SDS –PAGE Electrophoresis

Separates proteins according to their SIZE

BTEC 101

SDS PAGE Defined

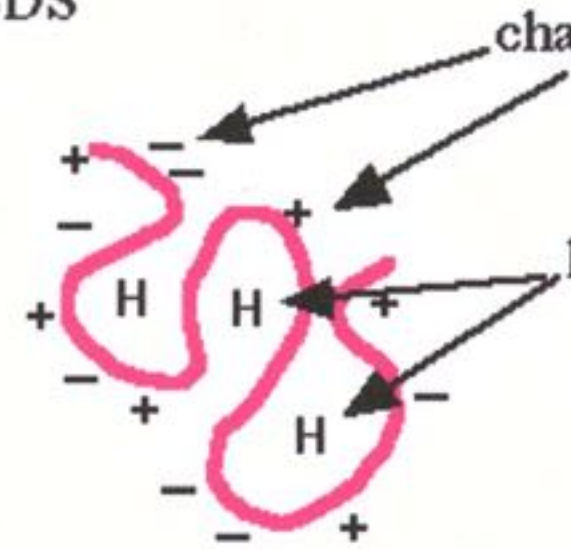
- **SDS** = sodium dodecyl sulfate, a **denaturing detergent** (soap) that can dissolve hydrophobic molecules but also has a negative charge (sulf**ATE**) attached to it. So when proteins are incubated with SDS, the proteins will be linearized by the detergent, plus all the proteins will be covered with many **negative charges**.

Shape & Mass affect movement through the environment

- Consider two proteins that are each 500 amino acids long but one is shaped like a closed umbrella while the other one looks like an open umbrella. If you tried to run down the street with both of these molecules under your arms, which one would be more likely to slow you down, even though they weigh exactly the same? Since we are trying to separate many different protein molecules of a variety of shapes and sizes, we first want to get them to be linear so that the proteins **no longer have** any secondary, tertiary or quaternary **structure** (i.e. we want them to have the **same linear shape**).

To convert all proteins to the same shape we use SDS

BEFORE SDS



Where nonpolar R-groups have collected in an attempt to get away from the polar water that surrounds the protein

AFTER SDS

The resulting protein has been denatured by SDS (reduced to its primary structure) and as a result has been linearized.

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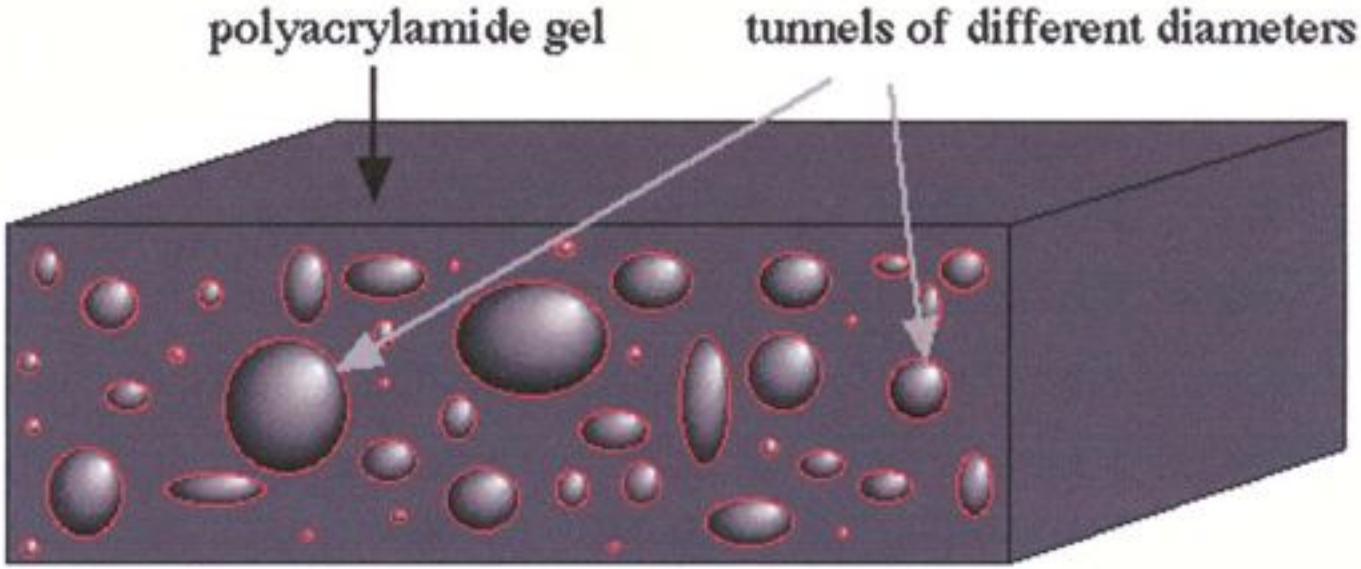
SDS PAGE Defined, cont.

- If the proteins are denatured & very negatively charged and put into an electric field, they will all move towards the positive pole at the same rate, with no separation by size. So we need to put the proteins into an environment that will allow different **sized** proteins to move at different rates.
- **PAGE** = PolyAcrylamide Gel Electrophoresis

Polyacrylamide gel is not solid but is made of a labyrinth of tunnels through a meshwork of fibers. We will use electricity to pull the proteins through the gel allowing different **sized** proteins to move at different **rates**.

A slab of polyacrylamide gel (dark gray) with tunnels (different sized red rings with shading to depict depth) exposed on the edge. Notice that there are many different **sizes** of tunnels scattered randomly throughout the gel.



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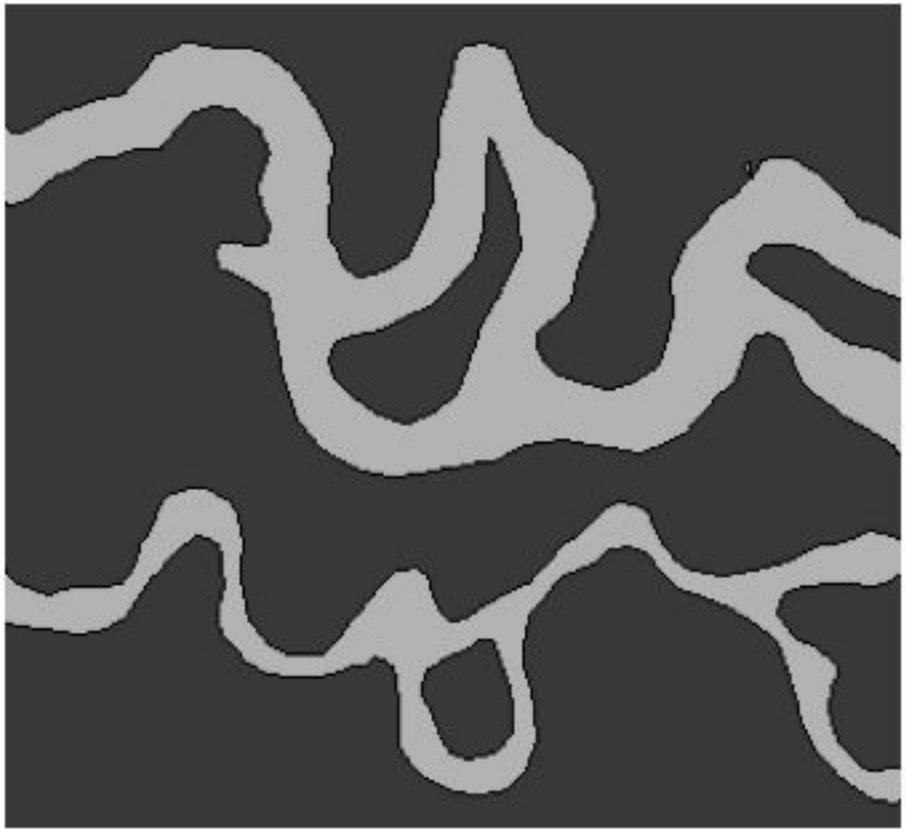
A top view of two selected tunnels (only two are shown for clarity of the diagram). These tunnels extend all the way through the gel, but they meander through the gel and do not go in straight lines. Notice the difference in **diameter** of the two tunnels.

Start

End

Which protein will travel faster?



- Pole

+ Pole

Protein movement ⟶

Apply denatured proteins of various lengths (sizes) to the gel and turn on the current.

Smaller proteins are more easily able to move through the gel; they can maneuver through the polyacrylamide tunnels **faster** than big molecules.

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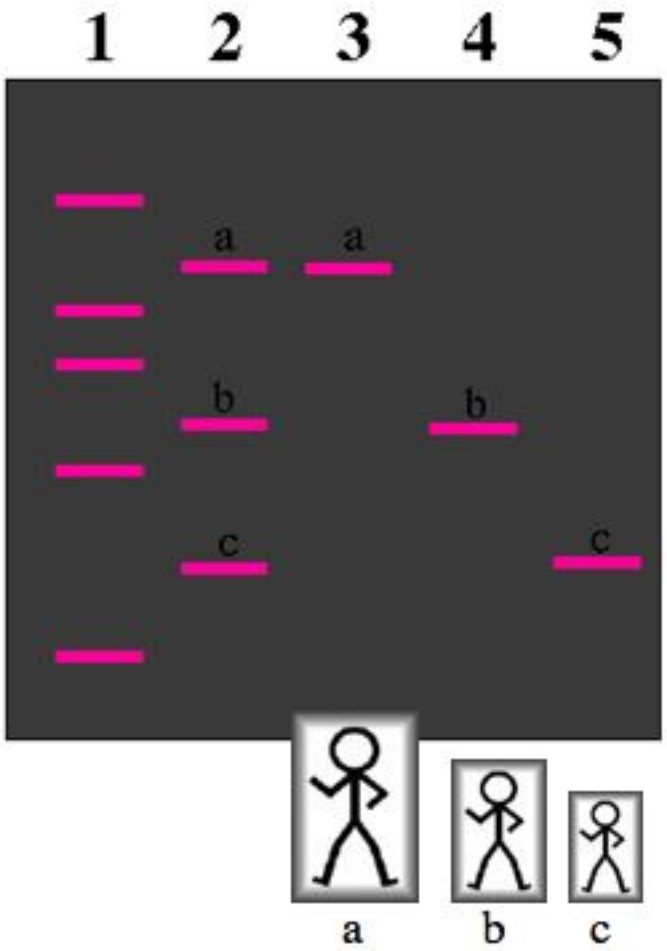
When we work with proteins, we work with many **copies** of each **kind** of protein. As a result, the collection of proteins (a bunch, or a band) of any given size tend to move through the gel at the same rate, even if they do not take exactly the same tunnels to get through.



If we were in a hot air balloon above the tunnels & watched 100 children, 100 teenagers, and 100 large adults running through the tunnels, we would see a collection (or band) of children moving quickly, a band of teenagers moving slower, and a third band made of adults plodding their way through the tunnels. Likewise, proteins tend to move through a gel in bunches, or **bands**, since there are so many copies of each protein & they are all the **same size**.

This gel has 5 numbered lanes where 5 different samples of proteins (many copies of each kind of protein) were applied to the gel. Lane:

1 = molecular weight standards of known sizes

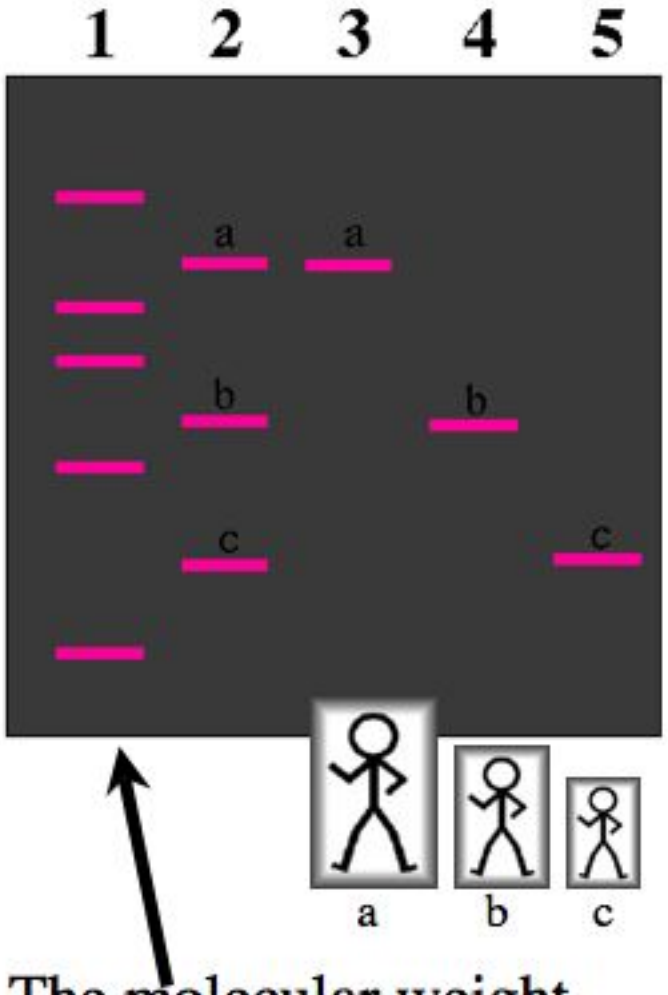
2 = a mixture of 3 proteins of different sizes

3 = protein "a" by itself

4 = protein "b" by itself

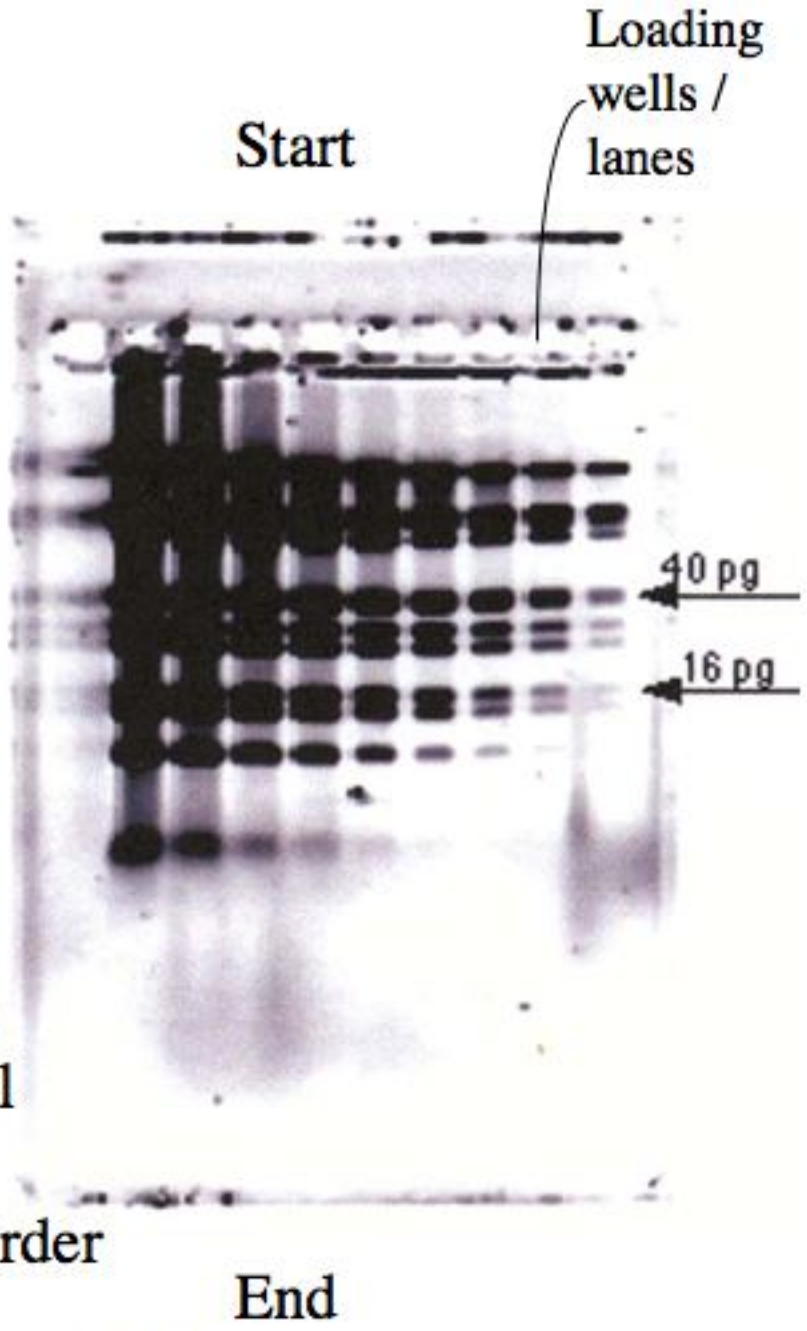
5 = protein "c" by itself

Proteins migrate the same distance in gel whether they are with other proteins (lane 2) or not (lanes 3-5).



The molecular weight standards are used to measure the relative sizes of the unknown proteins (a, b, and c).

When running an SDS-PAGE, we never let the proteins electrophorese (run) so long that they actually reach the other side of the gel. We turn off the current and then **stain** the proteins (normally they are colorless and thus invisible) and **see how far they moved through the gel**. Notice that the actual bands are equal in size, but the proteins within each band are of different sizes.



A variety of different proteins are being separated on this gel. This shows a serial dilution of the same protein sample to indicate how little protein is needed in order to be detected.

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SDS Page Gel Applications

- Molecular weight determination
- Determination of protein concentration
- First step in a Western Blot – Quantifies amount of your protein of interest
- Detection of protein degradation /modifications
- Analysis of protein purity

Old Days

- Had to make your own gels
- Problems that happened.

Now

- Buy pre-made gels
- Pre-made buffers
- Gets rid of dangers
- Gels of all percentages and even gradient gels are available.

Gels

- Gels are made of two parts:
- Stacking gel - where the combs are placed this is a lower percentage gel.
- Separating gel - is of a higher percentage or a gradient. This is where the greatest resolution will occur.
- The polyacrylamide is surrounded by glass or plastic plates and plastic spacers.
- Spacers and combs are always the same size.

Optimal Resolution Ranges (Hames, 1981)

• 15% Gel	• 15 to 45 kd
• 12.5% Gel	• 15 to 60 kd
• 10% Gel	• 18 to 75 kd
• 7.5% Gel	• 30 to 120 kd
• 5% Gel	• 60 to 212 kd

Staining to Visualize Proteins

- Coomassie Blue: 0.1μg - 1μg per band. (Much cheaper and easier- more forgiving)

- Silver staining 2-10ng per band. More sensitive, for low concentration of proteins

Other types of PAGE

- Native PAGE
- Isoelectric Focusing and 2-D Gels

Protein Size

- Measured in kd (kilo Daltons)
- In native conformation (not denatured) affects progression through the gel
- SDS gels gives more of a true size
- SDS-PAGE separates proteins based on their primary structure of size but not amino acid sequence. Therefore, if we had many copies of two different proteins that were **both 500 amino acids long**, they would travel together through the gel in a **mixed band**. As a result, we would not be able to use SDS-PAGE to separate these two proteins of the same size from each other.