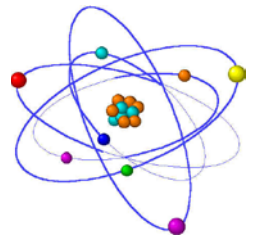
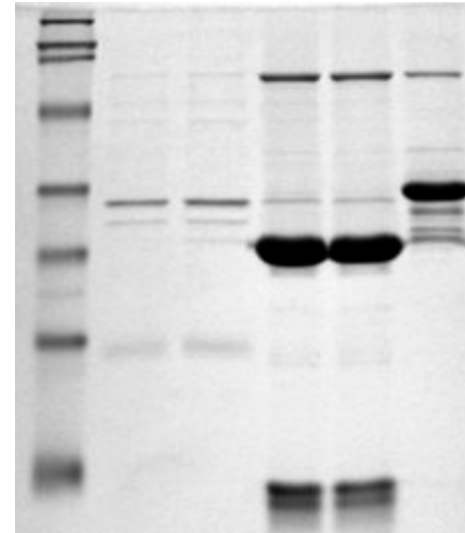
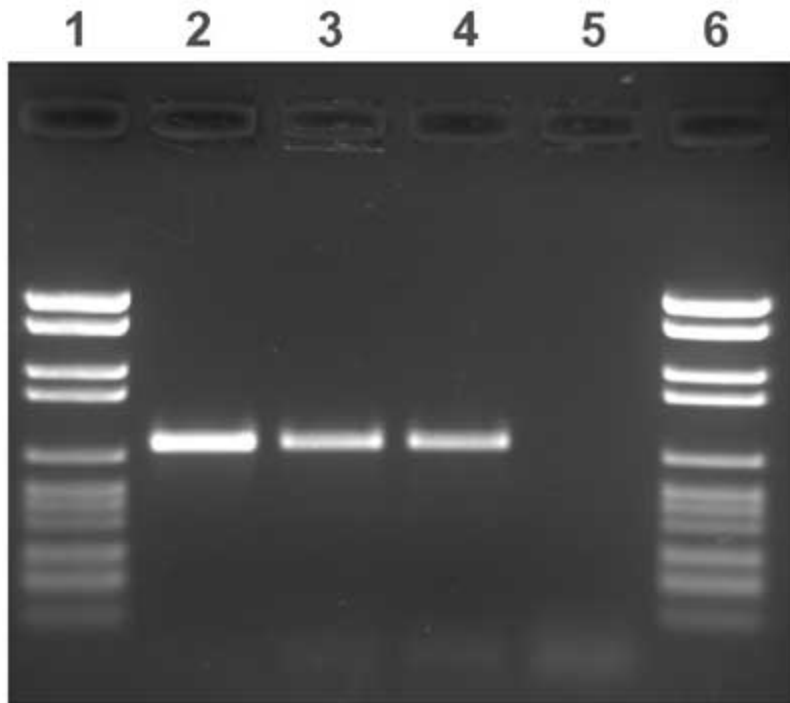


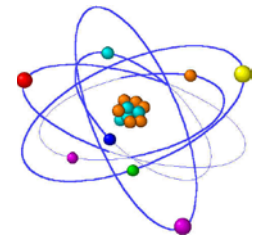
Biochemistry Lab



Visualizing Biological Molecules

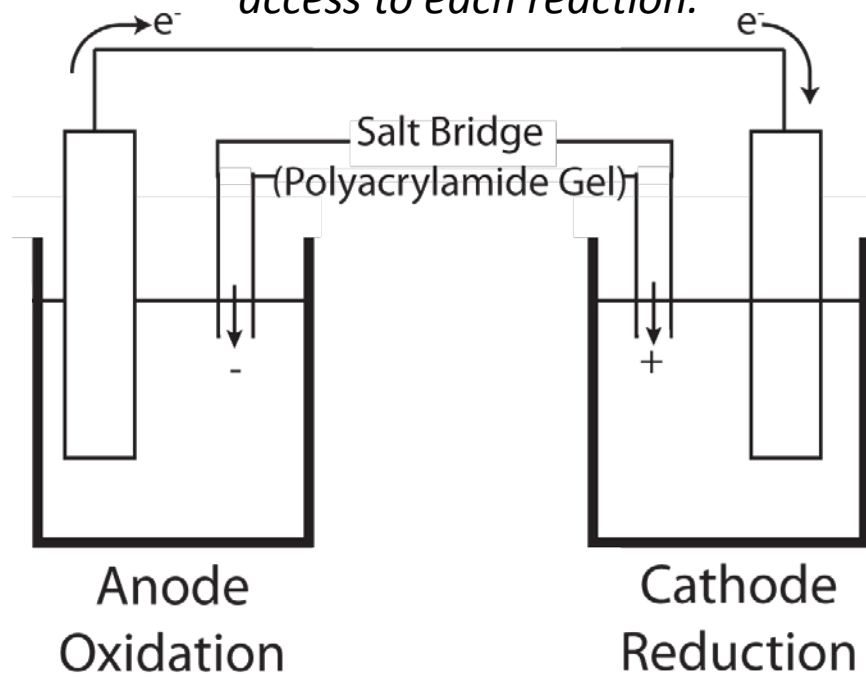


Electrophoresis



Controlled motion of charged molecules
Relies on an electrochemical cell

*The two chambers are connected
by a wire to allow electrons
access to each reaction.*

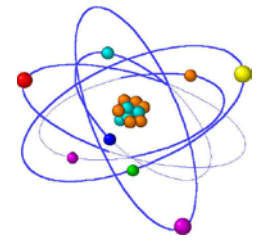


*Two chambers where
an electrochemical half
reaction can occur.*

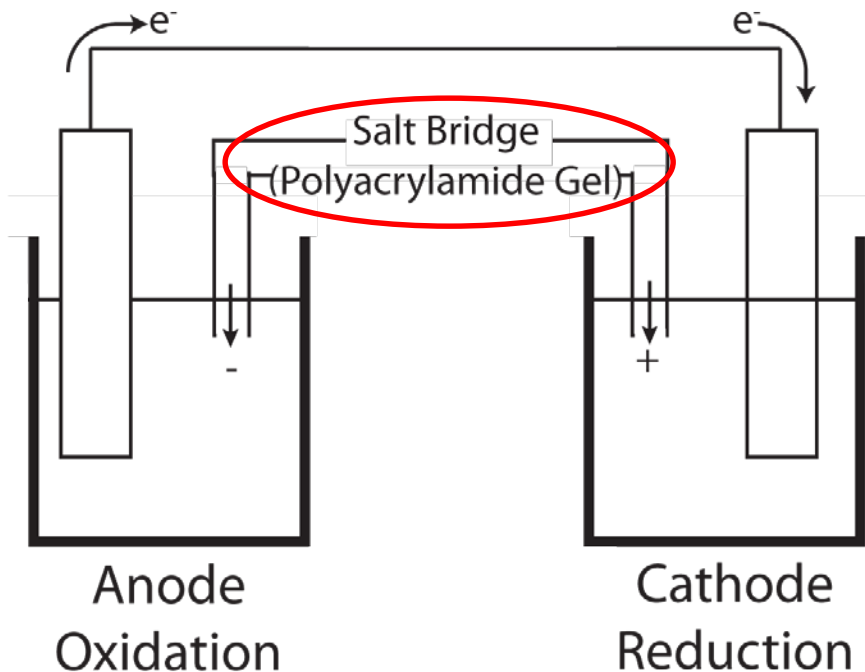
*A salt bridge is also
needed to maintain
charge balance.*

*Anions flow to Anode
Cations flow to Cathode*

Electrophoresis



Controlled motion of charged molecules

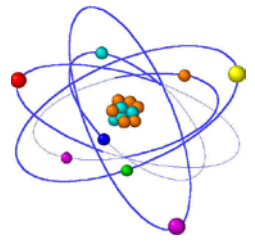


In electrophoresis, the salt bridge is made of a porous matrix that allows selective penetration of molecule based on size and shape.

A salt bridge is also needed to maintain charge balance.

*Anions flow to Anode
Cations flow to Cathode*

Electrophoretic Mobility

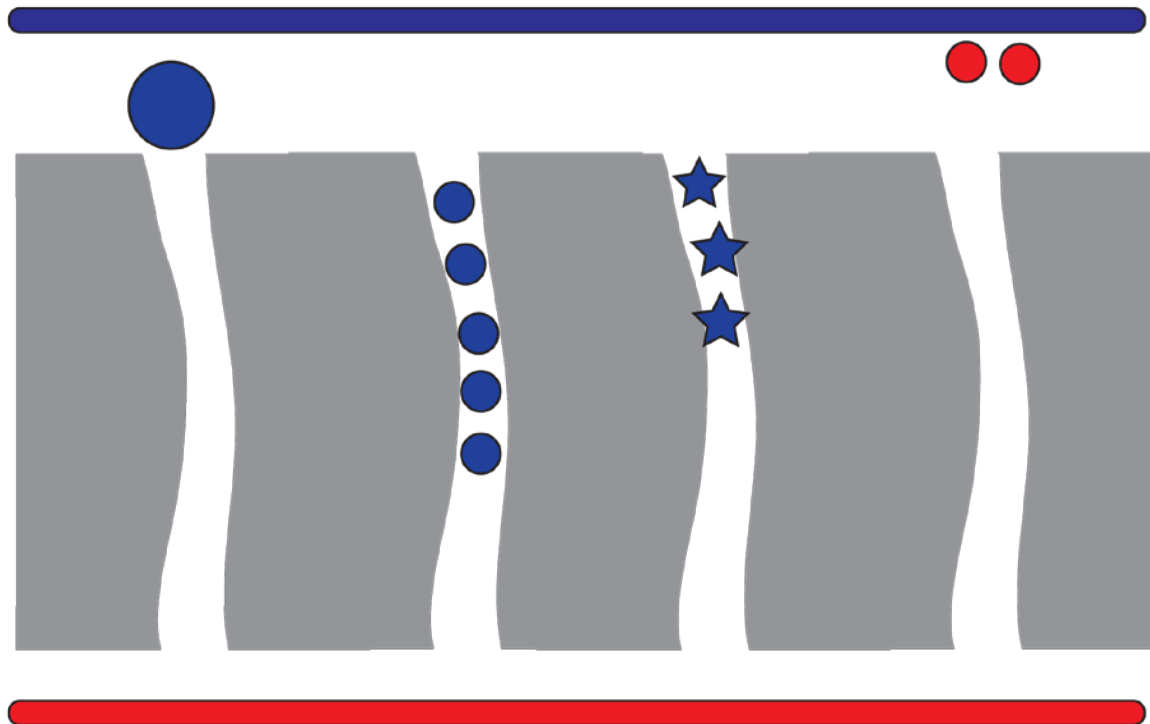


How far a protein can travel down a gel

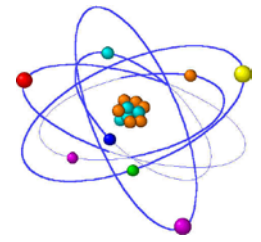
Size – Can it easily fit through the pores?

Shape – Does it have structural components that make it appear bigger?

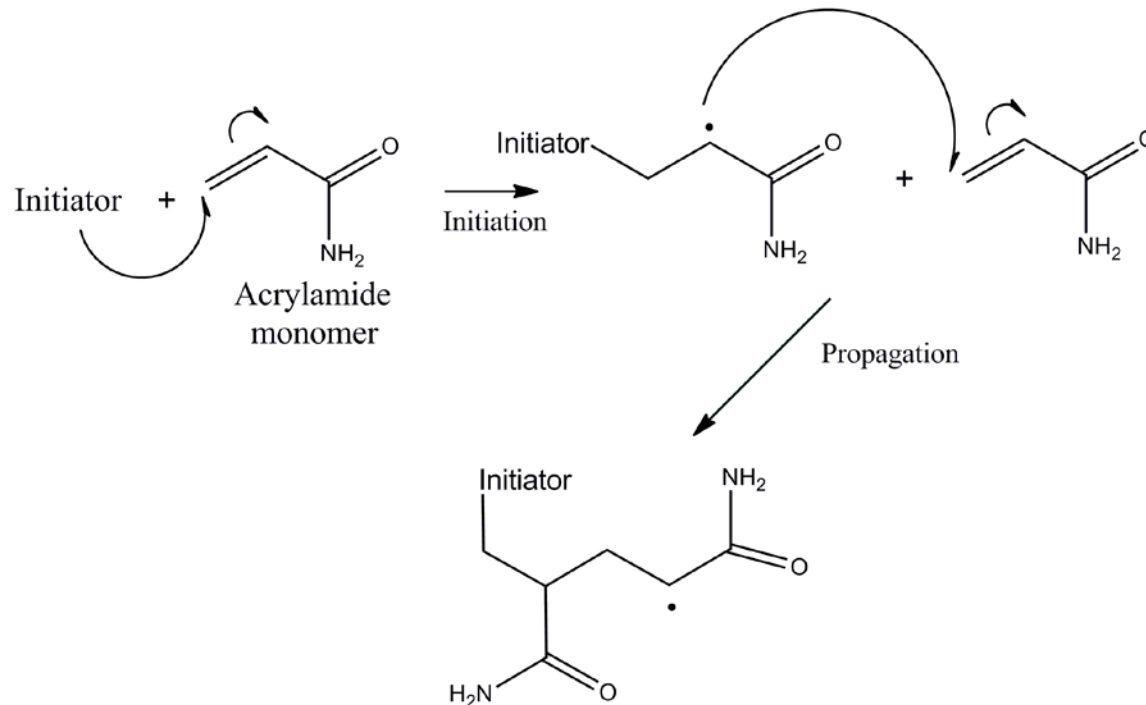
Charge – Will it travel toward the anode or cathode?



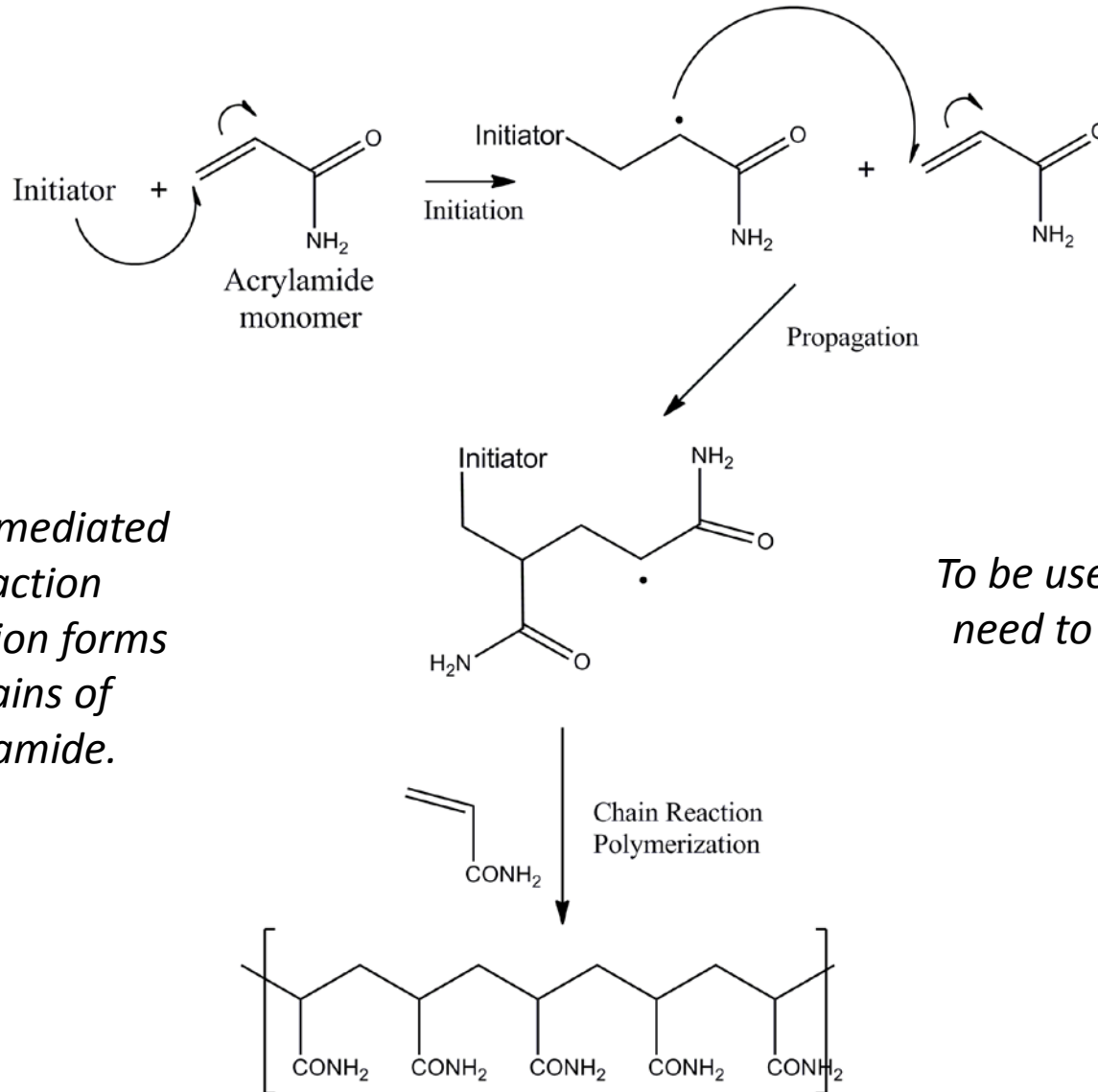
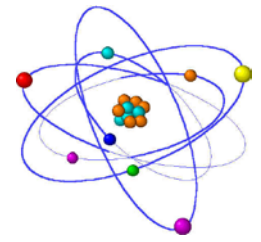
Polyacrylamide Gel Electrophoresis



Protein electrophoresis almost always uses polyacrylamide to form the resolving matrix



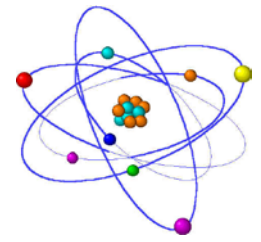
Polyacrylamide Gel Electrophoresis



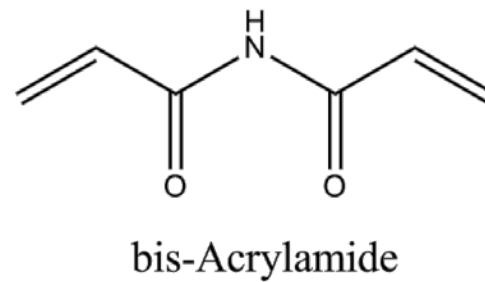
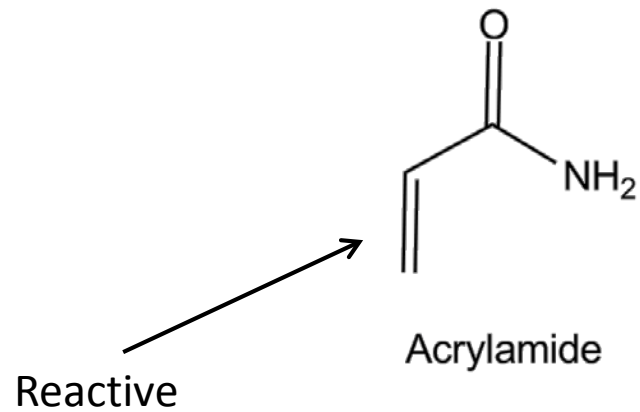
This radical mediated chain reaction polymerization forms linear chains of polyacrylamide.

To be useful, the chains need to be connected

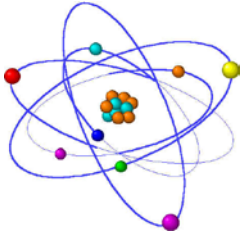
Polyacrylamide Gel Electrophoresis



What do we need for a crosslinking molecule?

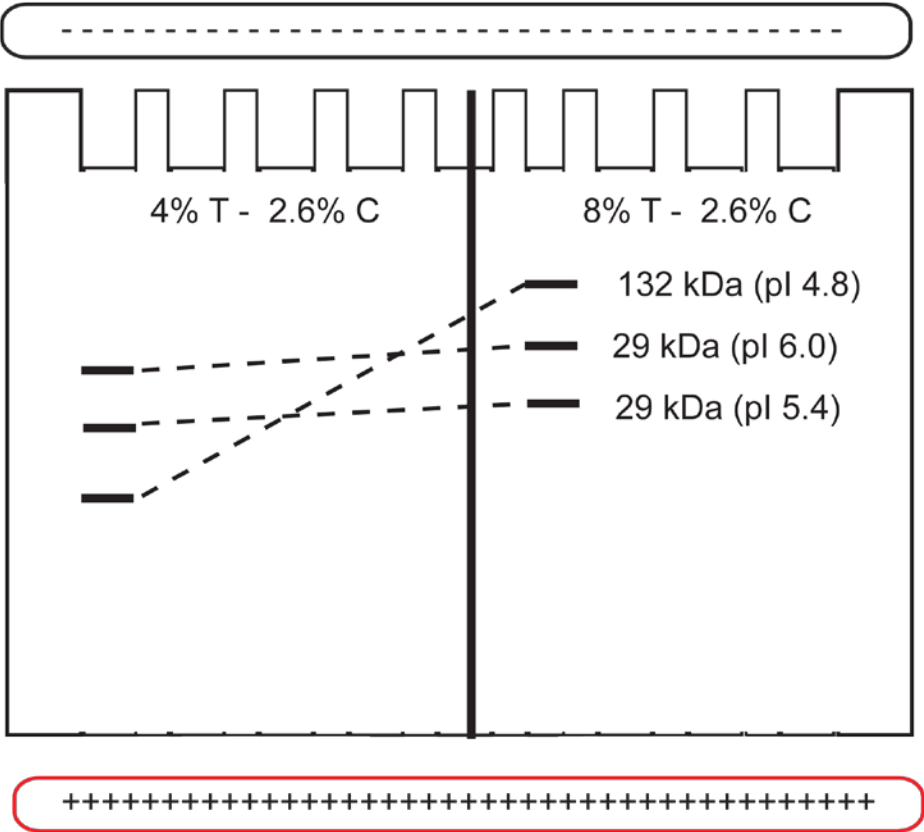


Size Matters

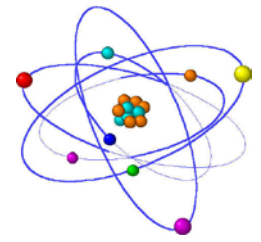


Large pores
Small %T or %C

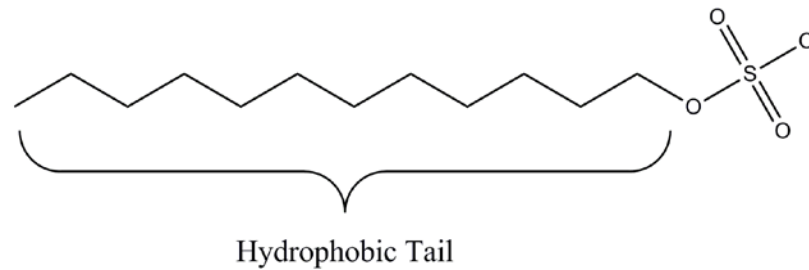
Small pores
Big %T or %C



Denaturing Condition



We want to see use PAGE to estimate size! This is NOT possible for folded protein.



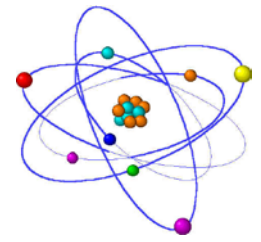
Adding a detergent to proteins induces an unfolding transition with the apolar tail coating the hydrophobic regions of proteins



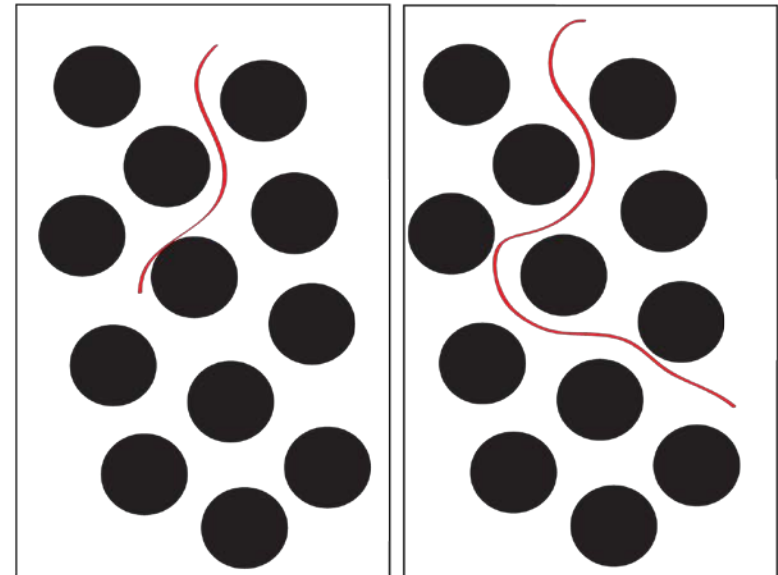
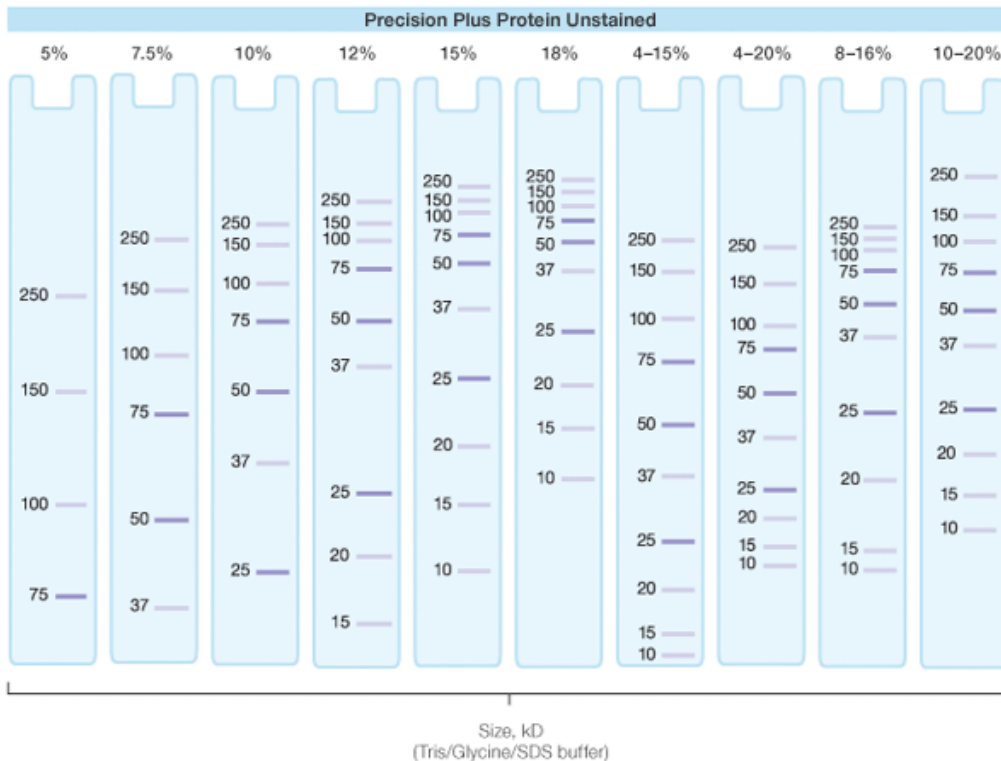
→ SDS



Denaturing Condition



Such a process allows proteins to be separated based primarily on polypeptide chain length.



Long Chains experience more drag while winding through a pores.