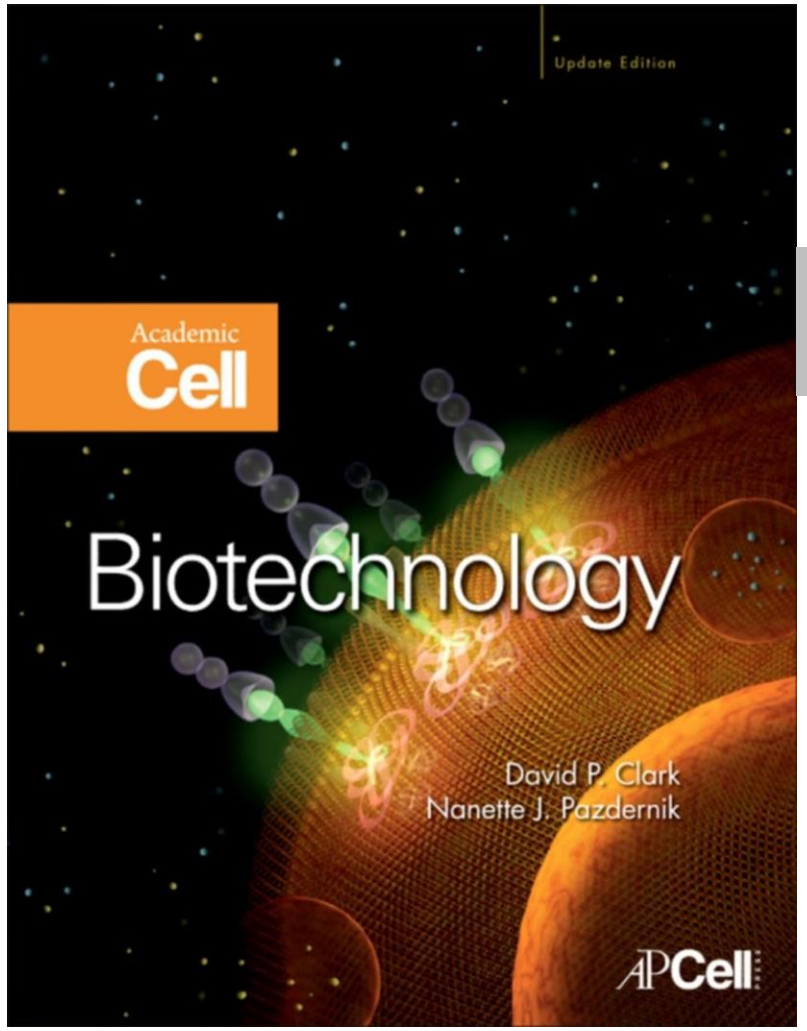
دانشگاه علوم پزشکی و خدمات بهداشتی،درمانی کرمانشاه
Kermanshah University Of Medical Sciences




# Chapter review

# Proteomics in biotechnology

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**Monday (1402/5/2) 12:30 p.m**

# Outlines

- Introduction
- Gel Electrophoresis of Proteins
- Western Blotting of Proteins
- High-Pressure Liquid Chromatography Separates Protein Mixtures
- Digestion of Proteins by Proteases
- Mass Spectrometry for Protein Identification
- Preparing Proteins for Mass Spectroscopy
- Protein Tagging Systems
- Phage Display Library Screening
- Protein Interactions: The Yeast Two-Hybrid System

# Intruduction

- Proteome refers to the entire protein complement of an organism
- Proteomics refers to the global analysis of proteins
- Protein profiling refers to the methods used for identification of proteome
- Translatome refers to the complement of proteins expressed under specific circumstance.
- the translatome is dynamic and changes when environmental conditions change
- The relationships among the genome, proteome, and translatome

# Gel electrophoresis of proteins

- First step in identification of proteins is separation
- Electrophoresis is used to separate proteins based on their size
- PAGE (polyacrylamide gel electrophoresis) usually for protein
- Agarose for DNA
- polyacrylamide has smaller pores than agarose and is thus suitable for proteins because they are generally smaller than DNA molecules
- Unlike DNA, most proteins do not have a net negative charge; therefore, protein samples are treated by boiling with sodium dodecyl sulfate (SDS)

# Gel electrophoresis of proteins

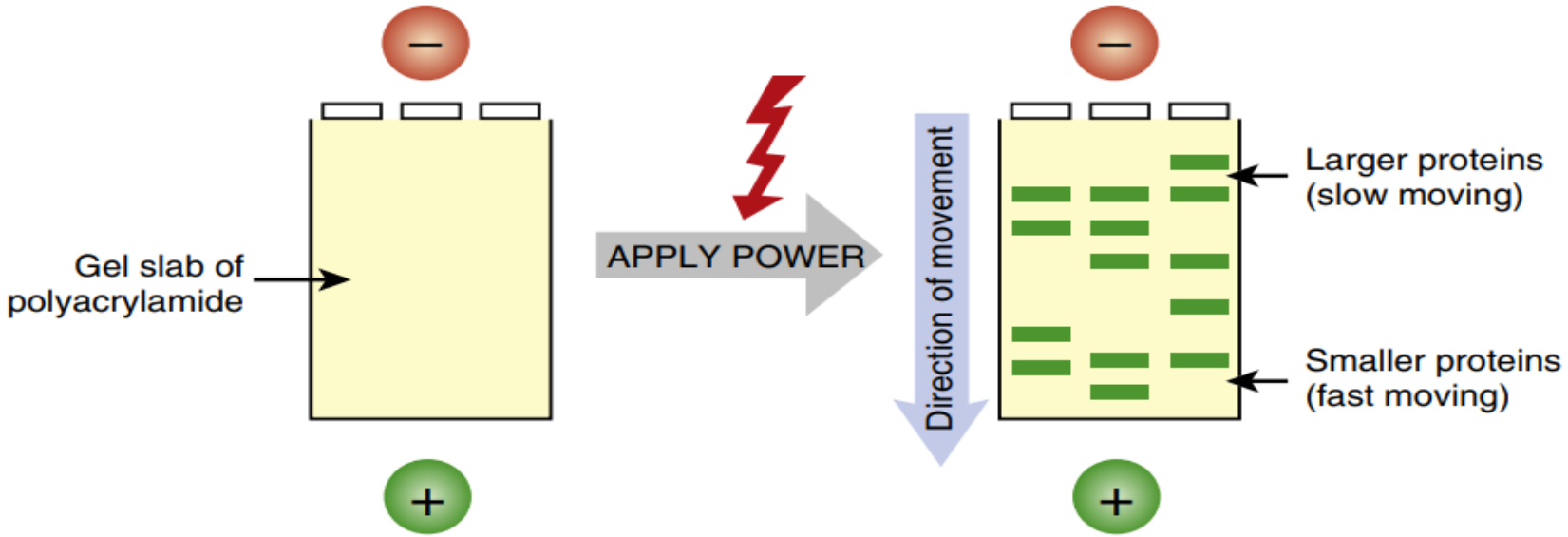
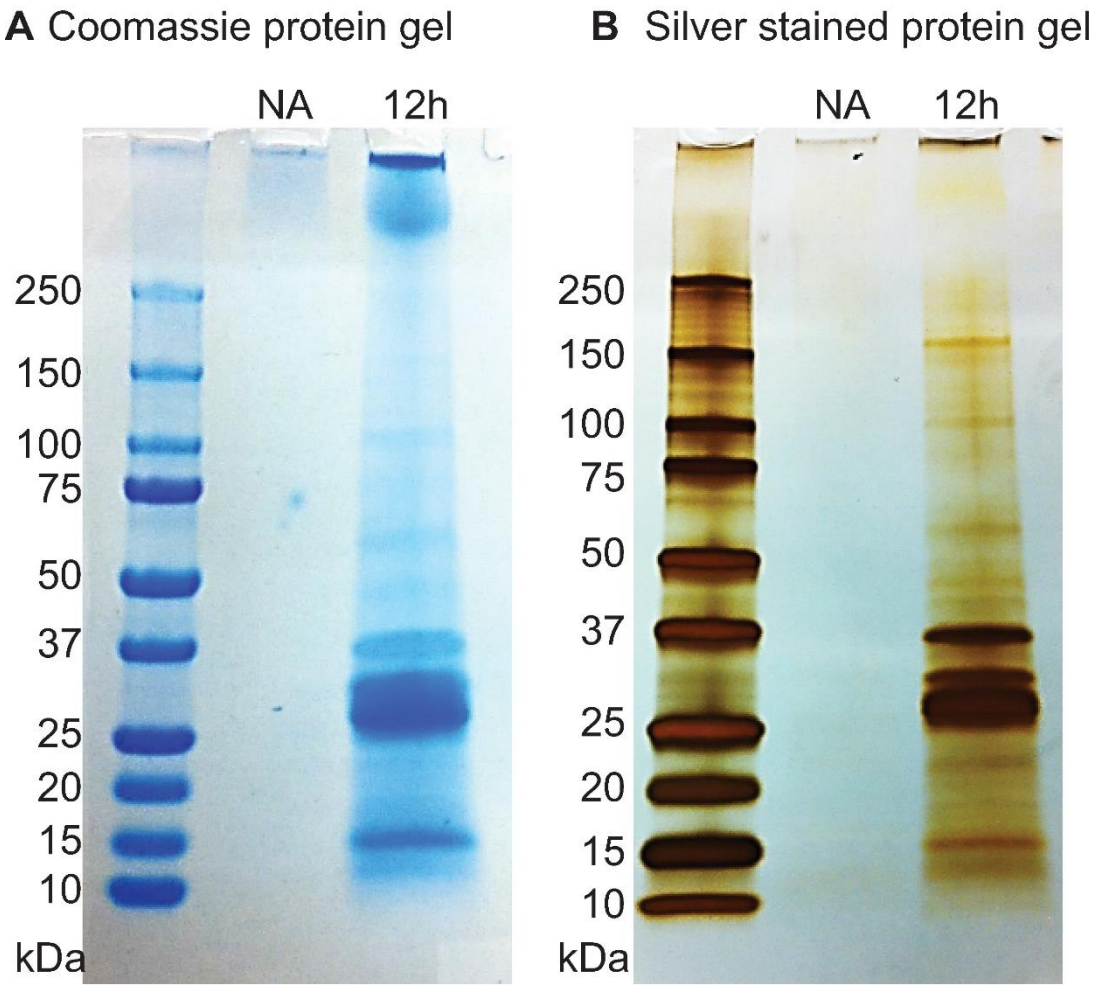



**FIGURE 9.1 SDS Polyacrylamide Gel Electrophoresis**
Proteins are denatured by boiling in buffer with SDS, which coats the surface of the unfolded amino acids to give the protein a net negative charge. The total charge correlates to the size of protein. After being loaded into a sample well of a polyacrylamide gel, the proteins migrate away from the negative pole and toward the positive pole. The sieving action of the gel allows the smaller proteins to move faster than the larger. The distance traveled in a given time is proportional to the log of the molecular weight.

# Staining of bands on gel

Finally, the separated proteins are visualized using either Coomassie blue, a dark blue dye, or the more sensitive silver stain, both of which bind tightly to all proteins.

Coomassie can detect only proteins that are present above the picomole amount, whereas silver stain can detect proteins that are in the femtomole order



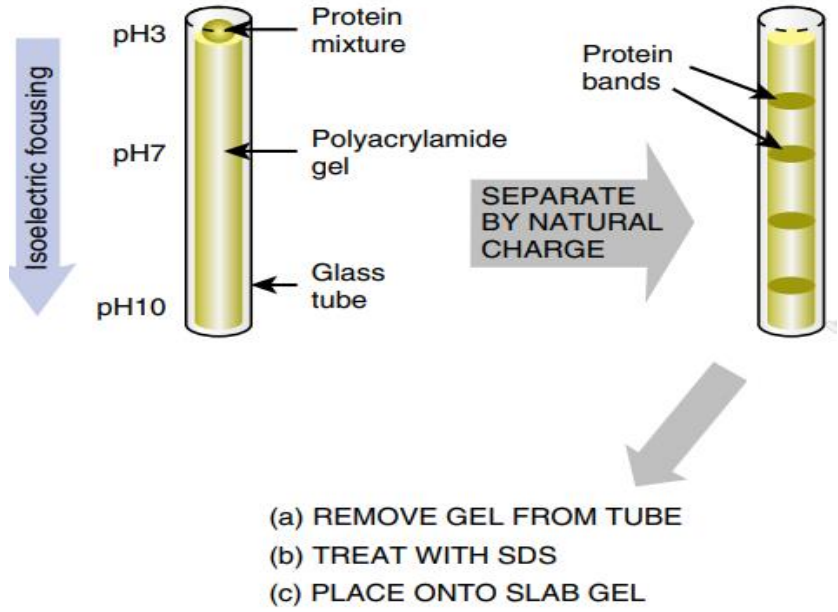
# Two dimensional - PAGE

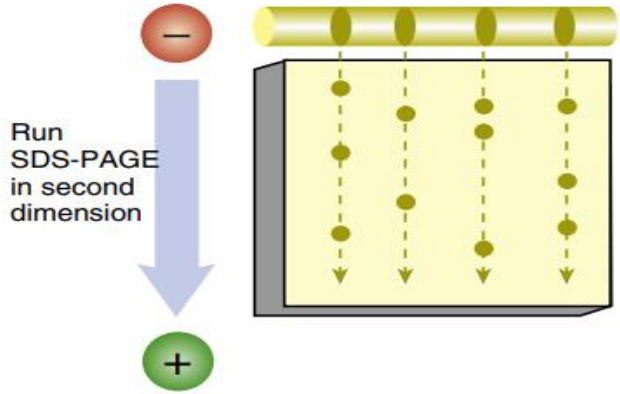
Isoelectric focusing is the term for separating proteins by their native charge.

All proteins have an inherent natural charge due to the side chains of their amino acid residues

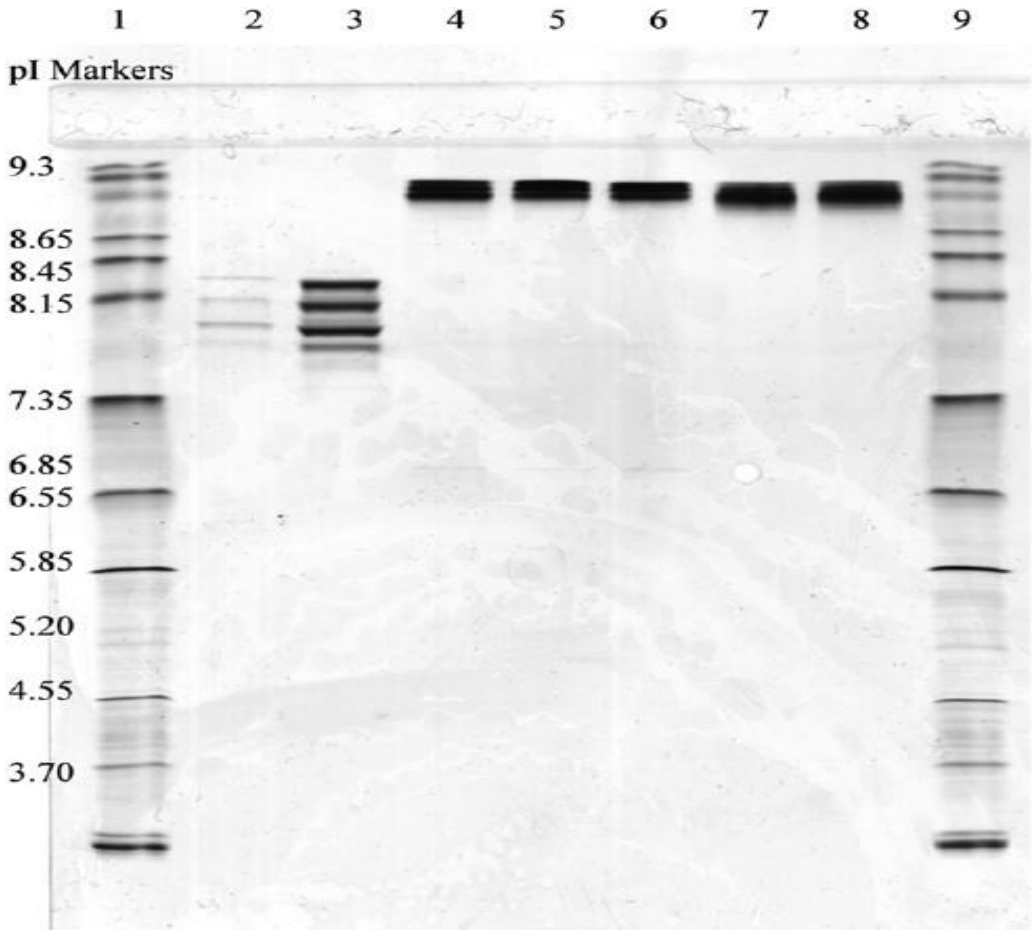
For separation by charge, the sample is loaded into the top of a gel with a pH gradient

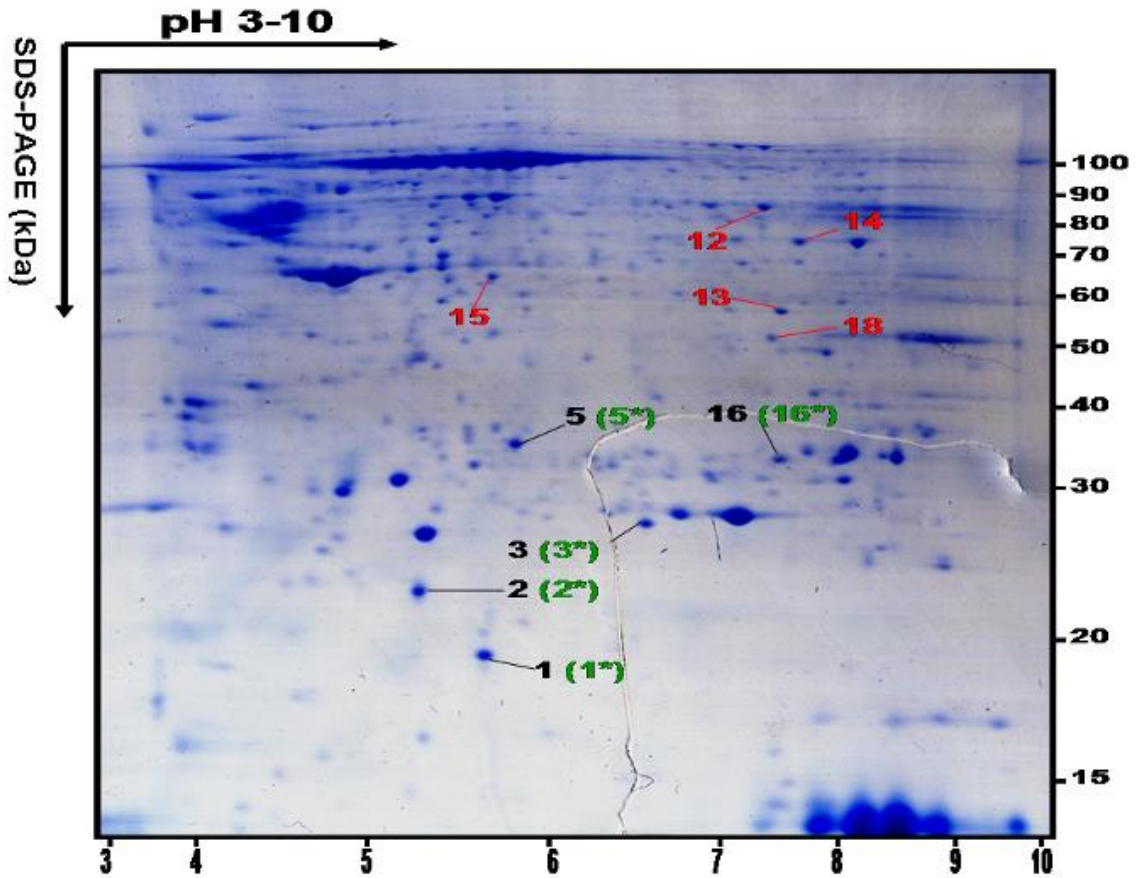
After this step is run, the gel is removed from its tube. The second dimension of 2D-PAGE is separation by size.



# Two dimensional - PAGE



# DIGE ( Differential gel electrophoresis )

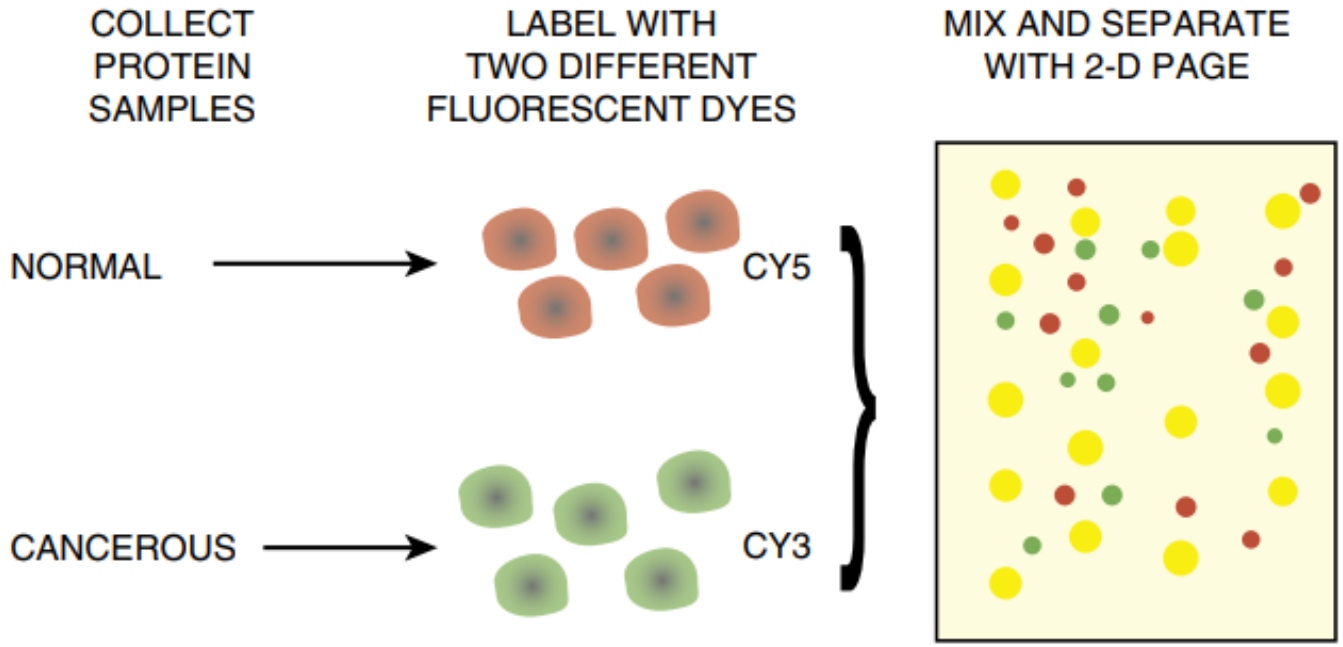
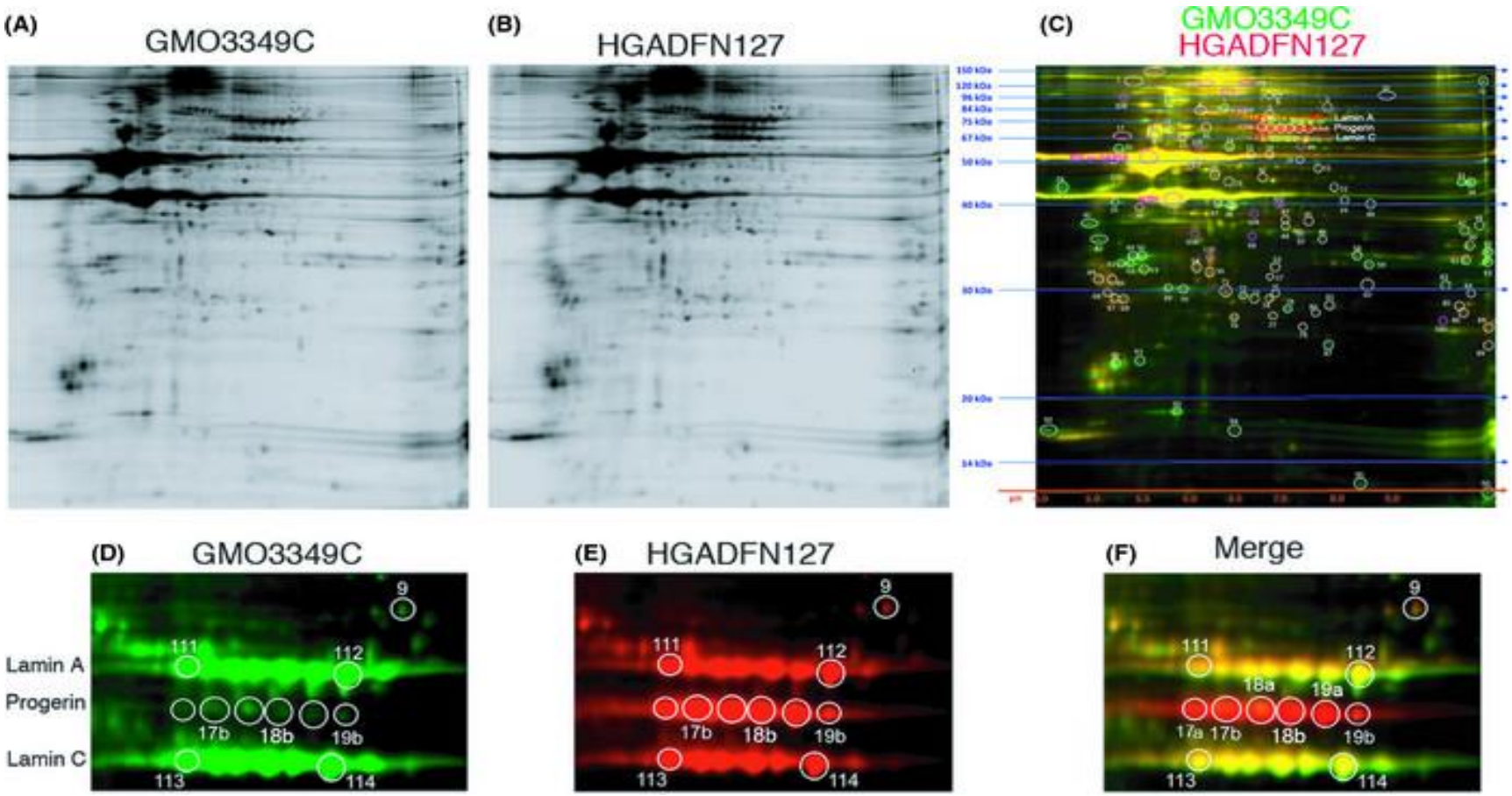



**FIGURE 9.3 Two-Color 2D-Gel**

Proteins from two different conditions (e.g., normal and cancerous) can be compared directly on the same gel by labeling each with a different fluorescent dye. When the gel is visualized to see the dyes, the proteins found only in normal tissue form red spots, the proteins found only in cancerous tissue form green spots, and proteins found in both normal and cancerous tissue look yellow because green and red fluorescent dyes appear yellow when mixed.

# DIGE ( Differential gel electrophoresis )




Hutchinson–Gilford progeria syndrome (HGPS)

# Western blotting

- The first step is to separate the proteins by size by using either standard SDS-PAGE or 2D-PAGE
- The proteins are then transferred from the gel to a type of membrane made of nitrocellulose
- Either way, the membrane must have a positive charge so that the negatively charged proteins will stick to its surface
- The proteins are moved from the gel to the membrane with an electric current

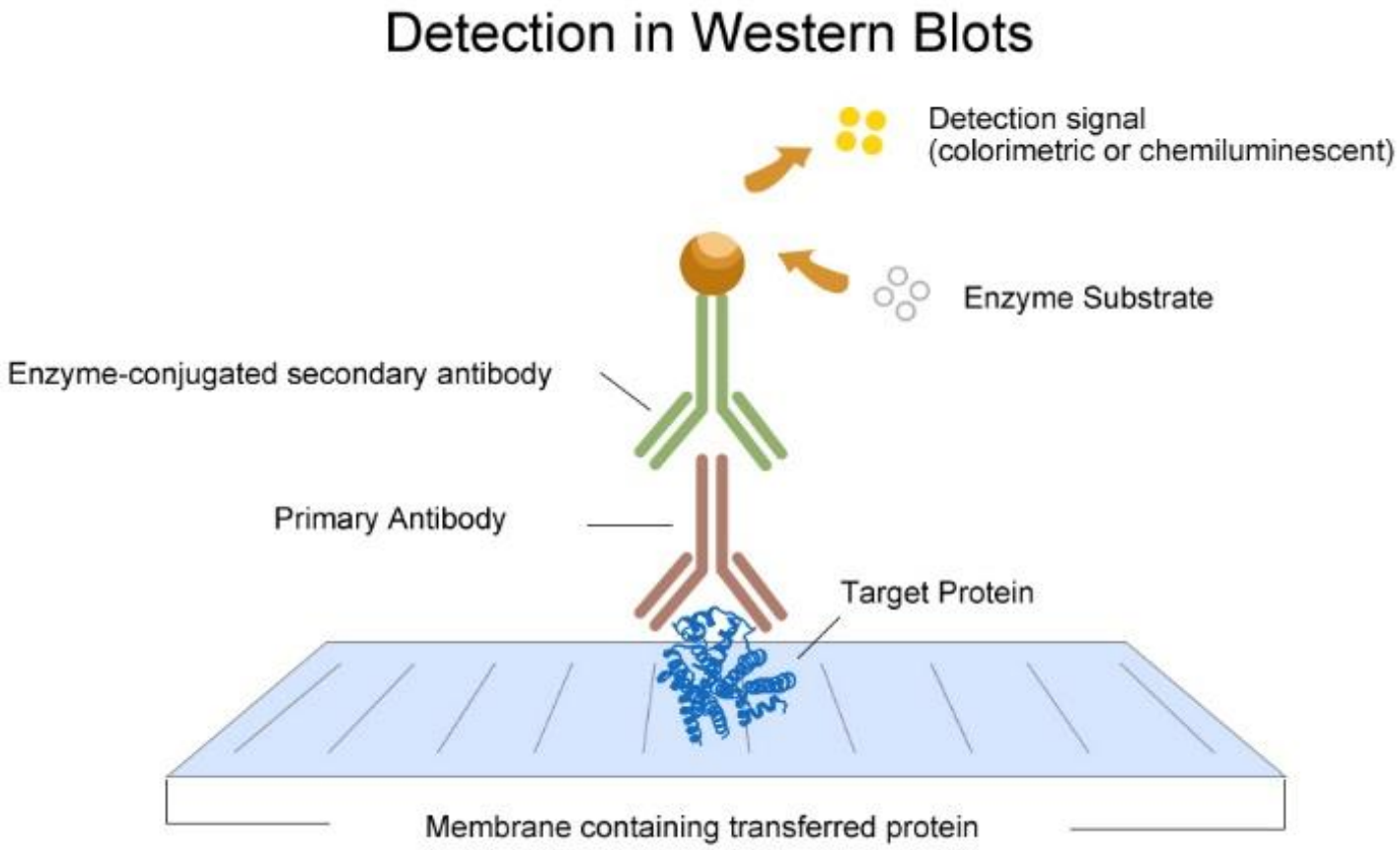
# Western blotting

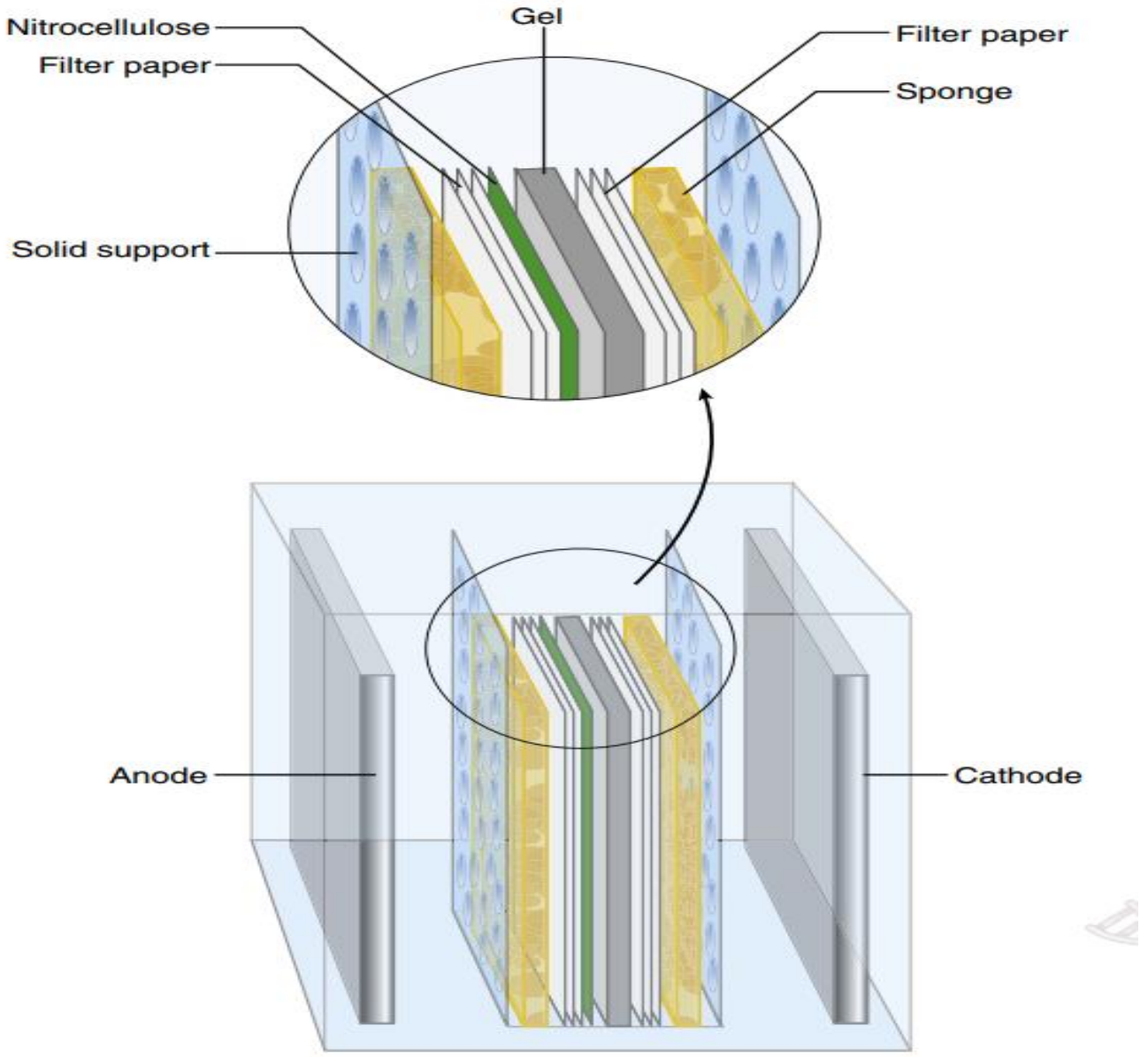
- After the proteins are attached to the nitrocellulose membrane, many areas of the membrane will not have any protein bound because the corresponding area of the protein gel was empty
- These blank areas are positively charged and can bind nonspecifically to the antibody. Therefore, these sites must be blocked
- Often, the membranes are soaked in reconstituted nonfat dry milk. The milk proteins mask the unused sites on the membrane and will not bind to the antibody
- Next, the antibody is added to a buffer solution and swirled around the membrane for a few hours. The antibody will bind only to its target protein

# Western blotting

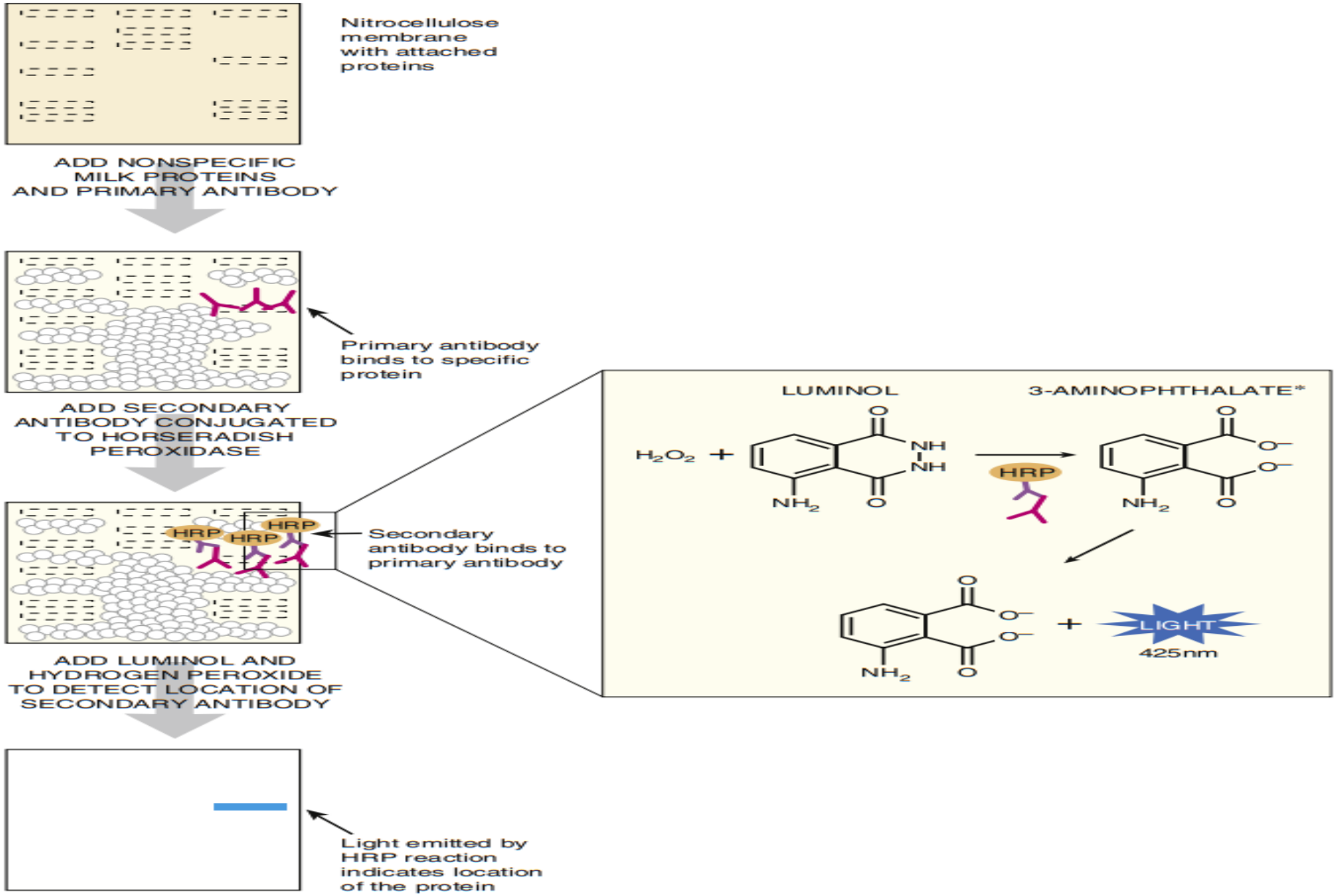
- The next step is to visualize the location of the primary antibody, thus revealing the location of the target protein
- To achieve this result, researchers add a secondary antibody conjugated to a tag or label
- Often the tag is horseradish peroxidase, which oxidizes luminol with hydrogen peroxide to form an excited state of 3-aminophthalate that decays to emit a pulse of light at 425 nm

# Western blotting






Components of western blot sandwich




# Western blotting procedure

# HPLC

- Chromatography is a general term for many separation techniques
- where a sample of molecules, the analyte, is dissolved in a mobile phase and then forced through a stationary phase
- In HPLC, the mobile phase is forced through a chromatography column, that is, a narrow tube packed with the stationary phase, under high pressure
- As the mobile phase exits the column, a detector emits a response to molecules in the eluting sample and draws a peak on the chromatogram.
- HPLC has many applications including separation, identification, purification, and quantification of proteins or other analytes

# HPLC

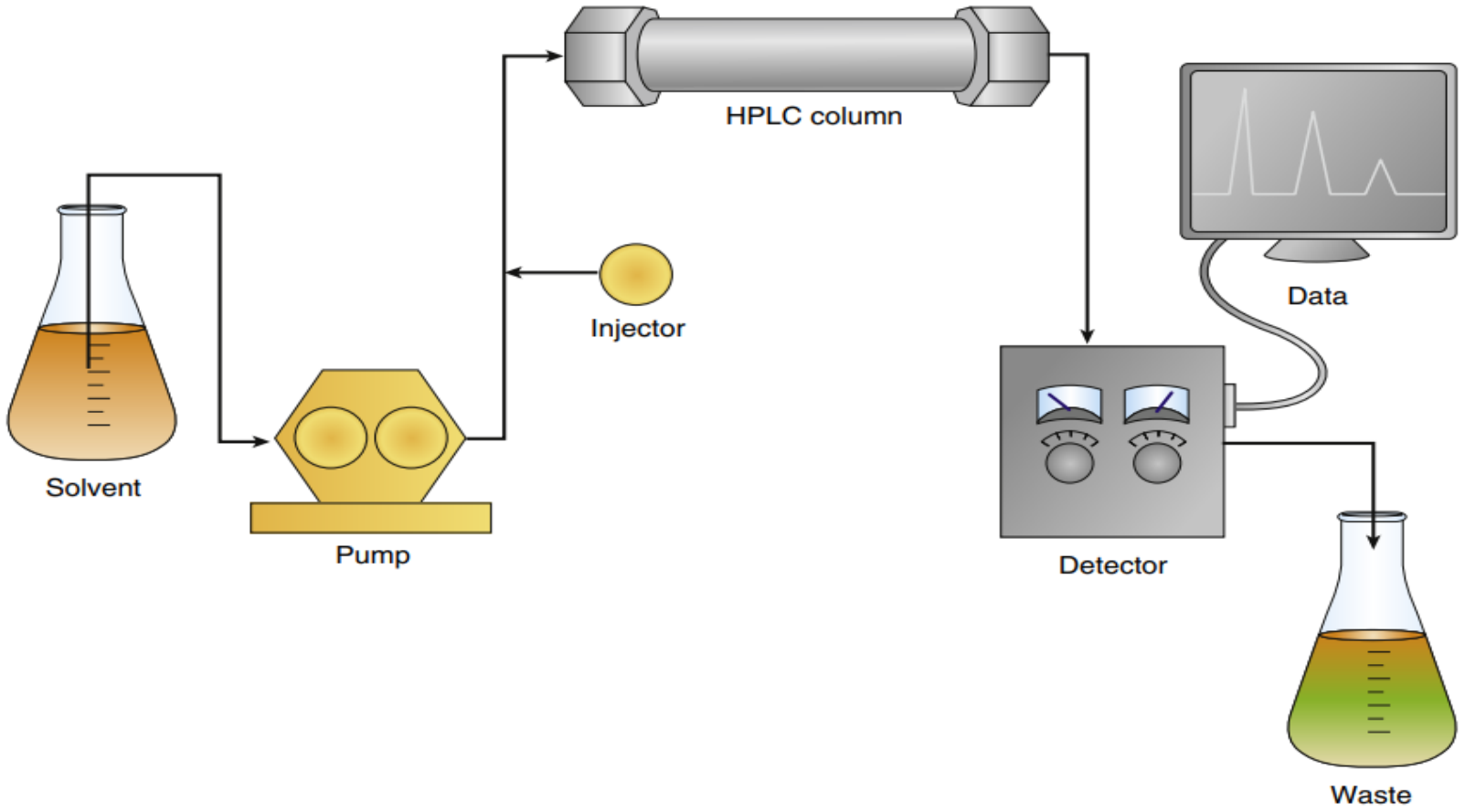



**FIGURE 9.6 High-Pressure Liquid Chromatography**
The mobile phase (far left) is pumped into the HPLC column with the protein sample, which is added through the injection port. The column separates the protein sample into different fractions that are measured by the detector. The fractions containing the protein of interest are saved, and the remaining are sent to waste. The computer records the data.

# Types of HPLC

- Size exclusion chromatography
- Reverse-phase HPLC
- Ion-exchange HPLC
- affinity HPLC

# Types of HPLC’s detector

- Refractive index detectors
- Ultraviolet detectors
- Fluorescence detectors
- radiochemical detectors
- electrochemical detectors

# DIGESTION OF PROTEINS BY PROTEASES

- Proteases (also known as proteinases or peptidases) hydrolyze the peptide bond between amino acid residues in a polypeptide chain
- Proteases may be specific and limited to one or more sites within a protein, or they may be nonspecific, digesting proteins into individual amino acids.
- The ability to digest a protein at specific points is critical to mass spectrometry

# DIGESTION OF PROTEINS BY PROTEASES

- Proteases are classified by three criteria :

1. the reaction catalyzed
2. the chemical nature of the catalytic site
3. their evolutionary relationships

# DIGESTION OF PROTEINS BY PROTEASES

- Classes of proteases :

1. Endopeptidases
2. Exopeptidases
3. Caboxypeptidases
4. Serine proteases
5. Cysteine proteases
6. Aspartate proteases
7. Metaloproteases
8. Thereonine proteases

# MASS SPECTROMETRY

- Mass spectrometry is a technique to determine the mass of molecules
- In mass spectrometry, a molecule is fragmented into different ions whose masses are accurately measured.
- The ions generate a spectrum of unique peaks which therefore determines the identity of the original molecule.

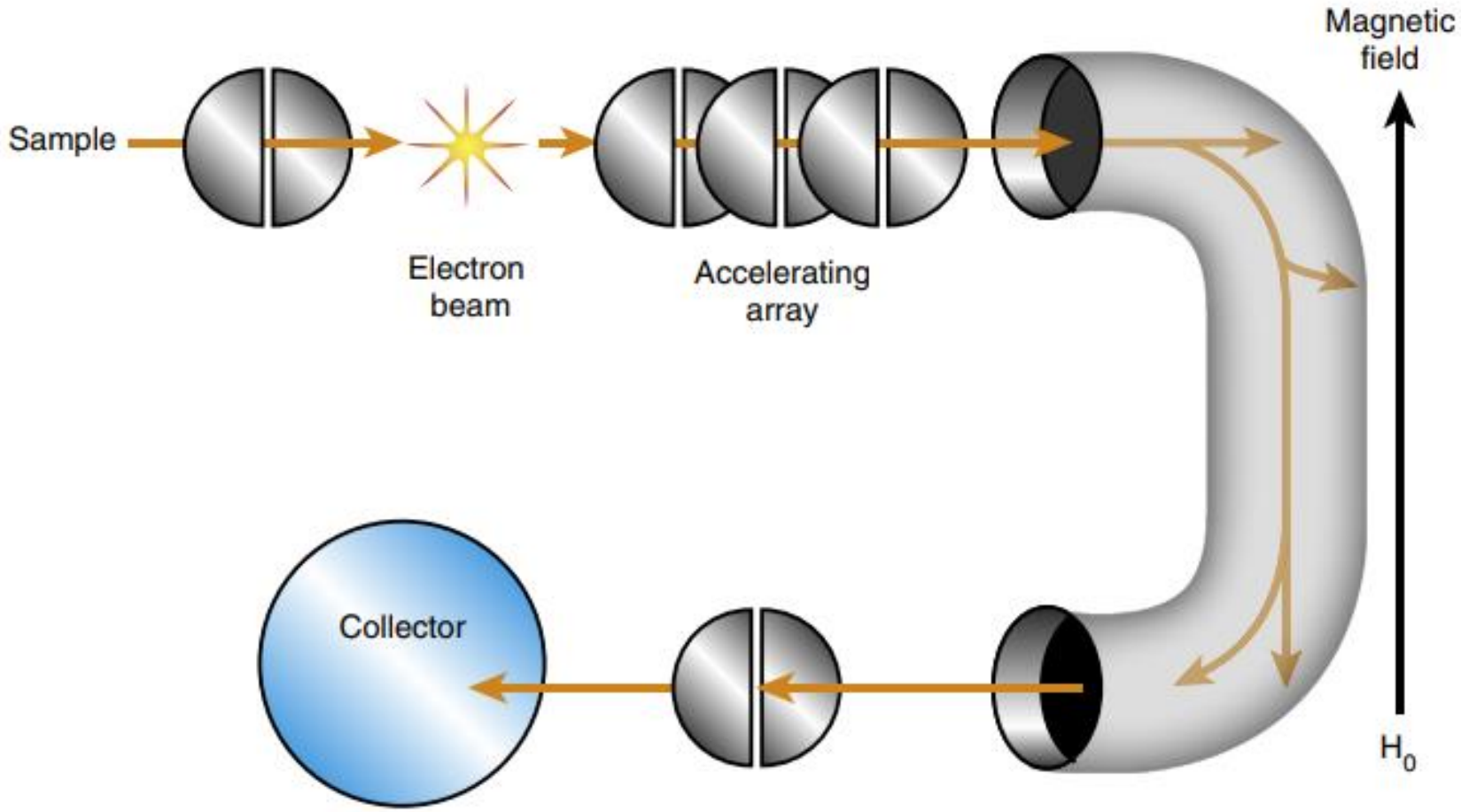
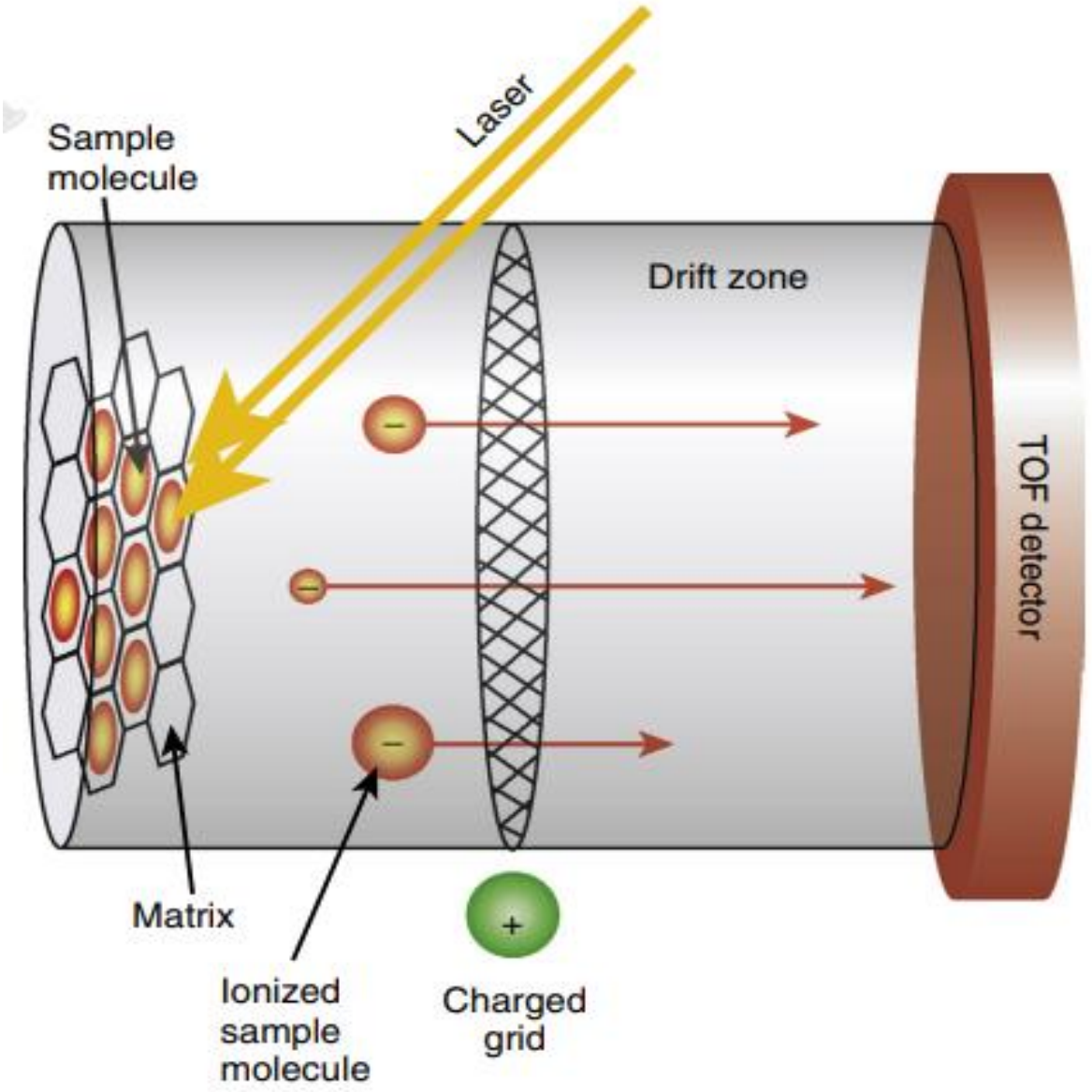
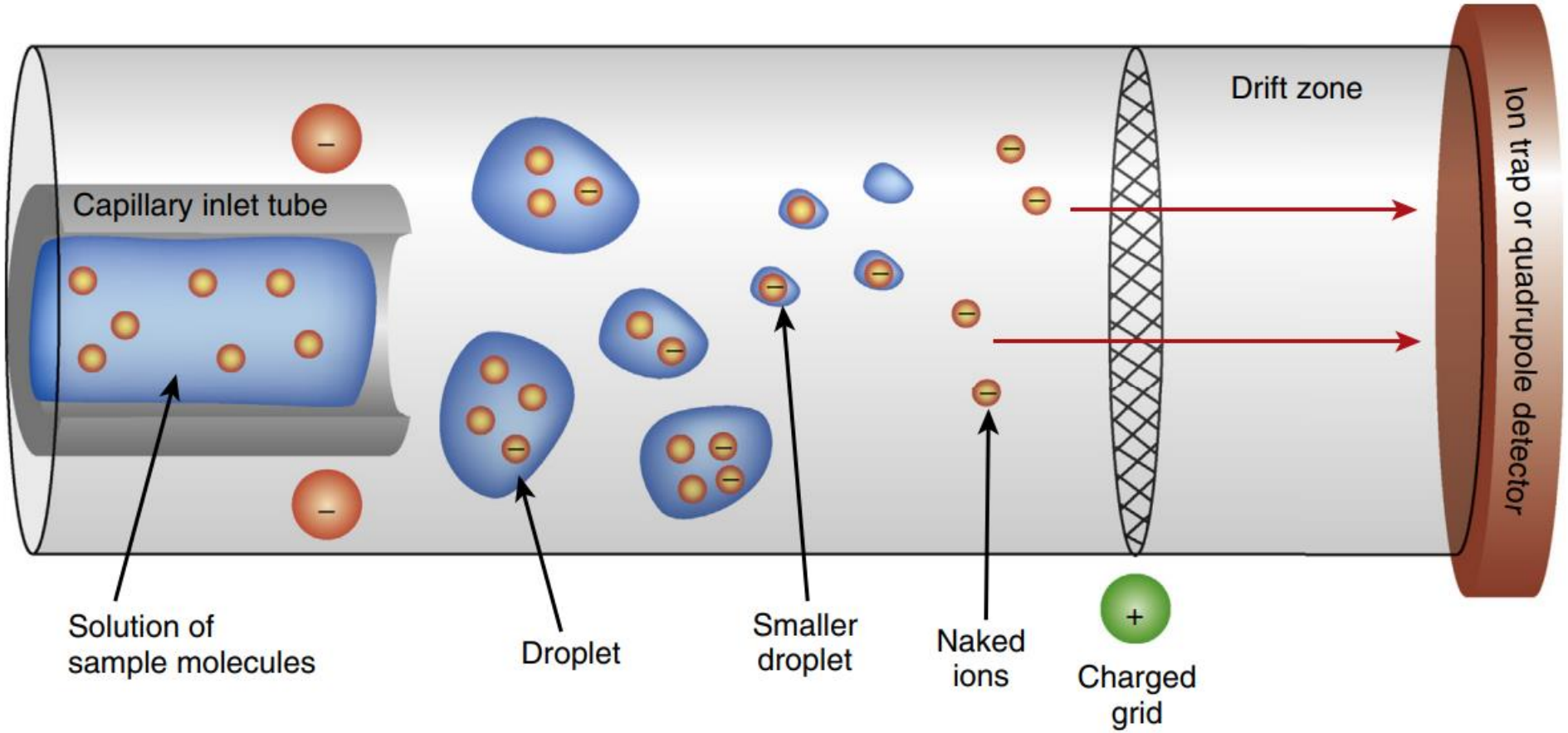


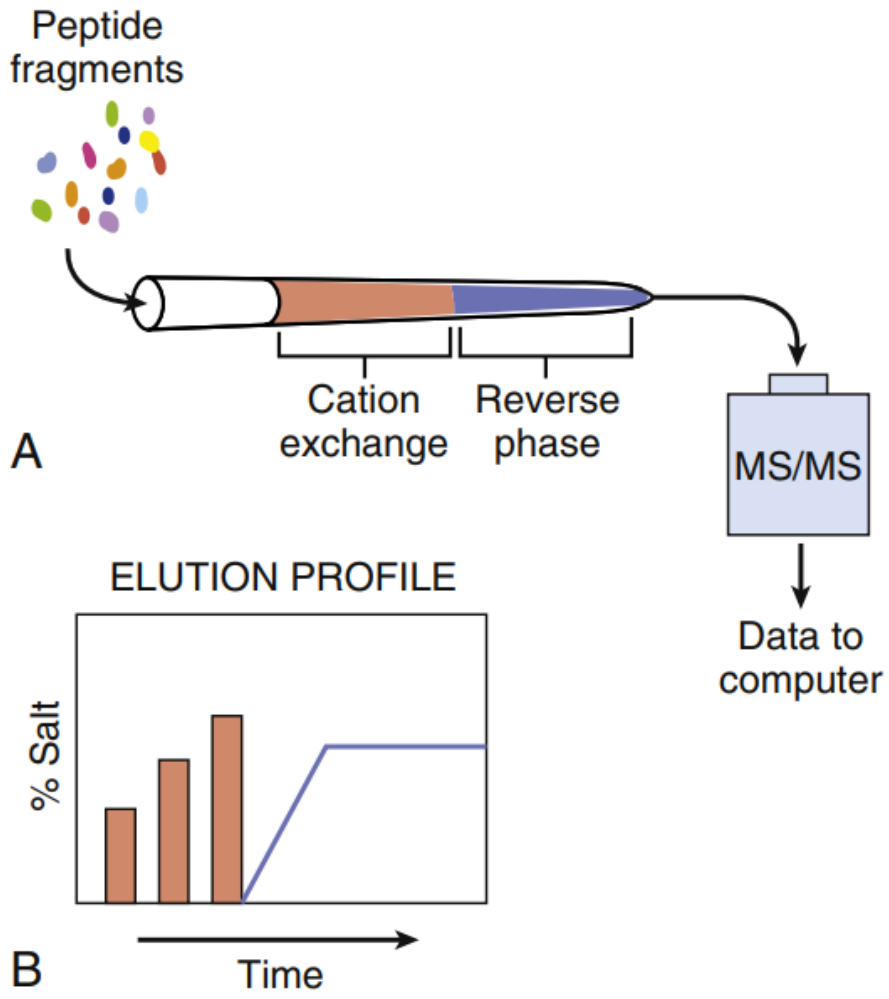

**FIGURE 9.9** Schematic Diagram of a Mass Spectrometry Tube




MALDI-TOF mass spectrometry



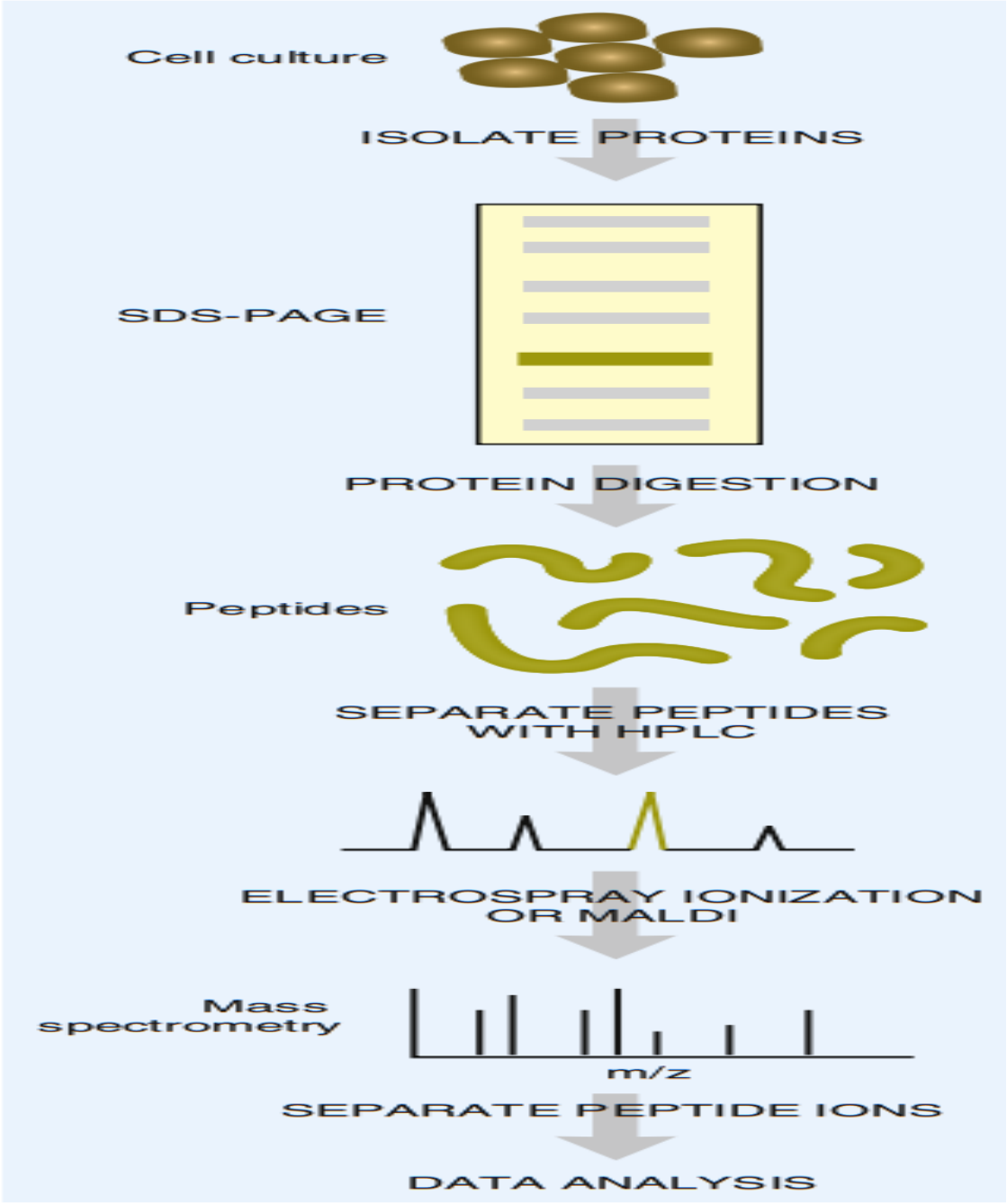

ESI mass spectrometry


Peptide fragments
Cation exchange
Reverse phase
A
MS/MS
Data to computer
ELUTION PROFILE
% Salt
Time
B

MuDPIT

# PREPARING PROTEINS FOR MASS SPECTROSCOPY

- To determine the sequence of a peptide, researchers must obtain a pure sample of the protein either by cutting a spot from a two-dimensional gel or by HPLC purification
- the proteins are treated with reducing agents to break apart any disulfide bridges
- To keep the –SH group from reforming a disulfide bridge, the proteins are also alkylated.
- The protein is then digested into fragments using a protease such as trypsin, which cuts proteins on the carboxy-terminal side of arginine and lysine
- Cutting a protein into peptides helps reduce undesirable characteristics of the entire protein


Cell culture
ISOLATE PROTEINS
SDS-PAGE
PROTEIN DIGESTION
Peptides
SEPARATE PEPTIDES WITH HPLC
ELECTROSPRAY IONIZATION OR MALDI
Mass spectrometry
m/z
SEPARATE PEPTIDE IONS
DATA ANALYSIS

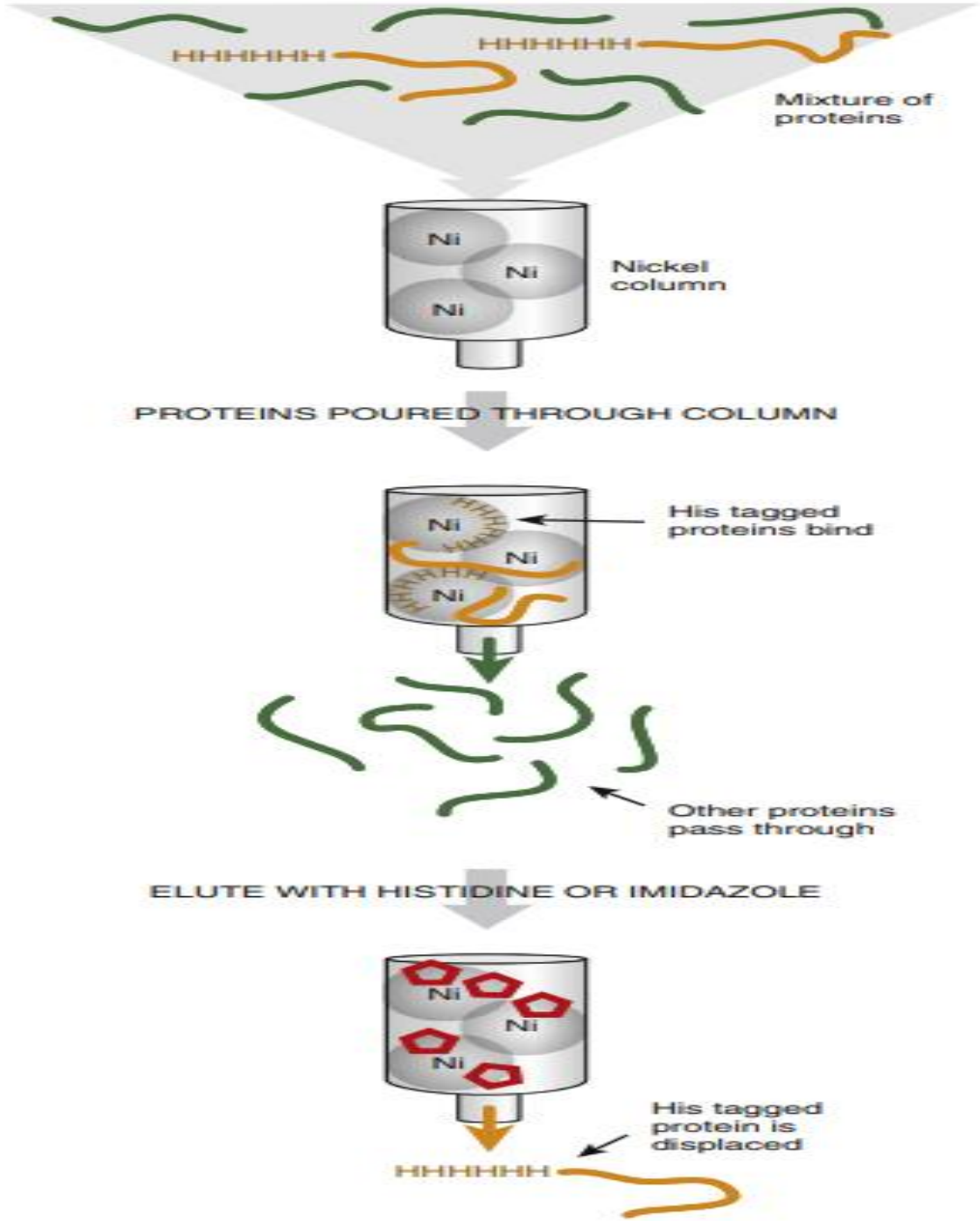
# PREPARING PROTEINS FOR MASS SPECTROSCOPY

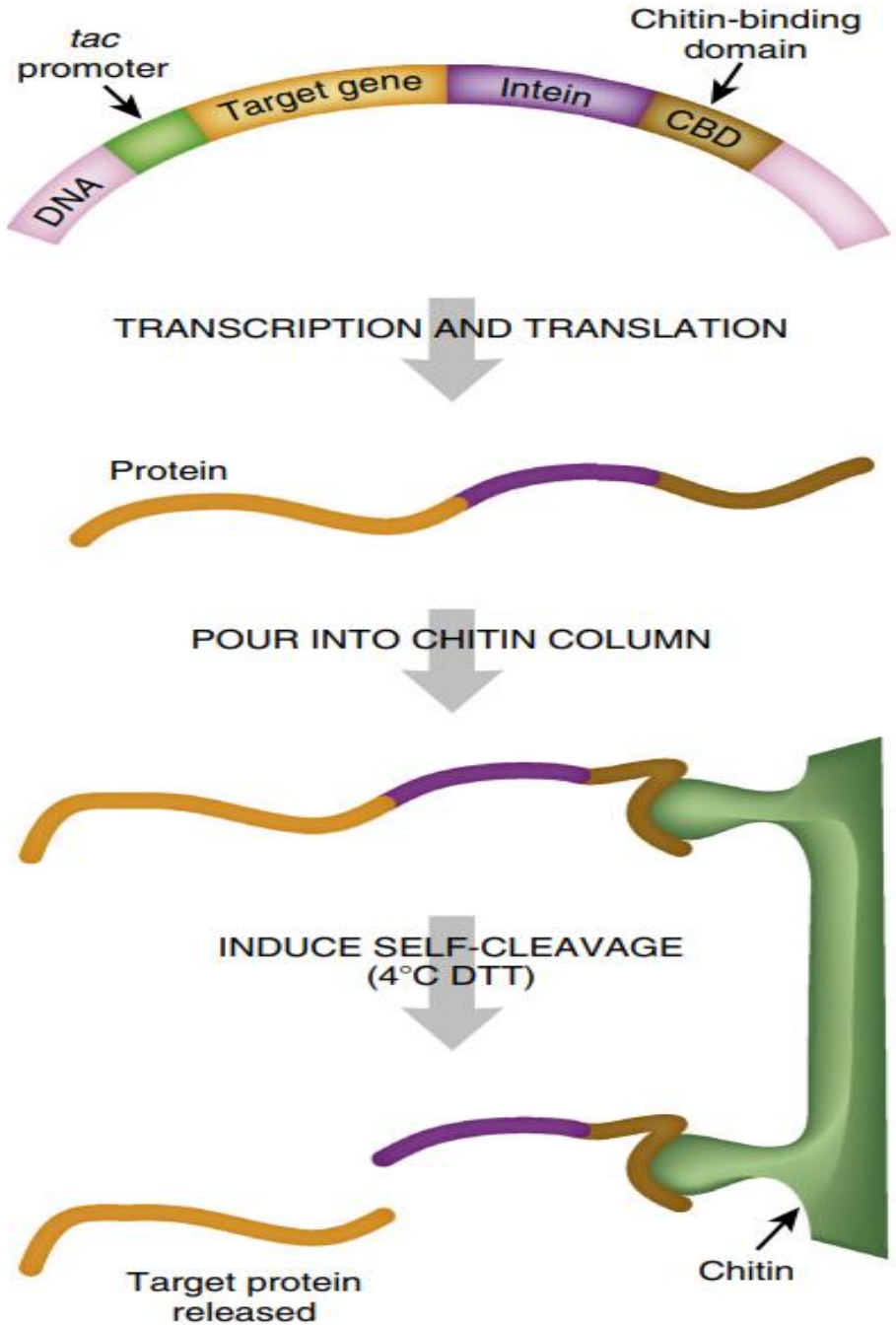
- To determine the peptide sequence, researchers use two rounds of mass spectroscopy
- This is called tandem mass spectroscopy because one ion is produced in the first round of mass spectroscopy
- then that ion is fragmented by collision with a gas such as hydrogen, argon, or helium
- As before, the ion fragments are separated based on their mass to charge ratio
- ach peak usually varies by one amino acid
- and the size difference between the peaks determines the amino acid sequence

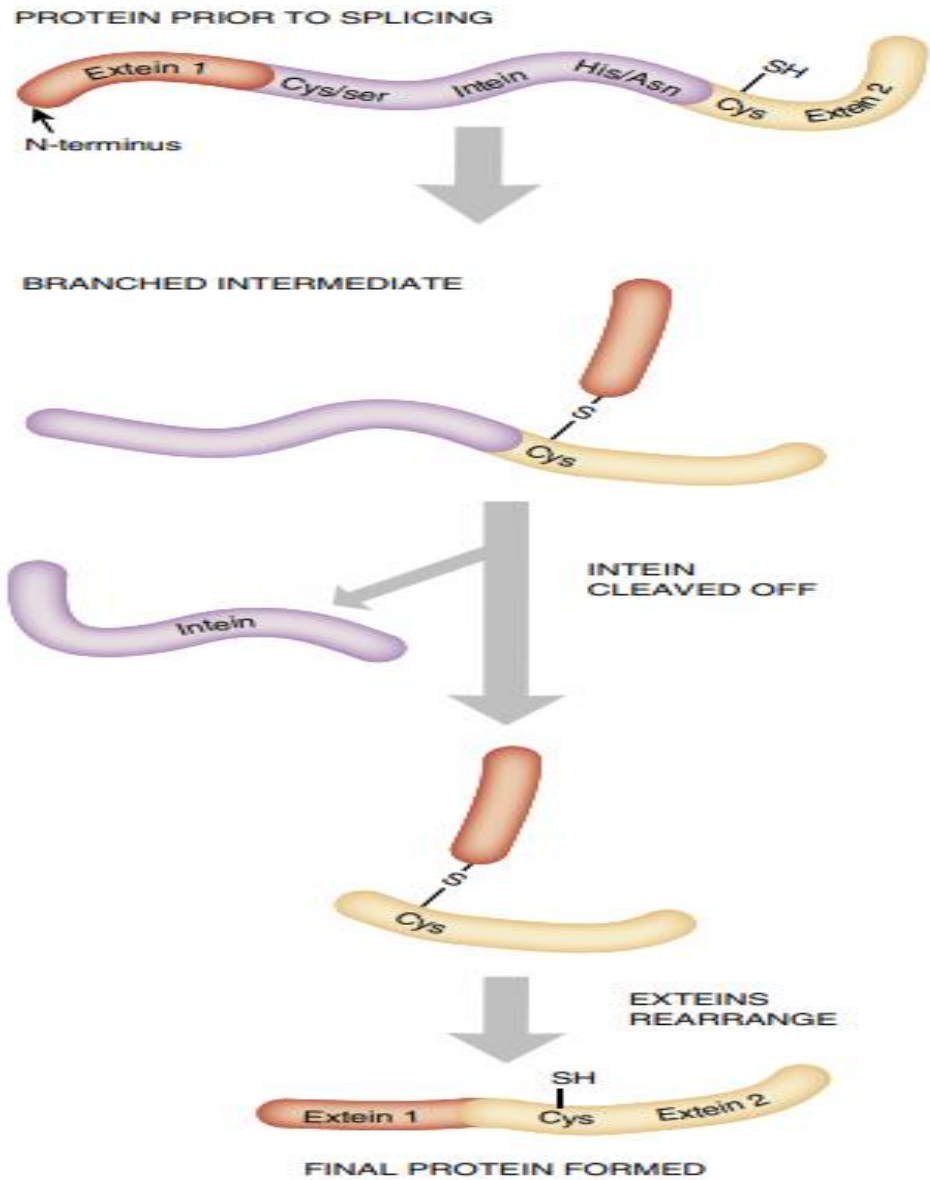
# PROTEIN TAGGING SYSTEMS

- Protein tagging systems are tools for the isolation and purification of single target proteins from a mixture

- For this purpose the protein of interest genetically fused to a tag coding sequence, creating a hybrid gene, then this gene will insert in an appropriate vector and introduced to a host cell.
- Examples : His tag, FLAG, Strep, GFP, protein A, GST, MBP

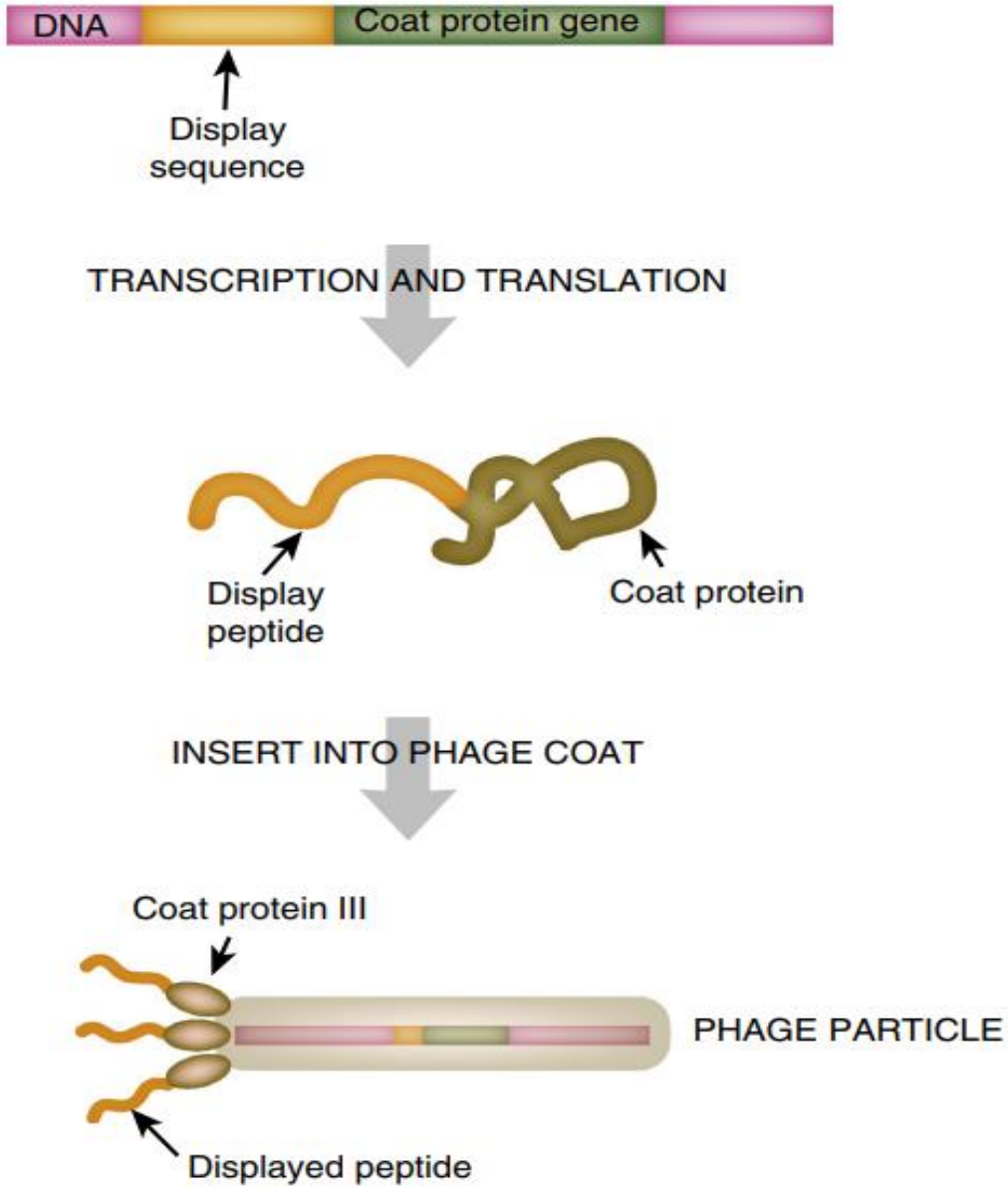

HHHHHHH
HHHHHHH
Mixture of proteins
Ni
Ni
Ni
Nickel column
PROTEINS POURED THROUGH COLUMN
His tagged proteins bind
Other proteins pass through
ELUTE WITH HISTIDINE OR IMIDAZOLE
His tagged protein is displaced
HHHHHHH


tac
promoter
Target gene
Intein
Chitin-binding
domain
CBD
DNA
TRANSCRIPTION AND TRANSLATION
Protein
POUR INTO CHITIN COLUMN
INDUCE SELF-CLEAVAGE
(4°C DTT)
Target protein
released
Chitin


PROTEIN PRIOR TO SPLICING
Extein 1
Cys/ser
Intein
His/Asn
SH
Cys
Extein 2
N-terminus
BRANCHED INTERMEDIATE
O
Cys
Intein
INTEIN
CLEAVED OFF
O
Cys
EXTEINS
REARRANGE
SH
Extein 1
Cys
Extein 2
FINAL PROTEIN FORMED

# Phage display library screening

- A phage display library is a collection of bacteriophage particles that have segments of foreign proteins protruding from their surface
- The outer coat of M13 bacteriophage has about 2500 copies of the major coat protein (gene VIII protein) and about five copies of the minor coat protein (gene III protein)
- Gene III protein is located at the end of the cylindrical bacteriophage particle with its N-terminus facing outward
- One popular phage display system fuses the foreign sequence to the N-terminus of the gene III protein


DNA
Coat protein gene
Display sequence
TRANSCRIPTION AND TRANSLATION
Display peptide
Coat protein
INSERT INTO PHAGE COAT
Coat protein III
PHAGE PARTICLE
Displayed peptide

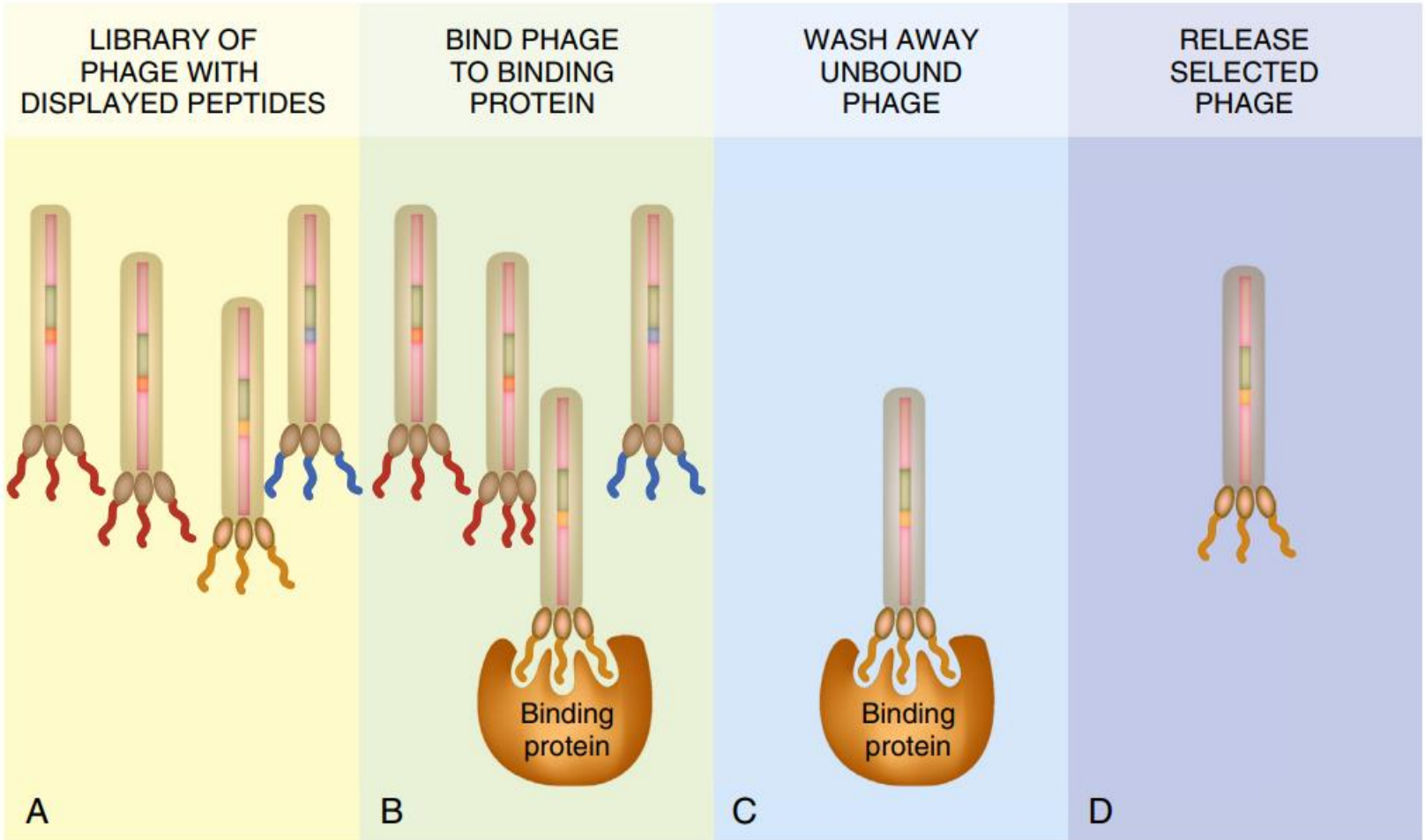
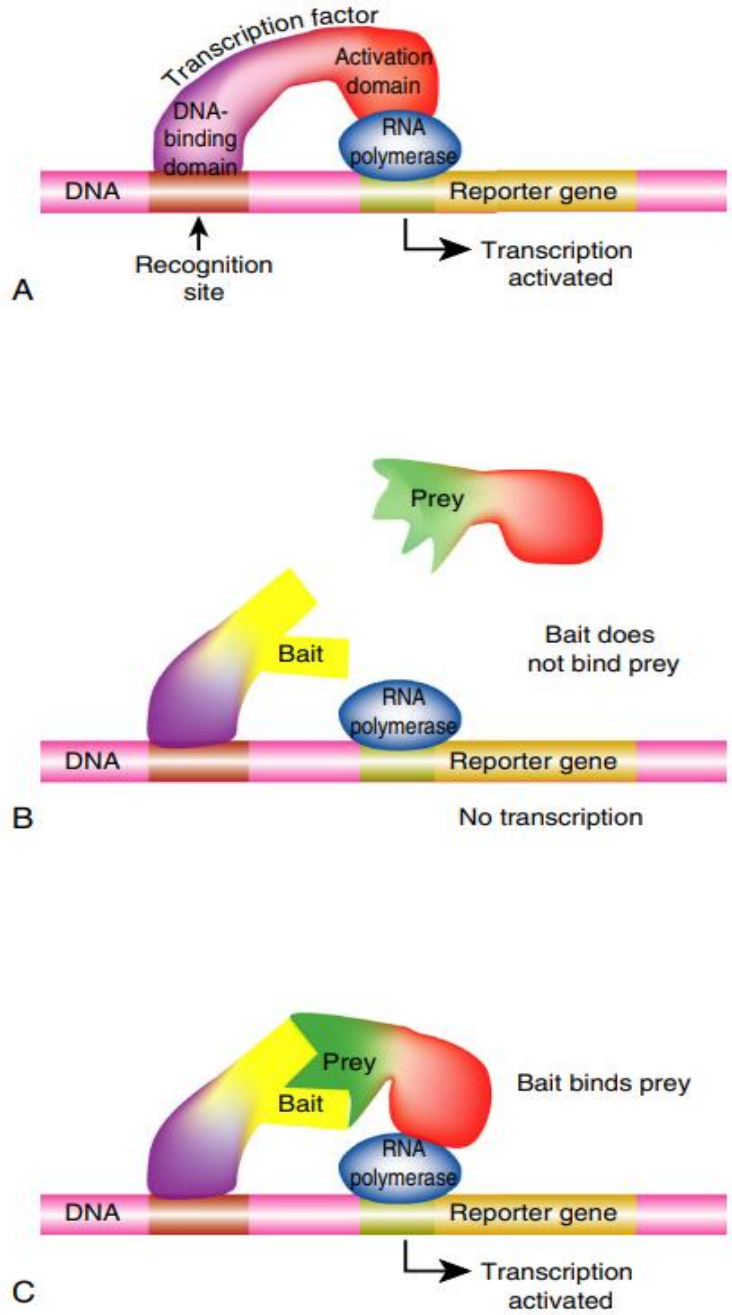


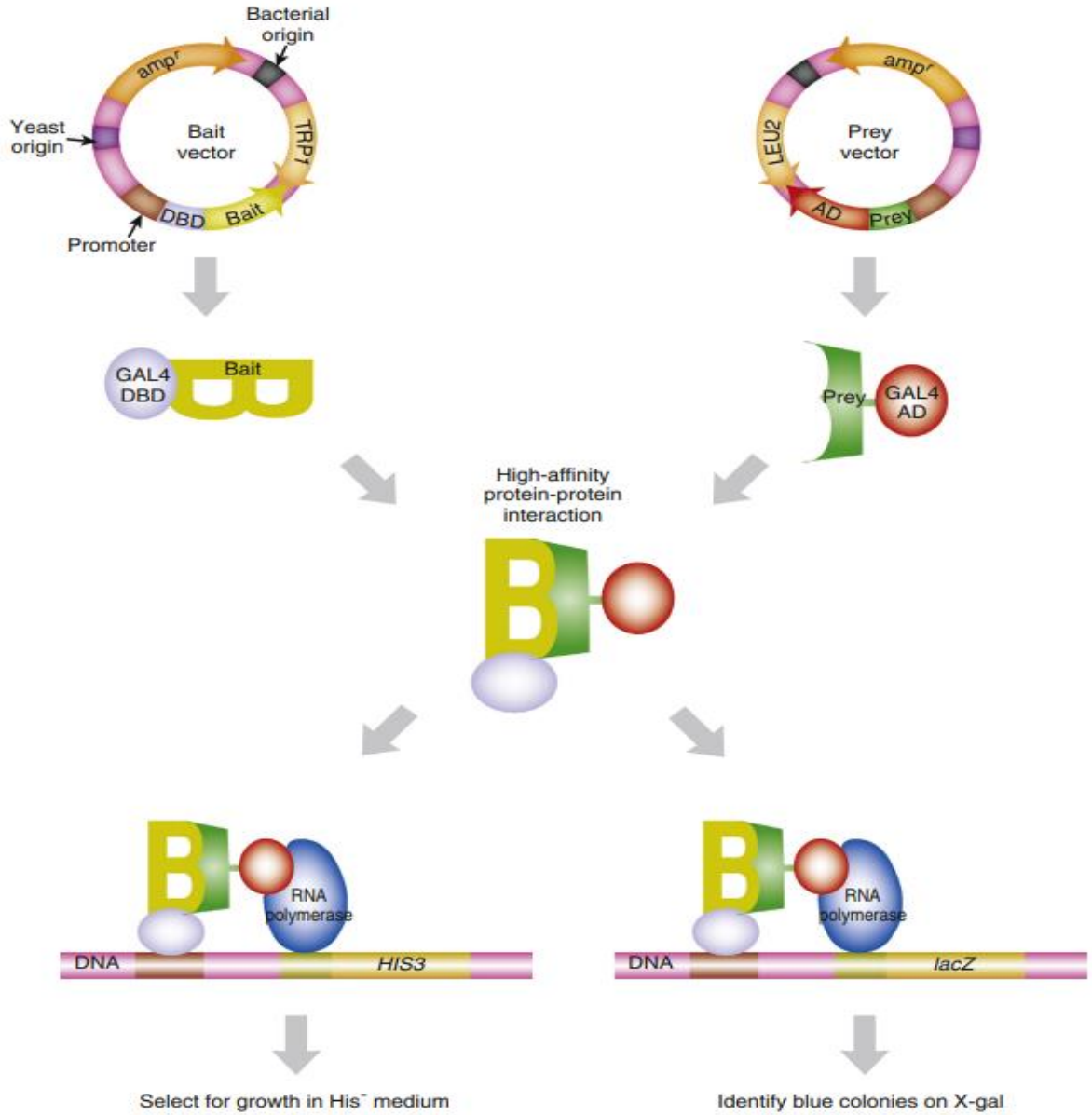

**FIGURE 9.21 Biopanning of Phage Display**

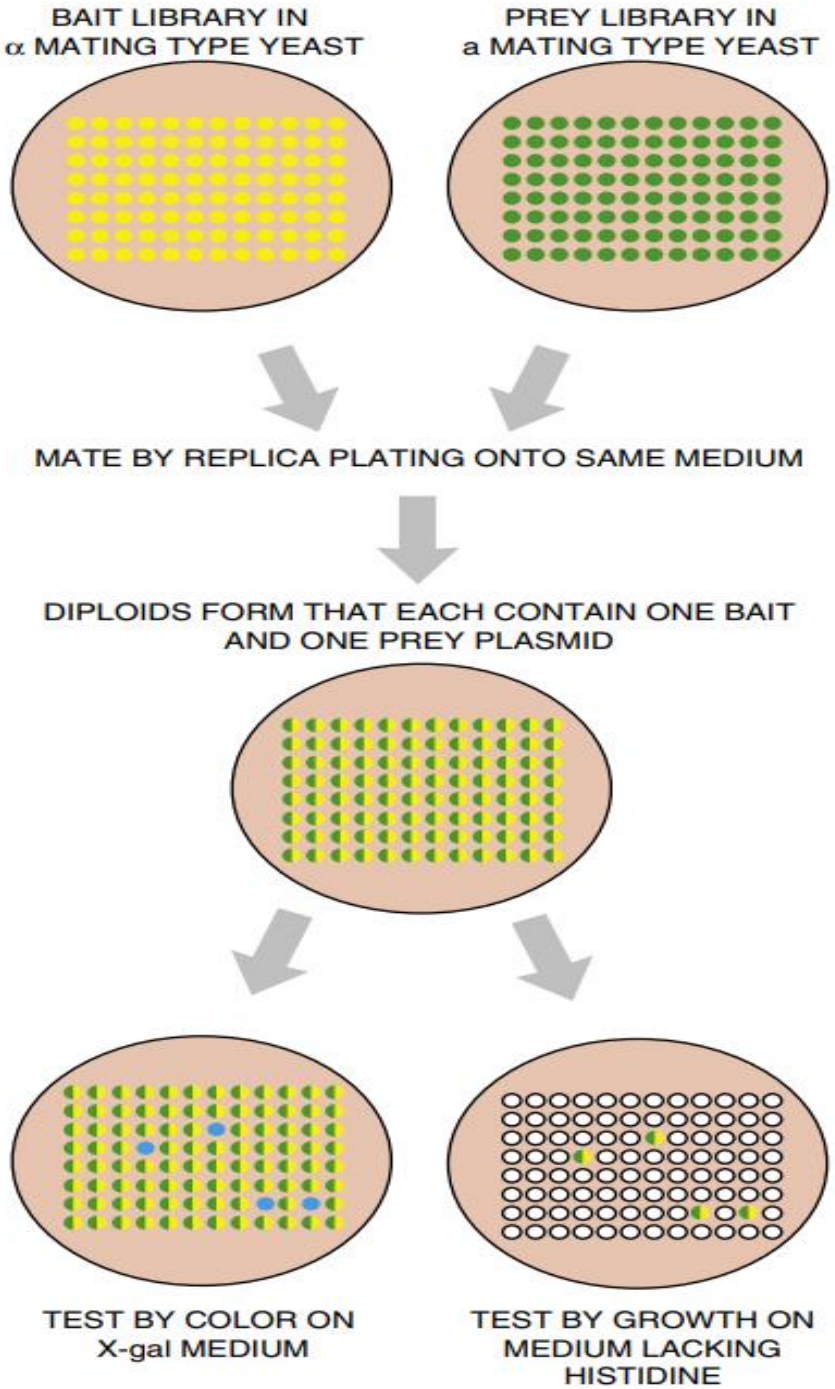
Biopanning is used to isolate peptides that bind to a specific target protein, which is usually attached to a solid support such as a membrane or bead. The phage display library (A) is attached to the binding protein (B). Those phages that bind to the target protein will be retained (C), but the others are washed away. The phage that does recognize the binding protein can be released, isolated, and purified.

# The yeast two-hybrid system

- It is used to examine relevant interactions between proteins.
- the total of all protein– protein interactions is called the protein interactome.
- In the two-hybrid system, the two domains are each fused to different proteins by creating hybrid genes. The bait is the DBD genetically fused to the protein of interest, and the prey is the AD fused to proteins that are being screened for interaction with the bait. When the bait and prey bind, the DBD and AD activate transcription of the reporter gene.


Transcription factor
Activation domain
DNA-binding domain
RNA polymerase
DNA
Reporter gene
Recognition site
Transcription activated
A
Prey
Bait
Bait does not bind prey
RNA polymerase
DNA
Reporter gene
B
No transcription
Prey
Bait
Bait binds prey
RNA polymerase
DNA
Reporter gene
Transcription activated
C


Bacterial origin
ampr
Yeast origin
Bait vector
TRP1
DBD
Bait
Promoter
Prey vector
ampr
LEU2
AD
Prey
GAL4 DBD
Bait
Prey
GAL4 AD
High-affinity protein-protein interaction
RNA polymerase
DNA
HIS3
RNA polymerase
DNA
lacZ
Select for growth in His- medium
Identify blue colonies on X-gal


BAIT LIBRARY IN
α MATING TYPE YEAST
PREY LIBRARY IN
a MATING TYPE YEAST
MATE BY REPLICA PLATING ONTO SAME MEDIUM
DIPLOIDS FORM THAT EACH CONTAIN ONE BAIT
AND ONE PREY PLASMID
TEST BY COLOR ON
X-gal MEDIUM
TEST BY GROWTH ON
MEDIUM LACKING
HISTIDINE

# Thanks for your attention