Molecular virology
- Laboratory exercises -

SDS-PAGE ANALYSIS OF VIRAL PROTEINS

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CHARACTERISTICS OF POLYACRYLAMIDE GELS:

- Synthetic polymer
- Chemically inert
- Irreversible chemical polymerisation
- Pore size is well defined – good resolution
- Good mechanical properties
- Applicable for analysis of proteins and nucleic acids

% Acrylamide	MW Range (kDa)
7	50 – 500
10	20 – 300
12	10 – 200
15	3 - 100

Advantages of PAGE in NA analysis

- Higher sample load per well
- Better NA purity for further analysis
- 0.2% resolution

Composition of polyacrylamide gel (10 mL, 8%):

6.63 mL EF-buffer
1.93 mL **AA** (40%)
1.33 mL **BIS** (2%)
100 µL **APS** (10%)
10 µL **TEMED**

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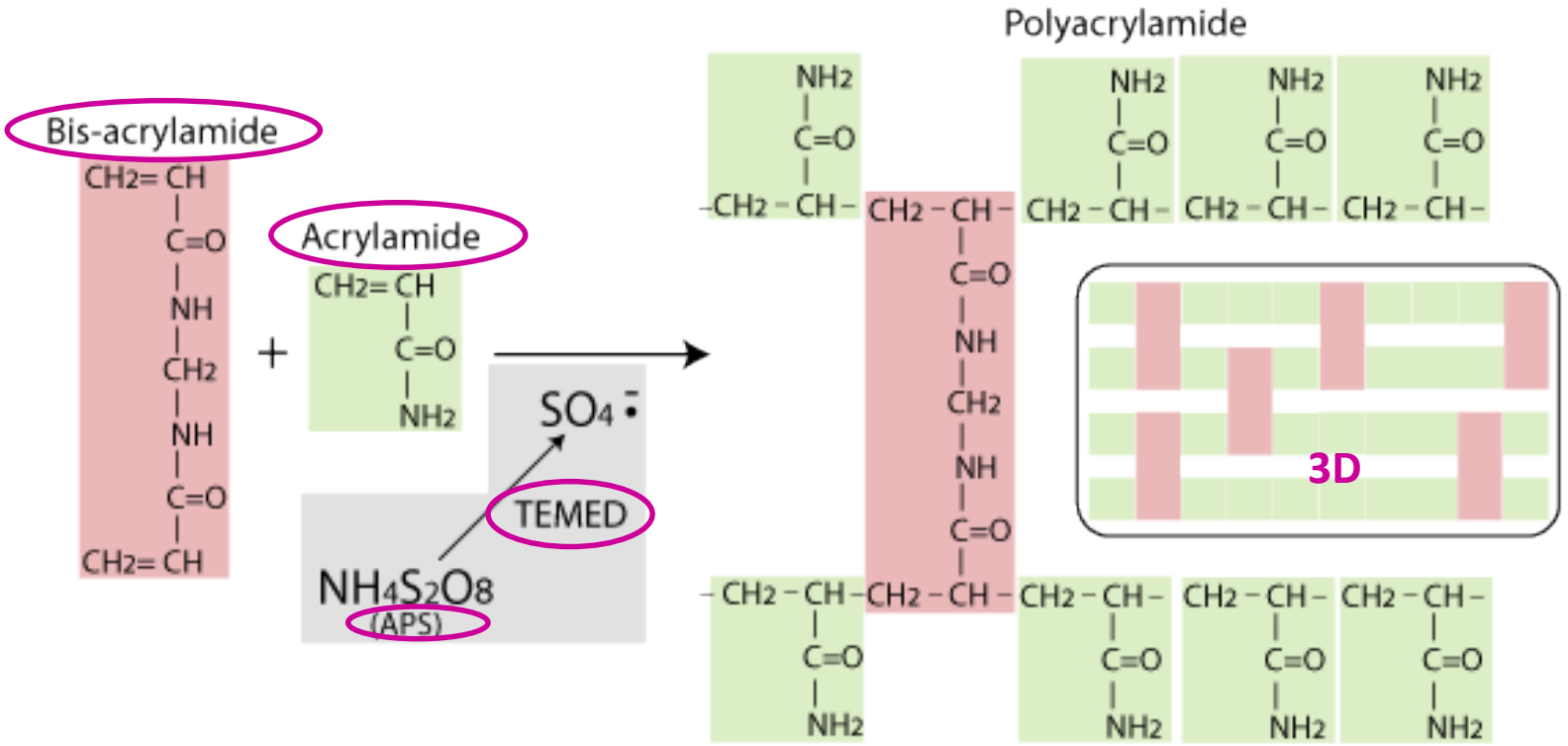
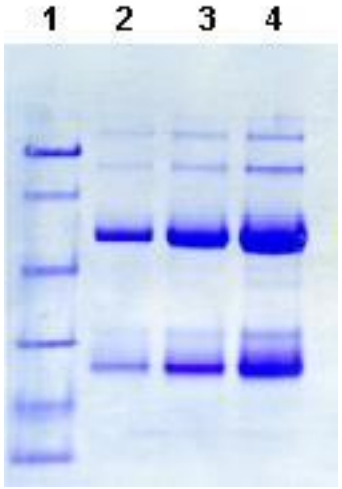
polymerisation for **15 min** at room temperature

EF buffer = electrophoresis buffer
AA = acrylamide
BIS = bis-acrylamide
APS = ammonium persulfate
TEMED = tetramethylethylenediamine



APS – source of free radicals for polymerisation
TEMED – catalyst, induces the production of free radicals

APPLICATION IN VIROLOGY:



- **Identification** of specific viral proteins
  (structural or functional, e.g. reverse transcriptase)

➡ often followed by **western blot**
e.g. detection of specific antibodies in serum (HIV)

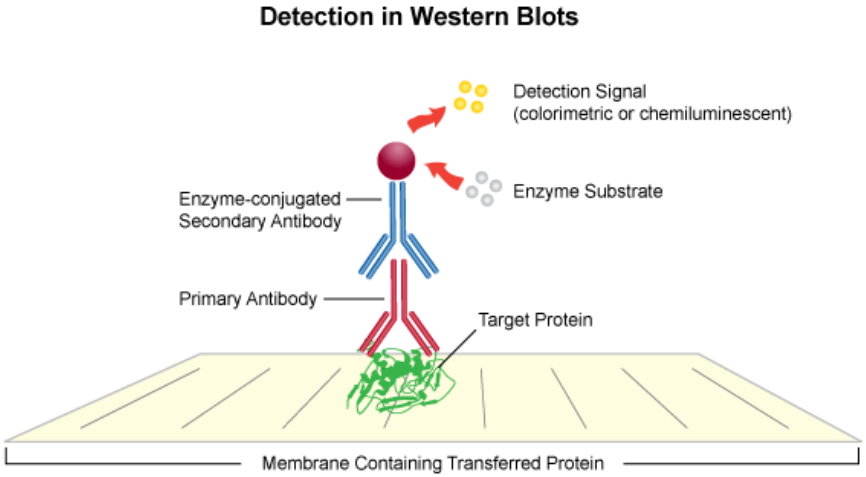


Diagram 2: *Illustration of detection in Western Blots.*

SDS-PAGE vs NATIVE PAGE

Electrophoretic mobility depends on:

charge (dependent on buffer pH)

shape

size

mass of molecules

Sodium dodecyl sulphate (SDS) or **sodium lauryl sulphate** (**SLS) –** anionic detergent; acts as a denaturing agent and a surfactant, binding to the denatured protein and **masking** its charge

1 g of protein binds 1.4 g of SDS

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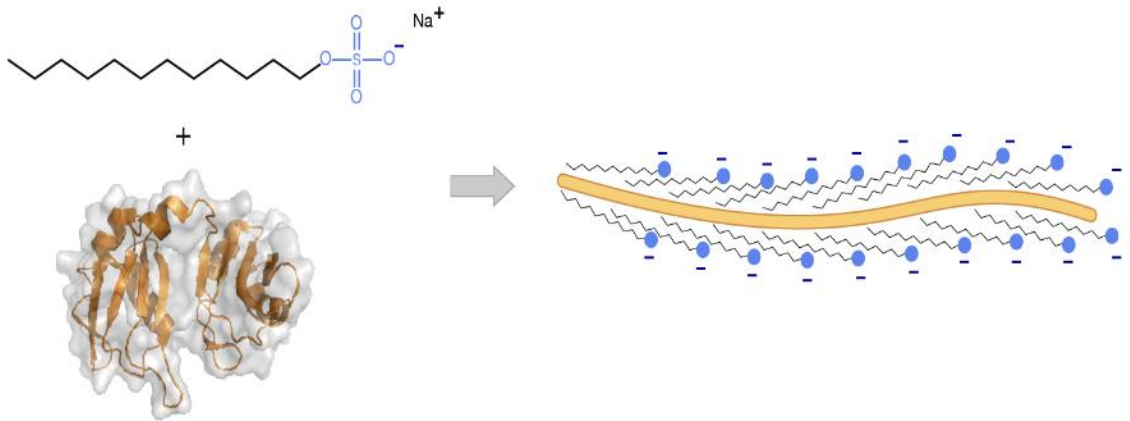
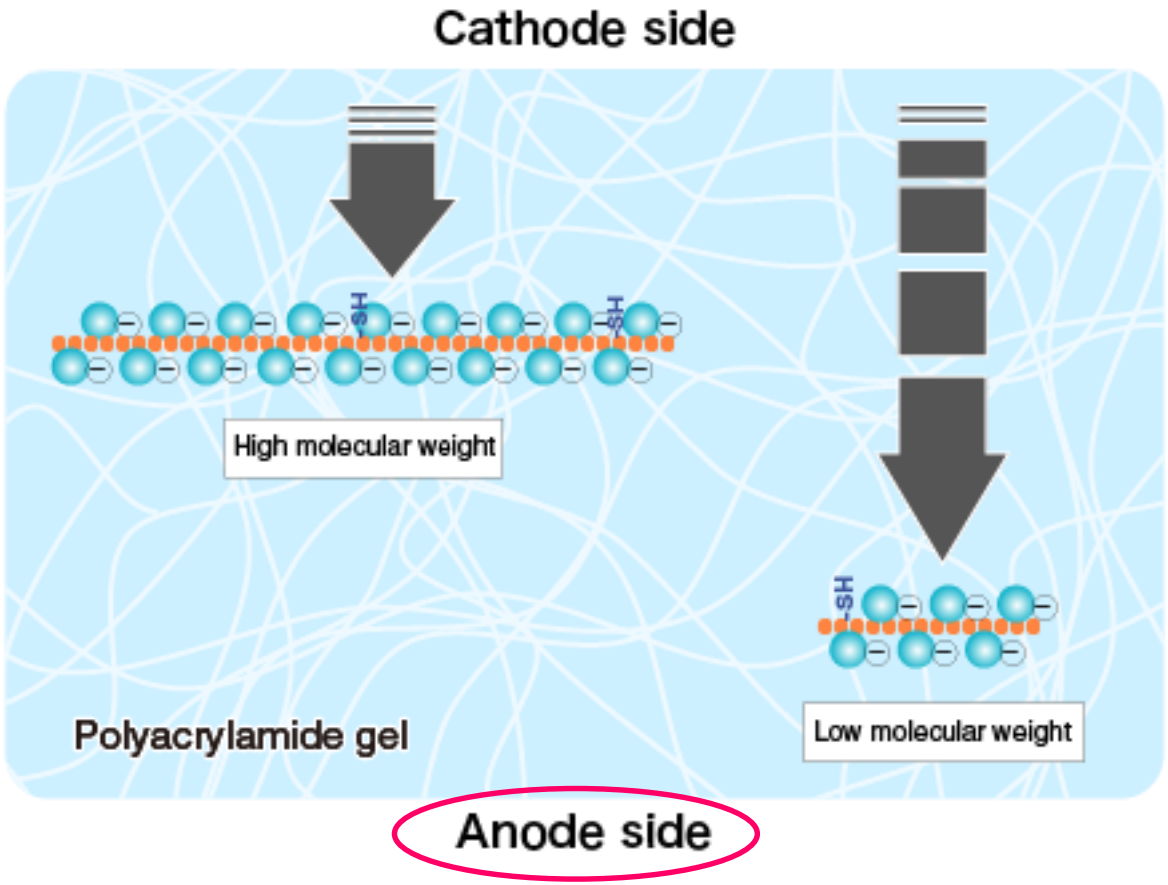
constant charge-to-mass ratio



Proteins are separated based on their polypeptide chain length by electrophoresis in a polyacrylamide gel with an appropriate mesh size.



Separation based on difference in molecular mass

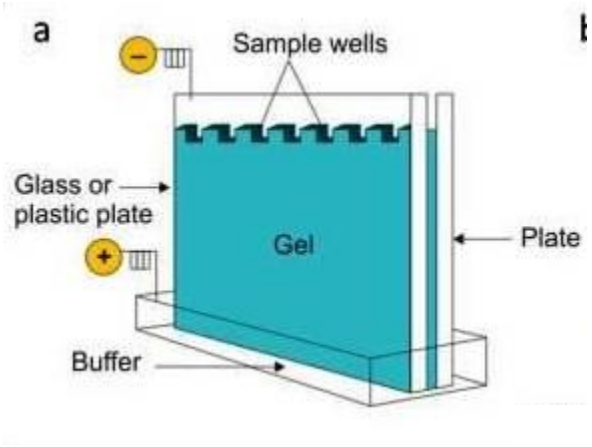
PROCEDURE:

- preparation of the gel
- preparation of samples
- electrophoresis
- staining

SAMPLES:

- freshly purified TMV, negative control, lyophilised TMV-protein
- freshly purified : PVX, CMV, TBSV…
- Protein marker 10-250 kDa (New England Biolabs)

Composition of polyacrylamide gel (10 mL, 8%):



6.63 mL EF-buffer
1.93 mL AA (40%)
1.33 mL BIS (2%)
100 µL APS (10%)
10 µL TEMED

Polymerisation for **15 min** at room temperature

Composition of electrophoretic buffer (1 L, 10x concentrated, pH 8.3):

30.3 g TRIS
144 g glycine
10 g SDS

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fill up to 1 L with deionised water

Composition of Laemmli hydrolysis buffer (2x conc., 100 mL):

12 mL Tris-HCl (1M, pH 6.8)
40 mL SDS (10%)
20 mL glycerol
10 mL β-mercaptoethanol
0.01 g bromphenol blue (BPB)
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fill to 100 mL with deionised water

Sample hydrolysis:

15 µl sample
15 µl Laemmli hydrolysis buffer
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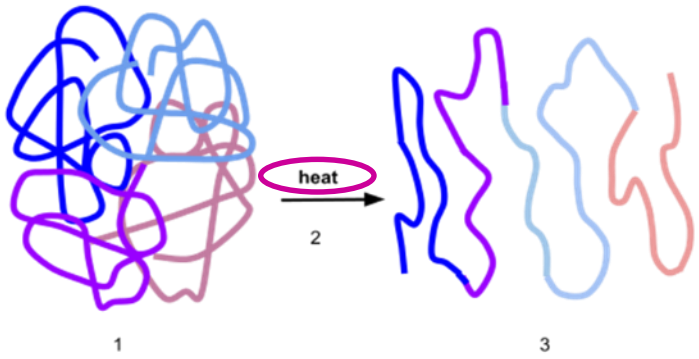
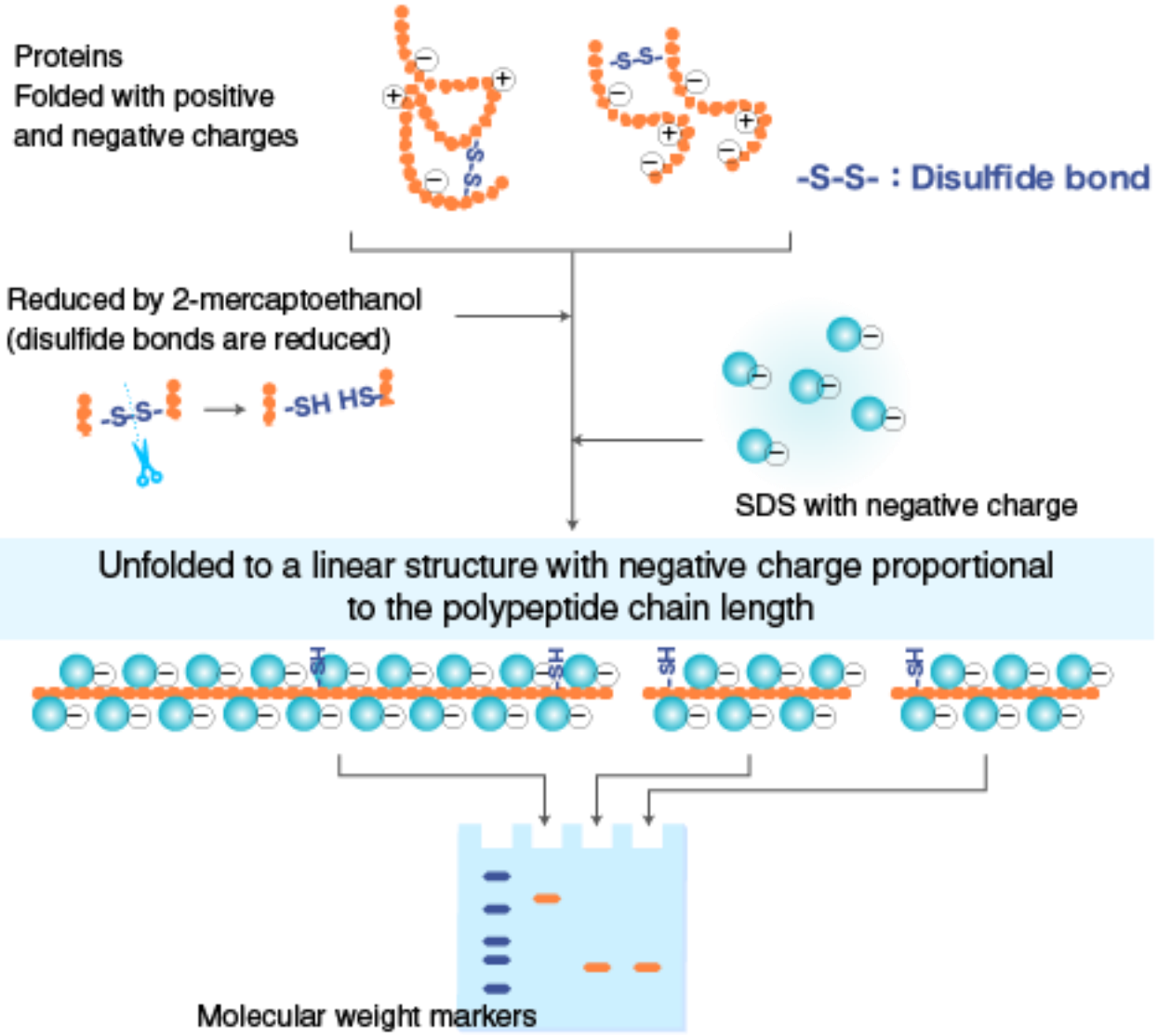
heat for **5 min at 95°C** and put on **ice**



SDS, β-mercaptoethanol and heat – protein denaturation
β-mercaptoethanol – reduces disulphide bonds
glycerol – increases sample density


Proteins
Folded with positive
and negative charges
-S-S-
-S-S- : Disulfide bond
Reduced by 2-mercaptoethanol
(disulfide bonds are reduced)
-S-S-
-SH HS-
SDS with negative charge
Unfolded to a linear structure with negative charge proportional
to the polypeptide chain length
Molecular weight markers

Sample laoding:

- protein marker – 7 µL per well (*ready to use*)
- virus samples – 20 µL per well

60-90 min at **70 V**

Composition of staining solution:

0.25% Coomasie Brilliant Blue
50% methanol
10% acetic acid
40% deionised water

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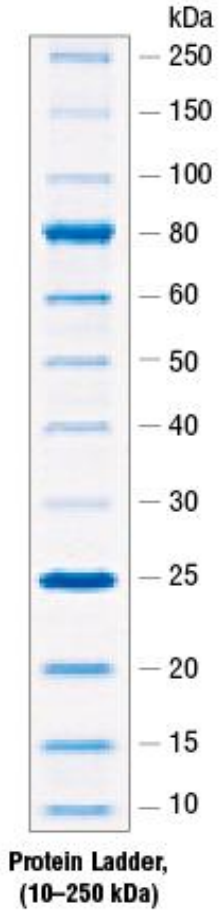
incubate the gel for **10 min**
rinse with deionised water 2x
boil briefly in a microwave oven 2x
rinse with deionised water 2x
leave to de-stain on orbital shaker overnight



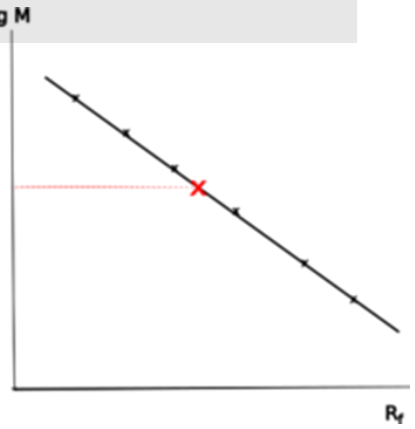
Protein Ladder,
(10–250 kDa)

Alternative staining methods:
- silver – best sensitivity
- amido-black…

EXCERCISE:

protein	Molecular mass (Da)	Logarithm of molecular mass	Electrophoretic mobility (mm)
albumin	66000	4.81954	3
ovalbumin	45000	4.65321	11
pepsin	34700	4.53237	15
trypsin	24000	4.38021	22
β - lactoglobulin	18400	4.25624	28
lysozyme	14300	4.14706	35

Protein X has electrophoretic mobility of **7.5**.
Calculate its molecular mass!



TASK:

Study all materials of virtual laboratory on link:
http://vlab.amrita.edu/?sub=3&brch=186&sim=319&cnt=1

and short video on apparatus assembly and gel casting:
https://www.youtube.com/watch?v=EDi_n_0NiF4