Techniques in Protein Biochemistry
Stryer Short Course
Chapter 5

Protein Analysis
• Isolation
• Purification
• Analysis 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

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

Isoelectric Focusing
Two-Dimensional Electrophoresis

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

![Estradiol molecule](image)

**Quantitation**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (unit)</th>
<th>Specific activity (units/mg)</th>
<th>Yield (%)</th>
<th>Purification factor</th>
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</thead>
<tbody>
<tr>
<td>Homogenization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel filtration</td>
<td>2.8</td>
<td>75,000</td>
<td>1,100</td>
<td>50</td>
<td>710</td>
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<td>Affinity chromatography</td>
<td>1.7</td>
<td>32,000</td>
<td>18,888</td>
<td>33</td>
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</tbody>
</table>
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
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