Proteomics Course

LECTURE-12
Gel-based Proteomics

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Outline

- Gel-based proteomics
- Electrophoresis
- One dimensional electrophoresis
- Two dimensional electrophoresis
  - Work-flow

Steps involved in proteome analysis

- Separation of proteins present in sample
- Techniques may involve
  - Electrophoresis one- or two-dimensional
  - Chromatography

Gel-based proteomics

- Gel-based electrophoretic separation techniques
  - Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
  - Two dimensional gel electrophoresis (2-DE)
  - Fluorescence 2-D Difference In Gel Electrophoresis (DIGE)
  - Blue native PAGE (BN-PAGE)

Electrophoresis

- Several techniques have been developed to study structure and functions of proteins
- Electrophoresis, based on *principle of migration of charged proteins in an electric field*
  - Gold standard due to its ability to provide information on protein structure and properties
  - Powerful technique for finer protein separation and visualization of separated proteins

Electrophoresis: historical perspective

- Electrophoresis invented by Prof. Arne Wilhelm Kaurin Tiselius in 1930
- He developed moving boundary method to study electrophoresis of proteins
- Rapid developments in electrophoresis and it was widely adopted in 1960s

Electrophoresis: historical perspective

Dr. Arne Wilhelm Kaurin Tiselius
Father of electrophoresis

Nobel Prize (1948)

One dimensional electrophoresis

One dimensional electrophoresis

- Separation based on charge-to-mass ratio and molecular weight of protein
  - Smaller proteins migrate a further distance through gel pores
- Commonly employed 1-D techniques
  - Sodium Dodecyl Sulphate-PAGE (SDS-PAGE)
  - Native Polyacrylamide Gel Electrophoresis (PAGE)

Electrophoresis for protein characterization

- Subunit composition
- Molecular weight of subunits
- Native molecular weight
- Post-translational modification

1-D electrophoresis: limitations

1-D electrophoresis: limitations

- Electrophoresis in a single dimension is useful for separation of few proteins simultaneously
  - But large number of proteins can not be separated with good resolution
  - Complex mixtures e.g. serum, cell lysate can't be separated

1-D electrophoresis: limitations

- Need technique to provide better resolution at proteome level
  - led to development of two dimensional gel electrophoresis (2DE)

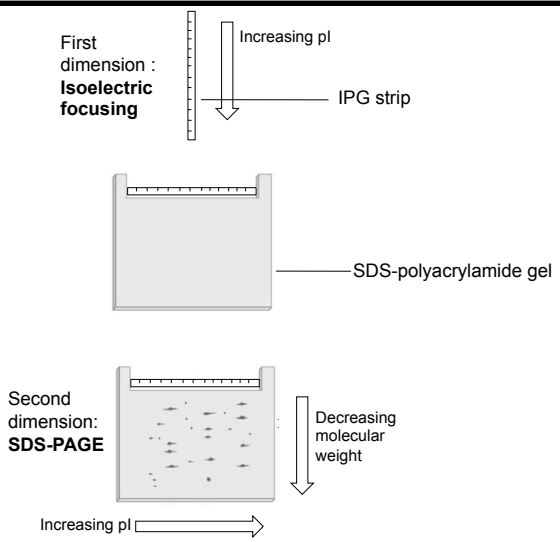
Two-dimensional electrophoresis (2DE)

Remains a core technology choice to separate complex protein mixtures

Two dimensional electrophoresis

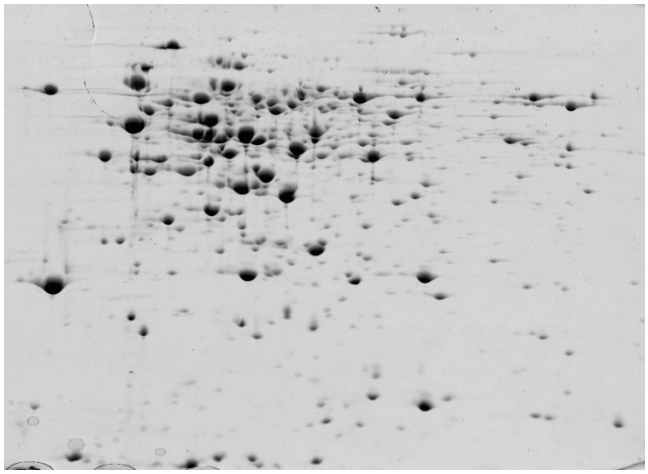
- First dimension:
  - Separates proteins on pH gradient based on isoelectric point (pI) using isoelectric focusing
- Second dimension:
  - Following IEF, proteins are resolved according to their molecular weight using SDS-PAGE

2-DE: combining IEF and SDS-PAGE



2-D gel image

pI



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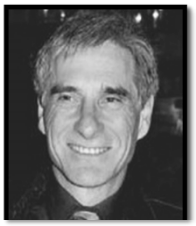
2-DE core technique in proteomics

- A powerful technique for simultaneous separation of thousands of proteins
- Relative easy to handle and affordable
- High-sensitivity visualization of proteins

2-DE technique: historical perspective

- 2-DE was used by Klose (1975) to investigate heterogeneity of mouse lactate dehydrogenase isozymes
- Independently, O'Farrell *et al.* (1975) resolved complex proteins in crude extracts of *E. coli*

2-DE technique: historical perspective



Prof. Patrick O'Farrel

Prof. Joachim Klose

2-DE was tedious in 1970s

- Protein separation in first dimension involved casting of polyacrylamide gels containing ampholytes in glass tubes
- Considerable care and attention required to prepare tube gels
- Gel to gel reproducibility was major concern!

How 2-DE became popular?

How 2-DE became popular?

- Recent advancement
  - development of immobilized pH gradients
  - solubilization of hydrophobic proteins
  - gel casting and electrophoretic apparatus
  - staining advancements
  - image analysis software

Immobilized pH gradient (IPG) strips

- Development of immobilized pH gradient (IPG) strips eliminated inconsistencies associated with isoelectric focusing
- IPG strips - computer-controlled gradient formation, pH gradient is covalently incorporated into acrylamide matrix and immobilized
- Supplied ready to use and made 2-DE more efficient

Advantages of IPG strips over tube gels

- IPG strips are more stable and durable
- Higher resolution, improved reproducibility for inter-laboratory comparisons
- Higher loading capacity for micropreparative 2DE
- Separation of basic proteins under equilibrium conditions

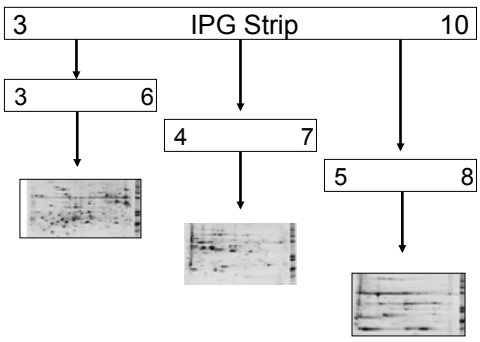
Advanced 2-DE technology: IPG strips

Prof. Angelika Görg

Advantages of IPG strips over tube gels

- Concentrated protein samples can be applied without causing gradient degradation
- Immobilized strips are dehydrated which allows rehydration of the strips directly with the sample to be separated
- Eliminates many problems associated with top loading of a carrier ampholyte IEF tube gel which is more sensitive to overloading than IPG

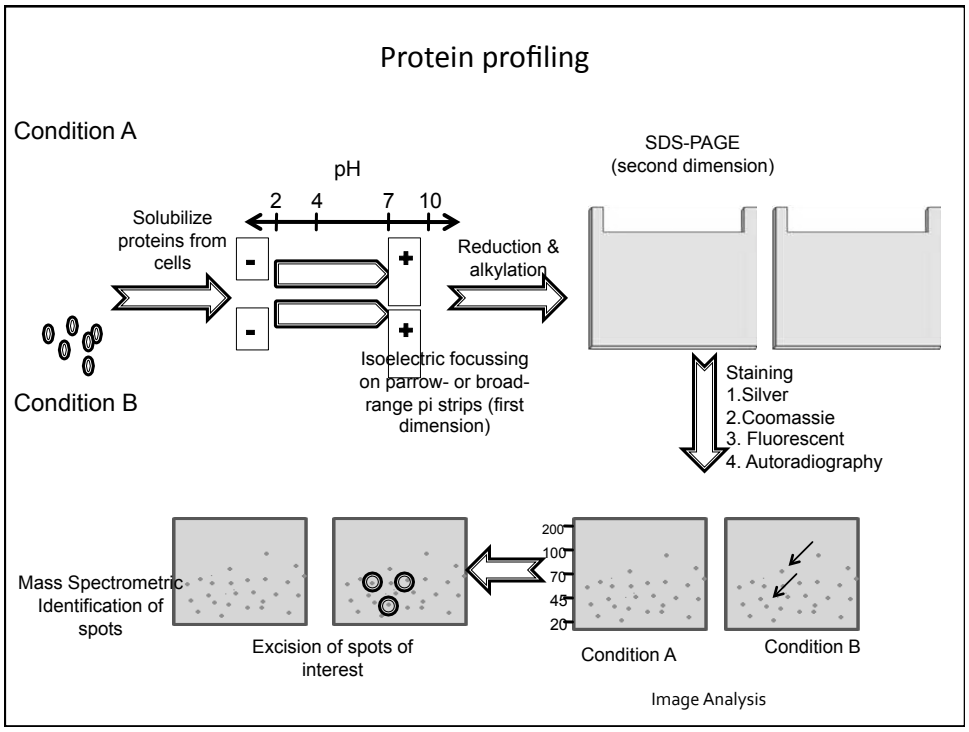
IPG Strip selection: based on experimental requirement

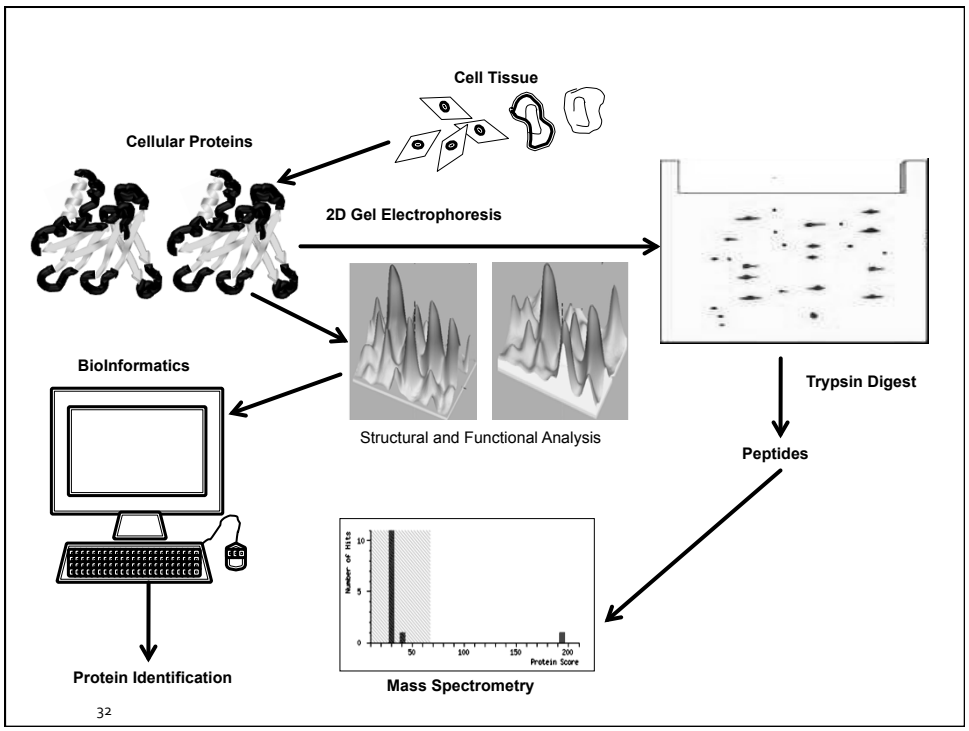


2-DE applications

2-DE technique: objective

- Study global protein expression
- Study differential protein expression
- Resolve proteins from complex mixtures
- Isoforms, post-translational modifications
- Visual analysis of proteins


Protein profiling
Condition A
pH
2 4 7 10
SDS-PAGE
(second dimension)
Solubilize
proteins from
cells
Reduction &
alkylation
Isoelectric focussing
on narrow- or broad-
range pi strips (first
dimension)
Condition B
Staining
1.Silver
2.Coomassie
3. Fluorescent
4. Autoradiography
200
100
70
45
20
Mass Spectrometric
Identification of
spots
Excision of spots of
interest
Condition A
Condition B
Image Analysis


Cell Tissue
Cellular Proteins
2D Gel Electrophoresis
BioInformatics
Trypsin Digest
Structural and Functional Analysis
Peptides
Protein Identification
Mass Spectrometry
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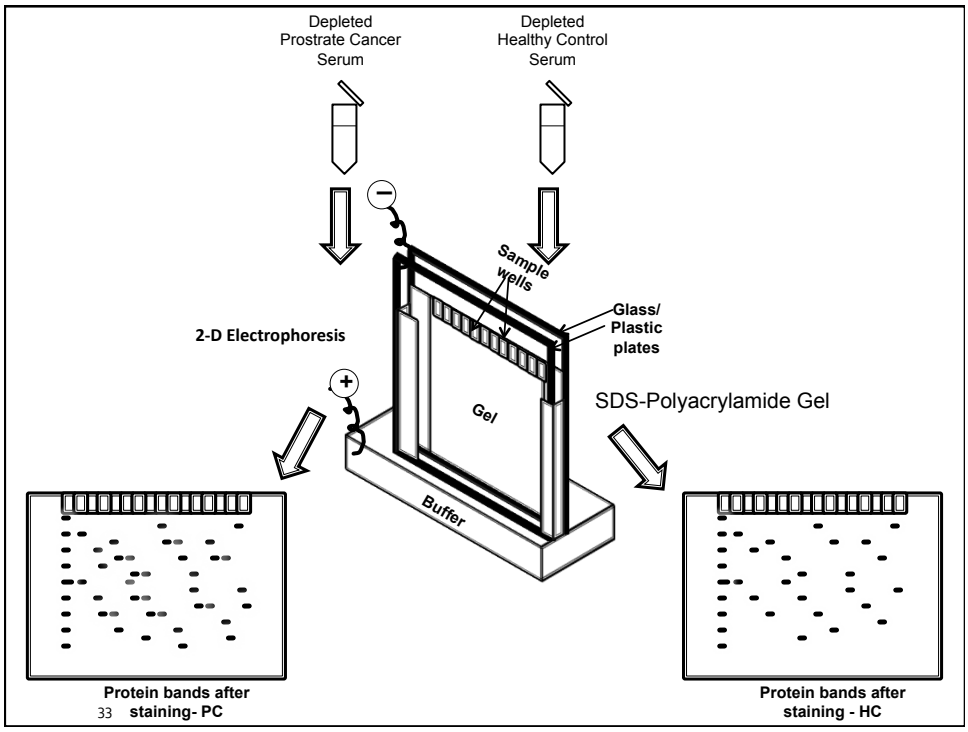

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Two-dimensional Electrophoresis: EXPERIMENTAL WORK FLOW

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Work-flow

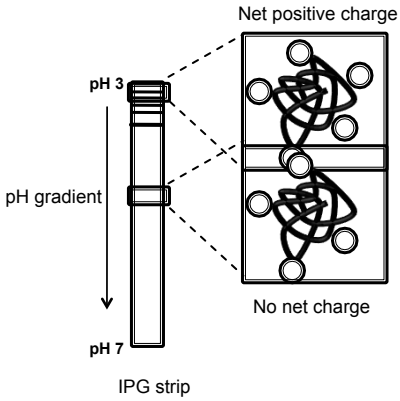
1. Isoelectric focusing (first dimension)
2. Equilibration of IPG strips
3. SDS-PAGE (second dimension)
4. Staining – gel visualization
5. Image analysis
6. Spot picking
7. Enzymatic digestion
8. MS analysis

1. Isoelectric Focusing (IEF)

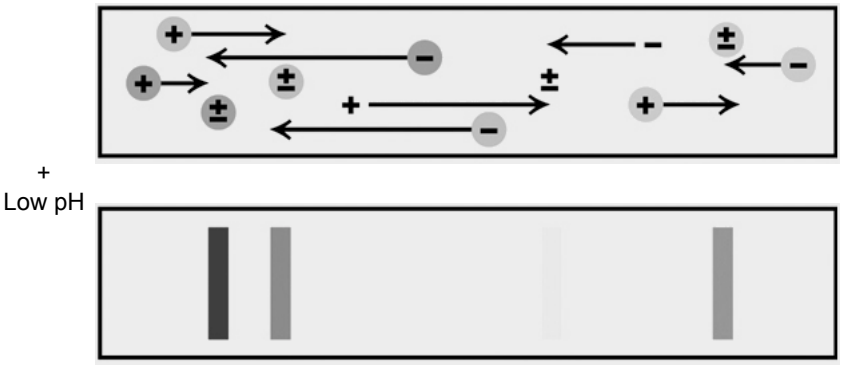
Isoelectric focusing (IEF)

- Protein separation according to isoelectric point
- Proteins introduced into immobilized pH gradient
- Electric field is applied in which protein migrates according to its charge
- Protein reaches Isoelectric point (pI)
  - pH = pI protein does not move in electric field owing to the lack of charge

Isoelectric focusing (IEF)



Isoelectric focusing (IEF)



Rehydration

- Protein extracted from previous step
- Rehydrate IPG strips overnight in a reswelling tray at RT using solution containing the extracted protein in buffer (rehydration/IPG buffer)
- Passive rehydration – no voltage applied
- Active rehydration – apply low voltage
- Overlay mineral oil on rehydrated strips

IPG strips used for IEF

- IPG strips different pH ranges (e.g. pH4-7, 3-10 etc.)
- IPG strips length are between 7-24 cm
- IEF units are capable of accommodating IPG strips of different length (7-24 cm)
- Large gels are recommended to resolve spots better
- However, handling large gels is tedious

IPG strips: protein loading and volume

- 7 cm
  - Coomassie (100-300 µg; 160 µl)
  - Silver/SYPRO Ruby (10-100 µg; 160 µl)
- 17 cm
  - Coomassie (250-1000 µg; 350 µl)
  - Silver/SYPRO Ruby (100-1000 µg; 350 µl)

Summary

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  - Work-flow

REFERENCES

- Liebler CD. *Introduction to proteomics: tools for the new biology*. 2002. Humana Press. ISBN 0-89603-991-9 (HC), ISBN 0-89603-992-7 (PB).
- Issaq HJ., Conrads TP., Janini GM. and Veenstra TD. *Methods for fractionation, separation and profiling of proteins and peptides.* Electrophoresis 2002, 23, 3048–3061.
- Berg J., Tmyoczko J. & Stryer L., Biochemitry fifth ed., W. H. Freeman & company, 2002. ISBN: 0716746840.
- Nelson D. & Cox M., Lehninger, Principles of Biochemistry fourth ed., 2004. W. H. Freeman and company. ISBN: 023022699X.
- Voet D. & Voet J., Biochemistry fourth ed., Wiley, 2000. ISBN: 047158651X.