Techniques in Protein Biochemistry

Stryer Short Course

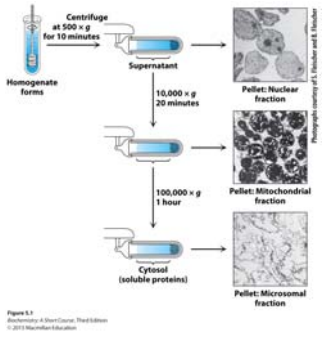
Chapter 5

Protein Analysis

- Isolation
- Purification
- Anaylsis of purity
- Activity Assay
- Sequencing

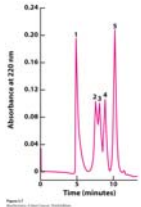
Isolation

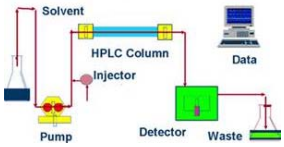
- Homogenate
- Fractions
- Differential centrifugation
- Crude extract



Purification

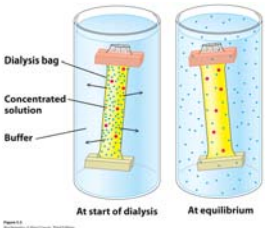
- Solubility
- Chromatography
  - Size
  - Charge
  - Affinity
- HPLC





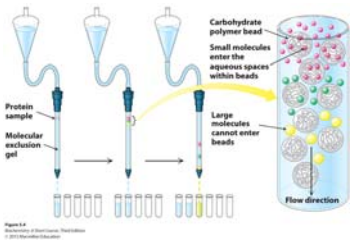
Separation by Solubility

- Salting-in and salting-out
- Competition for water of hydration
- dialysis



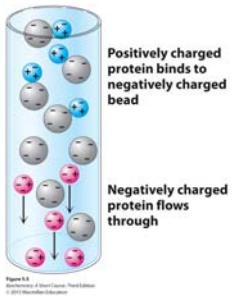
Gel Filtration Chromatography

- Size exclusion
- Porous polyacrylamide or agarose beads



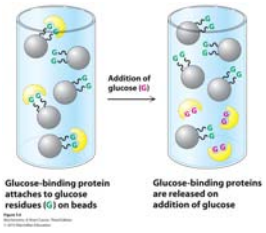
Ion-Exchange Chromatography

- Choose charge of beads based on net charge of protein of interest
- Elute using more concentrated ion buffer



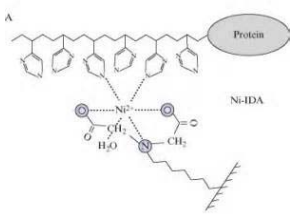
Affinity Chromatography

- Most specific
- Most selective
- Most difficult
- Once developed, can be a highly effective purification technique



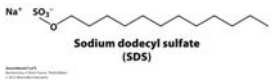
Affinity Chromatography

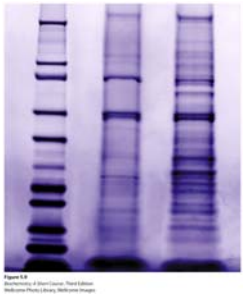
- Immunoglobins
- Poly-His tail
- Bound inhibitors
  - Serine proteases
- Biotin/streptavidin
- Calmodulin binding proteins
- And many others



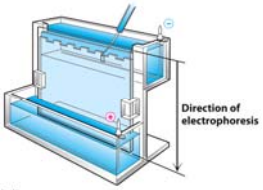
Qualitative Purity Analysis

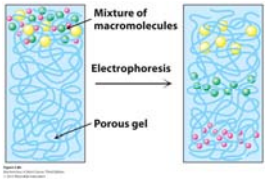
- Electrophoresis and staining
- Approximate size (kDa)
- Relative purity
- Denature protein
  - Charge it evenly with SDS





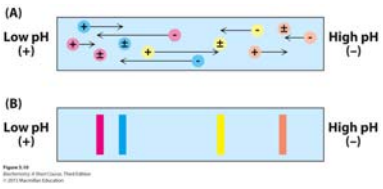
SDS-PAGE





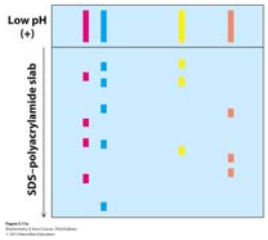
Isoelectric Focusing

- Based on isoelectric point
- Buffer gradient
- Electrophoresis with no SDS



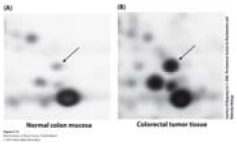
Two-Dimensional Electrophoresis

- Separate based on pI and on size
- First, isoelectric focusing
- Second, gel laid horizontally on SDS-PAGE
- Separation of hudreds of proteins



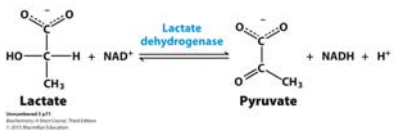
Application

- Find relative increase in protein concentration in disease state or physiological response



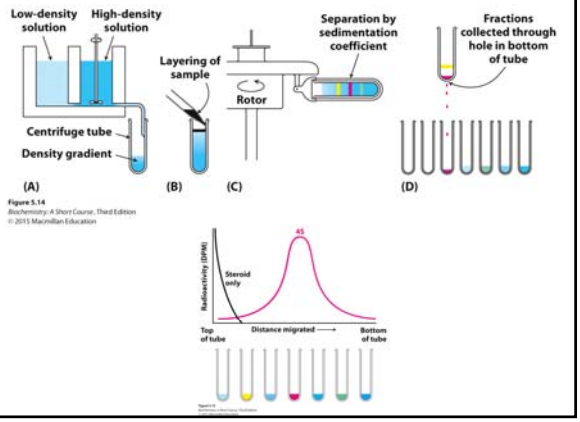
Quantitative Analysis

- Activity assay
  - Enzymatic or binding
  - Colorimetric, radiolabel
  - Continuous/discontinuous
- Total protein (Bradford, Lowry)



Binding Assay

- Example: estrogen receptor
- Binds tightly to radiolabeled estradiol
- Ultracentifugation



Quantitation

Table 5.1 Quantification of a purification protocol for a hypothetical protein

Step	Total protein (mg)	Total activity (units)	Specific activity (unites mg^{-1})	Yield (%)	Purification level
Homogenization	15,000	150,000	10	100	1
Salt fractionation	4,600	138,000	30	92	3
Ion-exchange chromatography	1,278	115,500	90	77	9
Gel-filtration chromatography	68.8	75,000	1,100	50	110
Affinity chromatography	1.75	52,500	30,000	35	3,000

Table 5.1
Biochemistry: A Short Course, Third Edition
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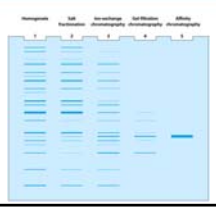
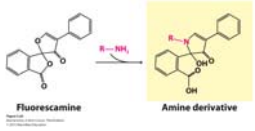
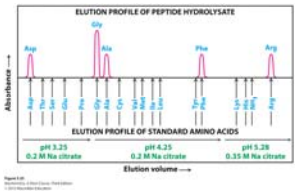


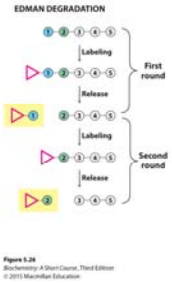
Traditional Sequencing

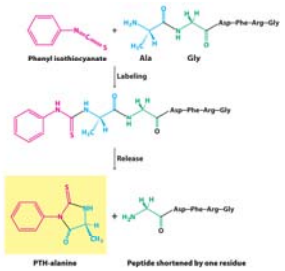
- Full hydrolysis
- Amino acid composition





Edman Degradation



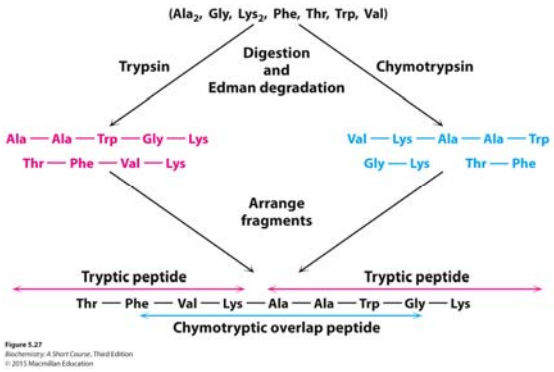


Sequencing Longer Polypeptides

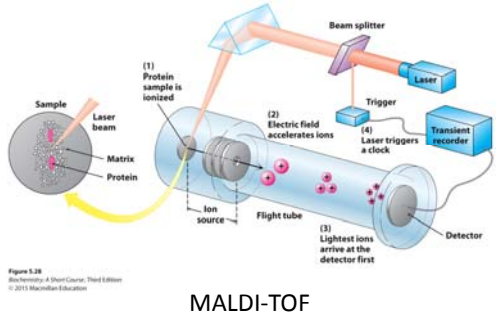
- Edman limited to about 50 residues
- Can cleave longer peptides into shorter ones with specific hydrolysis
- Then overlap

Table 5.3 Specific cleavage of polypeptides

Reagent	Cleavage site
Chemical cleavage	
Cyanogen bromide	Carboxyl side of methionine residues
O-Iodosobenzoate	Carboxyl side of tryptophan residues
Hydroxylamine	Asparagine-glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
Enzymatic cleavage	
Trypsin	Carboxyl side of lysine and arginine residues
Clostripain	Carboxyl side of arginine residues
Staphylococcal protease	Carboxyl side of aspartate and glutamate residues (glutamate only under certain conditions)
Thrombin	Carboxyl side of arginine
Chymotrypsin	Carboxyl side of tyrosine, tryptophan, phenylalanine, leucine, and methionine
Carboxypeptidase A	Amino side of carboxyl-terminal amino acid (not arginine, lysine, or proline)



Modern Techniques: MS



MALDI-TOF

Identify Known Proteins

- Fast
- Reliable
- Automated
- Technician-level

Sequencing Unknown Proteins

- Tandem Mass Spectroscopy
- After precursor ion analyzed, can be bombarded and fragmented
- Analysis of fragments by second mass analyzer