CONCEPT: SDS-PAGE

●*SDS-PAGE* (Sodium Dodecyl Sulfate-PolyAcrylamide Gel Electrophoresis): separates proteins _____ based on *mass*.

□ *SDS*: a highly _____ detergent with a *negative* charge that denatures proteins.

□ _____ gel matrices are commonly used to separate proteins.

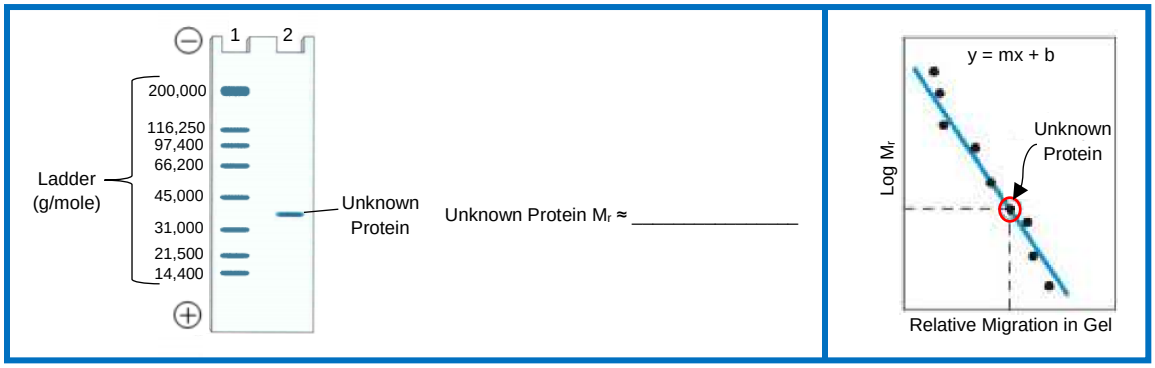
□ Recall: Larger proteins travel _____ through the gel.

●Ladder/Marker: control reference proteins of _____ molecular size & quantity.

□ Size & _____ of unknown proteins can be approximated by comparisons to the ladder.

□ A plot of log M_r (molecular weight) vs. relative-migration in the SDS-PAGE gel is a _____ relationship.

EXAMPLE: SDS-PAGE.



PRACTICE: By adding SDS to a protein and performing gel electrophoresis, it is possible to:

a) Determine a protein's isoelectric point.

b) Determine the amino acid composition of the protein.

c) Separate proteins exclusively based on molecular weight.

d) Preserve a protein's native structure and biological activity.

How SDS Works

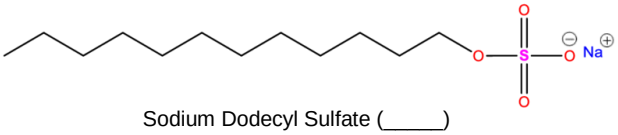
●SDS binds to proteins approximately _____ to molecular weight (~1 SDS per amino acid residue).

□ Nonpolar, negatively charged SDS _____ proteins & _____ any native charges on a protein.

□ Results in all proteins having unfolded *shapes* & very _____ *charge-to-mass* ratios.

□ SDS denatures _____ structure as well, but it does *not* cleave _____ bonds linking subunits.

EXAMPLE: SDS.

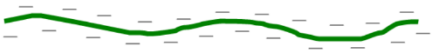


Sodium Dodecyl Sulfate (_____)

Before SDS

_____ Protein
(with native charges/shape)

After SDS



_____ Protein
(with unfolded shape & negative charge proportional to mass)

PRACTICE: True or false: Protein subunits linked via disulfide bonds appear as separate bands on an SDS-PAGE gel.

a) True. b) False.

CONCEPT: SDS-PAGE

PRACTICE: Which of the following statements are true regarding the treatment of proteins with SDS?

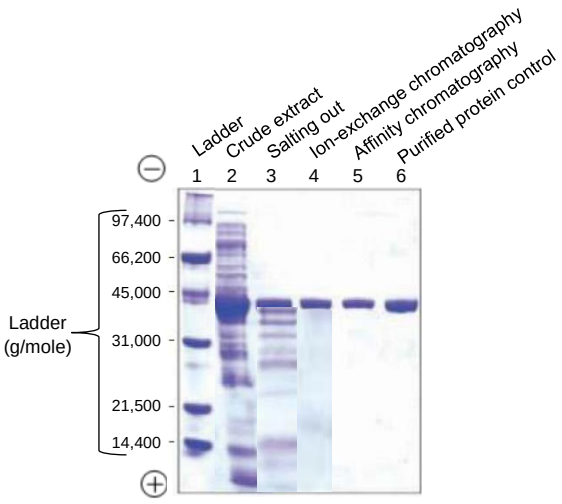
i) Only proteins with native net charges acquire an overall net negative charge.
ii) Proteins denature due to a disruption of the hydrophobic interactions stabilizing the core of their structures.
iii) All protein subunits can be separated via SDS-PAGE.

a) i, ii, iii. b) i, ii. c) ii, iii. d) i, iii. e) ii.

Visualizing Protein Purification on SDS-PAGE

●Unlike chromatography, SDS-PAGE allows numbers/quantities of proteins to be _____ on the gel.

EXAMPLE: Visualizing effectiveness of purification techniques with SDS-PAGE.



PRACTICE: Suppose you purify a protein from liver cells and the SDS-PAGE results after different purification steps are shown. You then take the affinity purified sample and run it through a cation exchange column. The 2nd SDS-PAGE shows the results for the flow through and eluate from the cation exchanger. Based on this data, what conclusions can you draw from the results in lanes #5, 7 & 8?

Lane #5:

Lane #7:

Lane #8: