Announcements

- Chapter 7 post-lab grades released sometime next week
- Chapter 6 and 8 template released today
- You will get your groups soon for the creativity project
- Long incubation step for lab this week
- Next week's protocol to y'all by EOD tomorrow

Chapter 8C: In vitro transcription and translation

Objectives

- To learn how to cast and run SDS-PAGE gels.
- To analyze and observe the synthesized proteins by in-gel fluorescence

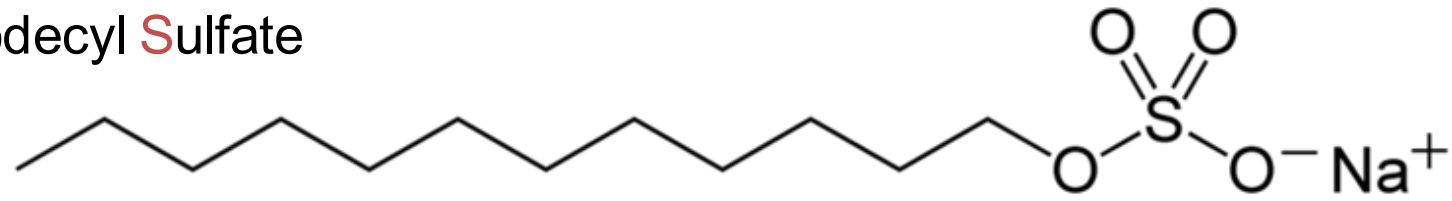
Procedures

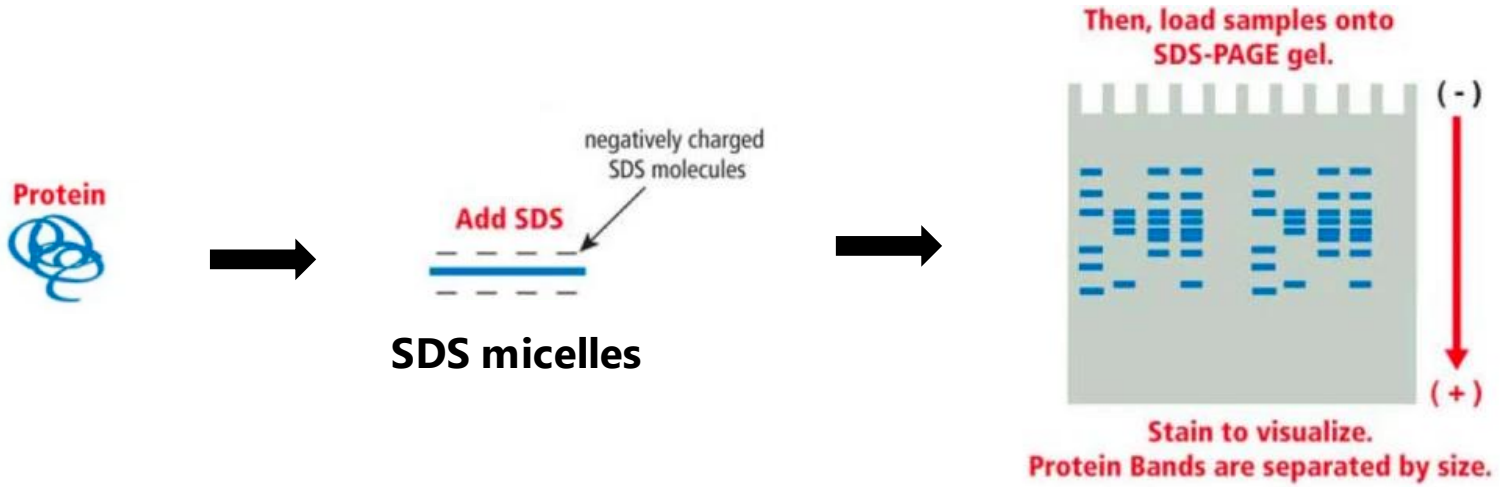
- Run **SDS-PAGE gels** to ***analyze synthesized proteins***
- Cast **SDS-PAGE gels** (running and stacking gel layers)

REMINDER: Recall in Chapter 5…

SDS-PAGE: Denaturing Poly-Acrylamide Gel Electrophoresis

Sodium Dodecyl Sulfate





All proteins will have **same mass/charge ratio**

Proteins are separated based only on **size**

(Image: Edvotek®)

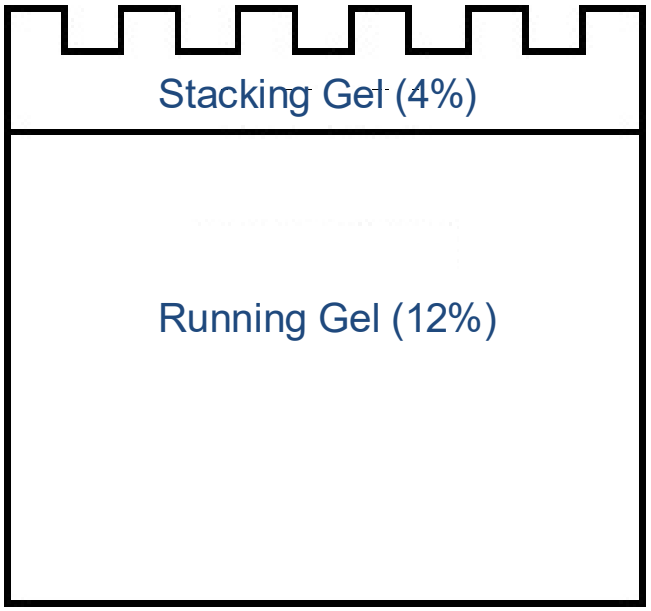
WHY TWO GELS?

Discontinuous Electrophoresis

- Two gel layers
  - 1) first for stacking
  - 2) second for separation/sieving

  Each has a different - **pH, pore size**
  - *(See pp. 175-180)*

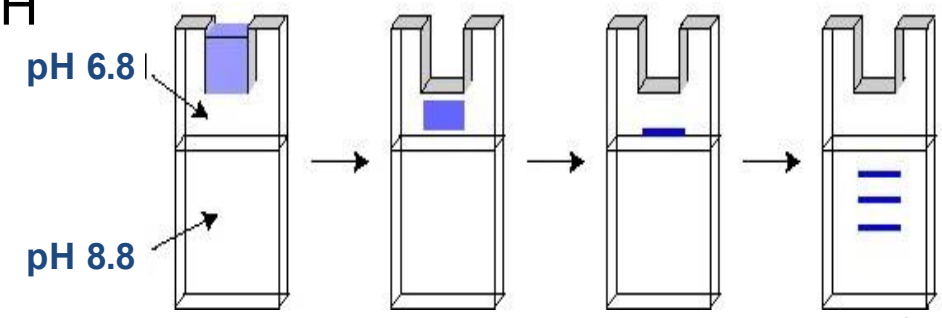
Whole gel:



- **Stacking Gel** – top layer; lower pH (6.8) & lower acrylamide percentage (4-5%)
  - Proteins "stack" into a tight bands due to a voltage gradient set up by glycine in the running buffer undergoing a change in mobility due to the pH changes

Single well:

- **Running Gel** – bottom layer; high pH (8.8), higher acrylamide percentage
  - proteins resolved based on by size due to "Sieving" effect

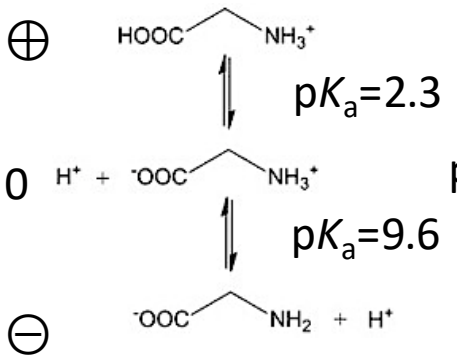


(Image: University of Utah)

Laemmli, UK (1970) *Nature* **227**:680-685.

How does discontinuous SDS-PAGE work?

Glycine



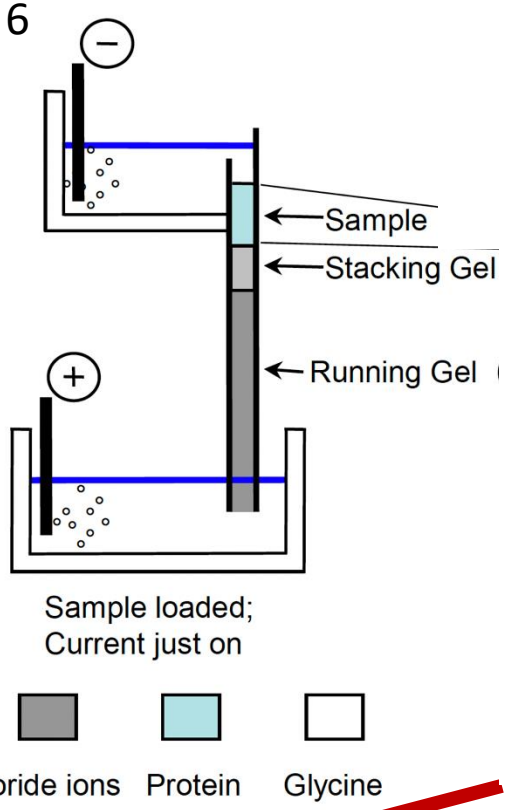
p*I* = 6

Net charge at 6.8: only very slightly negative (–0.0015)

Net charge at 8.8: –0.15

Stacking gel pH 6.8

Running gel pH 8.8



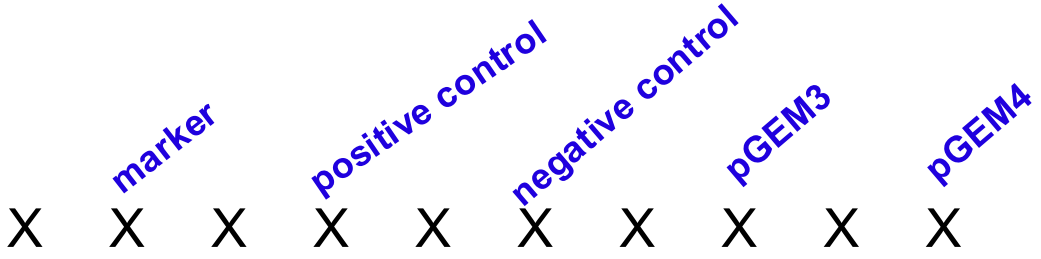
(slower) **Glycine < Proteins < Dye < Chloride** (faster)

(slower) **Proteins < Glycine < Dye < Chloride** (faster)

Chapter 8: Procedure

Gel Loading and Running:

- 2 groups per running box - 1 gel per small group
- NEB ladder is ready to use, no need to denature
- Load 6 µL of ladder
- Use gel loading tips found at the reagents bench
- Record your gel loading scheme in notebook



Page ***267*** in lab manual:
M_r of luciferase enzyme is 61 kDa
M_r of REL protein is about 65 kDa

Chapter 8C Pre-lab clarification

- Use either the 15-mL or 50-mL conical tube to prepare your running and stacking gel solution
- After you add the running gel solution, add a layer of isopropanol over the gel. Drain the isopropanol once the running gel layer have solidified.

Chapter 8C Post-lab clarification

- Chapter 8 post-lab write-up won't be due until week of Feb 25 - Mar 2

Chapter 8C

Before the lab period, you should have:

- ✓ Completed your Pre-lab Write-up and submit on Gradescope
  - ✓ Title, purpose and procedures

At the end of lab, you should have:

- ✓ A sketch of your SDS-PAGE gel loading scheme
- ✓ Electrophoresed samples and turn in washed gel to TF
- ✓ Prepared an SDS-PAGE gel for the next section and turned in to your TFs to check
  (prepared during your electrophoresis step)

Questions?

In-class activity
&
Discussion Quiz