

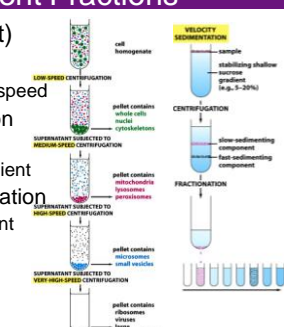
Chapter 8:
**Manipulating
Proteins, DNA,
and RNA**

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PURIFYING PROTEINS

Cells Can Be Separated into Their Component Fractions

- Homogenate (extract)
 - Cell fractionation
 - Progressively higher speed
 - Velocity sedimentation
 - Salt solution
 - Shallow sucrose gradient
 - Equilibrium sedimentation
 - Steep sucrose gradient

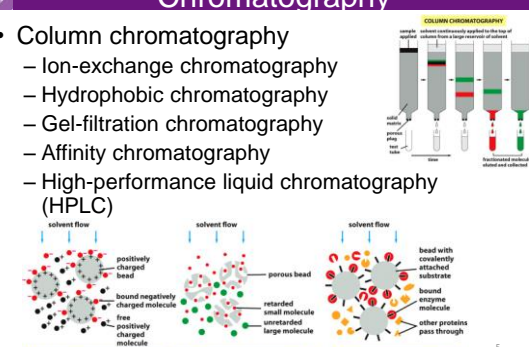


Cell Extracts Provide Accessible Systems to Study Cell Functions

- Functions of cell components
 - Mitochondria
 - Chloroplasts
 - Rough & smooth ER
- Cell division & movement of proteins in cells
 - *Xenopus laevis* oocytes

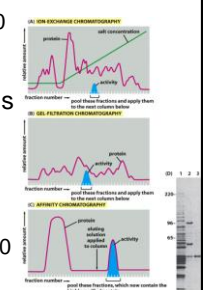
Proteins Can Be Separated by Chromatography

- Column chromatography
 - Ion-exchange chromatography
 - Hydrophobic chromatography
 - Gel-filtration chromatography
 - Affinity chromatography
 - High-performance liquid chromatography (HPLC)



Affinity Chromatography Exploits Specific Binding Sites on Proteins

- General columns
 - Increases desired protein < 20 fold
- Biological binding interactions (affinity)
 - Bound to inert beads
 - Substrate
 - DNA
 - Antibodies
 - Increases desired protein 1000 -10,000 fold



This is only a guideline topics discussed in-class as well as the assigned pages from the text and supplemental material may also be on the exam.

Genetically-Engineered Tags Provide an Easy Way to Purify Proteins

- Epitope added
 - Attaches to antibody
- Histidine string added
 - Attaches to nickel ions
 - metal affinity

Genetically-Engineered Tags Provide an Easy Way to Purify Proteins

- Fusin protein
 - Attaches to its substrate
 - Can find protein-protein interactions
- Tandem affinity purification tagging (tap-tag)
 - Two tags and a protease cleavage site

Purified Cell-free Systems are Required for the Precise Dissection of Molecular Functions

- Purified cell-free systems
 - homogenate able to translate RNA into protein
 - Fractionation to determine step by step process
- Responsible for details of:
 - DNA replication, DNA transcription, RNA splicing, Protein translation, muscle contraction, particle transport along microtubules , etc.

ANALYZING PROTEINS

Proteins Can Be Separated by SDS Polyacrylamide-Gel Electrophoresis

- Sodium dodecyl sulfate(SDS)
 - Negatively charged detergent
 - Unfolds hydrophobic regions
- β - mercaptoethanol
 - Breaks disulfide bonds

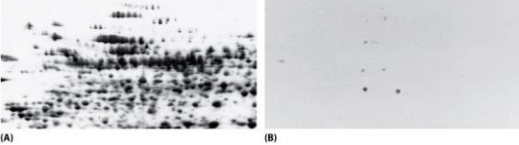
Proteins Can Be Separated by SDS Polyacrylamide-Gel Electrophoresis

- Bands form based on molecular weight
 - Detected with stain
 - Coomassie blue
 - Silver or gold

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Specific Proteins Can Be Detected by Blotting with Antibodies

- Western Blotting
 - Transfer proteins from gel to a solid matrix (nitrocellulose or nylon)
 - Soak membrane to a antibody coupled with radioactive isotope (or fluorescent dye)



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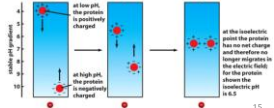
Mass Spectrometry Provides a Highly Sensitive Method for Identifying Unknown Proteins

- Mass spectrometry
 - Separates ions by mass-to-charge ratio

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Two-Dimensional Separation Methods are Especially Powerful


- 2D gel electrophoresis
 - Resolve up to 2000 proteins
- 1st step: Isoelectric focusing
 - Separate based on charge
 - Using nonionic detergent, β -mercaptoethanol and urea
 - Separation obtained using pH gradient
 - Isoelectric point – pH at which protein has no net charge
 - Will not migrate



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Two-Dimensional Separation Methods are Especially Powerful

- 2nd step: Electrophoresis
 - SDS added
 - Run at a right angle to first direction
- Resolving power
 - Can distinguish a single charged aa.



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