# Protein Purification

## Initial Questions

- How much and how pure?
  - application
  - source
  - feasibility
- Native configuration?
  - functional/structural (yes)
  - sequence (no)
  - antibody (maybe)
- Detection method?
  - functional assay
  - band on gel

**Why purify proteins?**

- functional and/or structural studies
- industrial or pharmaceutical applications
- generate antibodies
- partial sequence

# Developing a Protein Purification Scheme

- carry out small pilot experiments to evaluate various separation techniques
- start with rapid high capacity techniques (which are generally low resolution) and progress to high resolution low capacity techniques

# Capacity vs. Resolution

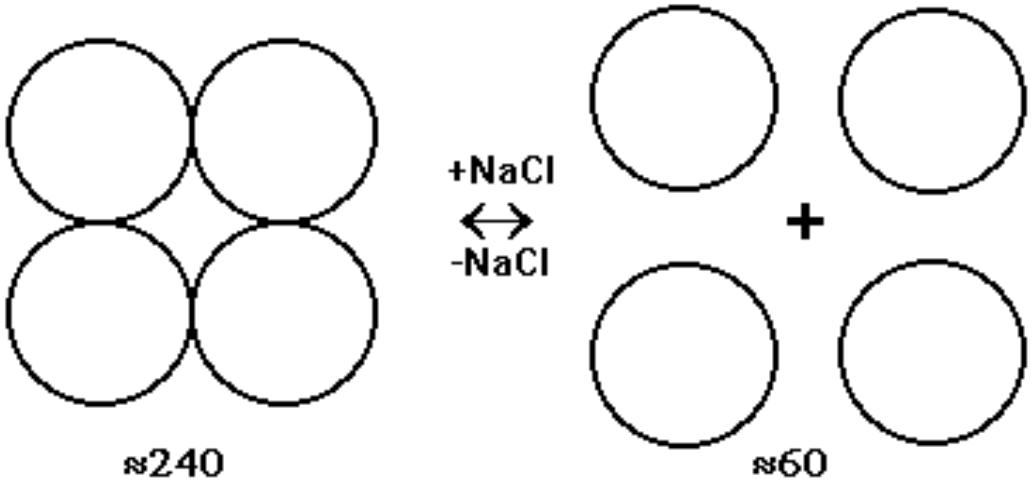
| Method | In general: capacity decreases | resolution increases | time & effort increase |
|---|---|---|---|
| • differential solubility<br>• ion exchange<br>• adsorption<br>• hydrophobic<br>• electrophoresis | ↓ | ↓ | ↓ |
| deviations | ♦ gel filtration (low capacity, low resolution)<br>♦ affinity (depends on ligand)<br>♦ HPLC/FPLC (↑ resolution, ↓capacity) | | |

# Developing a Protein Purification Scheme

- carry out small pilot experiments to evaluate various separation techniques
- start with rapid high capacity techniques and progress to high resolution low capacity techniques
- minimize time and number of manipulations whenever possible
  - eg, arrange methods to minimize buffer changes if other factors are equal
- exploit unique features

# Exploiting Unique Features

- affinity chromatography
- CaM ± $Ca^{2+}$/hydrophobic
- subunits vs. complex (gel filtration)

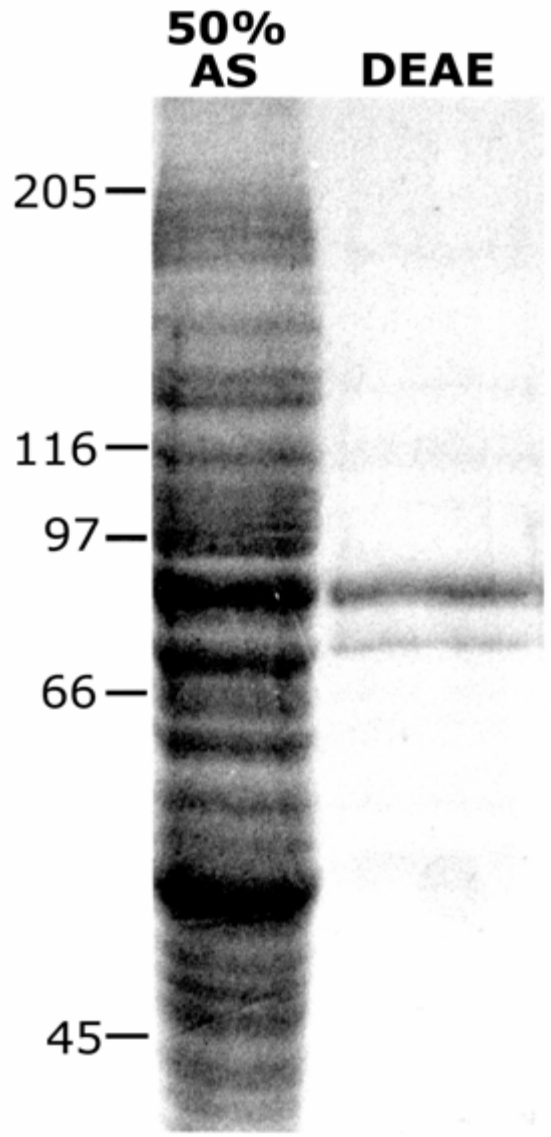


# Evaluation of Protein Purification

- qualitative (gel electrophoresis)
- quantitative
  - recovery (% yield)
  - fold-purification

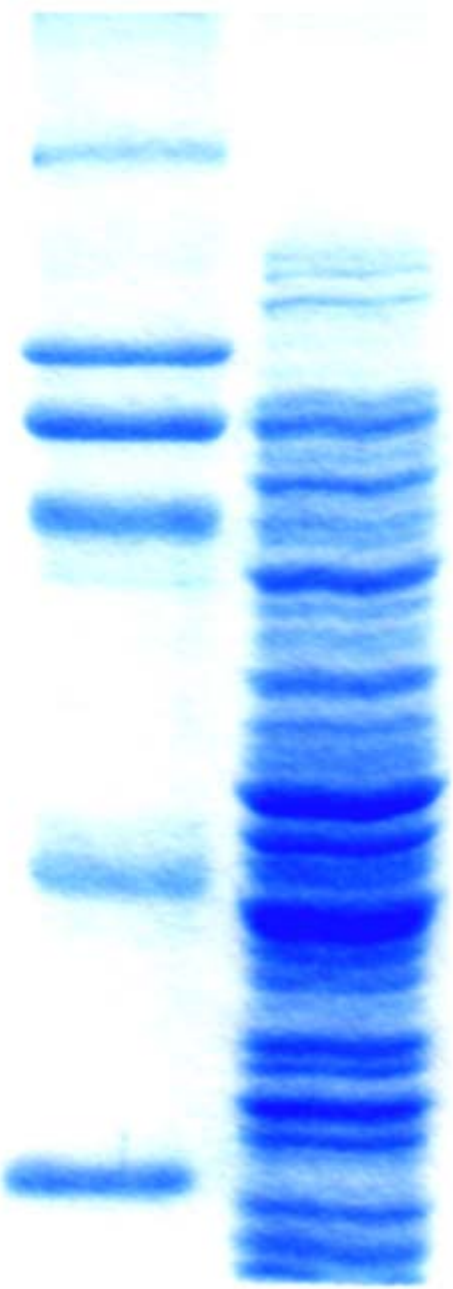
$$\%\ \text{yield} = \frac{\text{total act. recovered}}{\text{total starting act.}}$$

$$\text{fold-purification} = \frac{\text{sp. act. recovered}}{\text{starting sp. act.}}$$



# Protein Sequencing

- partial sequence data:
  - identify protein by homology
  - design DNA probes
  - assess purity
- automated Edman degradation
  - protein bound to solid support
  - N-terminal residues sequentially removed
  - identified by HPLC
- possible after gel electrophoresis

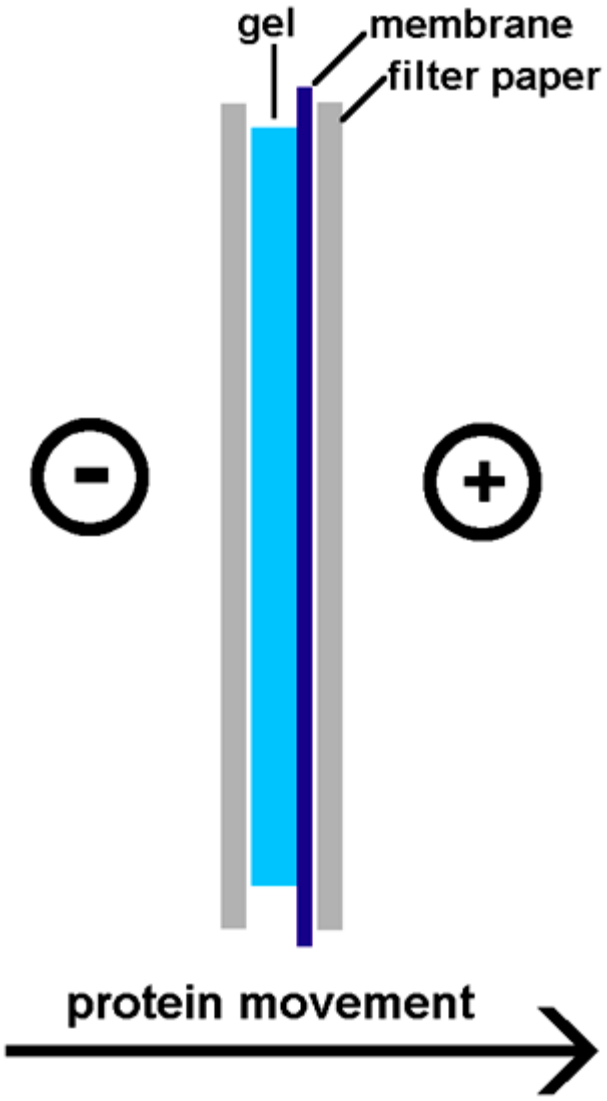


# Preparative Electrophoresis

- high resolution provides analytical information
- difficult to exploit in protein purification
  - capacity
  - recovery
- recovery of proteins from gels
  - diffusion
  - electroelution
  - immunization
  - transfer to membrane

# Protein Transfer

- electrophoretic transfer using special apparatus
- PVDF membranes
  - good protein retention
  - chemical resistance
- transfer in buffer with 10% MeOH to reduce SDS
- optimize transfer time
  - smaller proteins transfer faster
  - larger proteins retained better
  - 0.22 and 0.45 μm pore size
- produces replica of gel



# N-terminal Sequencing Needs

- relatively pure sample (>80%)
- 10-100 pmoles of protein
  - 0.5-5 μg for 50 kDa
- 'unblocked' N-terminus
- free of contaminants (Tris, glycine, SDS, acrylamide, etc.)

# Microsequencing Procedure

1. Purify protein so that it is a major band resolved from contaminants.
2. Gel electrophoresis (1- or 2-D)
3. Transfer protein to membrane support following electrophoresis.
4. Stain membrane and excise protein band of interest.
5. Wash membrane extensively with $H_2O$ before sequence analysis.
6. Submit membrane to sequencing service.

# Internal Sequencing

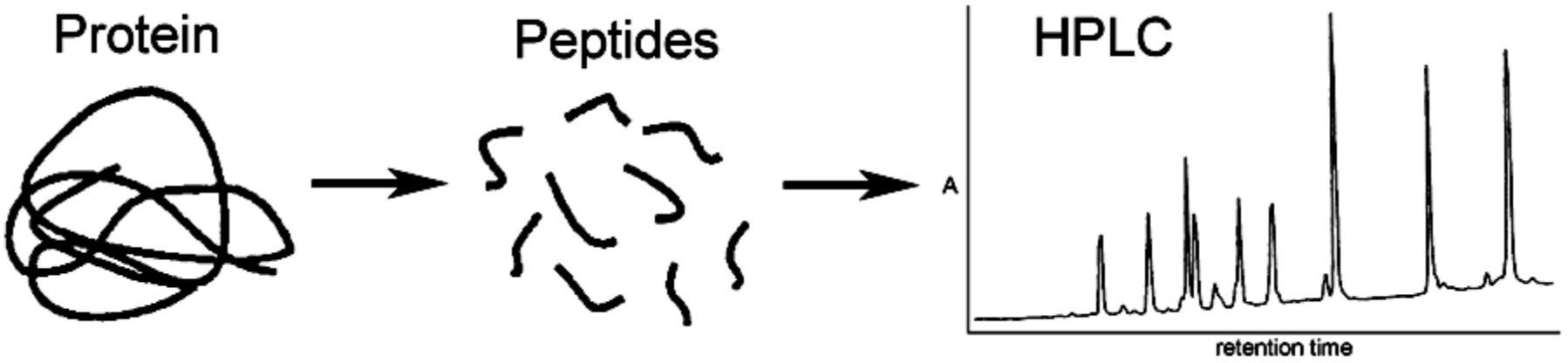
- treat protein with site-specific protease or chemicals

**Site Specific Cleavage of Proteins**

| | | |
|---|---|---|
| chemical | CNBr | Met-X |
| | Skatole | Trp-X |
| proteolytic | trypsin | Arg-X<br>Lys-X |
| | V8 | Glu-X<br>Asp-X |

# Internal Sequencing

- peptides generally isolated by reverse phase (HPLC) chromatography




- peaks dried onto glass fiber filters and sequenced