Announcements

- Log in to ResponseWare
- Quiz this weekend
- Study guide is up
- Reading questions for Monday and Wednesday will be up
  - May want to start sequence alignment early.
- pKa practice
- Structure practice
- Check Reading Grades

Protein Purification

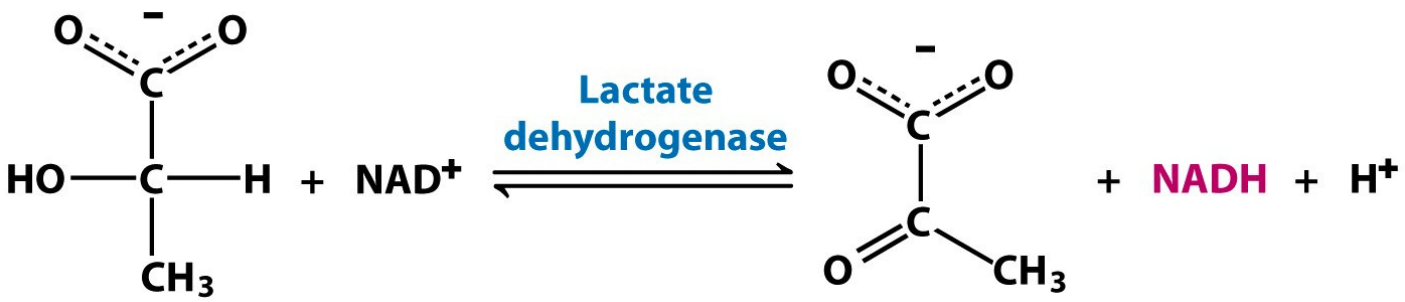
- Pure protein (lots of activity)
- lots of protein

Assay → how much of / how well the protein catalyzes a reaction or does its job

measure activity

find our protein

- enzyme assays → how well it catalyzes a reaction
- [protein] assays → how much protein / vol



Unnumbered figure pg 67
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enzyme assay – directly or indirectly measure the reaction

-specific for enzyme

[protein] assays – dyes that bind protein

-not specific for any one protein

Don't know how many molecules looking at how active a tube of molecules is

Specific activity = $\frac{\text{activity}}{\text{mg}}$ = $\frac{\text{Enzyme activity} \rightarrow \frac{\text{Enzyme units (Eu)}}{\text{mL}}}{\text{Concentration } \frac{\text{mg}}{\text{mL}}} = \frac{\text{Eu}}{\text{mg}}$

how active the individual molecules are

↳ higher the S.A. the more pure the protein

E.A. = 1 $^{eu}/_{mL}$

Beginning ||||||||||| | |||| |||| ← S.A. $\frac{1}{20}$ $^{eu}/_{mg}$

End | S.A. 1 $^{eu}/_{mg}$

E.A. = 1 $^{eu}/_{mL}$

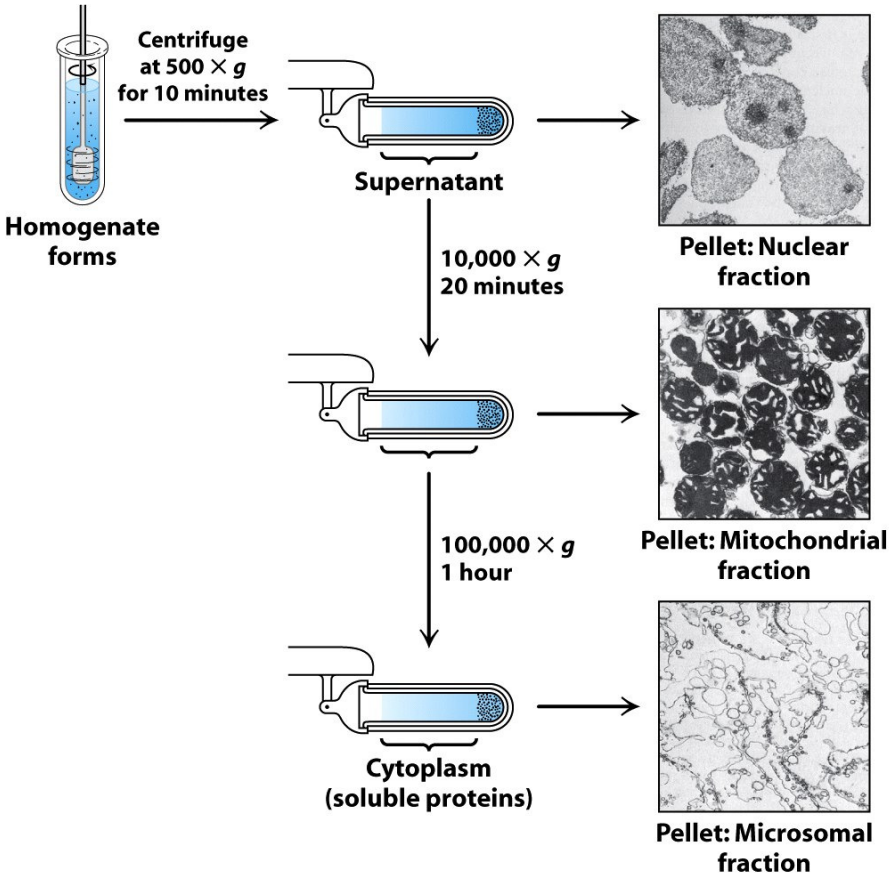

Figure 3-1
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1. Break cells

2. Centrifuge

2 parts

- soluble → cytosol
- insoluble → membranes
  nucleus

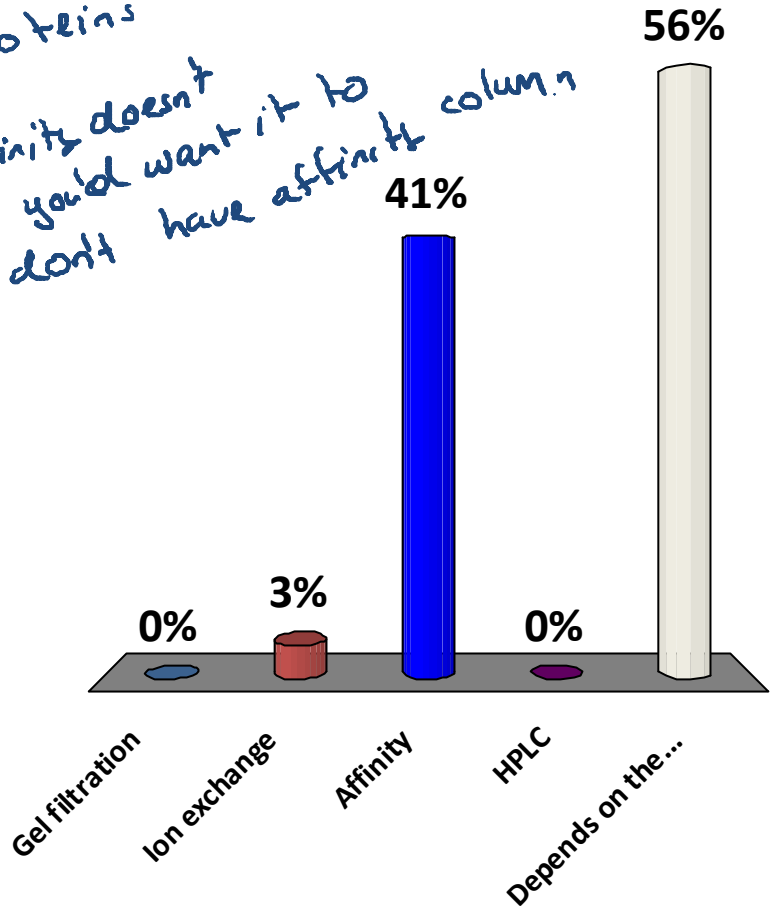
trying to separate our protein from the rest of the cytsolic proteins

Which of the following columns are best for purifying protein?

1. Gel filtration
2. Ion exchange
3. Affinity
4. HPLC
5. Depends on the protein.

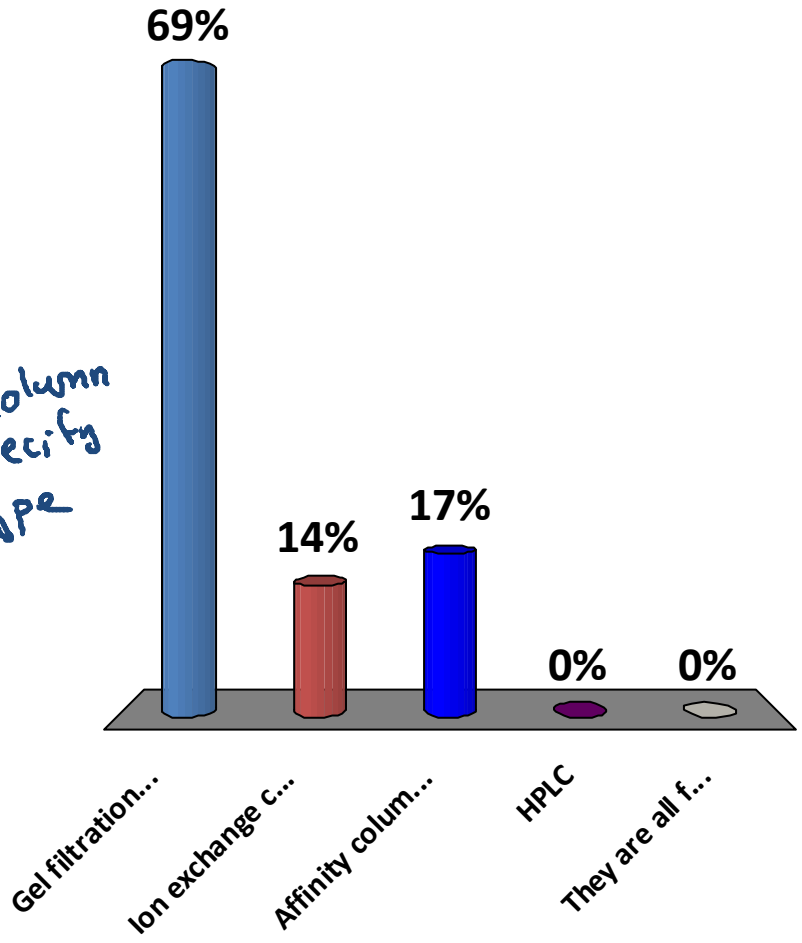
specific for the proteins

sometimes affinity doesn't work like you'd want it to or you don't have affinity column



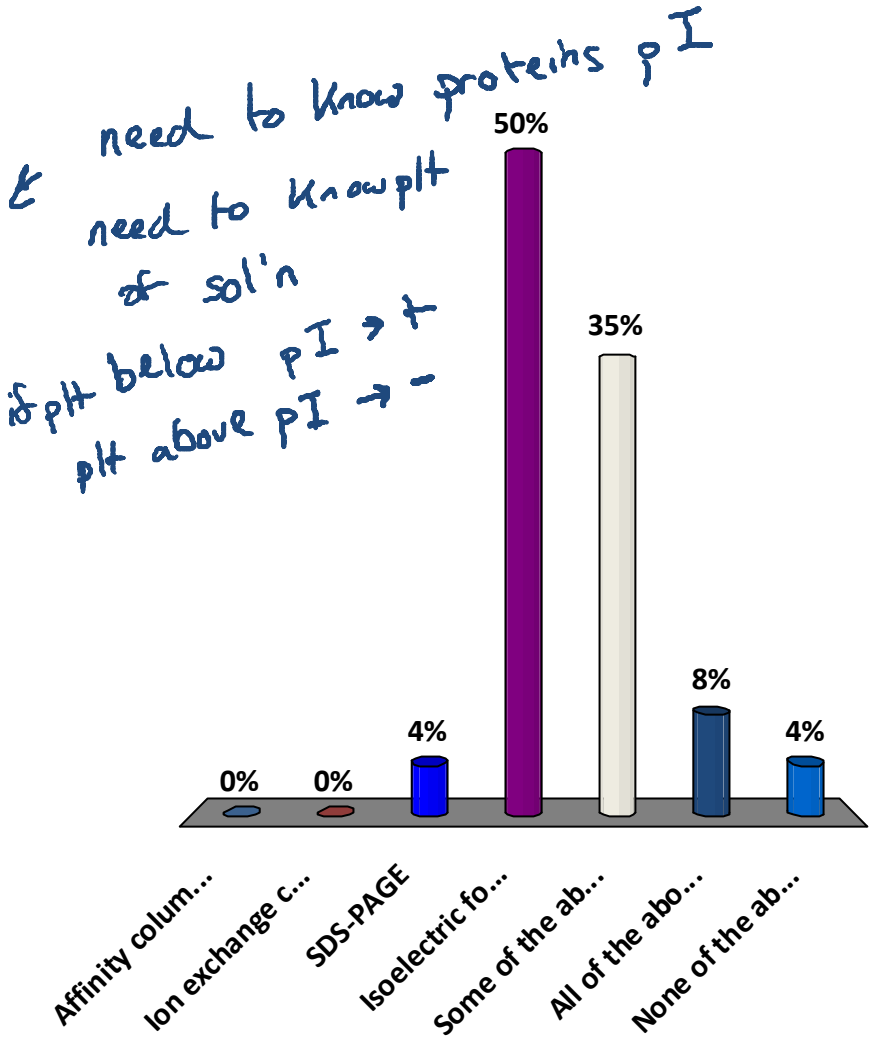
If you describe a purification scheme, listing which of the following would not give sufficient information about what you did?

1. Gel filtration column
2. Ion exchange column
3. Affinity column
4. HPLC → not specific to a column type have to specify column type
5. They are all fine



Which techniques make use of knowing a protein's pI?

1. Affinity columns
2. Ion exchange columns
3. SDS-PAGE
4. Isoelectric focusing
5. Some of the above
6. All of the above
7. None of the above



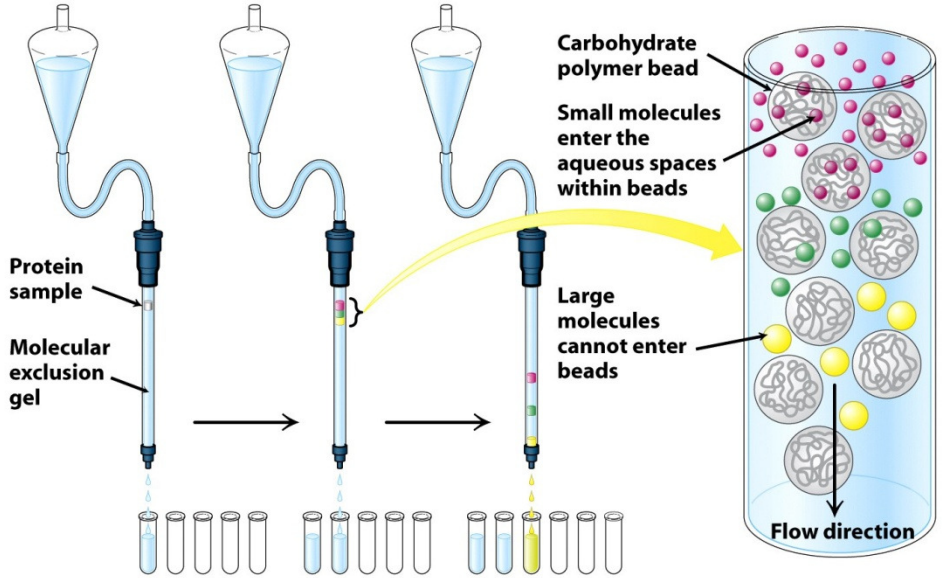


Figure 3-3
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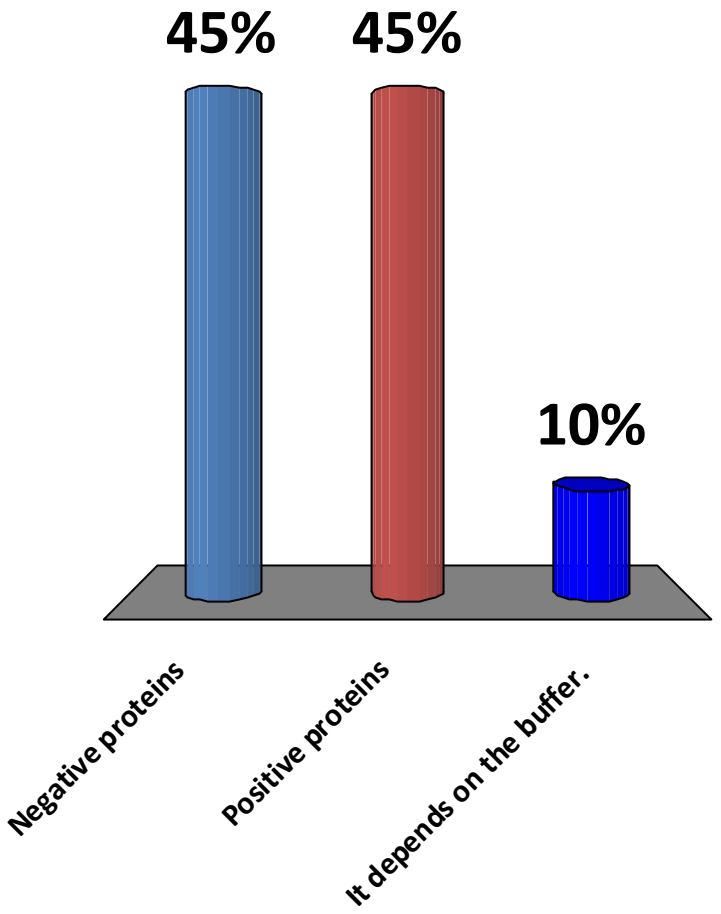
Size exclusion > same thing
gel filtration

Big molecules come out first b/c they can't go through the holes in the beads

Anion exchange columns bind

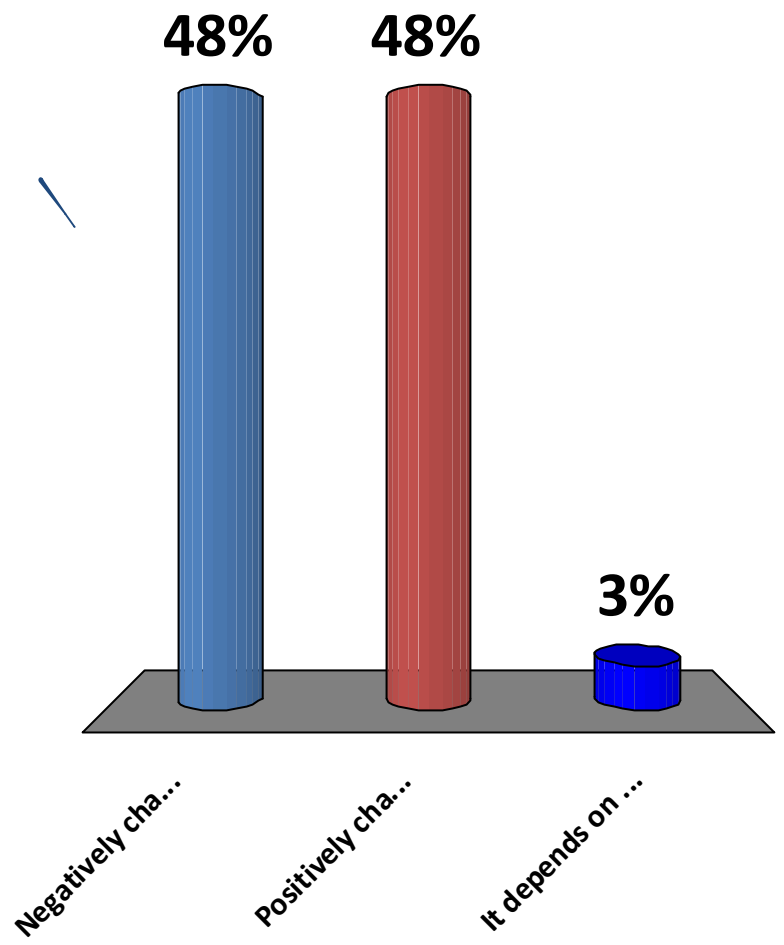
↳ are + charge

1. Negative proteins
2. Positive proteins
3. It depends on the buffer.



Cation exchange columns are

1. Negatively charged
2. Positively charged
3. It depends on the buffer.



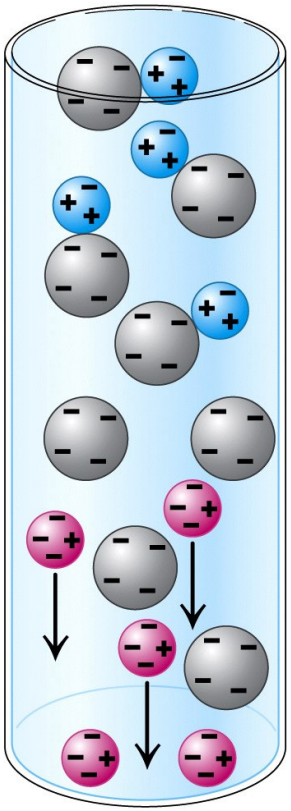


Figure 3-4
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Maybe a better way to ask:

SDS-PAGE, IEF, and 2-D gel electrophoresis all work to isolate proteins but they are not a good choice to use as a way to purify proteins for use in lab.

Why?

- denature → unfold protein
- not much protein

Because these processes do not occur under physiological conditions, and so even though the protein is isolated it is probably not in the correct form. Very often these processes denature the protein being isolated and purified.

These assays are not good techniques for true protein purification because they leave the proteins denatured. This causes the specific activity to be ~0, so almost all lab experiments would be invalidated if they used these denatured proteins. In addition, it seems as though it is very difficult to get a high yield from these techniques, as the proteins are not easily purified from the gels themselves.

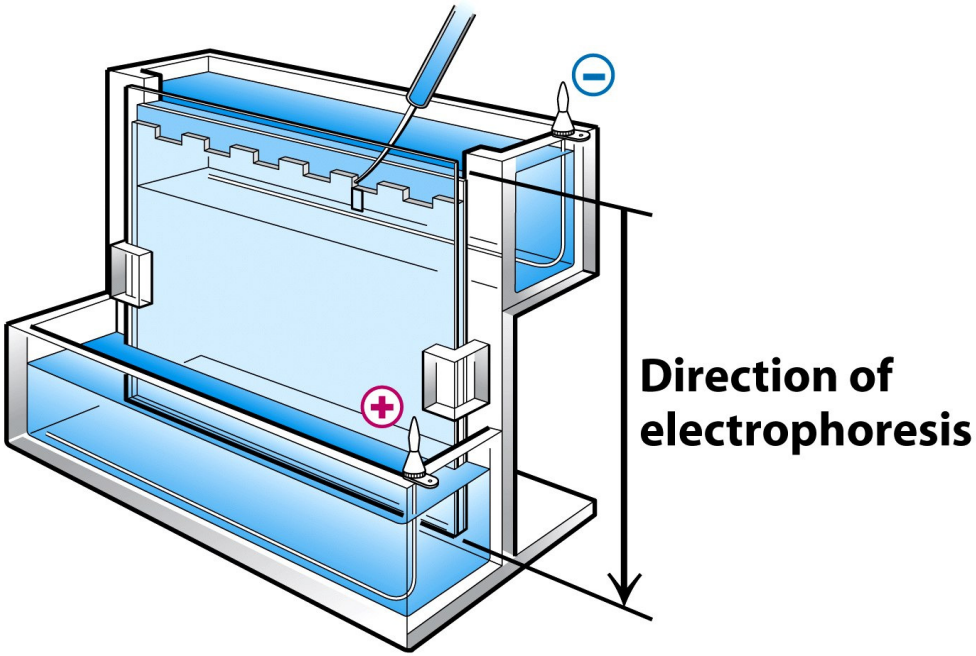

Figure 3-7a
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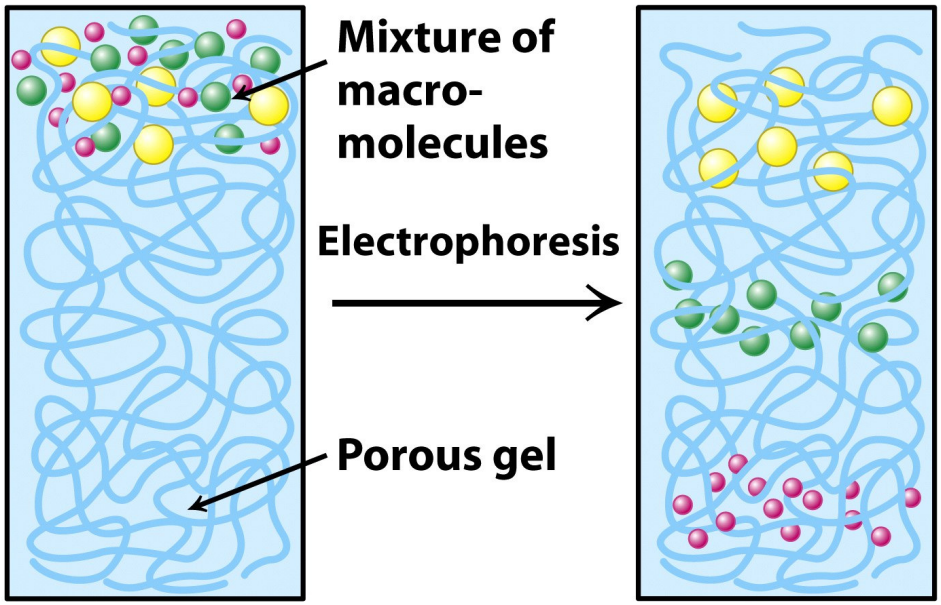

Figure 3-7b
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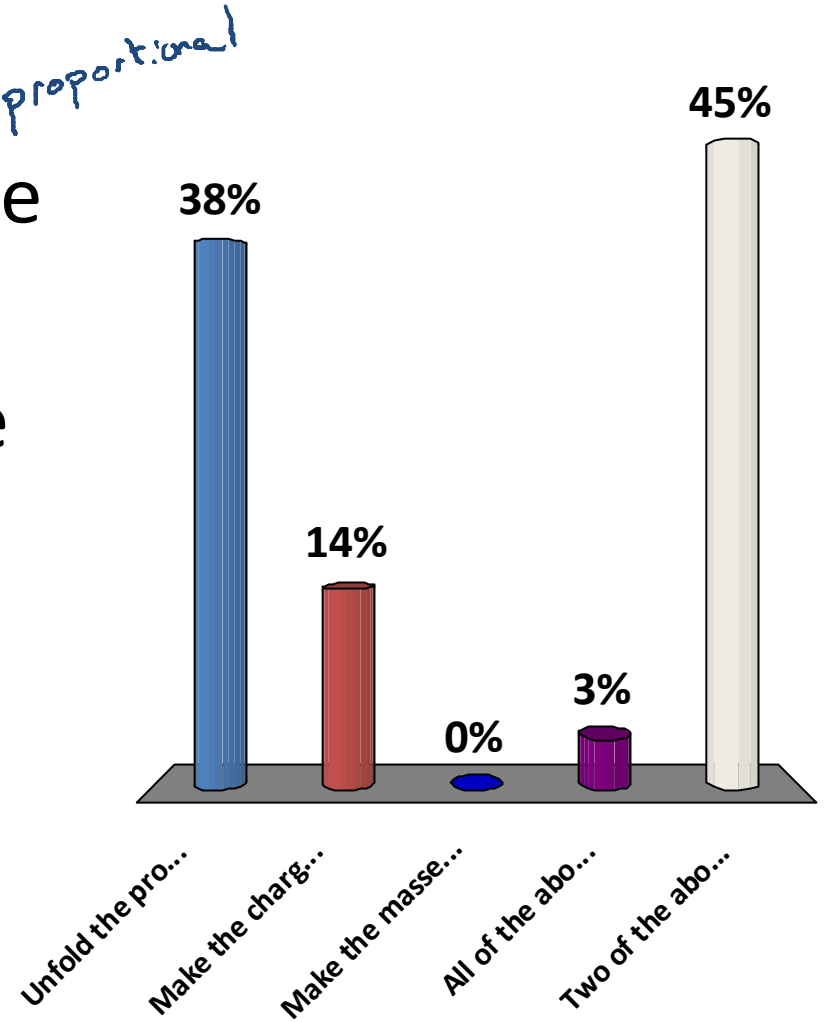
The role of SDS is to

1. Unfold the protein
2. Make the charges on the proteins the same
3. Make the masses of the protein the same.
4. All of the above
5. Two of the above



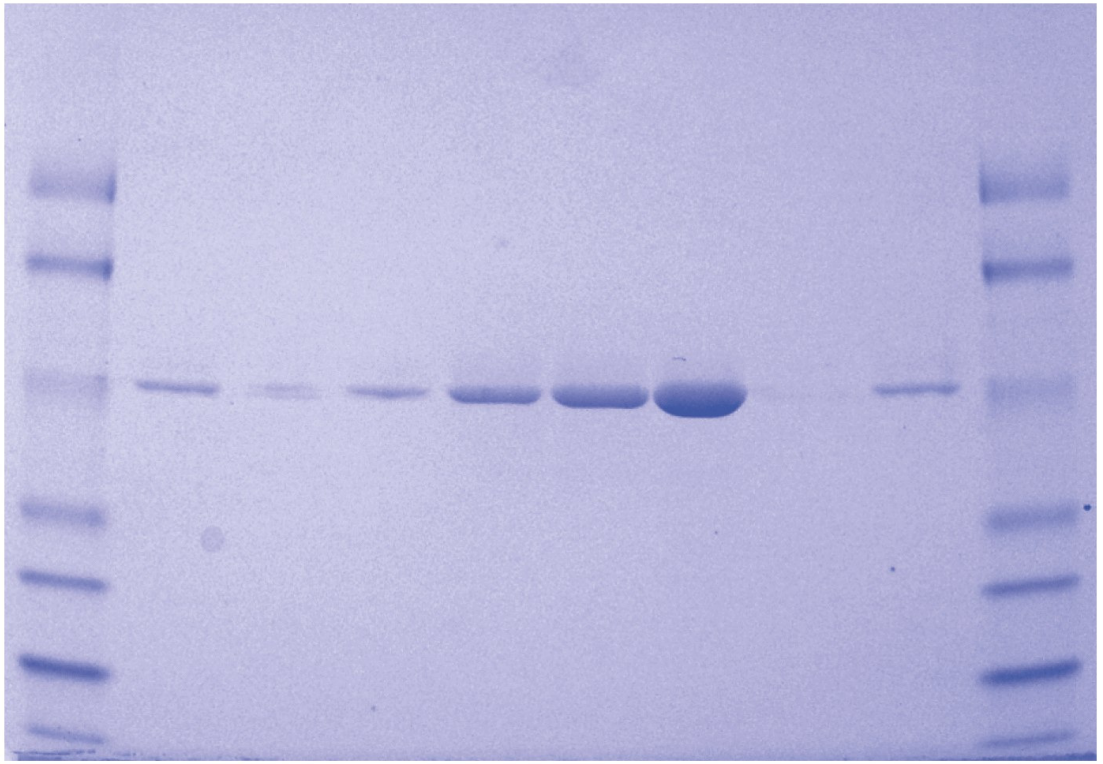


Figure 3-9
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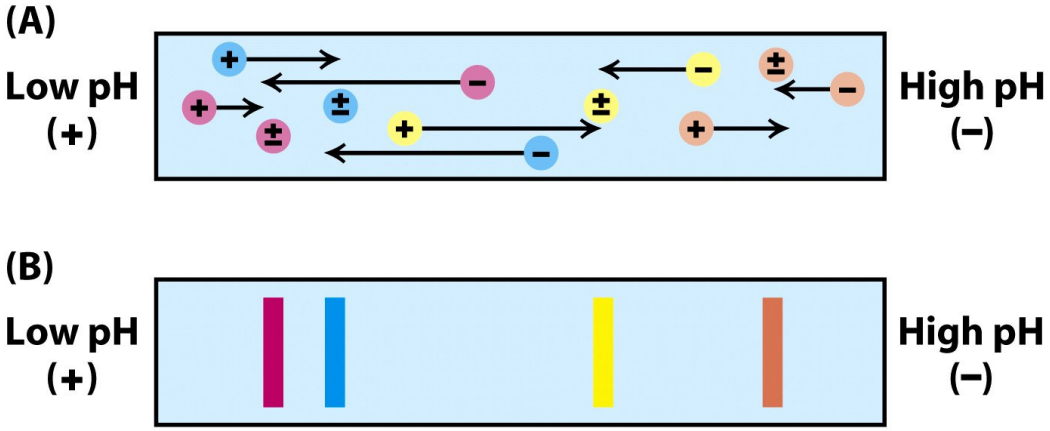

Figure 3-11
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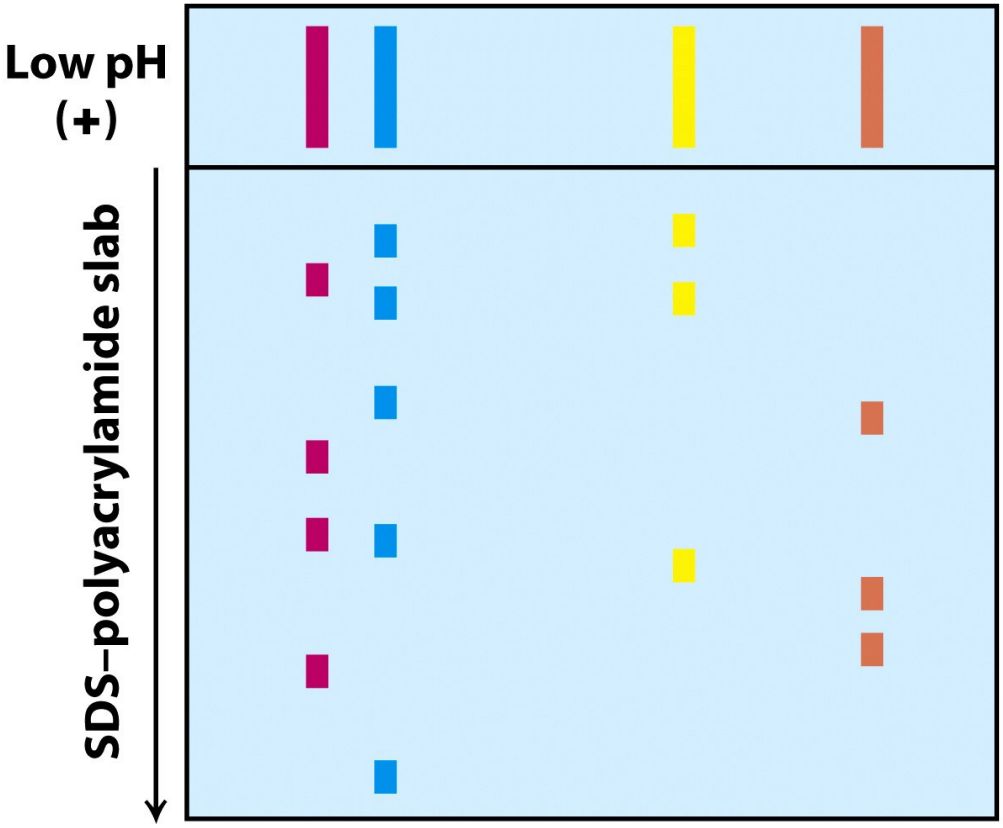
IEF

separates on charge



2 D

← IEF first

← SDS-PAGE 2nd

Figure 3-12a
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Isoelectric focusing

SDS-PAGE

Figure 3-12b
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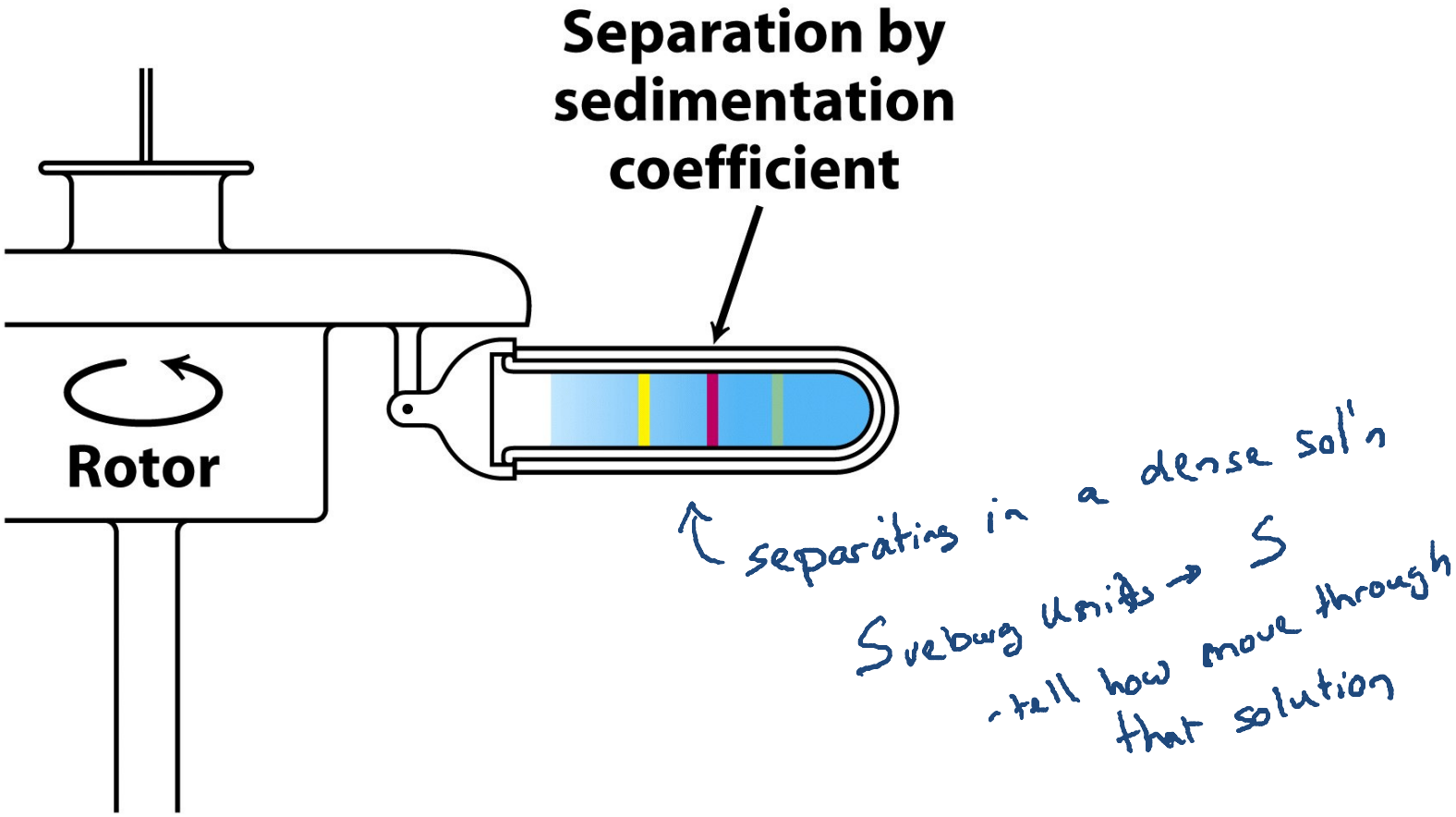

separating in a dense sol'n

Svedberg Units → S

- tell how move through that solution

Figure 3-15c
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