2-DE)

(1) Solubilise sample

(2) Separate by charge (first dimension)

- isoelectric focusing (IEF)
- immobilised pH gradients (IPG)

(3) Separate by molecular weight (second dimension)

- SDS-PAGE
- where focused IPG strip is the sample

(4) Stain gel

- Coomassie blue
- silver staining
- fluorescent staining (SyPRO dyes)

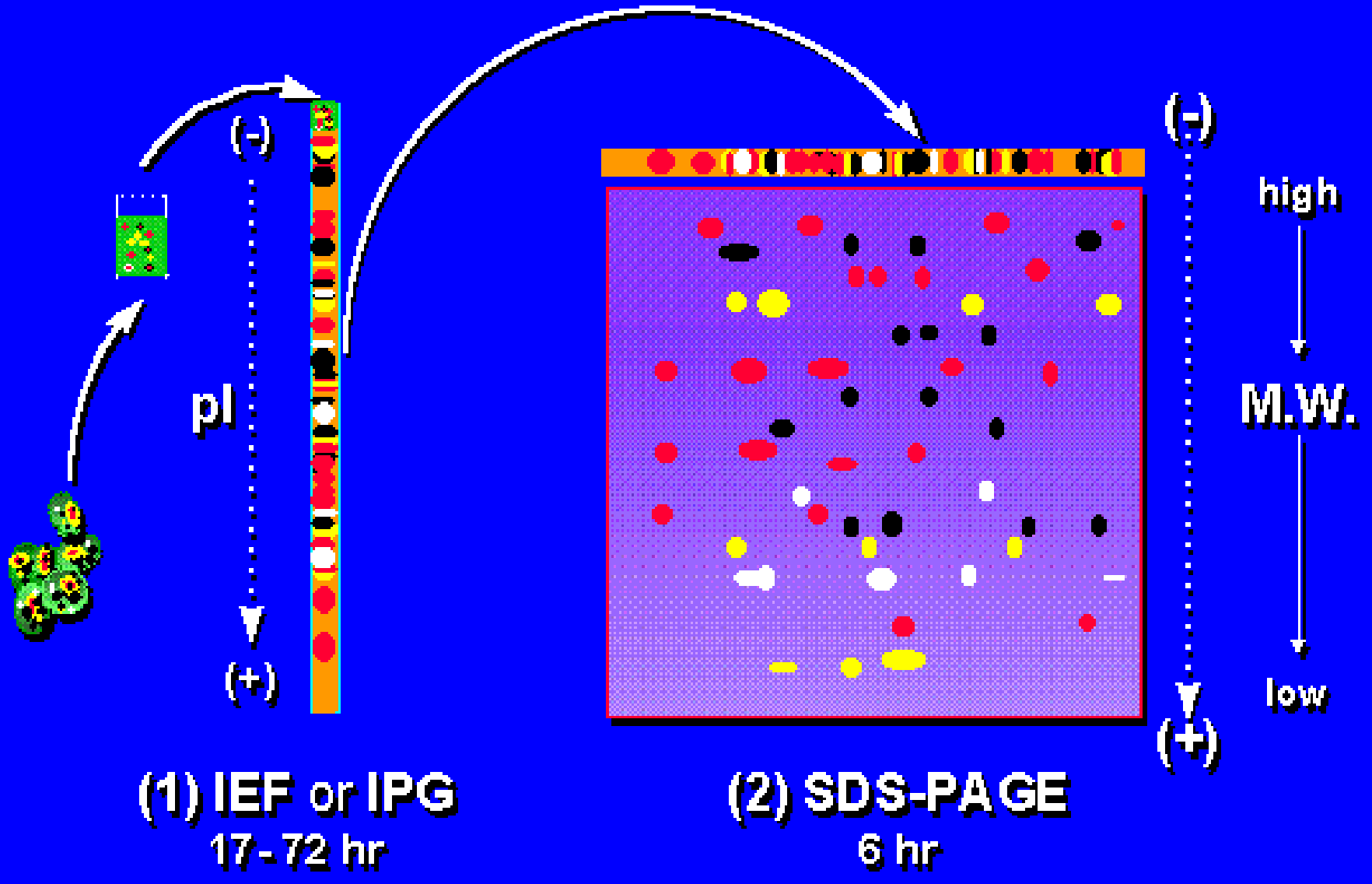
(5) Quantification

- scanning and analysis of image
- PDQuest, Image Master 2D elite

(6) Determine identity of spot of interest

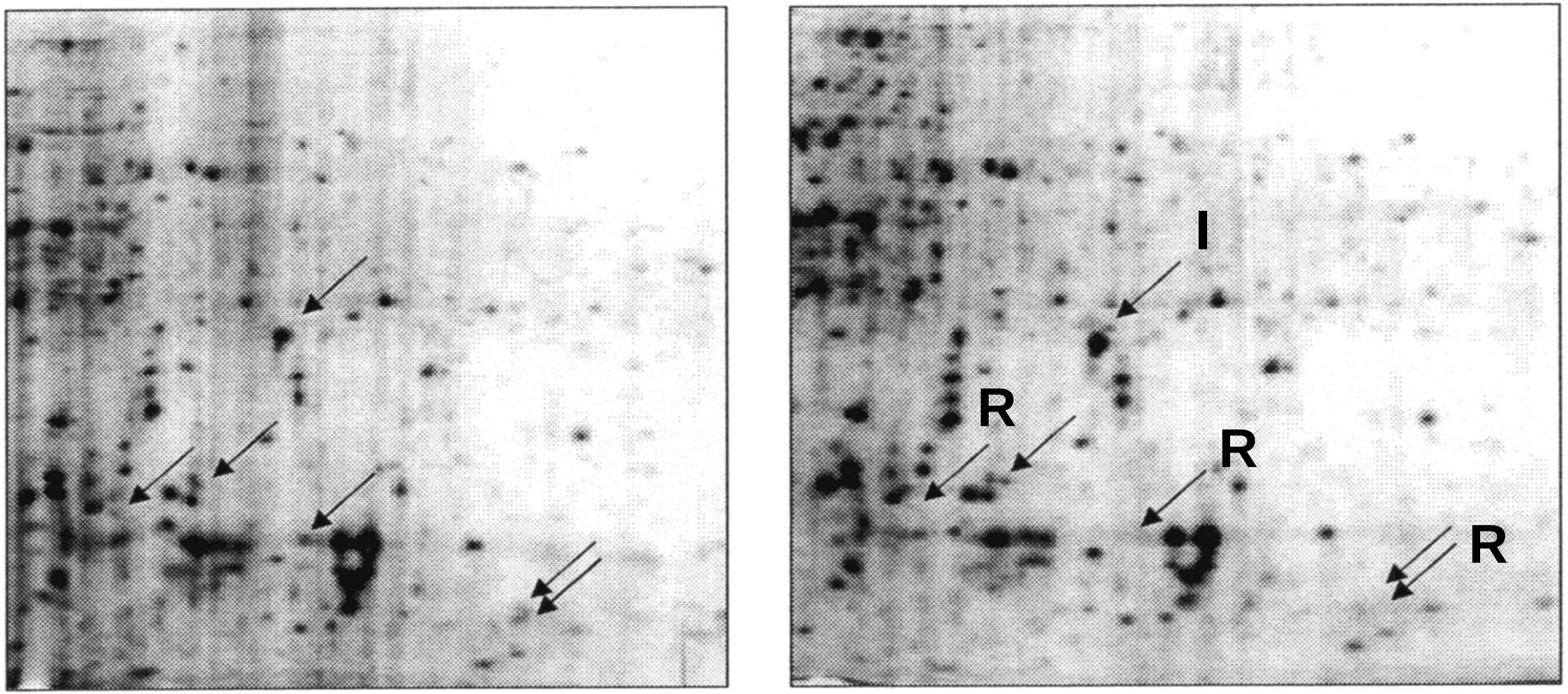
- mass spectrometry
- N-terminal sequencing

Two Dimensional Electrophoresis

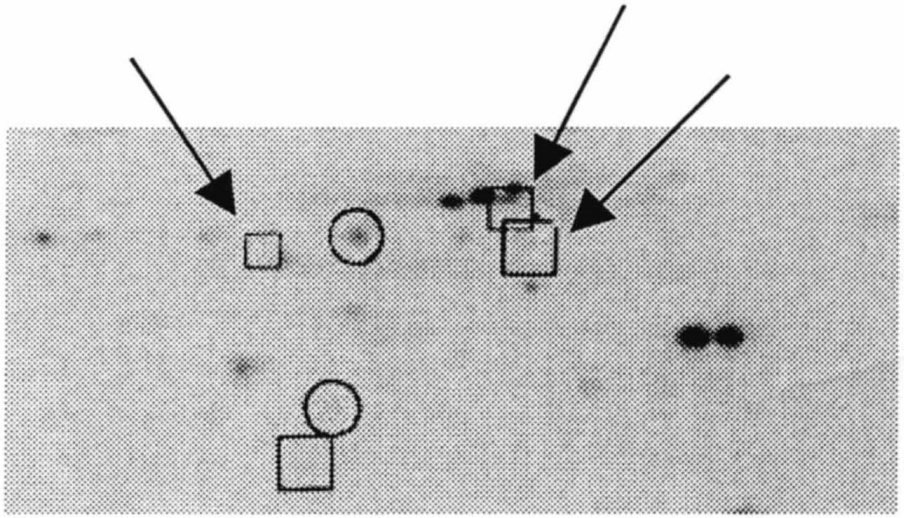


Control

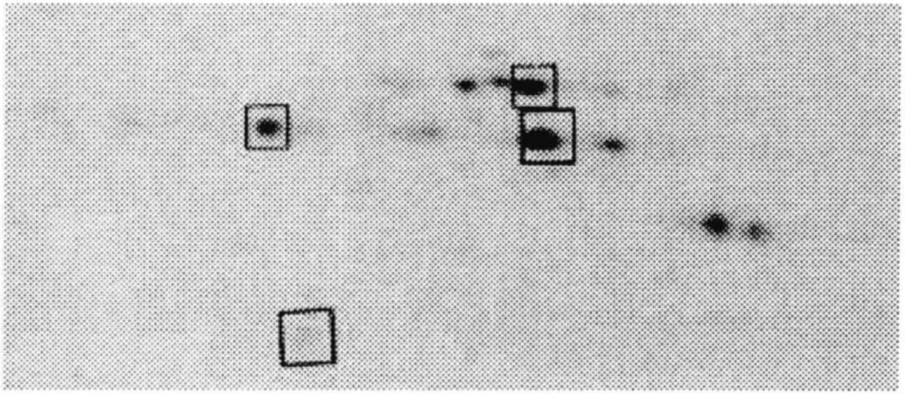
Treatment



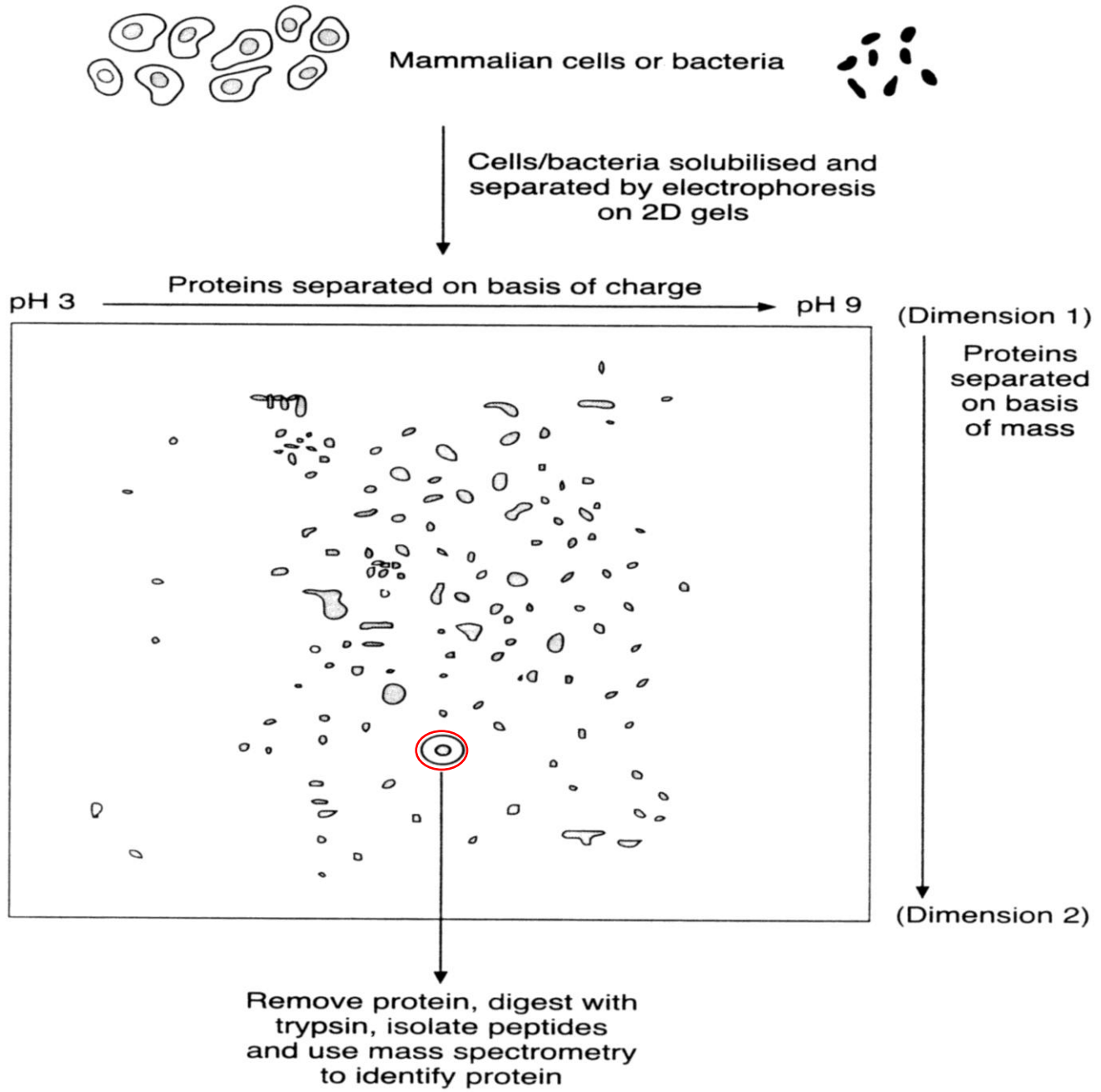
I Induced; R Repressed



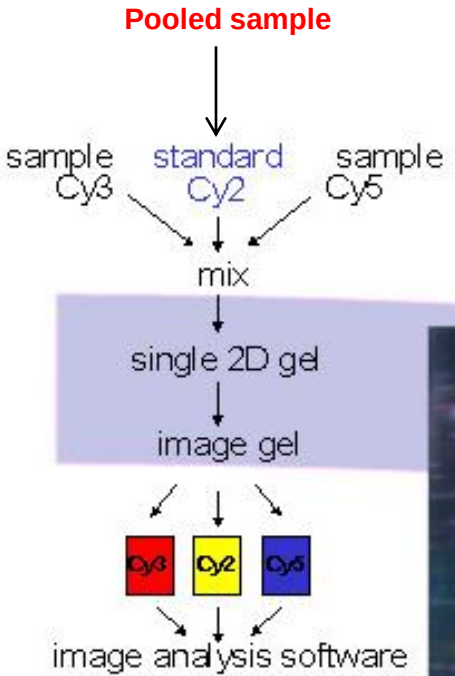
Control



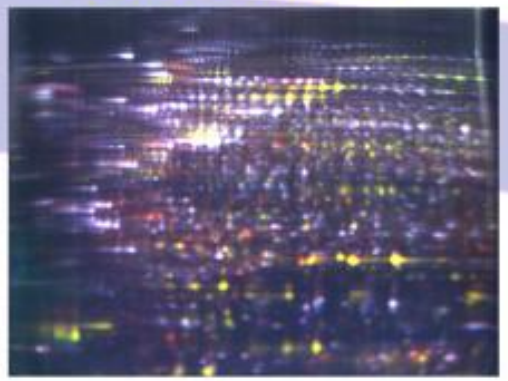
Treatment


Mammalian cells or bacteria
Cells/bacteria solubilised and separated by electrophoresis on 2D gels
Proteins separated on basis of charge
pH 3
pH 9
(Dimension 1)
Proteins separated on basis of mass
(Dimension 2)
Remove protein, digest with trypsin, isolate peptides and use mass spectrometry to identify protein

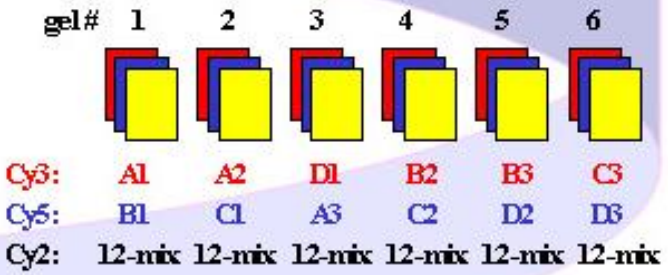
2D DIGE-MS



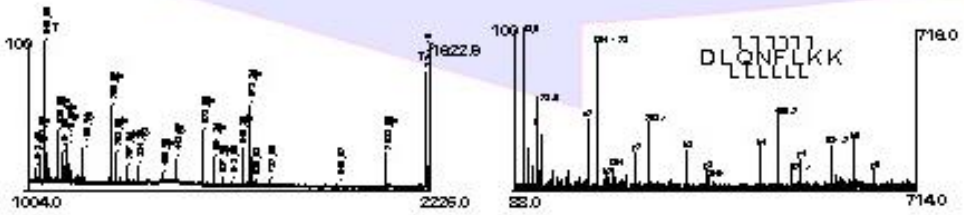
samples are co-resolved

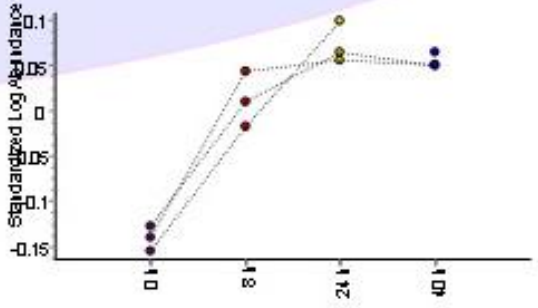


Quantitative inter-comparison of multiple samples (in replicate)



MALDI-TOF and TOF/TOF MS on targeted proteins

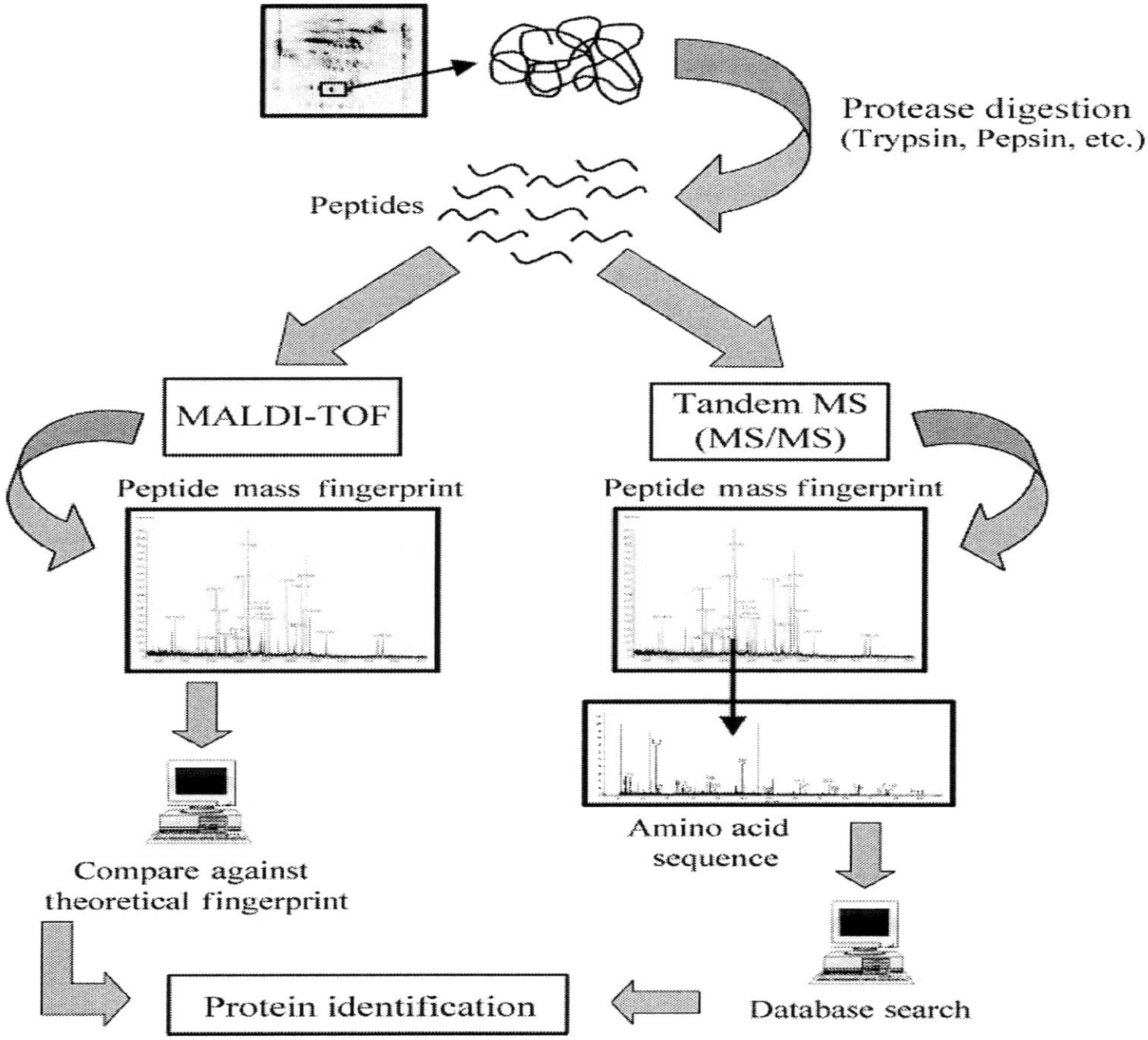




Identification of proteins

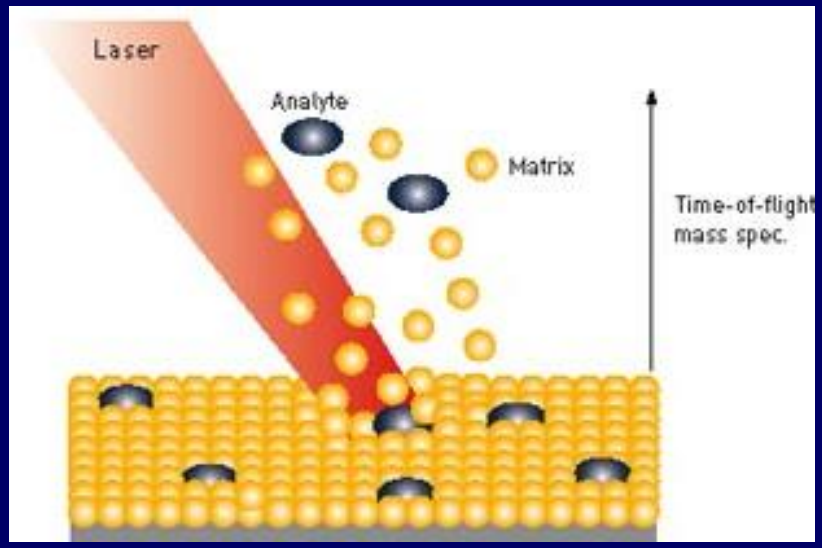
- (Database search by spot location)

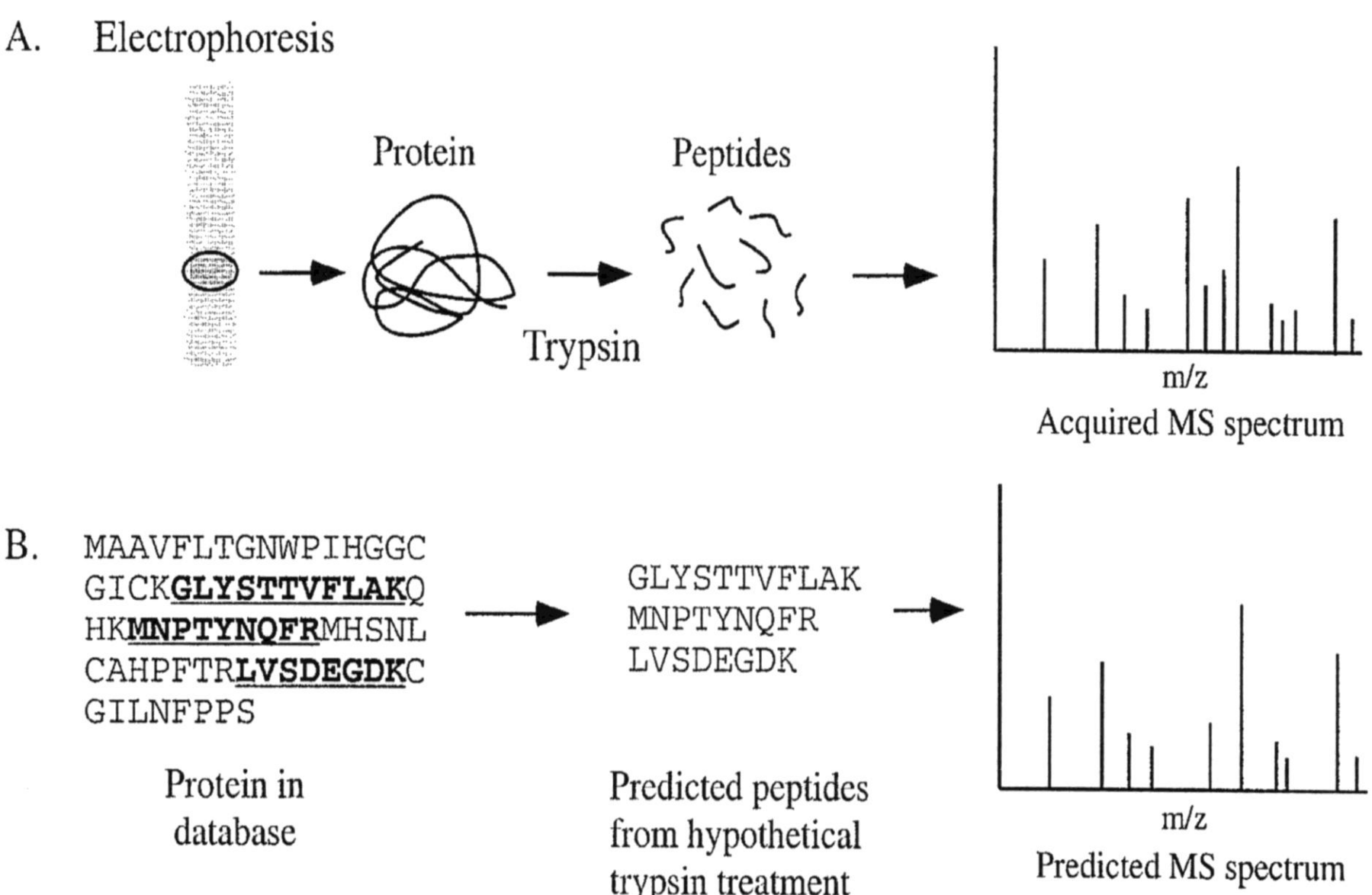
- Peptide digestion and mass spectrometry
  (1) Cut out band
  (2) Enzyme digestion (trypsin - Arg/Lys)
  (3) Extract peptides from gel piece
  (4) Analyse peptides using MALDI-TOF and/or Tandem MS/MS
  (5) Database searches


Protease digestion
(Trypsin, Pepsin, etc.)
Peptides
MALDI-TOF
Tandem MS
(MS/MS)
Peptide mass fingerprint
Peptide mass fingerprint
Amino acid
sequence
Compare against
theoretical fingerprint
Protein identification
Database search

MALDI-TOF (time of flight)

- Soft ionisation
  - tends to leave proteins intact
- Accuracy 0.1%
- Peptide digests and “whole” proteins

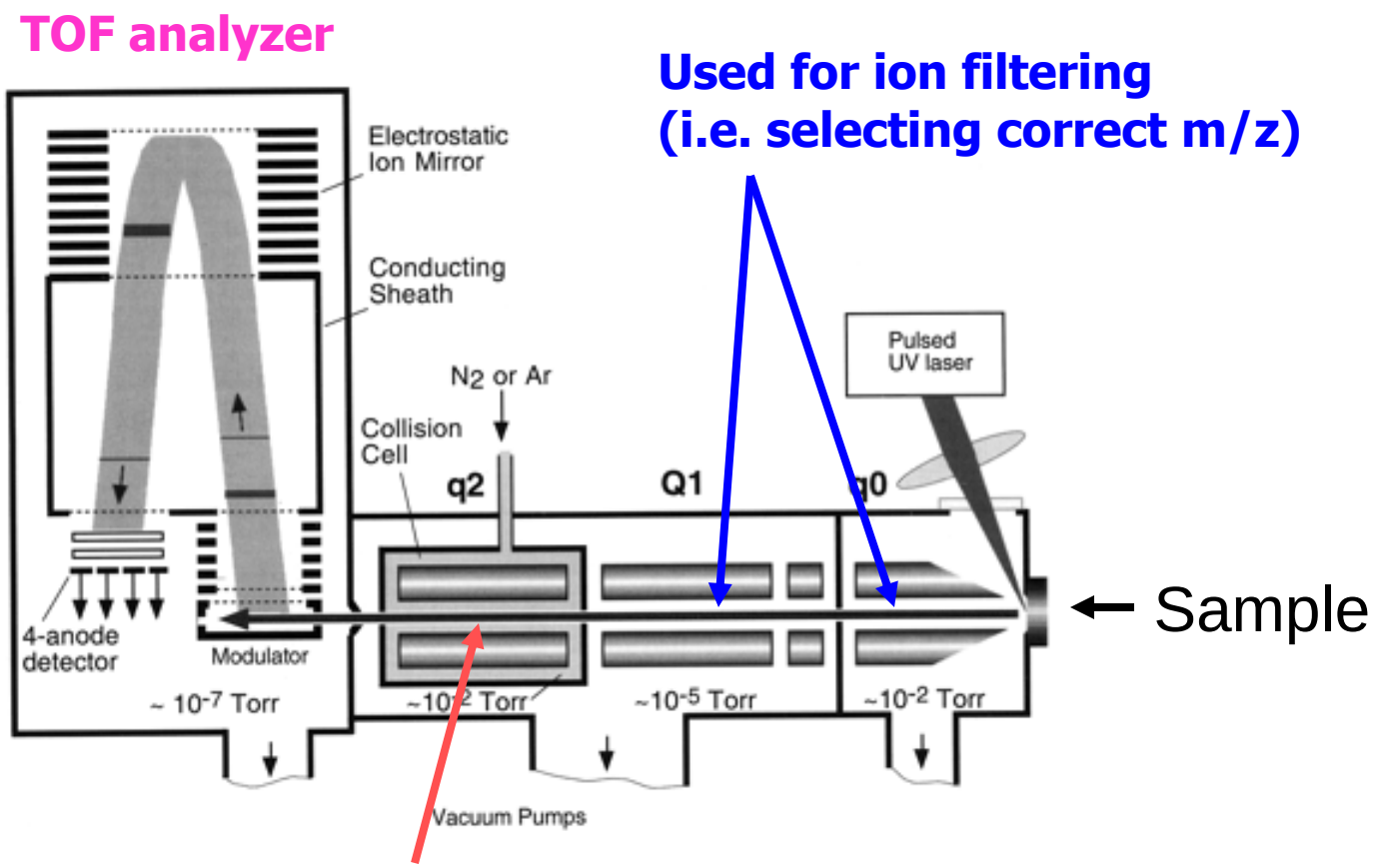



A.
Electrophoresis
Protein
Trypsin
Peptides
m/z
Acquired MS spectrum
B.
MAAVFLTGNWPIHGGC
GICKGLYSTTVFLAKQ
HKMNPTYNQFRMHSNL
CAHPFTRLVSDEGDKC
GILNFPPS
Protein in database
GLYSTTVFLAK
MNPTYNQFR
LVSDEGDK
Predicted peptides from hypothetical trypsin treatment
m/z
Predicted MS spectrum

Tandem MS/MS can be used for protein sequencing

- identifies amino acid sequence of small peptides

Tandem MS/MS e.g. QTof

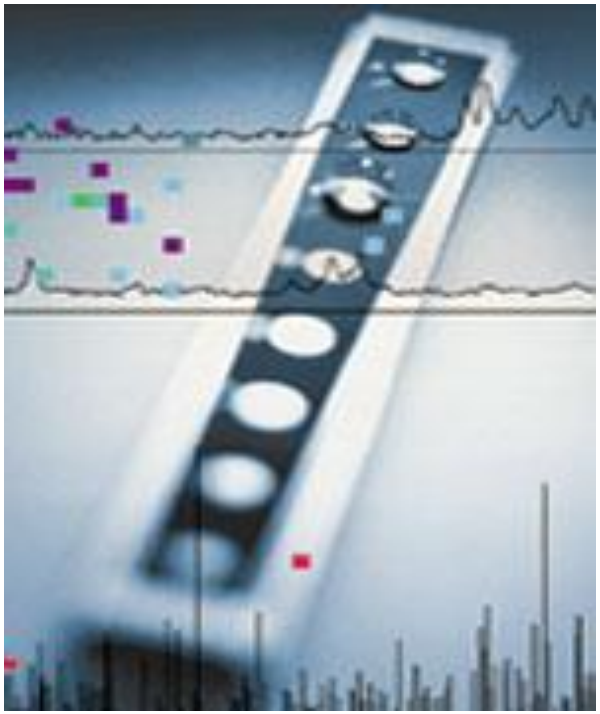


Problems of 2D-E

- Reproducibility
- Poor recovery of proteins (especially membrane or hydrophobic)
- Limited pH range
- Sensitivity of low abundant proteins
- Labour intensive
- In general automation difficult

Biomarker Discovery

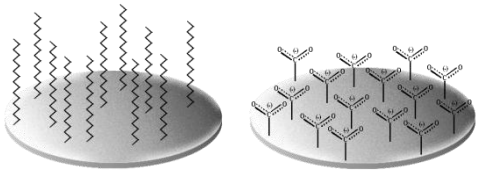
- Surface-Enhanced Laser Desorption Ionisation (SELDI) Mass Spectrometry



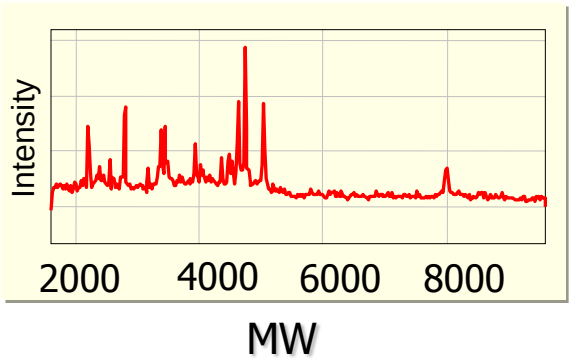
Overview of SELDI process

SELDI combines two techniques:

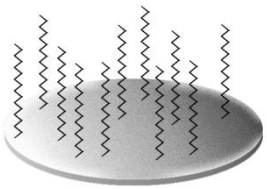
Retentate Chromatography



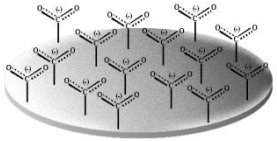
Mass Spectrometry



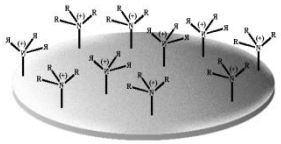
ProteinChip® Arrays



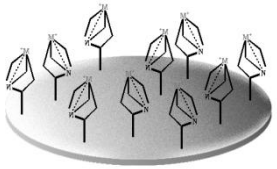
(Reversed Phase)



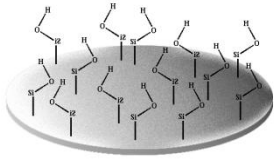
(Cation Exchange)



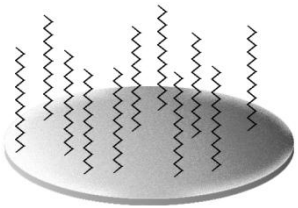
(Anion Exchange)



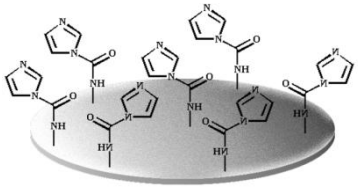
(Metal Affinity)



(Normal Phase)



(Neat Desorption)



(Reactive Surfaces)

(Gold)

Generates different protein profiles from the same sample

Slide courtesy of BIORAD

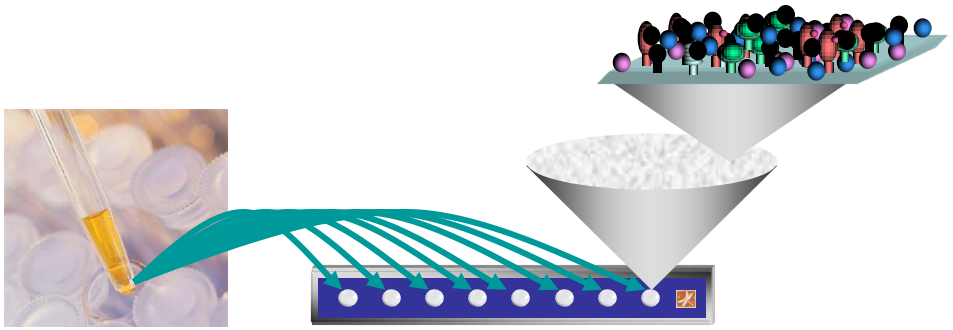
Sample Types

Mechanistic studies	Animal models	Clinical samples
■ Cell lysates	■ Tissue lysates	■ Serum or plasma
■ Cell culture media	• Liver	■ Urine
■ Laser capture microdissected cells	• Brain	■ CSF
■ Tissue lysates	• Kidney	■ Pleural effusions
	■ Urine	■ Laser capture microdissected cells
	■ Serum or plasma	■ Lavage/Sputum/Saliva
	■ Laser capture microdissected cells	■ Aspirates

ProteinChip® Preparation

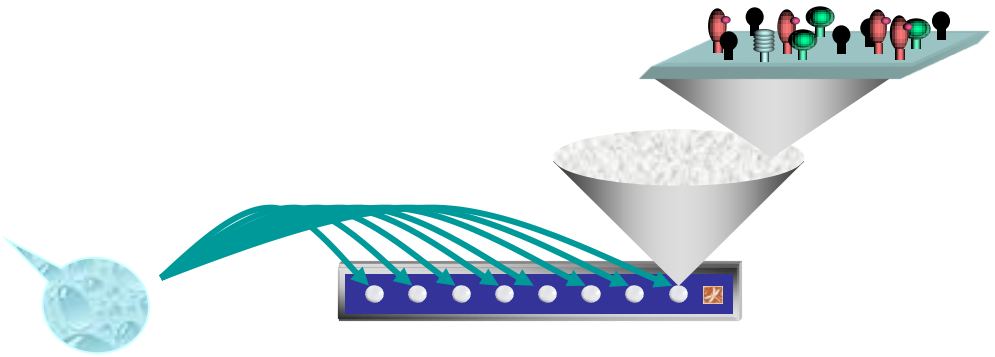
1. Apply Crude Sample

Proteins bind to chemical or biological “docking sites” on the ProteinChip® surface through an affinity interaction.



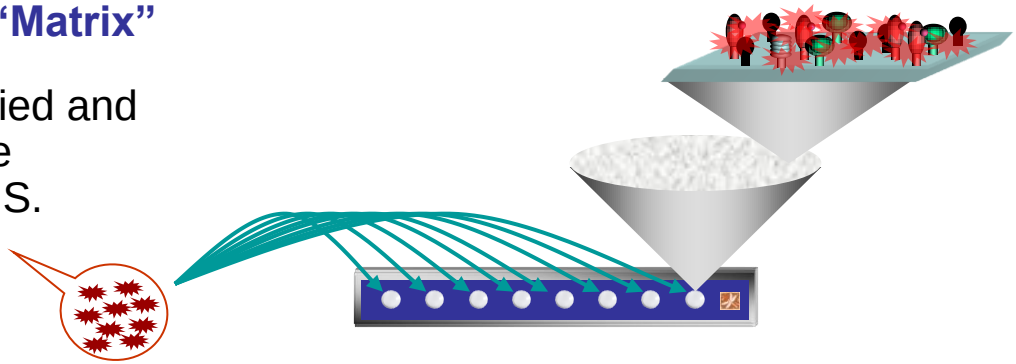
2. Wash ProteinChip®

Proteins that bind non-specifically, and buffer contaminants are washed away, eliminating sample “noise”.



3. Add Energy Absorbing Molecules or “Matrix”

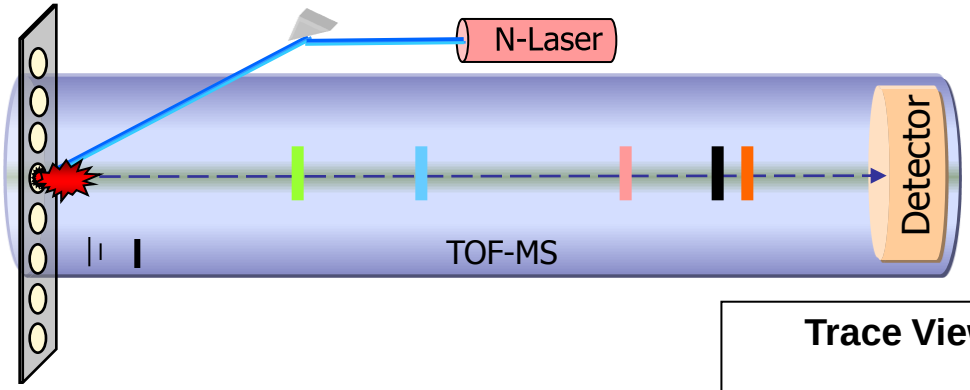
After sample processing the array is dried and EAM is applied to each spot to facilitate desorption and ionization in the TOF-MS.

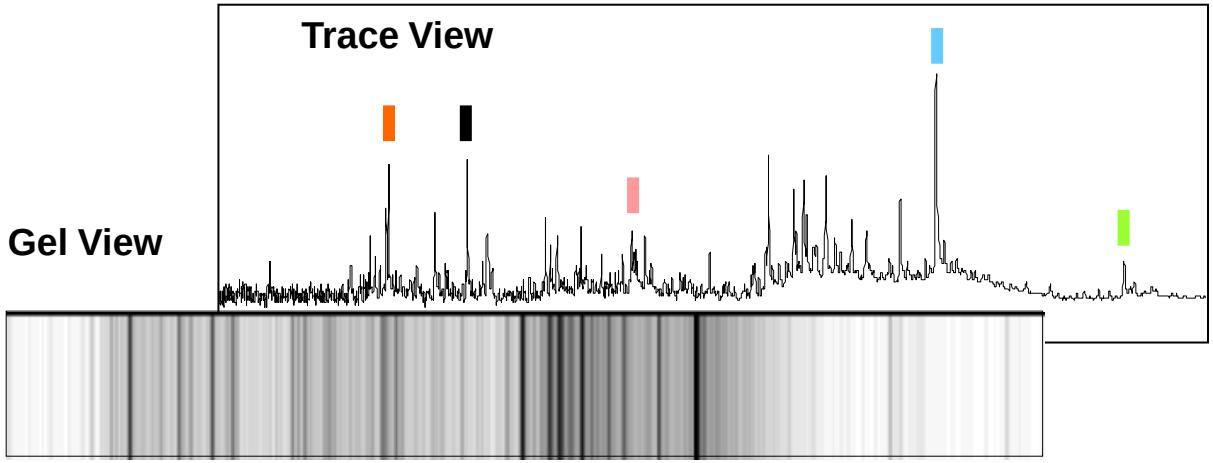


Slide courtesy of BIORAD

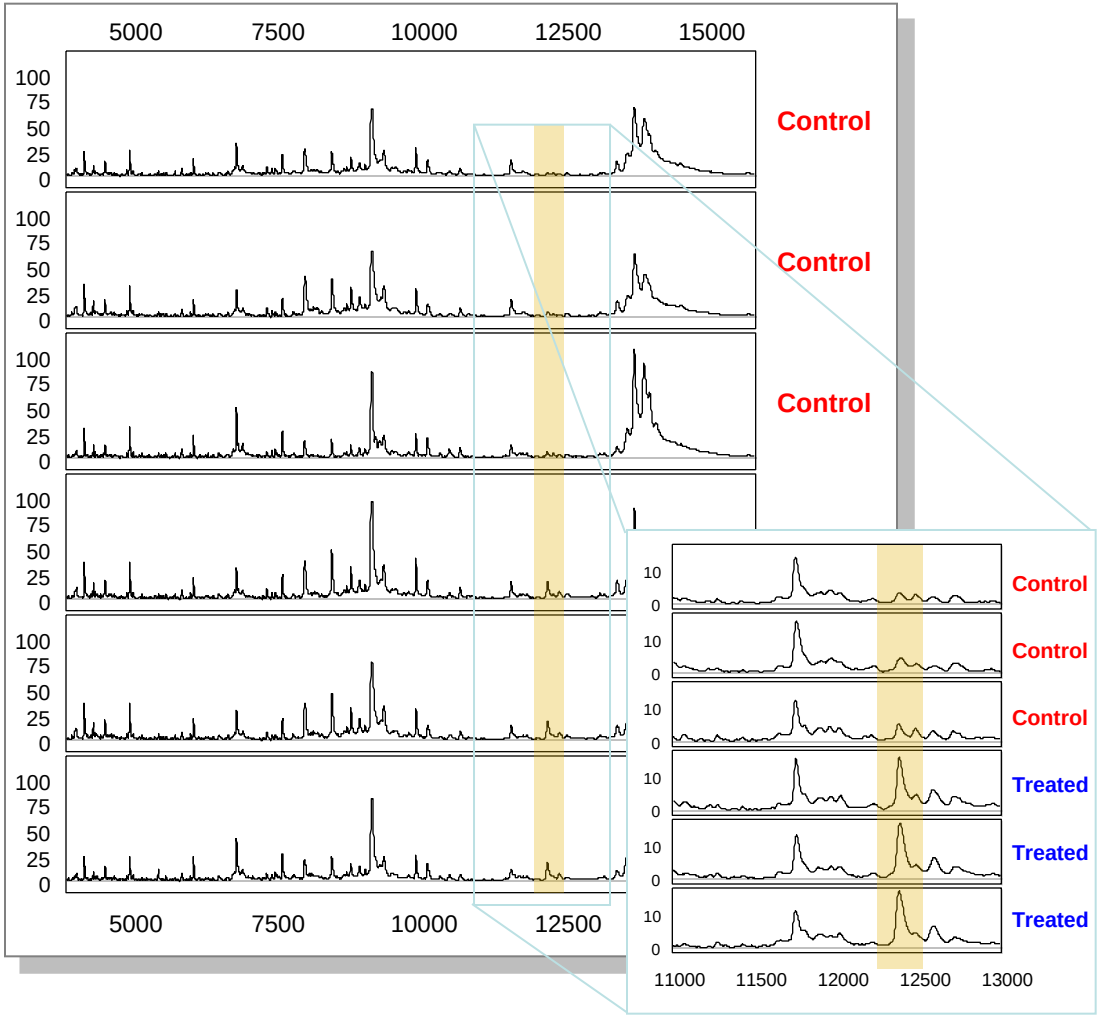
SELDI-TOF-MS Detection

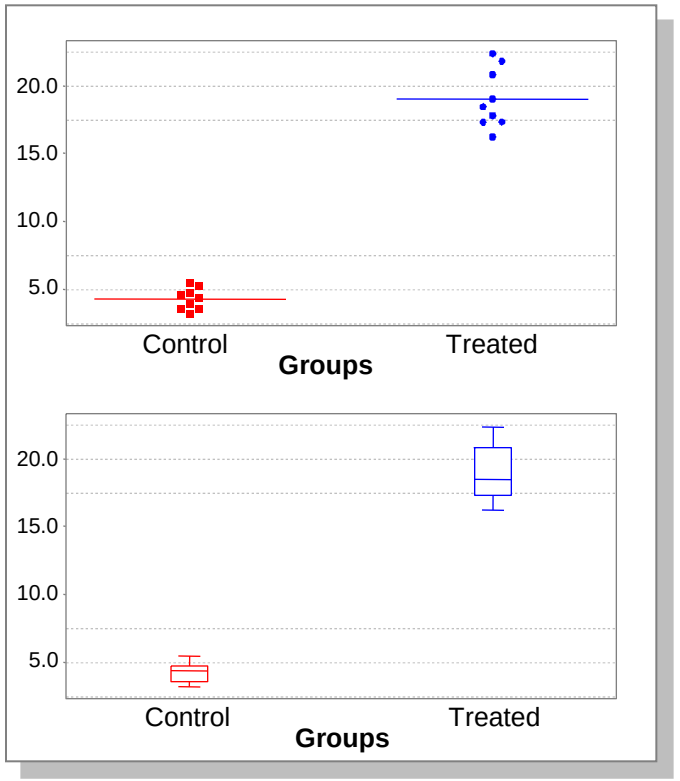
- Retained proteins are “eluted” from the array by the laser desorption - ionisation. It is a Surface Enhanced Laser Desorption / Ionisation (SELDI).
- Ionised proteins are detected and their mass is determined by time-of-flight mass spectrometry (TOF-MS).





Differential Expression





Under specific bind/wash conditions, look for statistically significant up and down regulation of protein signals.

SELDI in clinical medicine

- Predominant in the field of cancer but increasingly used in infectious disease
- A common pattern of inflammation
- Bacterial pathogens can induce distinct proteomic or genomic signatures

Disease or etiological agent	
Viral	Hepatitis B/ C
	SARS
	HIV
	HTLV
	BK virus
Bacterial	TB
	Intra-amniotic infection
	Endocarditis
Parasite	Trypanosomiasis

Hamilton & Langford 2010

SELDI and diagnosis of TB

Identification of diagnostic markers for tuberculosis by proteomic fingerprinting of serum

Dan Agranoff, Delmiro Fernandez-Reyes, Marios C Papadopoulos, Sergio A Rojas, Mark Herbster, Alison Loosemore, Edward Tarelli, Jo Sheldon, Achim Schwenk, Richard Pollok, Charlotte F J Rayner, Sanjeev Krishna

- Phase 1: Culture-proven TB and controls
  - Gambia and Uganda
  - 4 protein biomarkers
  - Sensitivity 94.4% and Specificity 91.8%
- Phase 2: UK cohort
  - Sensitivity 89% and Specificity 74%

Adult vs. childhood TB

Feature	Adults	Children
Disease type	Reactivation	Primary progressive disease
Symptoms	Cough, fever, chest pain, haemoptysis, weight loss	Negligible symptoms at time of primary infection
Infectivity	Smear positive pulmonary TB patients are infective to others	Paucibacillary disease, usually not infectious
Disease progression	Low risk of progressing to active disease	High risk of progressing to active disease

Estimates of Childhood Morbidity & Mortality from TB

- 1.7 million deaths per year
- 9 million new cases
- Despite current initiatives (DOTS) less than half of cases detected
  - Higher in children and HIV+ patients

- Current diagnostic tools inadequate (X-ray, Sputum, Tuberculin skin test)
- Misdiagnosis→ Increase in multi-drug resistant disease

Diagnostic methods - culture

Culture of samples from suspected sites of infection to determine the presence of *Mycobacterium tuberculosis*

Gold standard

Allows antibiotic sensitivity to be determined

Drawbacks:

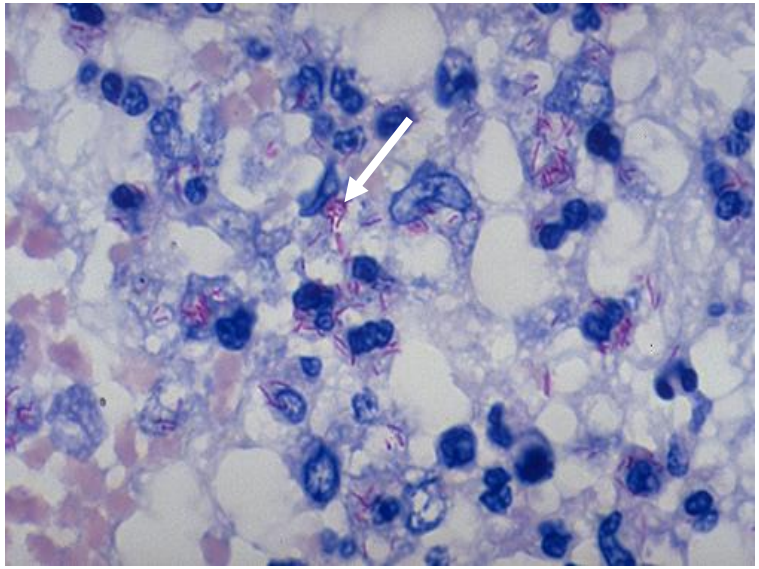
- Paucibacillary load
- Obtaining samples
- Gastric aspirates
- Loss of bacteria during the processing/clean-up of the sample
- Time consuming
- Costly

Diagnostic methods – sputum smear microscopy

Samples stained using Ziehl Neelson staining procedure in which bacilli retain stain following washing with strong acid and appear red under the microscope

Drawbacks:

- WHO estimated 35% sensitivity
- 10^4-10^5 bacteria
- Based on sputum so difficulty in detecting non-pulmonary disease
- Cannot always distinguish between *M tb* and other NTM



Diagnostic methods - TST

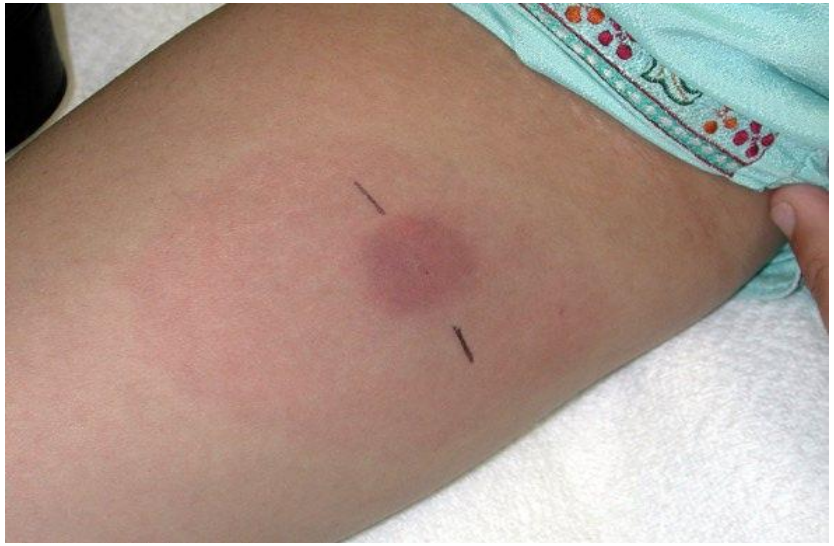
Intradermal injection of Purified Protein Derivative followed by assessment of the induration 48hrs later

Induration > 10 mm TB infection

Induration < 10 mm No TB infection

Drawbacks:

- False positives
  - Exposure to environmental strains
  - Previous immunisation w/ BCG
- False negatives
- Subjectivity and variability
- Boosting
- Convenience and resources

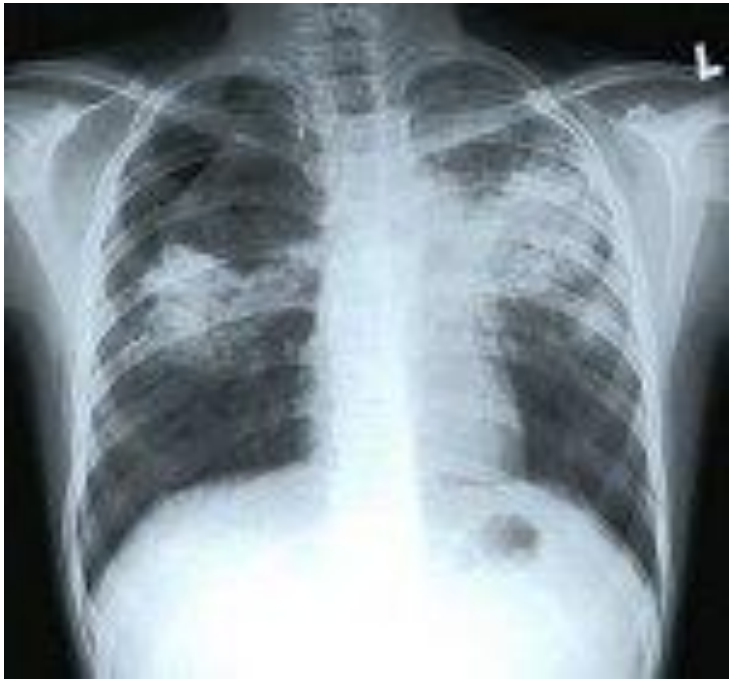


Diagnostic methods – chest X-Ray

Used to detect lung abnormalities in people who have symptoms of TB

Drawbacks:

- Cannot confirm active TB
- Scarring from previous infection
- Confounding infections
- Low ability to detect early stage TB

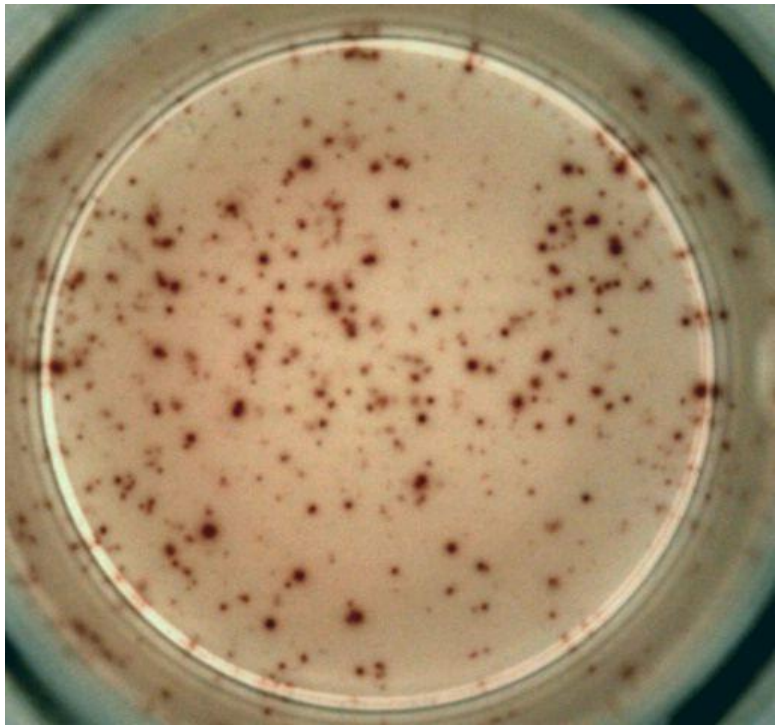


Diagnostic methods - IFN-γ

Whole-blood or enzyme-linked immunospot (ELISpot) assays to detect T cell-specific responses

Drawbacks:

- New vs. past infection
- Active vs. latent
- Age-dependant response



AIM

To find serological biomarkers of tuberculosis disease which could be used in the development of a new diagnostic test for paediatric TB

Why look for serum biomarkers?

- Blood test
- Small quantity of serum required
- Single visit
- Faster diagnosis
- Treatment commenced sooner

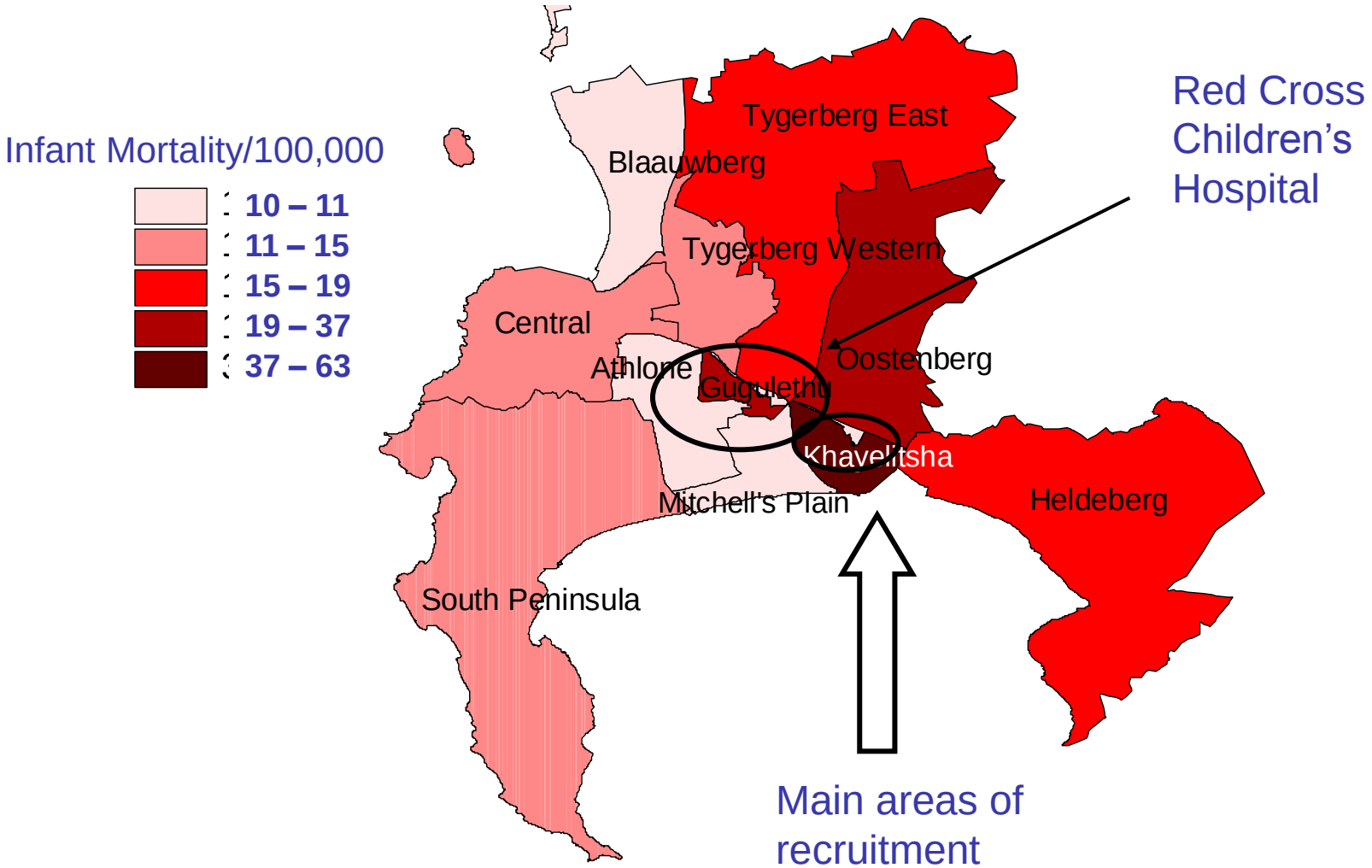
Epidemiology of TB in South Africa

- One of 13 countries accounting for 75% of world's TB cases & 80% of deaths
- Estimated no. new cases per year ~ 495/100,000*
- Cape Town TB incidence, 2003
  - Adult TB: 845/100,000†
  - Childhood TB: 441/100,000†
- Primary healthcare
- Secondary and tertiary level services

* WHO, 2001

† Int J Tuberc Lung Dis, 2004

Cape Metropole



Slide courtesy of Suzanne Anderson

Recruitment

Western Cape Study Sites

Malawi Study Sites

-High HIV/ TB co-infection

-Highest rates of TB

-Highest childhood TB

-Urban setting

-High rates of HIV

-High incidence of malaria

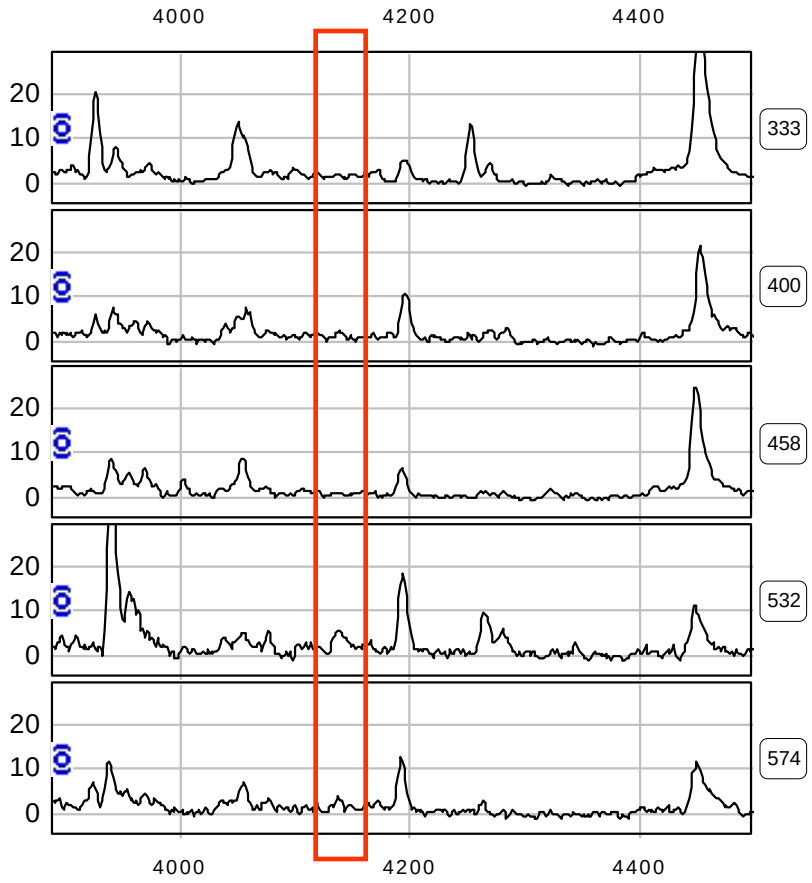
-Lower incidence of TB

-Rural setting

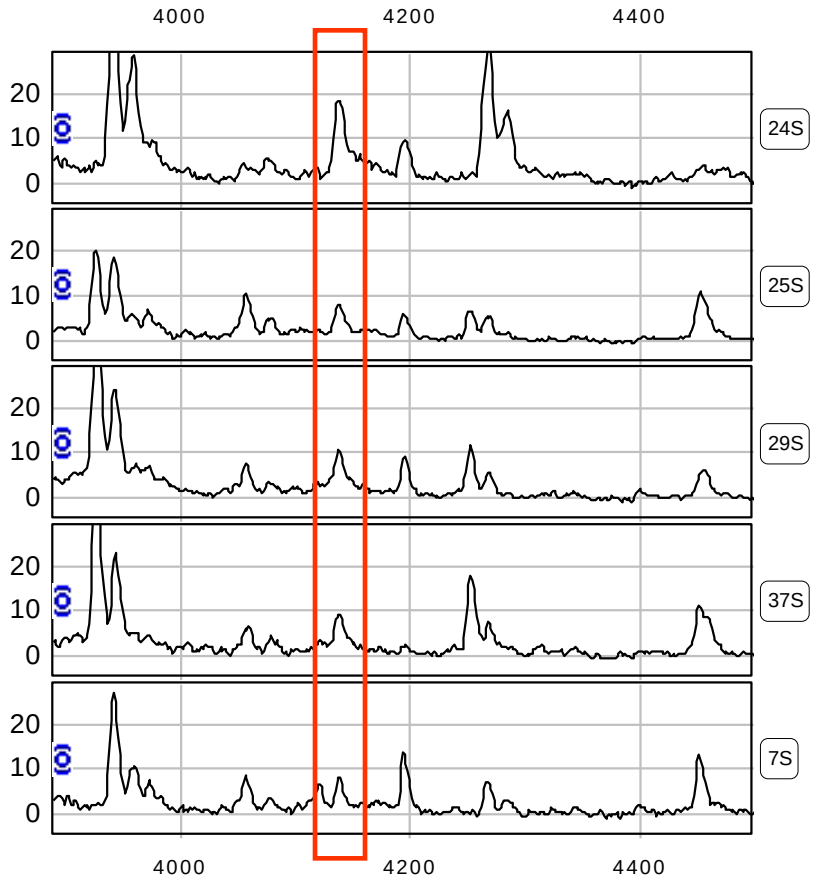
4 kDa protein biomarker

Positive or Negative biomarkers of infection

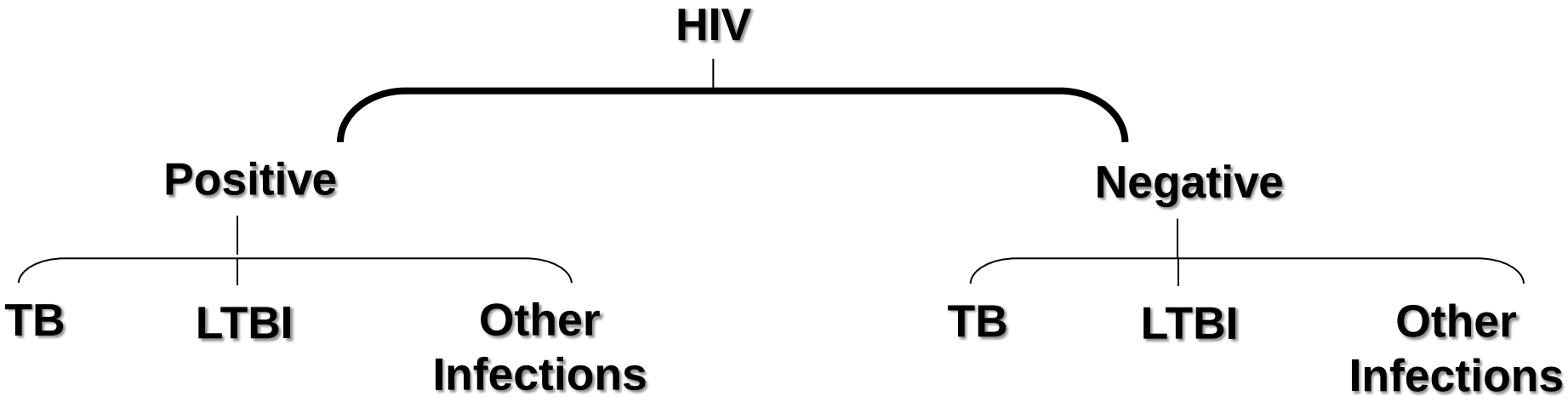
P/ EP



OI's

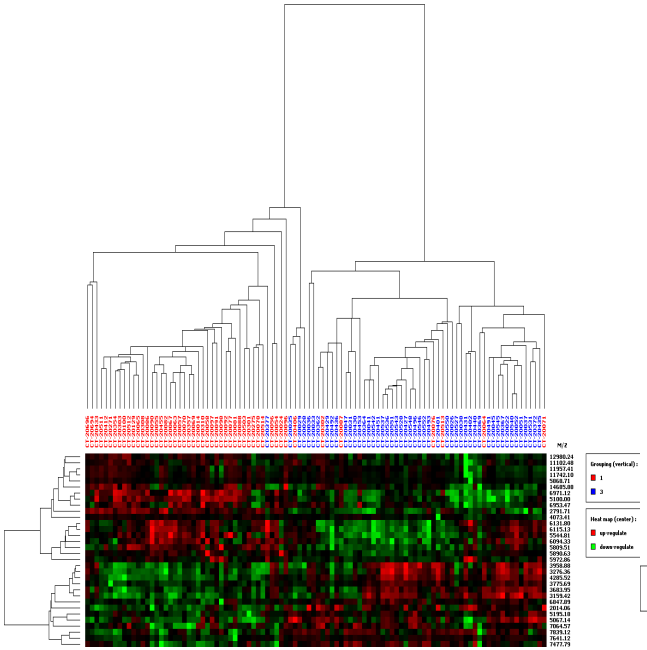


Current EU study

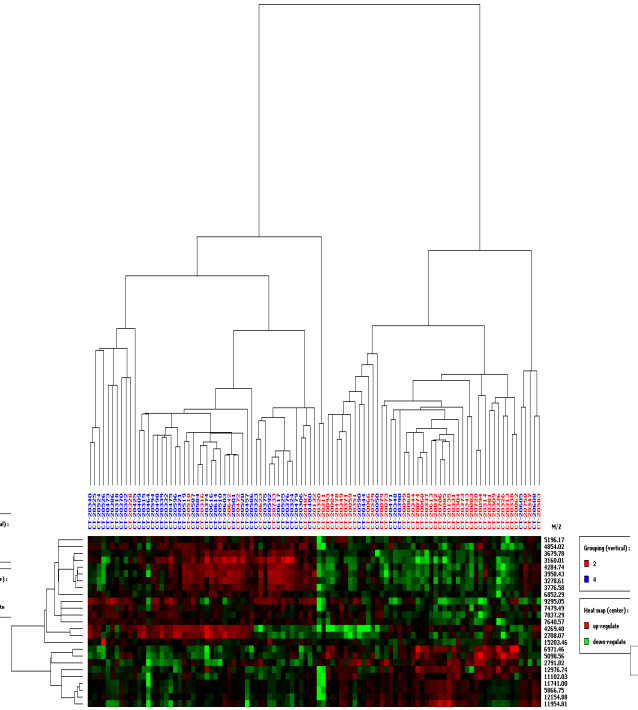


- Highly characterised cohorts
- Large number of patients and healthy controls (1500)
- Children and adults recruited
- Two study sites to ensure that biomarkers can distinguish between TB and other disease
- Validation on 1800 independent samples

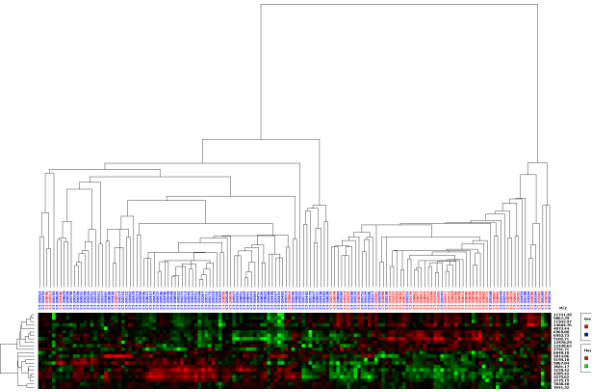
Cape Town
TB+ vs. LTBI (HIV-)



Cape Town
TB+ vs. LTBI (HIV+)

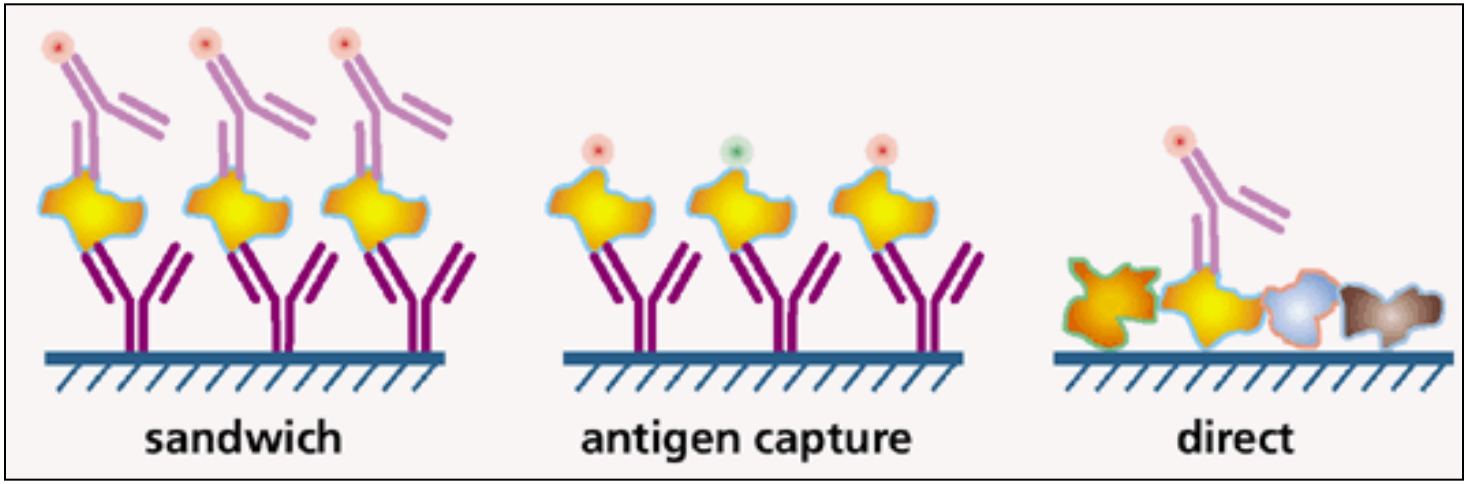


Cape Town & Karonga
TB+ vs. LTBI (HIV-)



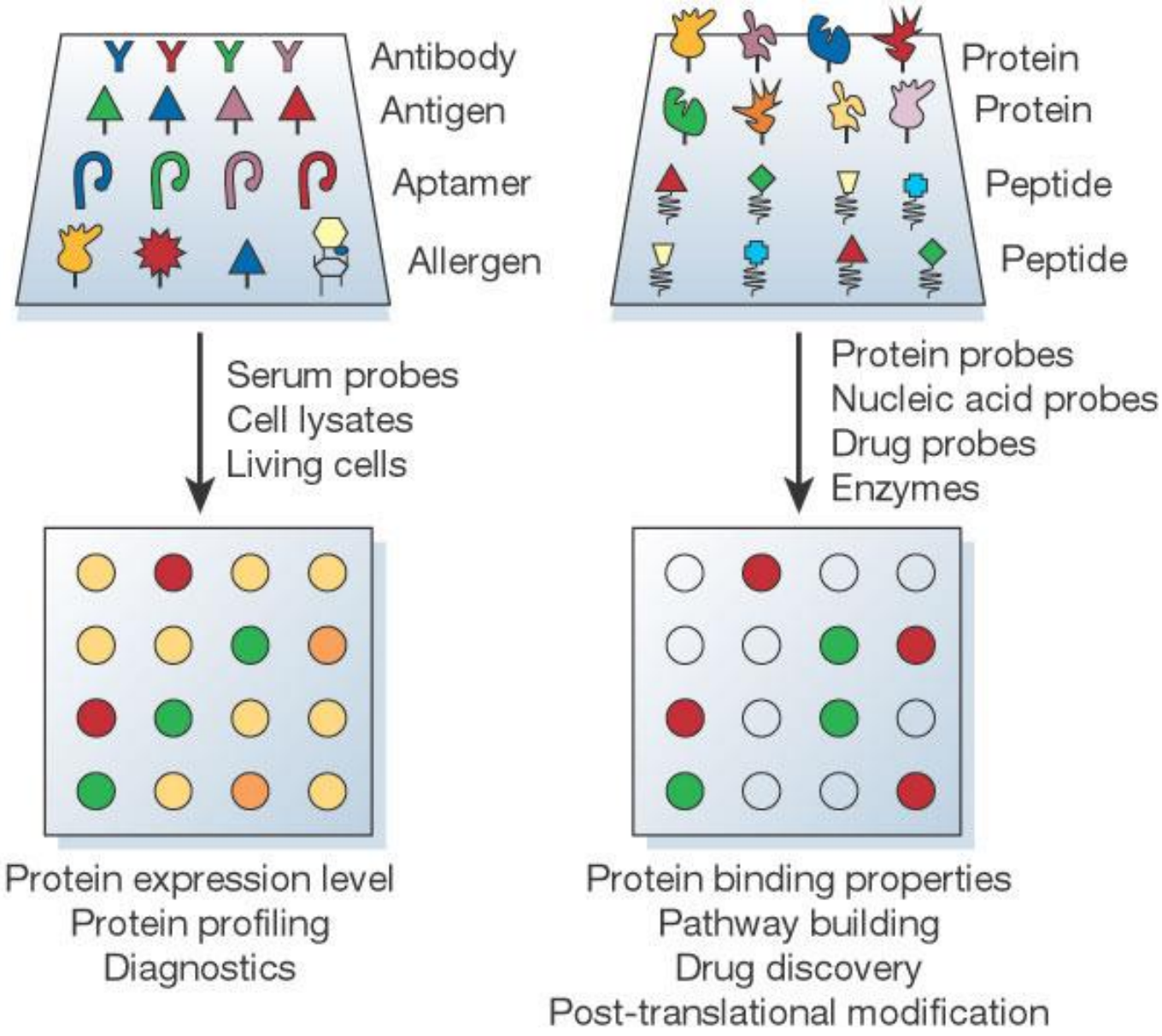
Next steps:

1. Sequence to identify these biomarkers of TB
2. Confirm protein ID in a second cohort by ELISA



Applications of proteomics in paediatric infectious disease

- Unlimited
- Vaccine discovery
- Antibiotic discovery
- Host response
- Pathogen response
- Host-pathogen interactive biology
- Clinical and clinical-science interface studies


Antibody
Antigen
Aptamer
Allergen
Protein
Protein
Peptide
Peptide
Serum probes
Cell lysates
Living cells
Protein probes
Nucleic acid probes
Drug probes
Enzymes
Protein expression level
Protein profiling
Diagnostics
Protein binding properties
Pathway building
Drug discovery
Post-translational modification
Analytical
Functional

Summary

- The ever increasing availability of completed genomic sequences and new technologies to examine the whole genome simultaneously
- Both genomic and proteomic methods can be used to study infectious disease (human and bacterial)
- Provide answers about:
  - Host response during infection
  - Bacterial evasion of host response
  - Identify new drug and vaccine targets
  - Better disease diagnosis