



Biochemistry

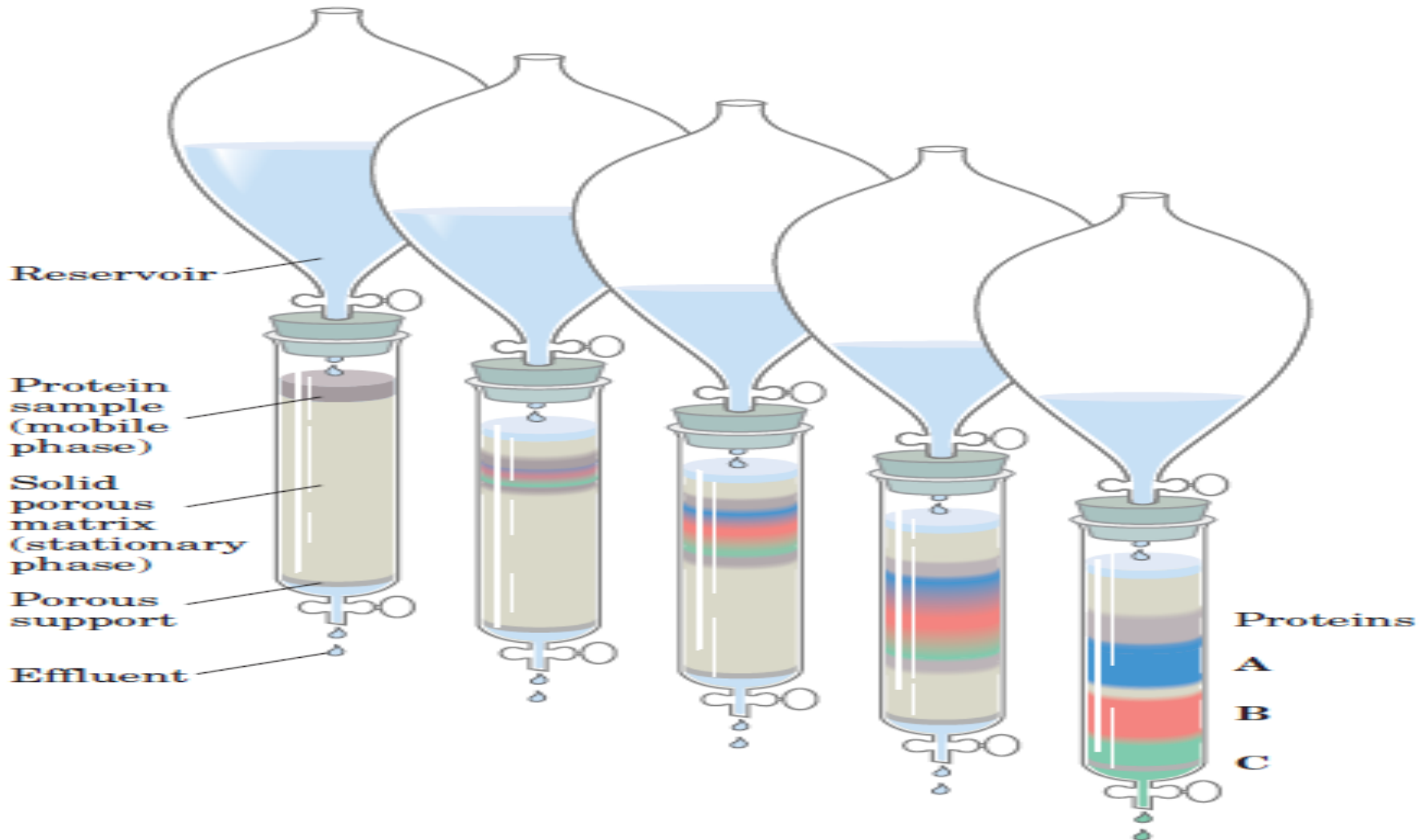
Lecture 3

Course prof.

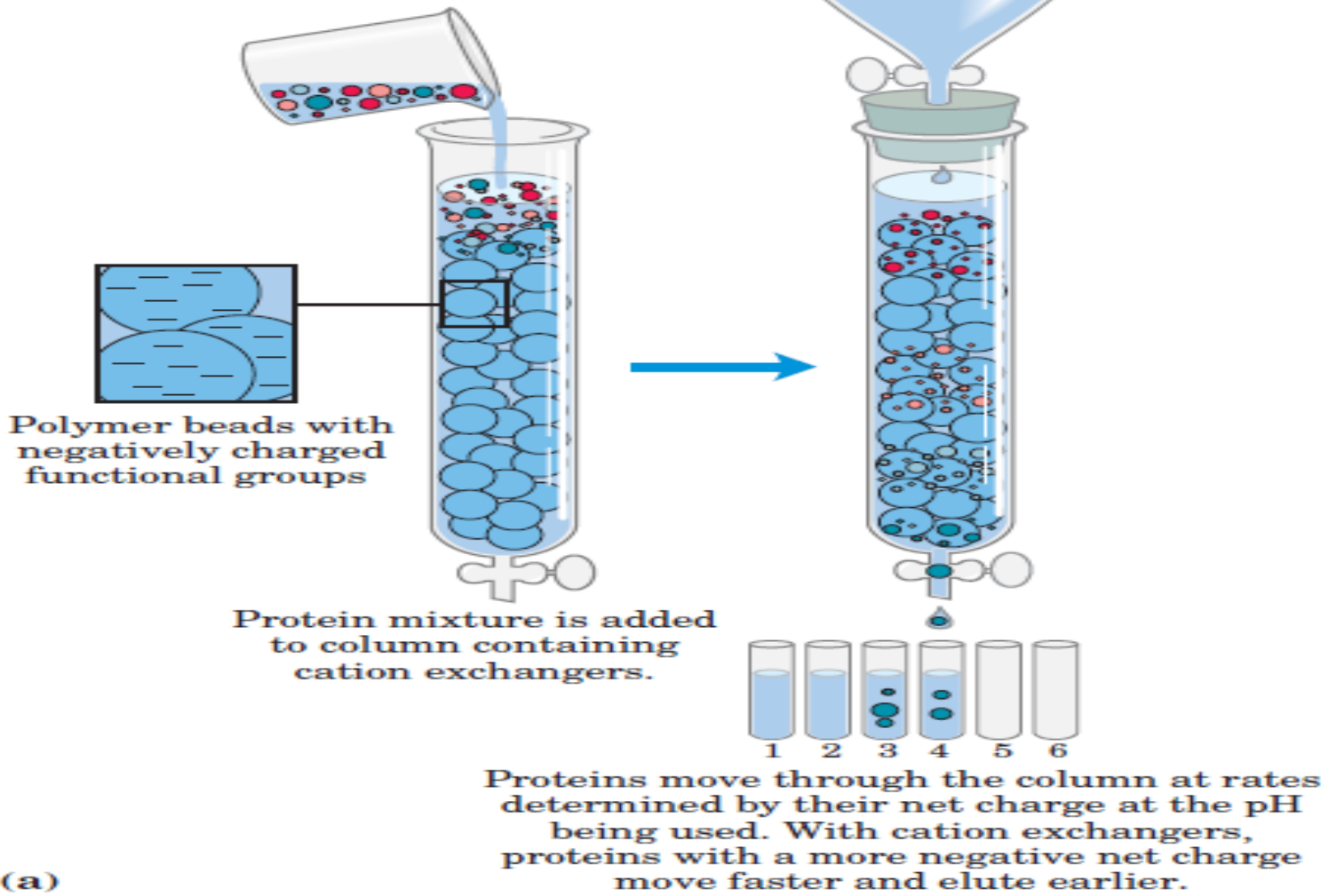
Dr. Ahmed Mohamed
Lecturer at Dep. Of
Biochemistry

CHEMISTRY 2

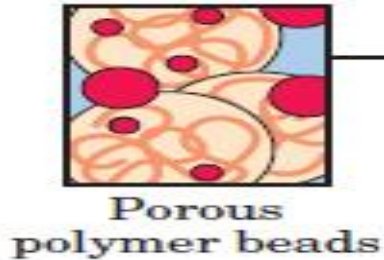
Proteins Can Be Separated and Purified



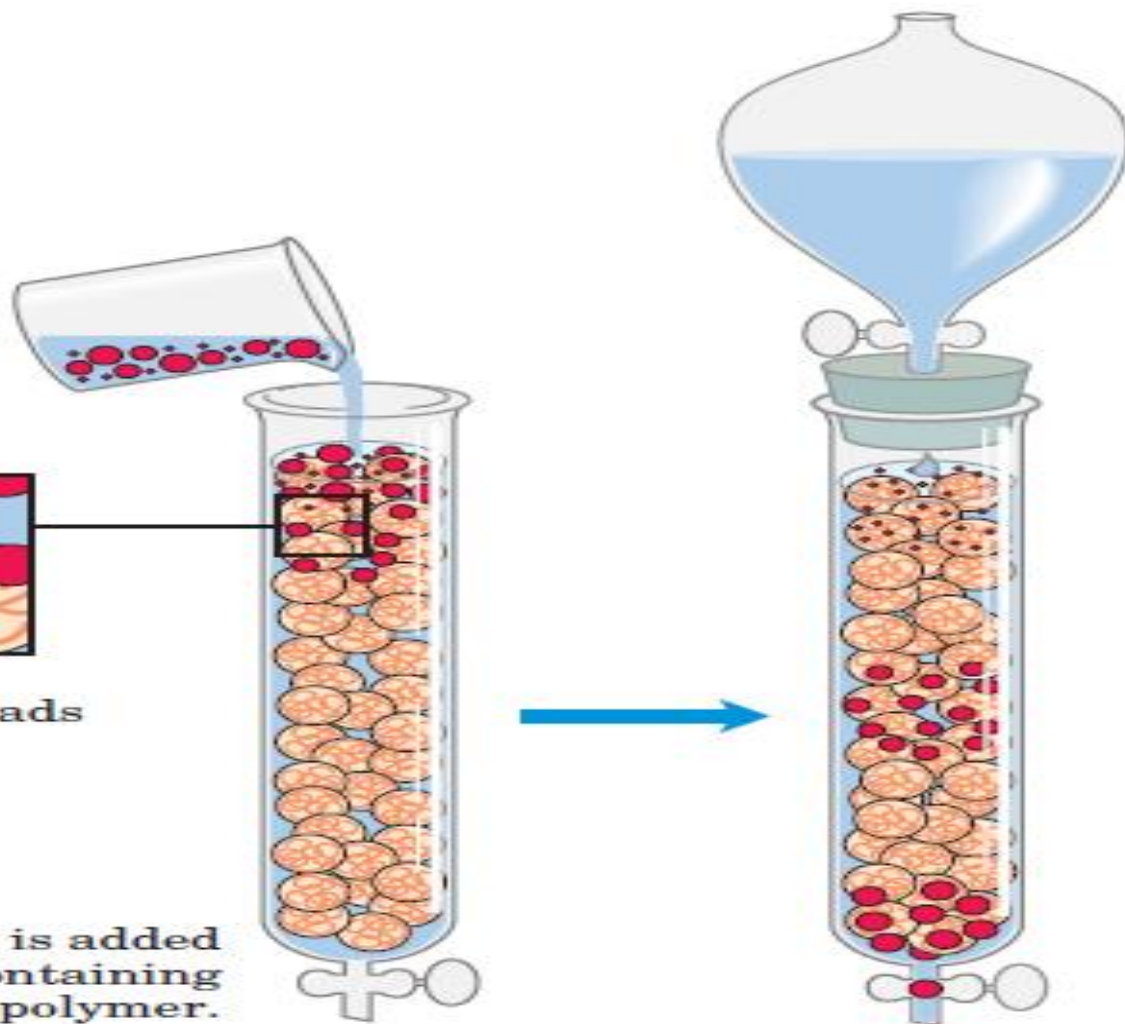
- Large net positive charge
- Net positive charge
- Net negative charge
- Large net negative charge



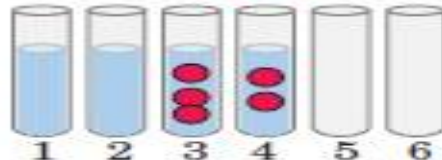
(a)



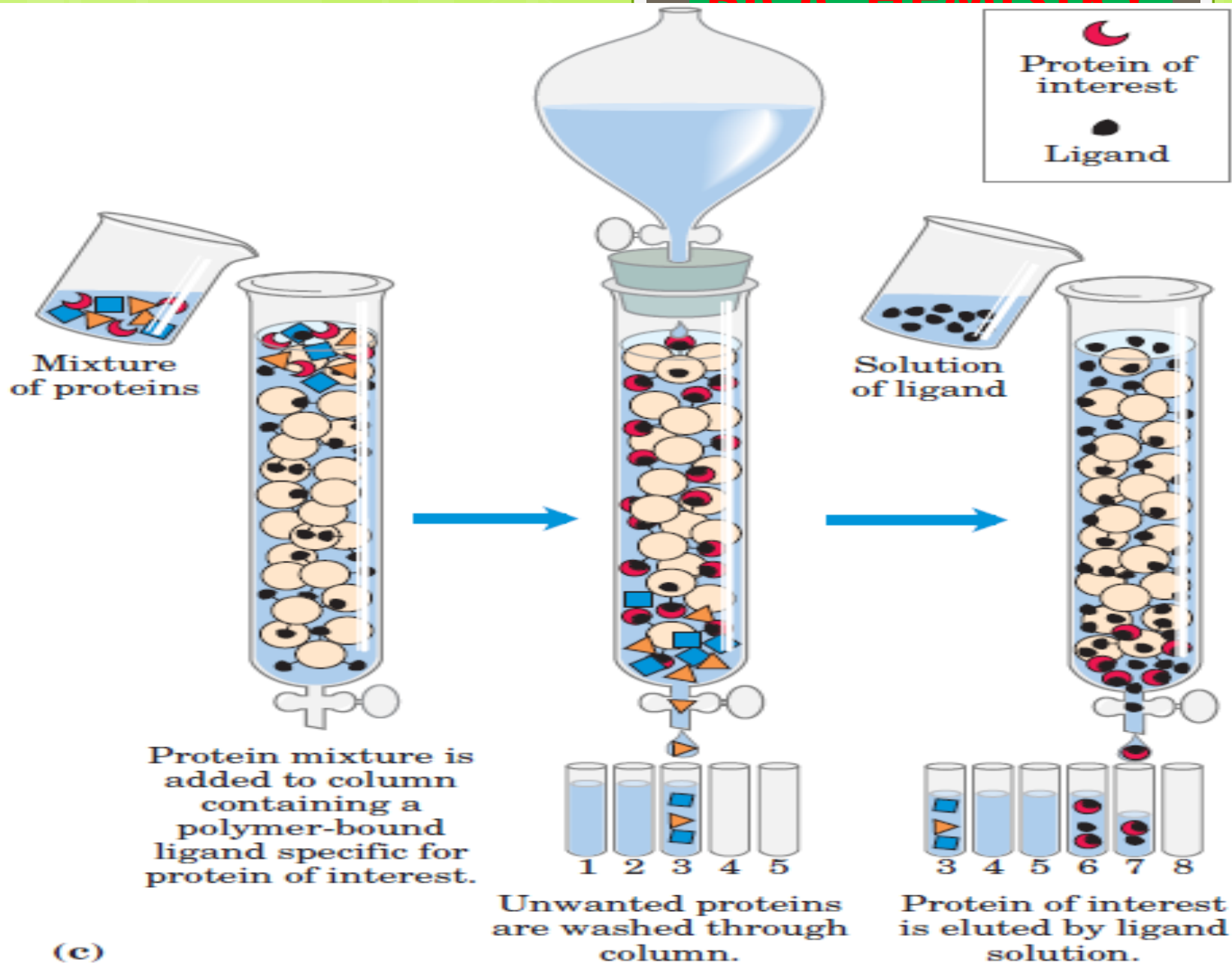
Protein mixture is added to column containing cross-linked polymer.



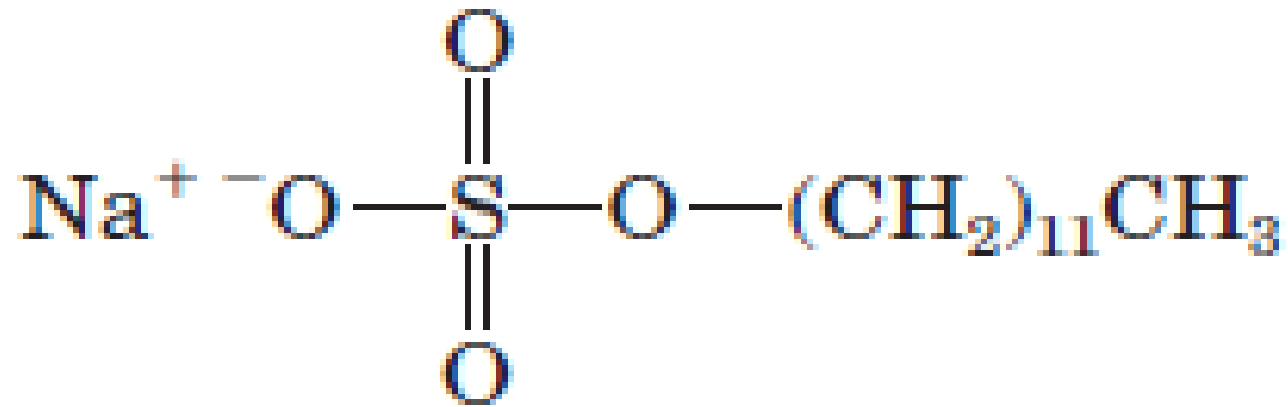
Protein molecules separate by size; larger molecules pass more freely, appearing in the earlier fractions.



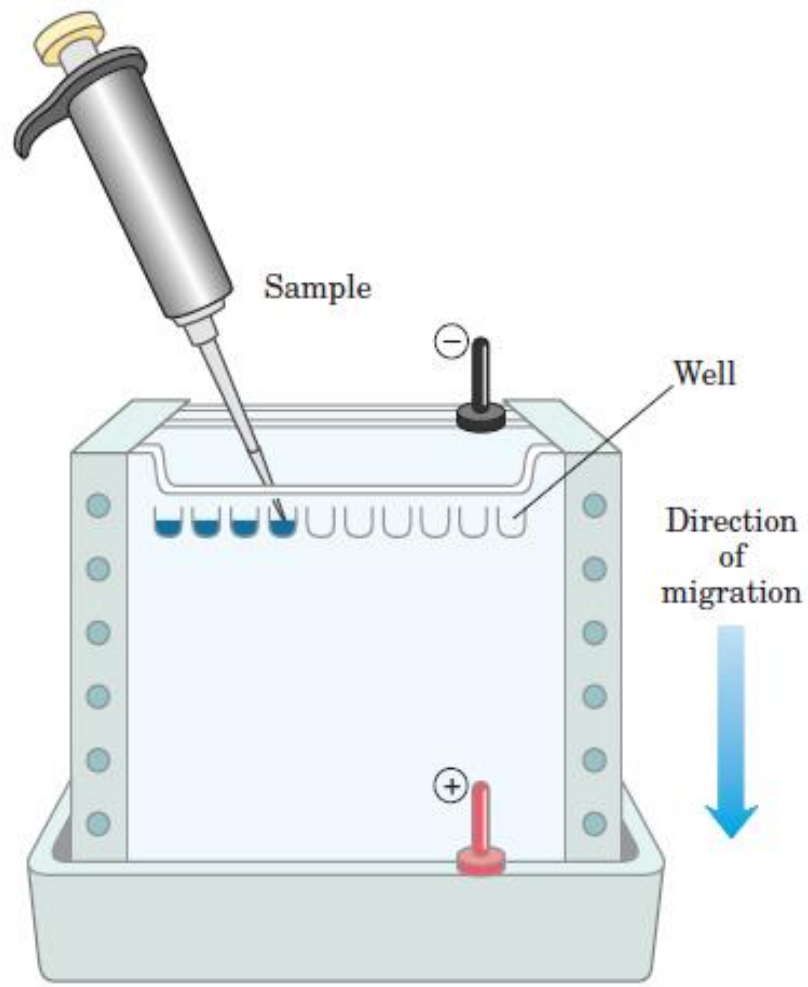
(b)



Proteins Can Be Separated and Characterized by Electrophoresis



Sodium dodecyl sulfate
(SDS)



<https://www.youtube.com/watch?v=PVvpEKeOzEM>
<https://www.youtube.com/watch?v=i4U4ndf2ayg>

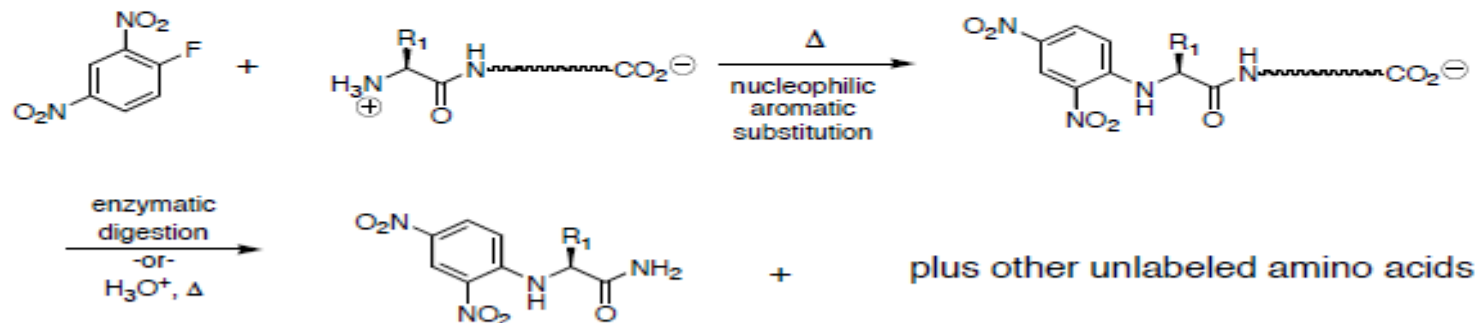
The Amino Acid Sequences of Millions of Proteins Have Been Determined

Short Polypeptides Are Sequenced Using Automated Procedures

Peptide and Protein Sequences:

primary (1°) structure- amino acid sequence

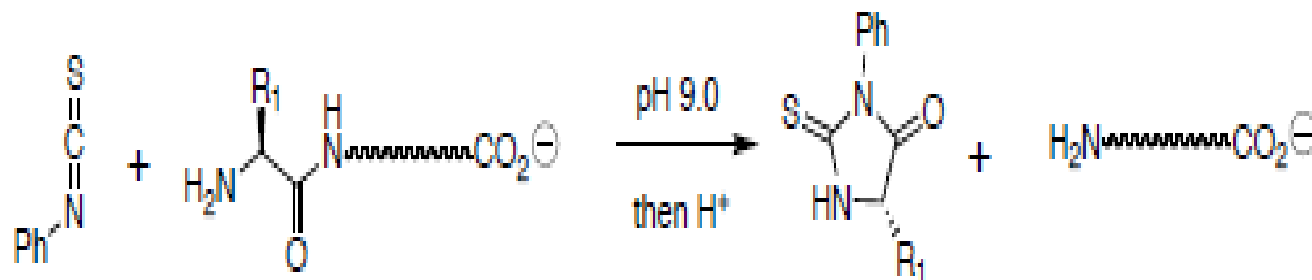
N-labeling with Sanger's reagent: Sanger's (2,4-dinitrofluorobenzene) reagent reacts with the N-terminal amino group and has a diagnostic UV absorbance that is detected after enzymatic digestion and amino acid analysis



N-terminal amino acid is specifically labeled with a unique UV chromophore

Edman Degradation: chemical method for the sequential cleavage and identification of the amino acids of a peptide, one at a time starting from the N-terminus.

Reagent: Ph-N=C=S, phenylisothiocyanate)

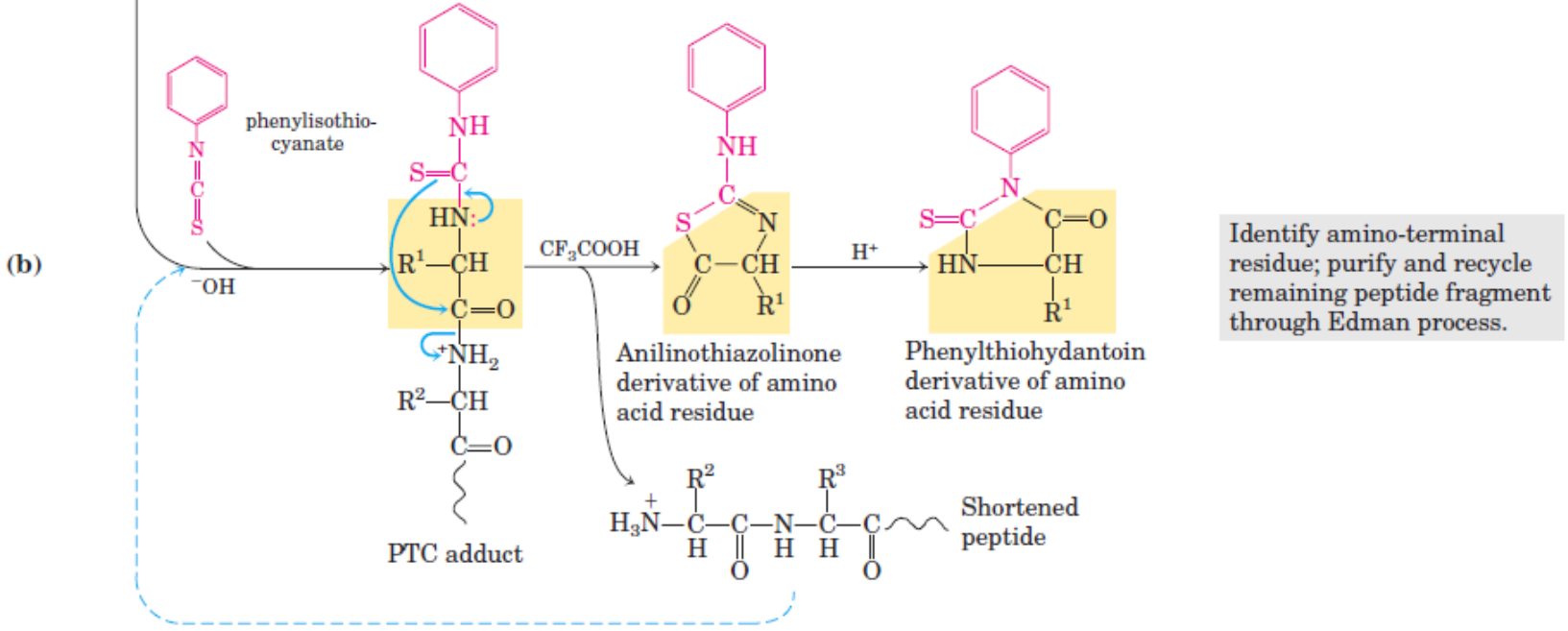
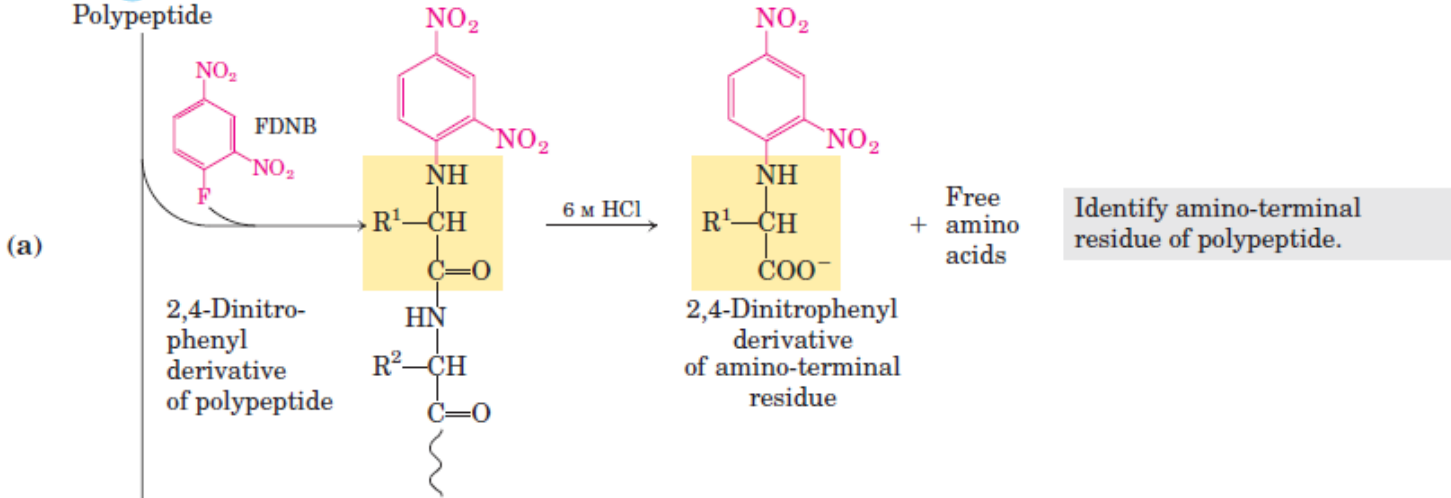


N-phenylthiohydantoin:
separated by HPLC,
detected by UV-vis

-1 peptide with a new
N-terminal amino acid
(repeat degradation cycle)

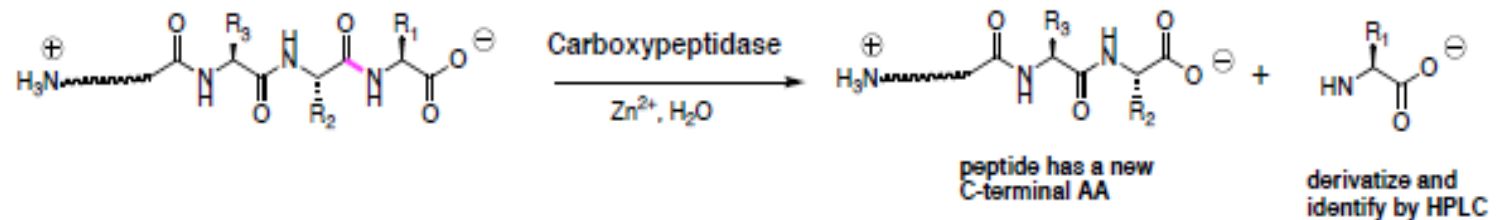


Polypeptide

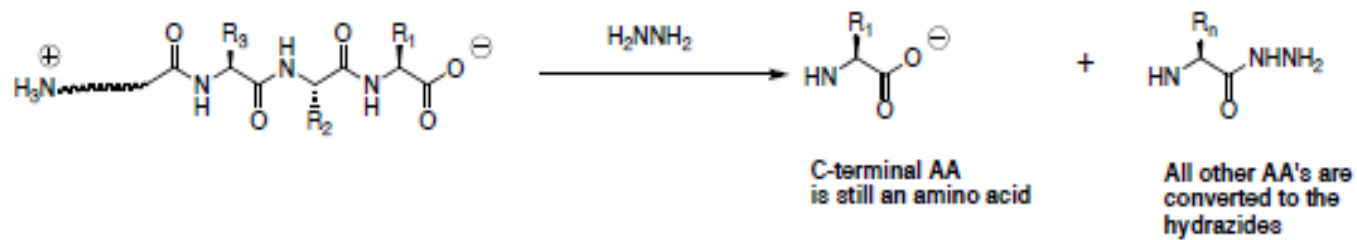


C-terminal sequencing:

Carboxypeptidase- enzyme that hydrolyzed amide bonds of a peptide or protein starting from the C-terminal end (exopeptidase)



Hydrolyze peptide with hydrazine ($\text{H}_2\text{N-NH}_2$)



References:

- Lehninger Principles of Biochemistry (Nelson W. H. Freeman. 4th Ed, 2004).
- Biochemistry (Reginald H. Garrett and Charles M. Grisham, University of Virginia 4th Ed, 2010).