Proteomics Course

LECTURE-13
Gel-based Proteomics
Two-dimensional electrophoresis workflow: Equilibration, SDS-PAGE, Staining

Dr. Sanjeeva Srivastava
IIT Bombay

1

Previous lecture

- Gel-based proteomics
- 2-DE work-flow

2-DE 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

Today's lecture

2 Equilibration of IPG strips
3 SDS-PAGE (second dimension)
4 Staining – gel visualization

2. Equilibration

Equilibration

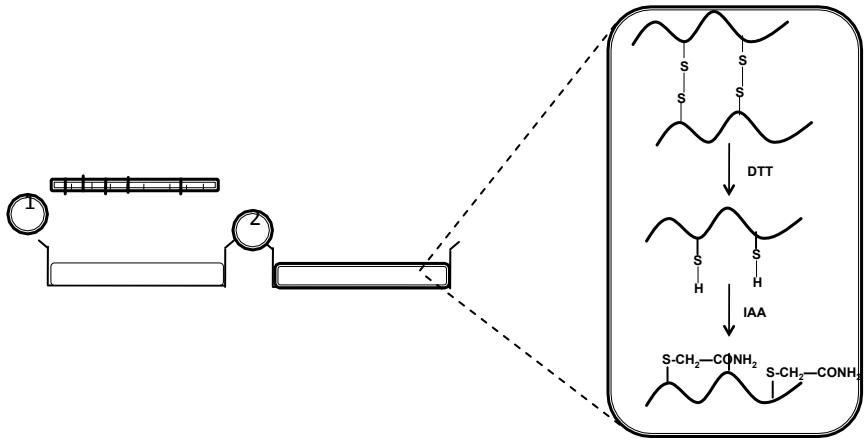
- After 1st dimension, a conditioning step is applied to proteins separated by IEF
- Coats protein with SDS for m.w. basis separation
- Cleaves inter, intra-chain disulfide bonds
- Alkylates sulfhydryl groups of cysteine residues

First equilibration

- Saturates IPG strips with SDS and reducing agents
- 6 M urea, 2% SDS, 0.375 M Tris-HCl, pH 8.8, 20% glycerol, 130 mM DTT

Second equilibration

- Alkylates residual DTT to minimize vertical streaking
- Iodoacetamide prevents protein re-oxidation
- 6 M urea, 2% SDS, 0.375 M Tris-HCl, pH 8.8, 20% glycerol, 135 mM iodoacetamide
- Equilibration twice for 10 minutes each



Focused strips treated with DTT reductant, followed by IAA which functions as scavenger of the excess reductant, alkylates and prevents protein re-oxidation

9

3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

10

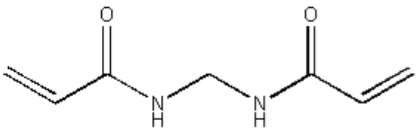
SDS-PAGE

- Widely used electrophoretic technique, which separates protein according to size
- SDS is negatively charged, it binds at a rate of 1.4g SDS/ 1g protein to provide proteins almost same charge to mass ratio
- Boiling proteins in SDS and thiol agent (β-ME), denatures protein and breaks S-S bond

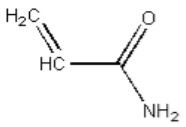
SDS-PAGE: role of components

- Acrylamide – matrix or gelling agent
- Bis-acrylamide – cross linking agent

N, N'-methylene-bis-acrylamide

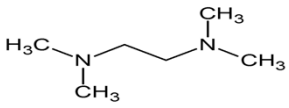


Acrylamide

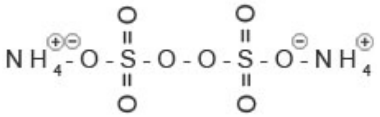


SDS-PAGE: role of components

- APS – initiator of polymerization
- TEMED – free radical stabilizer, promotes polymerization
- β-ME – breaks disulfide bonds

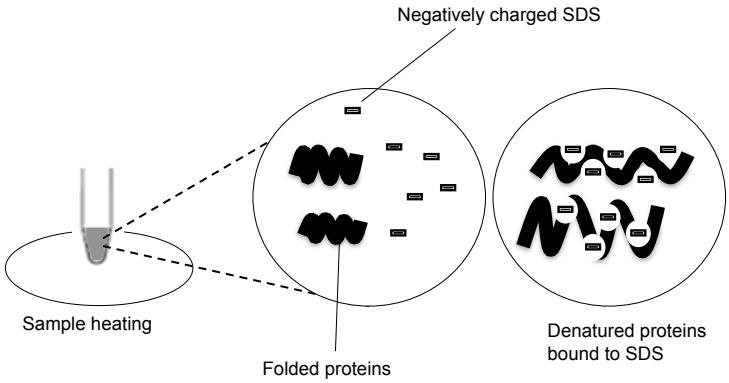


TEMED

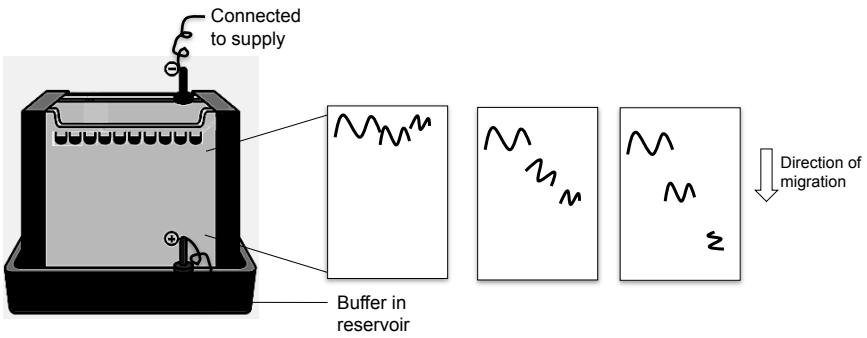


APS

SDS-PAGE



SDS-PAGE

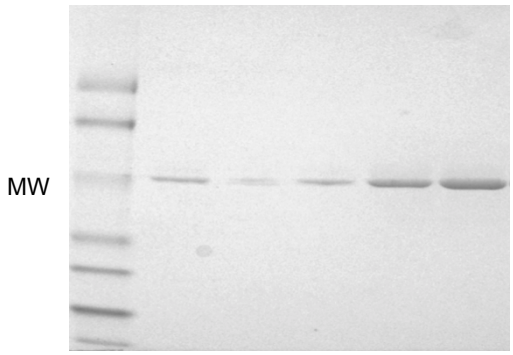


SDS-PAGE

- SDS-PAGE is commonly used for determination of molecular weight of unknown protein by running protein of interest along with protein markers (or standards) of known molecular weights

Protein	**Molecular weight, M (kD)**
Bovine serum albumin (BSA)	66.4
Lysozyme	14.7
Pepsin	35
Papain	38.9

SDS-PAGE



Blue-Native PAGE

Blue Native-PAGE

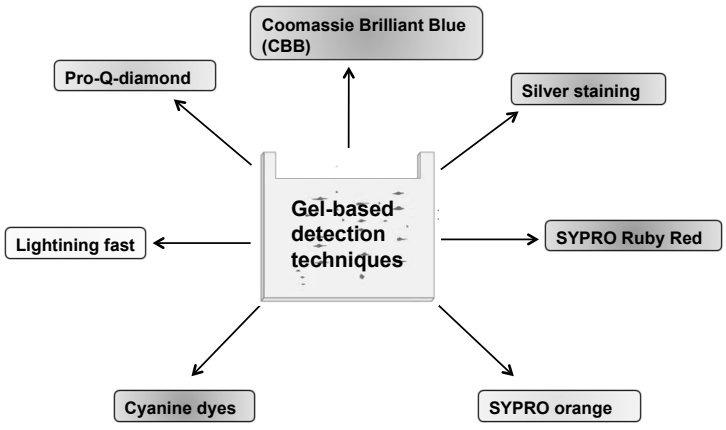
- Protein analyses under native conditions
- Protein sample mixed with Coomassie blue dye to provide the necessary charge to protein complexes and facilitate their separation in gel
- Unlike SDS, dye does not denature the proteins but binds to them in their native state

2D Blue Native-PAGE applications

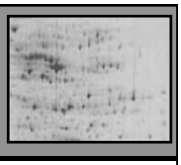
- Multi-protein complexes (MPCs) identification & analysis requires separation in native conditions
- In BN-PAGE electrophoretic mobility of MPCs determined by negative charge of bound Coomassie dye, and size & shape of complex
- Provides integrative view of protein function

4. Staining: gel visualization

An overview of staining techniques

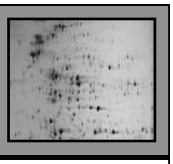


Staining: Coomassie blue



- Most commonly used stain for protein detection in polyacrylamide gels
- Biosafe Coomassie stain, ready to use, single-reagent protein stain
- Used stain can be disposed as non-hazardous waste

Staining: Silver



- More sensitive than Coomassie Blue dye
- Proteins fixation - methanol, acetic acid
- Staining
  - Silverammine complex bound to tungstosilicic acid
  - Ag ions transfer from tungstosilicic acid to proteins
  - Formaldehyde in the alkaline solution
  - Reduces silver ions to metallic silver
- Reaction stopped with acetic acid

Summary

- Two dimensional electrophoresis work-flow
- Equilibration
- One dimensional electrophoretic methods
  - SDS-PAGE
  - Native-PAGE
- Staining methods

REFERENCES

- 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.
- Walker JM. The proteomics protocols handbook.2005 Humana Press
- Dhingra et al. New frontiers in proteomics research: A perspective. International Journal of Pharmaceutics 2005. doi:10.1016/j.ijpharm. 2005.04.010
- Swamy et al. Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) for the Identification and Analysis of Multiprotein Complexes. Sci. STKE, 25 July 2006, Vol. 2006, Issue 345, p. pl4