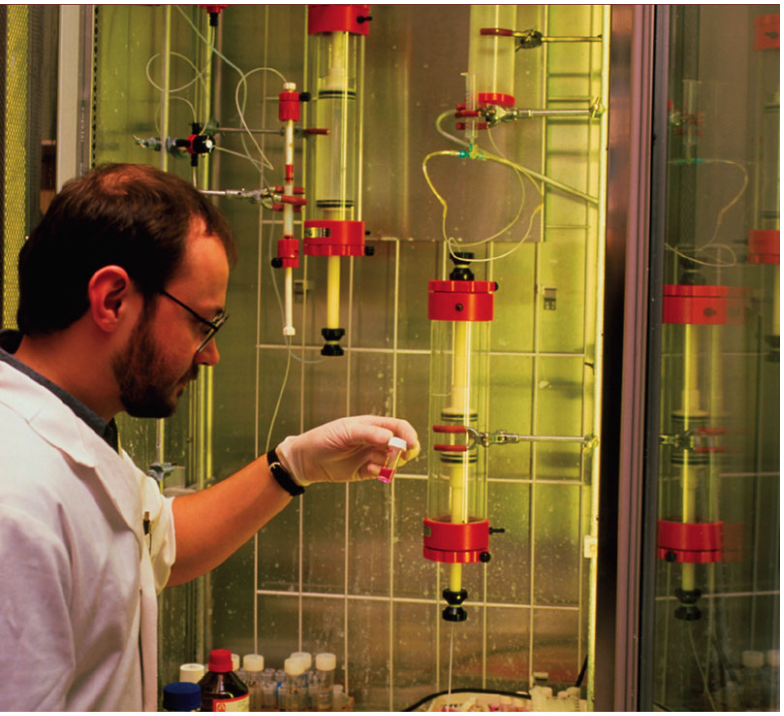
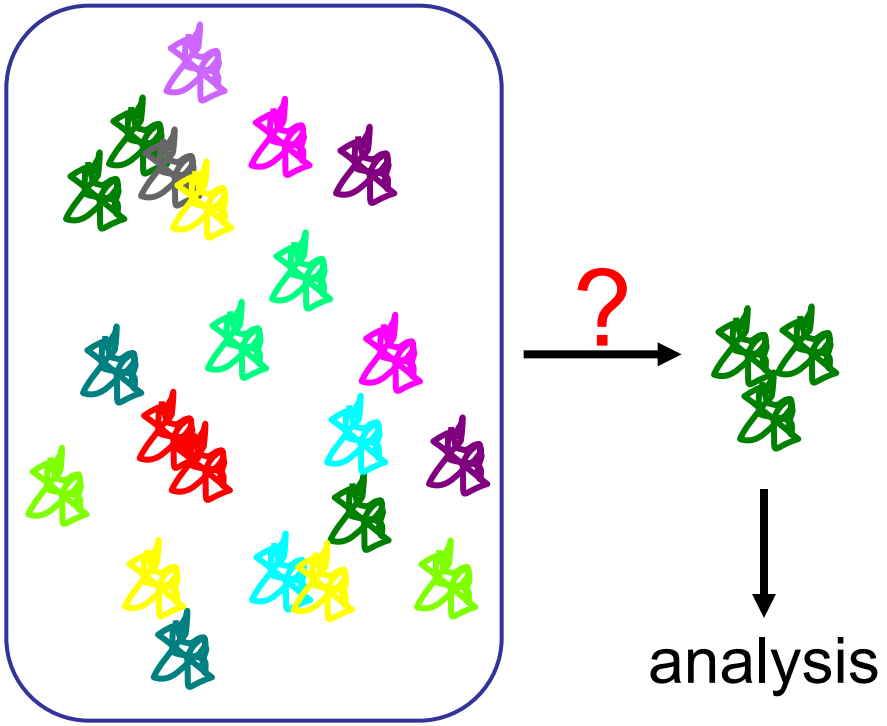
Protein Purification

In order to carry out detailed studies of a protein (structure, enzyme activity, amino acid sequence, etc) _____

How is a single protein isolated _____?





Protein Purification

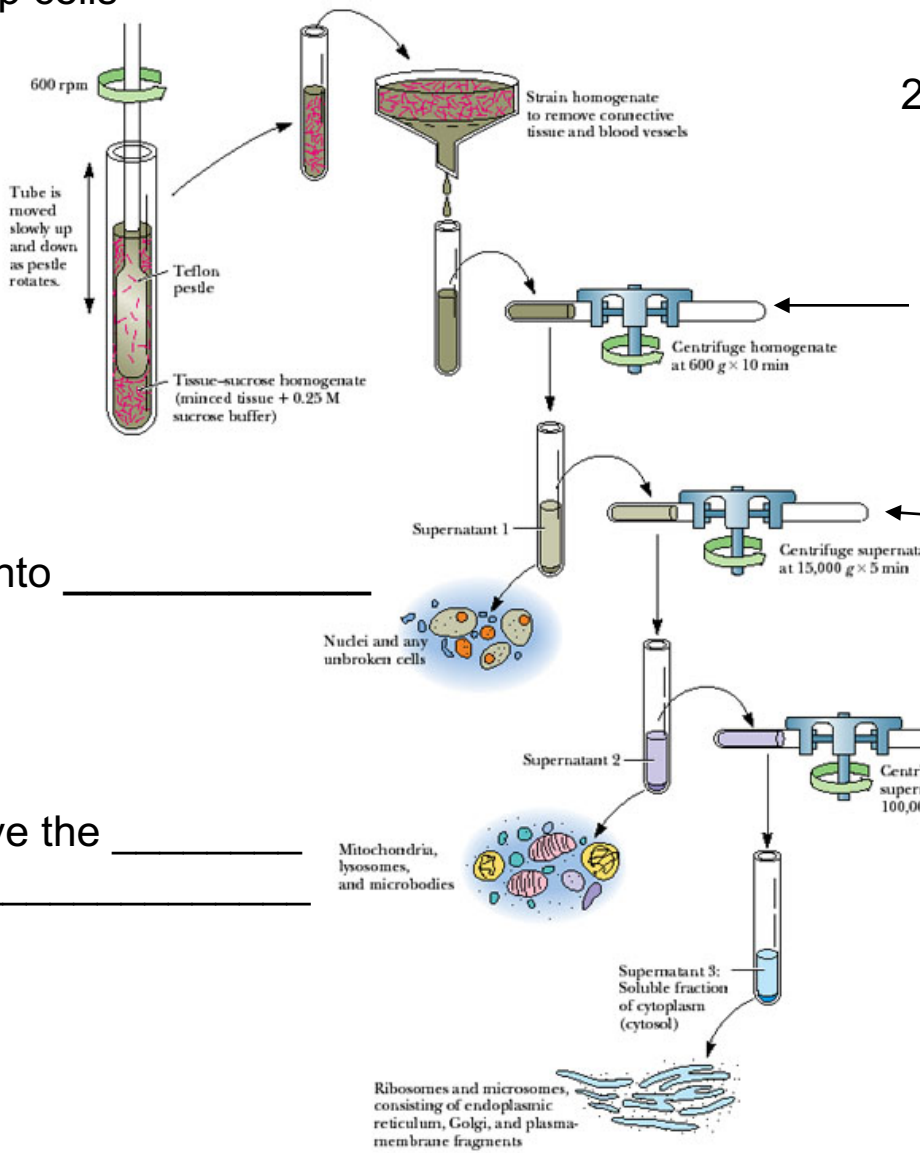
1. Extract _____
2. Separate extract contents by _____
   _____
3. Separate protein extract by _____
   - _____
   - _____
   - _____
4. Analyze purity of isolated protein _____

Protein Purification

Making protein extract

1. Grind tissue to break up cells

mortar & pestle



2. Centrifuge to

_____

_____

Low speed centrifugation to remove large contents, nuclei, cell wall debris (plants), etc.

Separate liquid sample into _____ and _____.

Higher speed centrifugation

_____

_____.

Supernatant: liquid above the _____

_____

Very high speed centrifugation to remove _____

_____

_____.

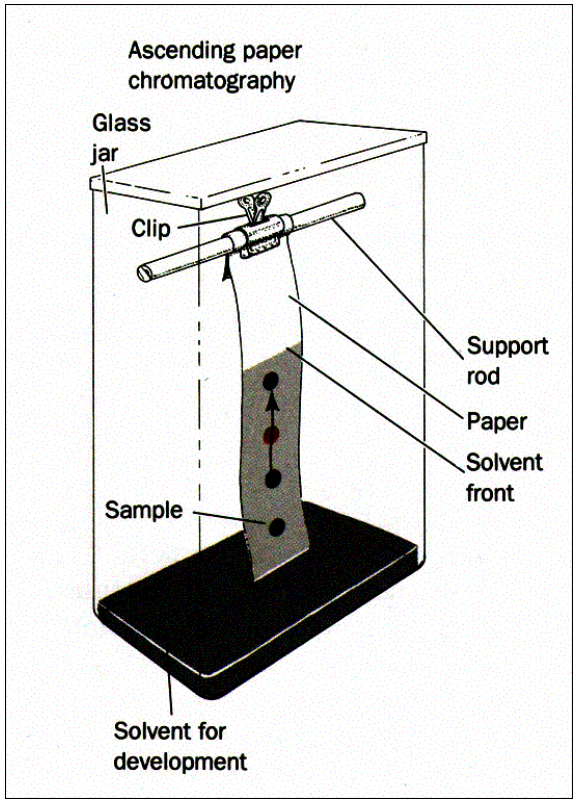
Protein Purification

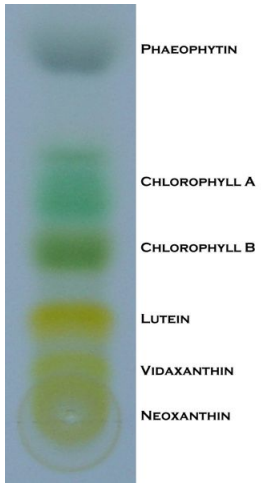
Chromatography

chroma - “color”
graphien - “to write”

Theory: separation of _____ within a sample based on their _____ as they pass through a medium.

First used to separate plant pigments





Phases:

stationary phase: a solid material over which _____ _____. Imparts separation of compounds.

mobile phase: a liquid solvent that carries _____ _____.

Protein Purification

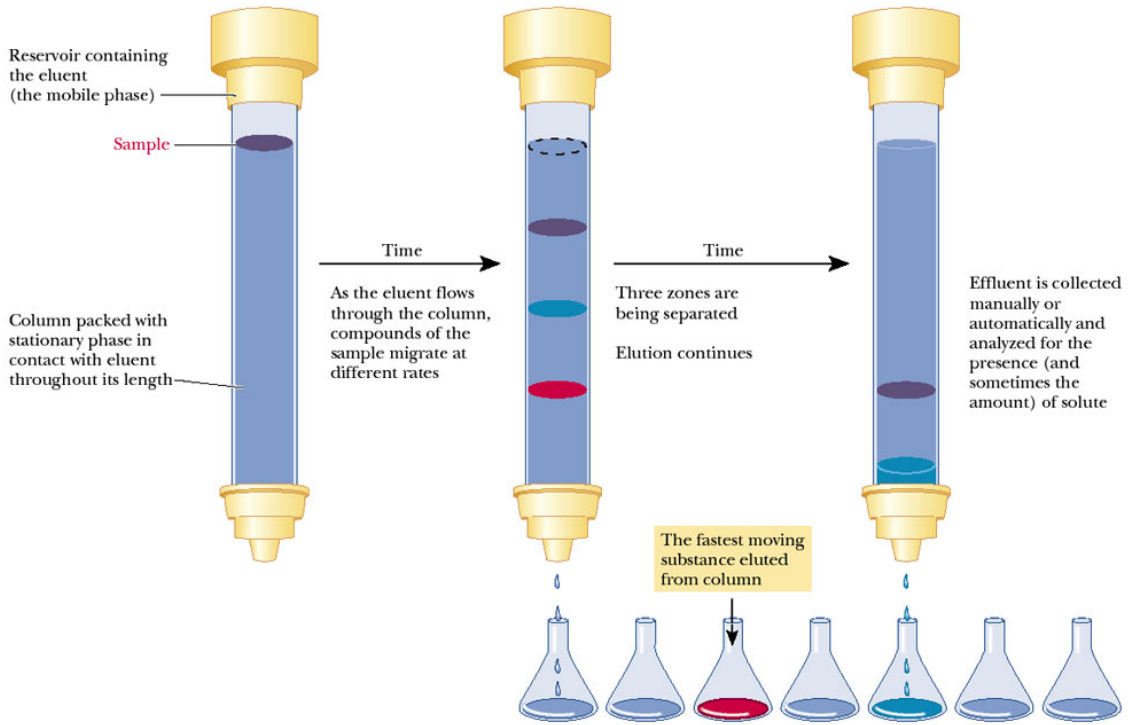
Column Chromatography

1. Pack column with _____mobile phase

2. Add sample to _____

3. Use pump or gravity to pass mobile phase over stationary phase thus _____

4. Collect samples as they _____



Protein Purification

Column Chromatography
Types of Stationary Phases

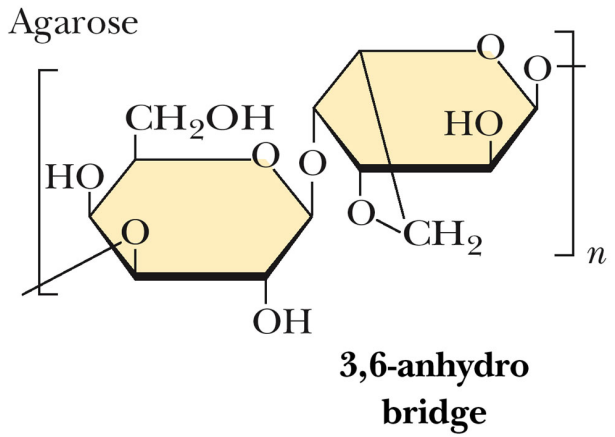
Size-exclusion/gel-filtration:

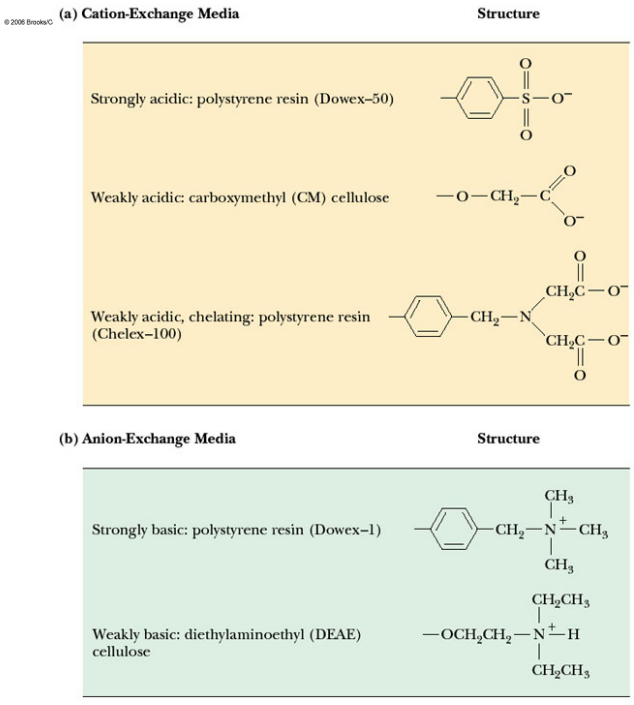
- dextran (Sephadex) - _____ _____ from certain bacteria.

- agarose (Sepharose) - a polysaccharide of _____ _____.

Ion-exchange:

- _____-exchange
- _____-exchange

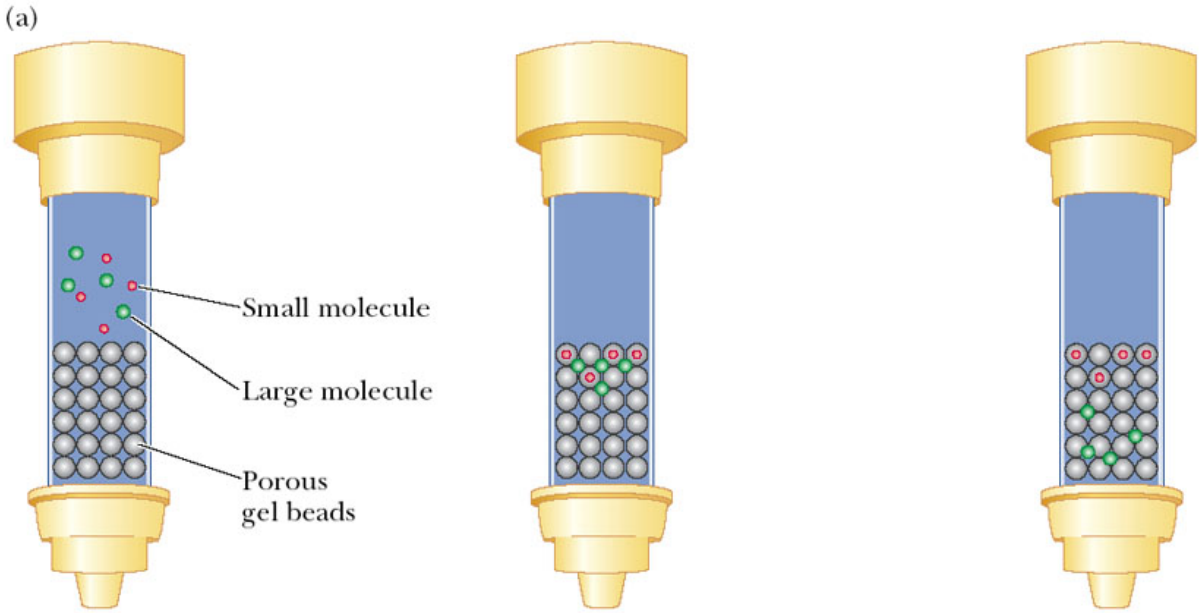


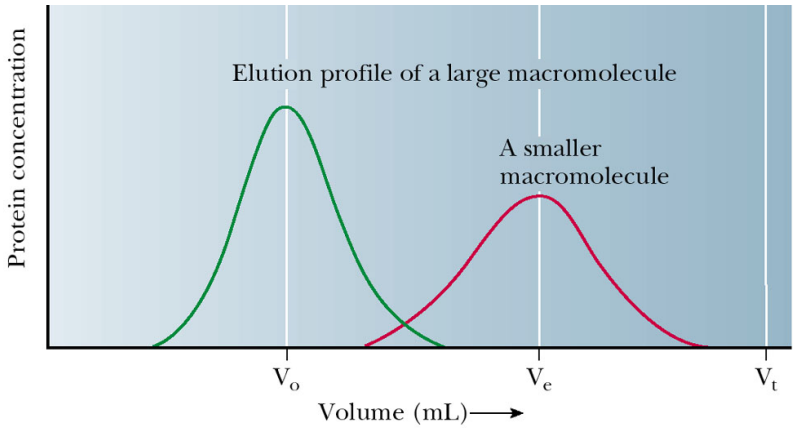


Protein Purification

Gel-filtration Chromatography

Principle: to separate _____

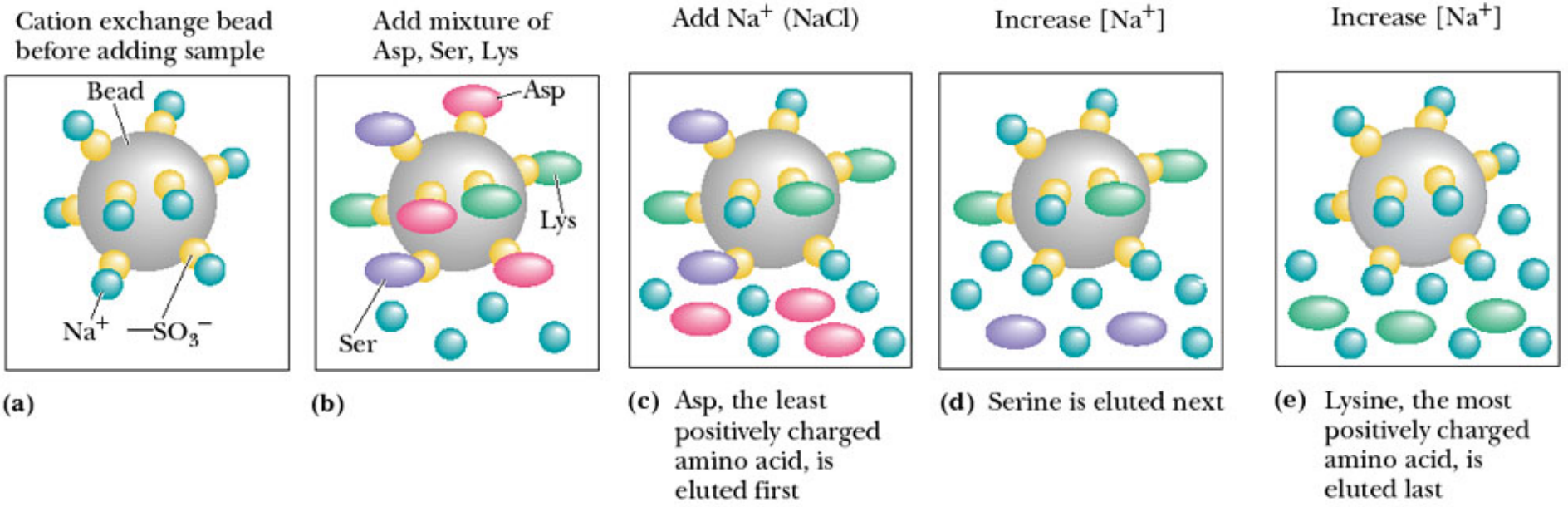




Protein Purification

Ion-exchange Chromatography

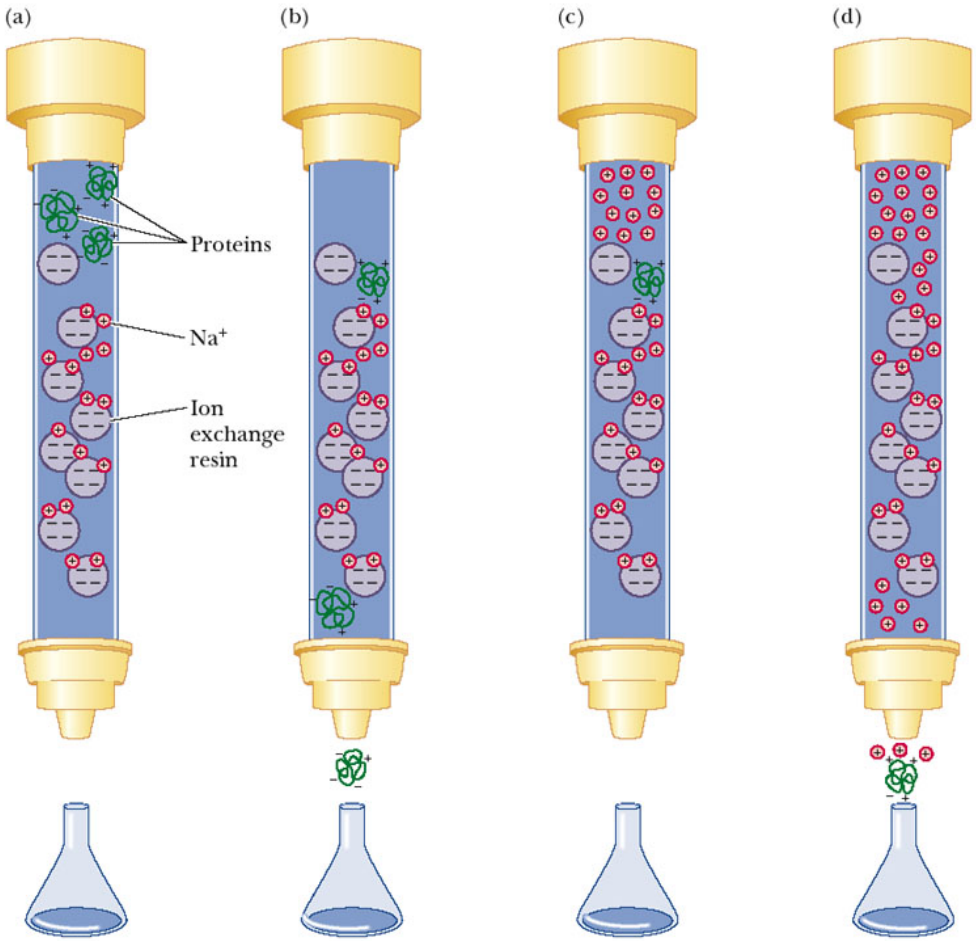
Principle: a charged ion bound to the column is displaced by _____. The protein is eluted from the column using _____ _____.



Protein Purification

Ion-exchange Chromatography

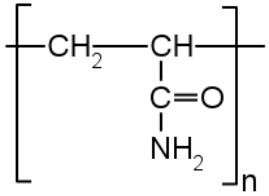
Principle: a charged ion bound to the column is displaced by the charged side groups on a protein. The protein is eluted from the column using increasing amounts of the ion.



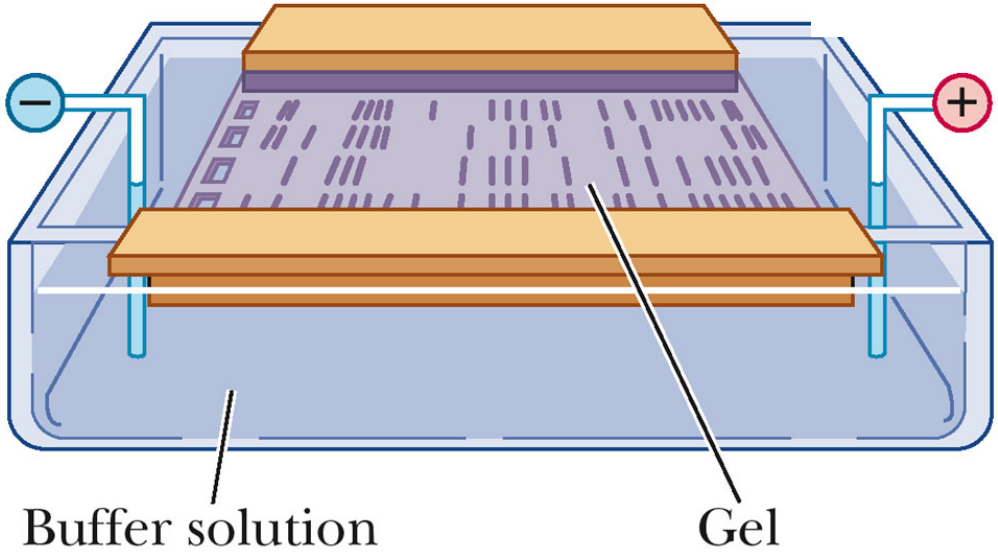
Protein Purification

Electrophoresis

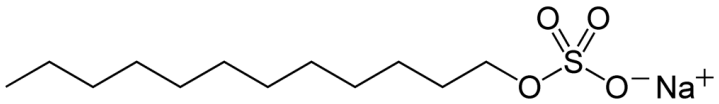
Principle: protein samples are separated through _____ _____. Proteins are "pulled" through the gel by an electric field.



Gel: polymer of _____ _____



Sodium dodecyl sulfate (SDS): a negatively charged _____ _____ _____ proteins and give a uniform "-" charge.



In the presence of _____ _____ will travel towards anode, "+"

SDS-PAGE: _____

Protein Purification

Electrophoresis

Cathode:

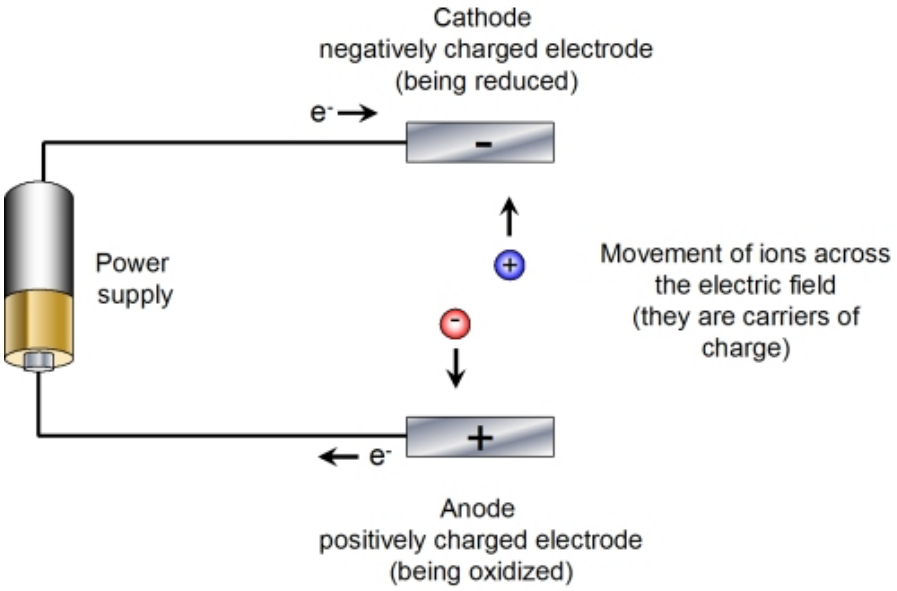
- The negatively charged electrode by which electrons enter an electrical device.
- The positively charged electrode of an electrical device that supplies current.

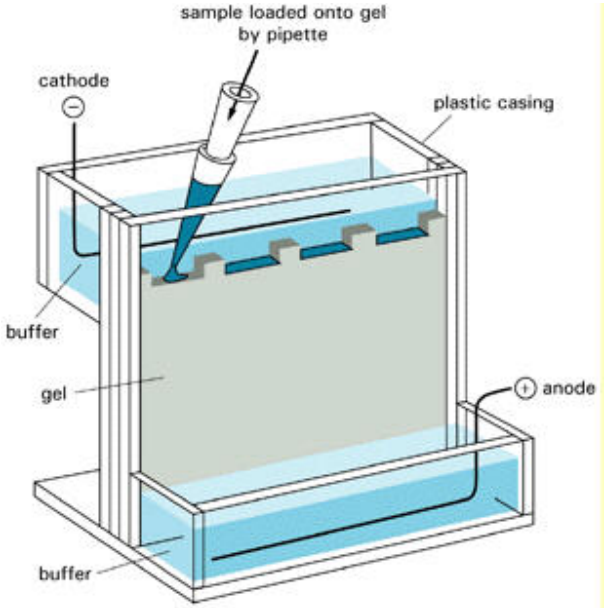
The cathode is negative in a device that consumes or uses power,
and the cathode is positive in a device that provides power

Anode:

- The positively charged electrode by which electrons leave an electrical device.
- The negatively charged electrode of a device that supplyies current.

the anode is positive in a device that consumes power,
and the anode is negative in a device that provides power





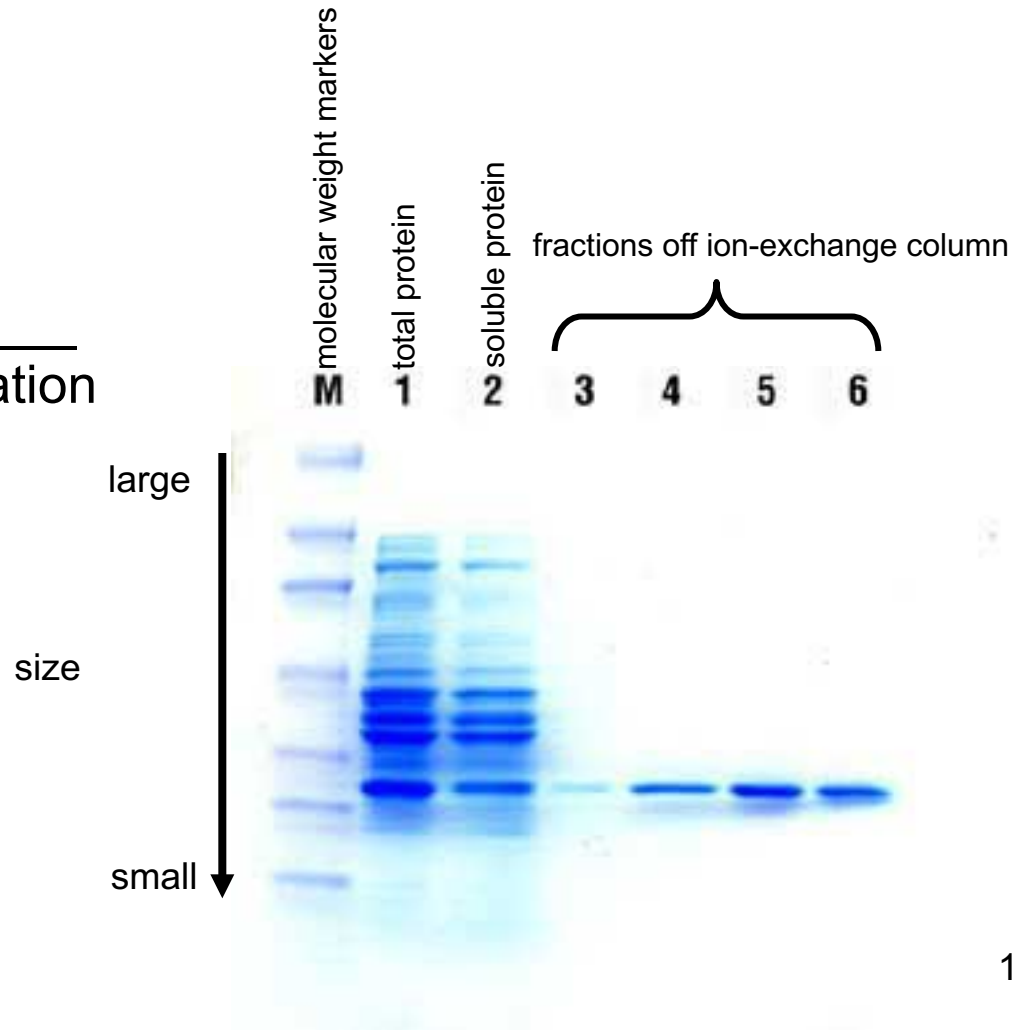
Protein Purification
Electrophoresis

SDS-PAGE: separates proteins within a sample _____.

large proteins migrate _____

small proteins migrate _____

Stain gel with _____ _____ for visualization



SDS-PAGE

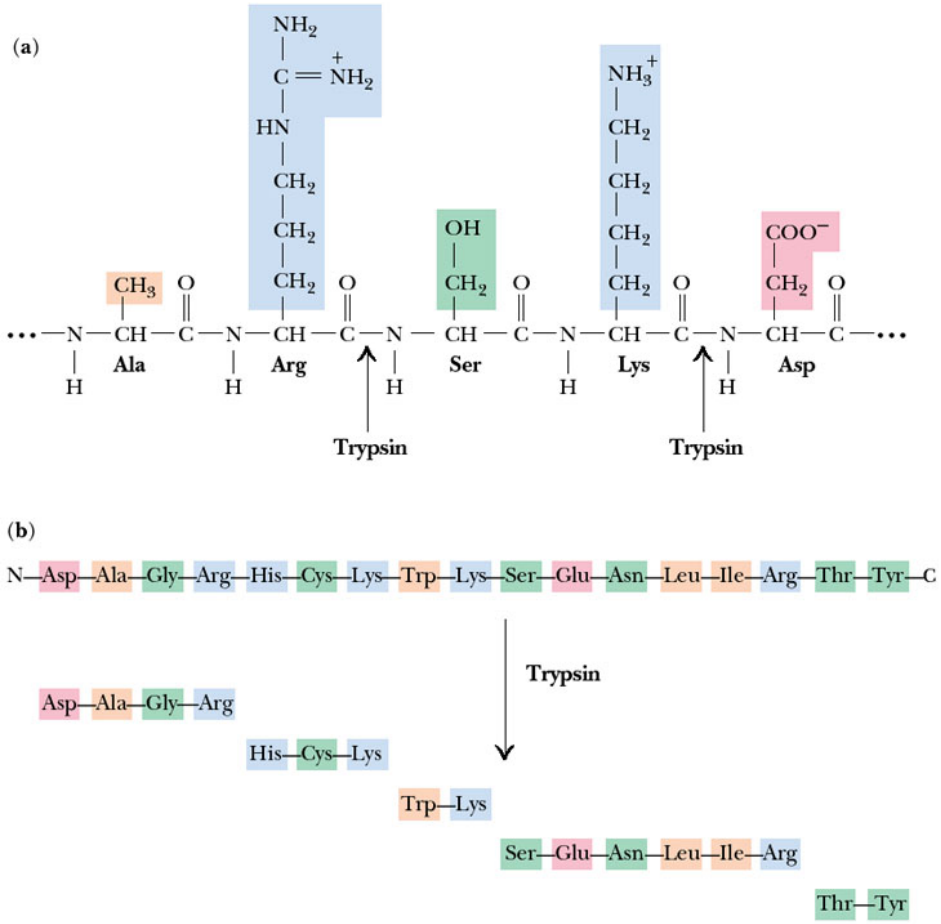
(http://www.youtube.com/watch?v=pjEHJIPRtlU)

Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Trypsin: enzyme that _____

_____

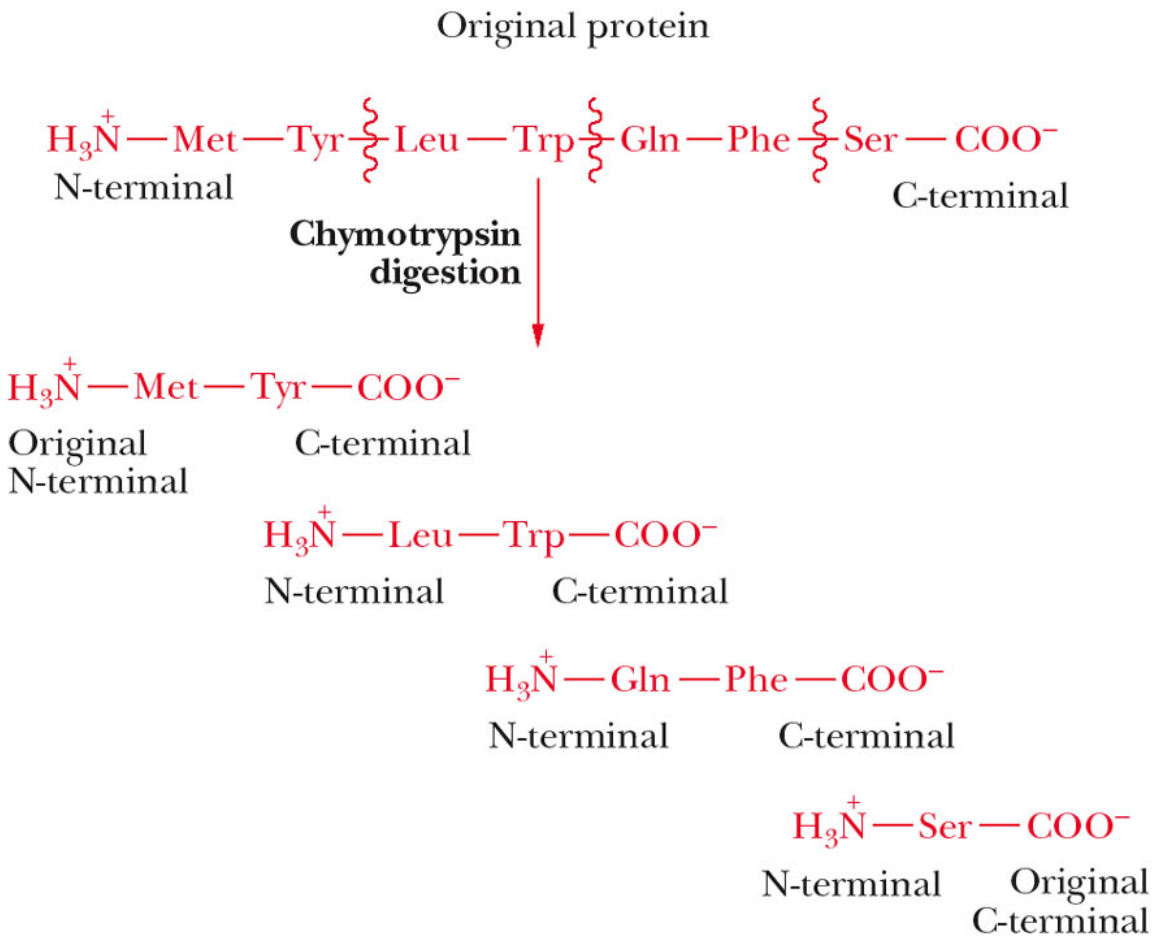


Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Chymotrypsin: enzyme that _____

_____



Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

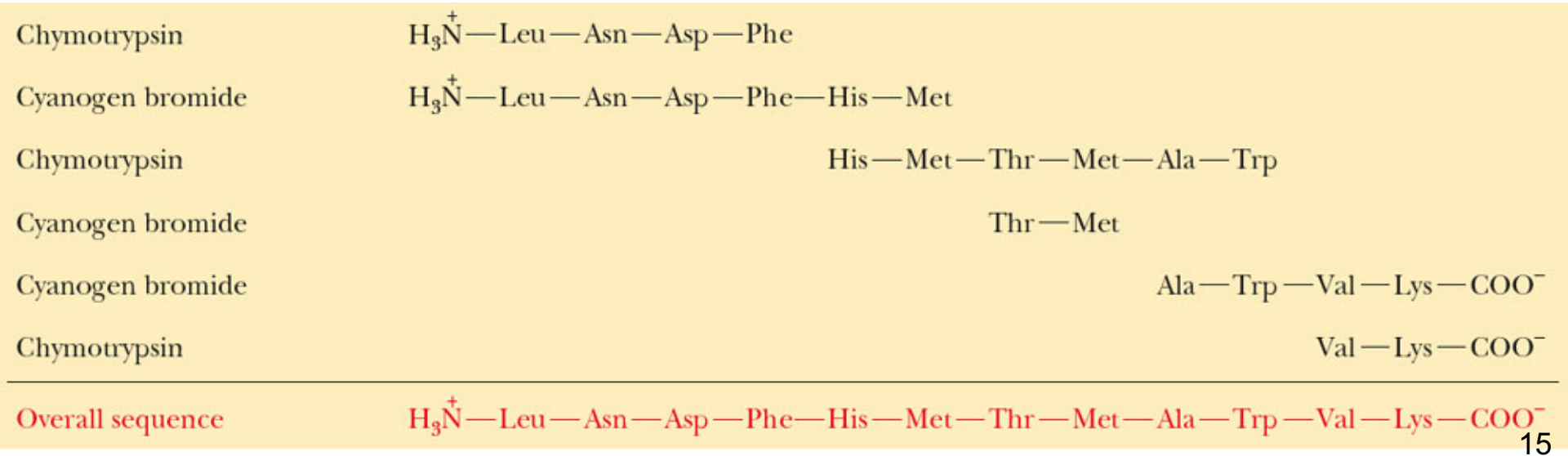
Cyanogen bromide: _____

1. _____ by several different methods, individually

2. Produces _____

3. Sequence peptides using _____

4. Arrange peptides to _____



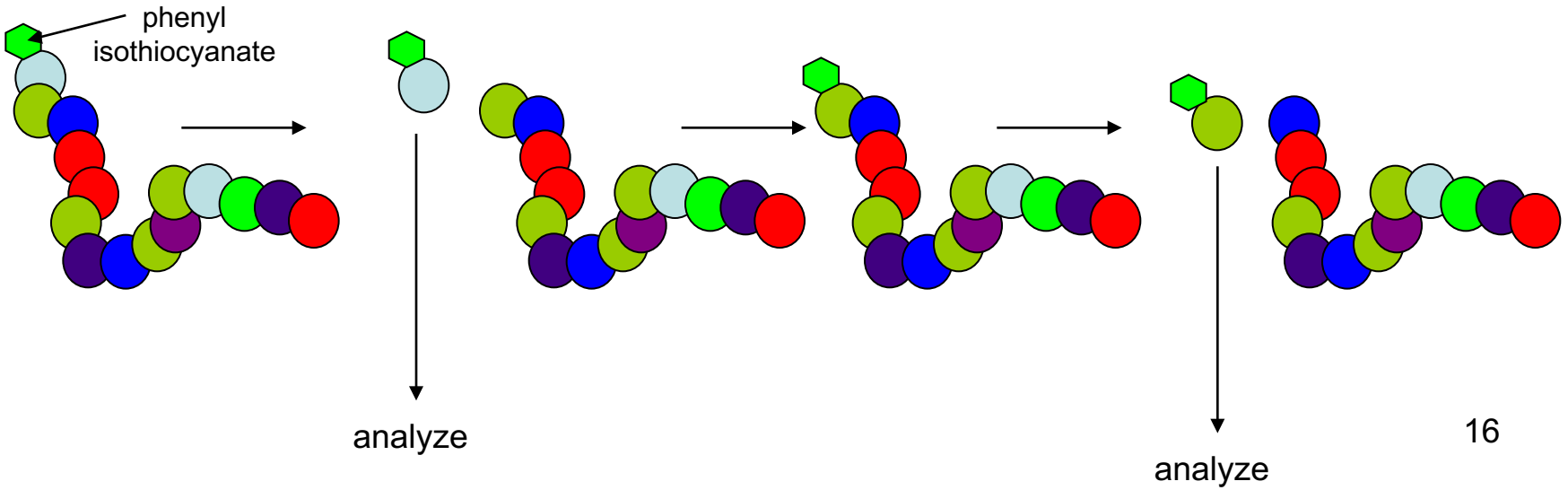
Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Edman degradation: a method used to determine _____

_____

1. Peptide is reacted with _____, bonds to N-terminal aa.
2. The modified aa is _____
3. Repeated until all _____.



Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Example: a protein sample is split into two equal samples. One is treated with trypsin and the other with chymotrypsin. Given the peptides found, determine the protein sequence.

Trypsin peptides: _____

and

_____

Chymotrypsin peptides: _____

and

_____

and

_____

Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Trypsin peptides: Leu - Ser - Tyr - Ala - Ile - Arg
and
Asp - Gly - Met - Phe - Val - Lys

Possibilities for protein sequence:

1. _____

or

2. _____

Protein Purification

Once a protein has been purified how is the primary structure (sequence) determined?

Chymotrypsin peptides: Val - Lys - Leu - Ser - Tyr
and
Ala - Ile - Arg
and
Asp - Gly - Met - Phe

1. Leu - Ser - Tyr - Ala - Ile - Arg - Asp - Gly - Met - Phe - Val - Lys

2. Asp - Gly - Met - Phe - Val - Lys - Leu - Ser - Tyr - Ala - Ile - Arg