Objectives

• Know the principles of electrophoresis and SDS-PAGE
  SDS – sodium dodecyl sulfate
  PAGE – polyacrylamide gel electrophoresis

• Describe how an SDS-PAGE gel is operated

• Understand how to determine molecular weight using
  SDS-PAGE

• Understand the components of the SDS-PAGE buffer and
  their functions

• Know how to prepare a polyacrylamide gel
General workflow for proteomic analysis

Sample

Sample preparation

Protein mixture

Sample separation

Peptides

Comparative analysis

Digestion

Mass spectrometry

Database search

MS data

Protein identification

Oct 2011 SDMBT
Principles of SDS-PAGE

Electrophoretic migration

\[ v \text{ (cm/h)} = E \text{ (V/cm)} \times u \text{ (cm}^2\text{/Vh)} \]

- \( v \) – migration velocity
- \( E \) – field strength
- \( u \) – mobility

Higher voltage – can go home faster but too high - heat

U - Property of molecule itself eg size, charge, shape –
Roughly proportional to ratio charge/radius of molecule

Smaller molecule – faster
More charged molecule - faster
Proteins are denatured, solubilised by SDS – i.e. protein surrounded by SDS molecules.

- The SDS-protein interaction is strong enough to make the composition of the SDS-protein complex essentially pH independent.

- The strong solubilizing effect of SDS make essentially all proteins negatively charged and can move in an electrical current.

- All protein-SDS complexes acquire the same rod-shaped conformation and differ only in size.

SDS – sodium doceyl sulfate

April 2006
Determination of Mr by SDS-PAGE

• There is a direct relationship between log $Mr$ and $R_f$ so that the determination of protein molecular weight can be made.

$R_f$ – the migration distance of the protein relative to that of the tracking dye.

$$R_f = \frac{\text{distance of protein migration}}{\text{distance of tracking dye migration}} \times \frac{\text{gel length before staining}}{\text{gel length after staining}}$$

• By calibrating the system with proteins of known molecular weight, this relationship can be used to determine the molecular weight of unknown proteins.
Fig. 5-3. A plot of log of molecular weight versus $R_f$ values.
SDS-PAGE markers

- Log (MW) of a protein $\propto$ migrating distance of the protein relative to the tracking dye

- MW determined by comparison to a set of standard protein markers of known MW
SDS-PAGE markers

(Biorad)
Since polyacrylamide gel electrophoresis (PAGE) separates proteins on the basis of their molecular weight.

Do glycosylation and phosphorylation affect the molecular weight of proteins?
Types of PAGE

SDS-PAGE
- Normal 1-D SDS PAGE
  For determining molecular weight
- SDS PAGE after IEF
  for separating proteins

Native PAGE
Types of PAGE

**SDS**

- Most common
- Can be reducing/non-reducing
- Proteins are separated by MW

**Native**

- To detect protein-protein interactions and protein multimers
- Proteins are separated by charge, conformation and MW

(Biorad)
# A typical recipe for 2x sample buffer for normal 1D SDS-PAGE

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5M Tris/HCl pH 6.8</td>
<td>2.5ml</td>
<td>Buffer/electrolyte</td>
</tr>
<tr>
<td>10% SDS</td>
<td>4.0ml</td>
<td>Detergent (see before)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.3ml</td>
<td>Increases density to allow sample to sink into well</td>
</tr>
<tr>
<td>β-mercaptoethanol</td>
<td>0.5ml</td>
<td>Reductant (reduces disulfide bonds)</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>1.0mg</td>
<td>Tracking dye</td>
</tr>
<tr>
<td>Water</td>
<td>0.7ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10ml</td>
<td></td>
</tr>
</tbody>
</table>

Sample + 2x sample buffer (1:1) and boil for 5-15 min and centrifuge
A typical recipe for SDS-PAGE gel

4.1 Laemmli\(^2\) resolving gel, 29:1 or 37.5:1 ratio

<table>
<thead>
<tr>
<th></th>
<th>Stack 4%</th>
<th>Resolving Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Acrylamide/Bis</td>
<td>3.3 ml</td>
<td>25 ml 40 ml 3.3(X%) = (A)(^+) ml</td>
</tr>
<tr>
<td>0.5 M Tris-HCl, pH 8.8</td>
<td>8.3 ml</td>
<td>– – –</td>
</tr>
<tr>
<td>1.5 M Tris-HCl, pH 8.8</td>
<td>–</td>
<td>25 ml 25 ml 25 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>250 µl</td>
<td>1.0 ml 1.0 ml 1.0 ml</td>
</tr>
<tr>
<td>Distilled deionized water</td>
<td>15 ml</td>
<td>48.5 ml 33.5 ml 73.5 - (A)(^+)</td>
</tr>
<tr>
<td>TEMED</td>
<td>25 µl</td>
<td>50 µl 50 µl 50 µl</td>
</tr>
<tr>
<td>10% APS</td>
<td>125 µl</td>
<td>500 µl 500 µl 500 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 ml</td>
<td>100 ml 100 ml 100 ml</td>
</tr>
</tbody>
</table>

(Biorad)          Oct 2011 SDBMT
A typical recipe for SDS-PAGE gel

- The formation of the polyacrylamide gel is an addition reaction of acrylamide and bisacrylamide monomers

Acrylamide monomer

Bisacrylamide (bis)

Polyacrylamide (soluble in water)

Chains of polyacrylamide
Cross linked by bis
A typical recipe for SDS-PAGE gel

- Ammonium persulphate (APS) provides the free radicals to initiate the addition polymerisation reaction.

- TEMED catalyses the rate of generation of free radicals from APS.
A typical recipe for SDS-PAGE gel

4.1 Laemmli² resolving gel, 29:1 or 37.5:1 ratio

- Acrylamide/bisacrylamide solutions usually come in 30 or 40% solutions with fixed ratios of 19:1, 29:1 or 37.5:1
- % is conc of acrylamide+bis w/v; the ratio 19:1 means 19 parts of acrylamide to 1 part bis
- 19:1 (5% C) solutions are for DNA sequencing, 37.5:1 (2.67% C) and 29:1 (3.33% C) are for protein separation
Determination of the total acrylamide and bisacrylamide concentration

\[ T = \frac{(A + B)}{V} \times 100\% \quad \text{and} \quad C = \frac{B}{(A + B)} \times 100\% \]

eg 12.5% gel

Higher T% - smaller pores – proteins don’t migrate so far

T (%) – the total acrylamide concentration.
C (%) – the concentration of the cross-linking agent (bisacrylamide)
A – the amounts of acrylamide (g).
B -- the amounts of bisacrylamide (g).
V -- the final volume of gelling solution.
T is usually in the range of 3 to 30%.
C is usually in the range of 1 to 25%.

Higher C% e.g. using 29:1 acrylamide/bis - also smaller pores

April 2006
% acrylamide determines the range of proteins well separated.
A typical recipe for SDS-PAGE gel

### 4.1 Laemmli\(^2\) resolving gel, 29:1 or 37.5:1 ratio

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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 M Tris-HCl, pH 8.8</td>
<td>25 ml</td>
<td>25 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>250 (\mu)l</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled deionized water</td>
<td>15 ml</td>
<td>48.5 ml</td>
</tr>
<tr>
<td>TEMED</td>
<td>25 (\mu)l</td>
<td>50 (\mu)l</td>
</tr>
<tr>
<td>10% APS</td>
<td>125 (\mu)l</td>
<td>500 (\mu)l</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 ml</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

(Biorad)

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Stacking gel

Note differences
1D-PAGE

**Stacking gel (T% = 4-6)**
Sharpens proteins into thin zones before resolution

**Resolving gel (T% usually 10-15)**
Separates proteins by MW

(Biorad)
Running of 1D SDS-PAGE

Sample previously boiled with 2x sample buffer

- Also available as horizontal format
- Available in different sizes

(Dr Frank Mari, Florida Atlantic University)
Running of SDS-PAGE as part of 2-D gel electrophoresis

- Sample previously separated on IEF gel (IPG dry strip)

- IPG dry strip is equilibrated with two types of buffer (one with DTT and the other with IAA)

- The SDS-PAGE gel has no stacking gel

- The SDS-PAGE has no wells
2D-PAGE – after IEF

Stacking gel (T% = 4-6)
IPG DryStrip (T = 4%, C = 3%) becomes the stacking gel

Resolving gel (T% usually 10-15)
Separates proteins by MW

(MW marker)

Immobilised by agar

Equilibration buffer provides tracking dye

(Technische Universität Münche)
Running of 2D SDS-PAGE

- Same concept as for 1D PAGE
- Larger tanks require cooling capacity
- Unlike 1D-PAGE, samples are not boiled in 2x sample buffer

(Biorad)
Buffers for SDS-PAGE

Vertical gel system

Run or tank buffer

(Dr Frank Mari, Florida Atlantic University)
A typical recipe for 10x running buffer for SDS-PAGE

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-base</td>
<td>30g</td>
<td>Buffer</td>
</tr>
<tr>
<td>Glycine</td>
<td>144g</td>
<td>Electrolyte</td>
</tr>
<tr>
<td>SDS</td>
<td>10g</td>
<td>Detergent</td>
</tr>
<tr>
<td>Water</td>
<td>1000ml</td>
<td></td>
</tr>
<tr>
<td>~1000ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Some Practical Differences between IEF and SDS-PAGE

- Sample buffer for IEF—low ionic strength, no SDS
- before staining – must fix with 20% TCA
- two electrode buffers – if carrier ampholytes are used
- much more sample can be loaded (horizontal gels)
- gels usually thinner and smaller acrylamide % and fixed onto plastic backing
- pI markers not molecular weight markers
- Voltage much higher, current typically drops to almost zero at end of run
- how to tell when run is over?