Principles of purification of macromolecules

Genetics 222
Method and Logic in Experimental Genetics

Reference

Protein Purification: Principles and Practice
Robert K. Scopes
Third Edition
Springer-Verlag
1994

Problem

- Often need one component in a cell in purified form
  - why purified?
  - what does "pure" mean?
  - sometimes, partial enrichment is enough
- Task is to separate desired component from a complex mixture
- It's usually important to maintain the activity of the component throughout the process
  - yield is important
  - but so is intactness, activity
  - achieving one is often at the expense of the other
- Components can be:
  - nucleic acids (DNA, RNA)
  - proteins
  - protein/nucleic acid complexes (snp's, transcription complexes)
  - large cellular complexes (ribosomes, spliceosomes)

Protein purification

- **Proteins**
  - huge number of proteins (> than the # of genes encoded in a genome)
  - most cell types in multicellular organisms express tens of thousands of different proteins
  - relative abundances of various proteins vary widely

- **Goals of protein purification**
  - obtain a particular protein free of others and other cell components
  - obtain a good yield (absolute amount and proportion of starting amount)
  - maintain the activity of the protein
    - Problems:
      - denaturation
      - proteolysis
      - in vitro mixing
  - a measurement of how well the process worked
  - some characterization of the purified protein

Methods for protein purification

1. Develop a quantitative assay

- quantitative measurement of activity
- quantitative immunoassay
- gel electrophoresis assay (immunoblot or gel activity assay)
- generally want to increase Specific Activity at each step

Definition of Specific Activity

- for proteins: units per milligram of total protein
- "unit" is defined by the researcher, and can be different when described by different people or different venders
- a unit is a quantitative measure of activity, usually associated with a turnover rate (for enzymes) or amount needed for stoichiometric binding (for receptors, ligands, DNA binding proteins)
- theoretical maximum for any given protein

Methods for protein purification

2. Obtain source of material

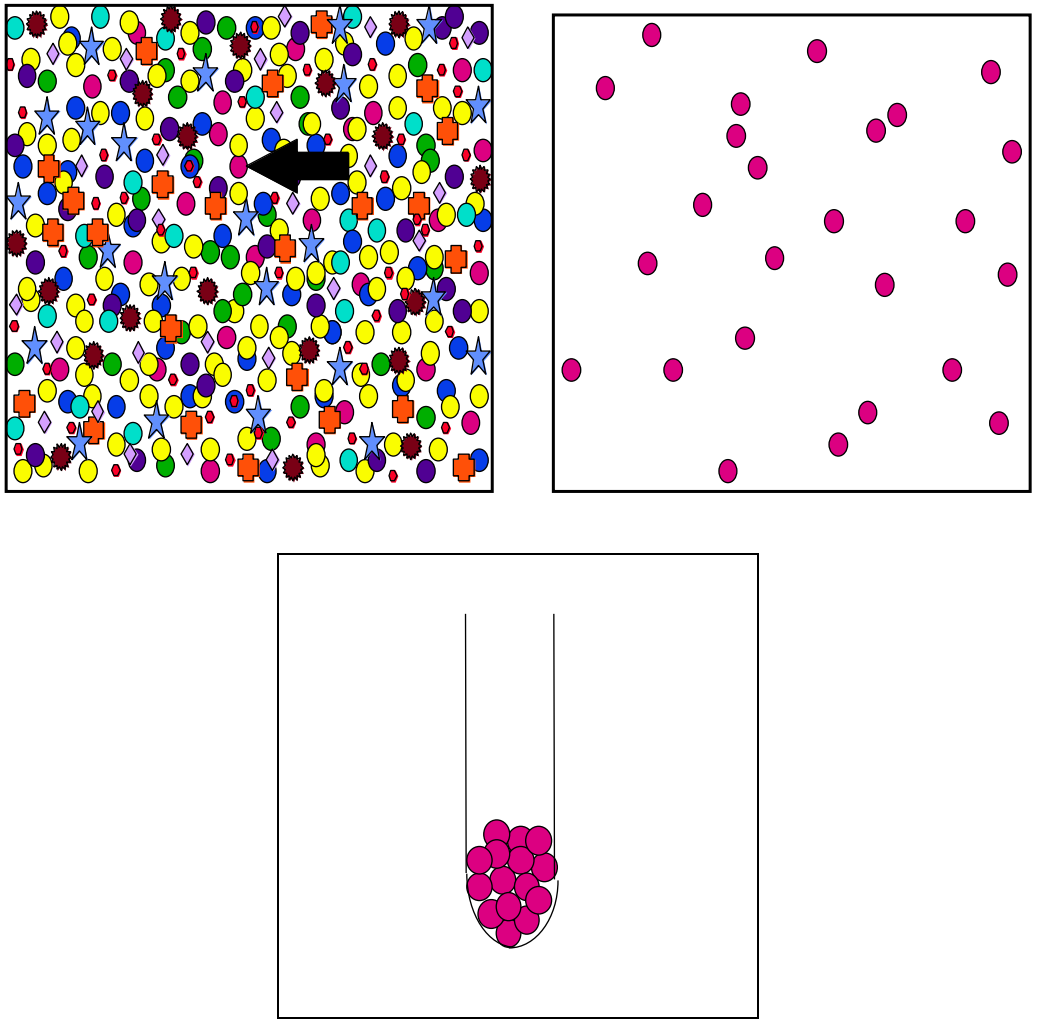
- whole organisms
- organs or tissues
- embryos
- tissue culture cells
- microorganisms

- need to understand and then weigh +'s and -'s of any particular source, e.g.:
  - how hard is it to obtain, grow, handle
  - amount of proteolytic activity
  - may sometimes be better to use a lower producing source that is cleaner
  - is the protein active in a particular source?
  - are inhibitors present in a particular source?
  - some organs and tissues have connective tissues that are hard to remove

- overproduction in a heterologous system (basis for much of biotechnology industry)

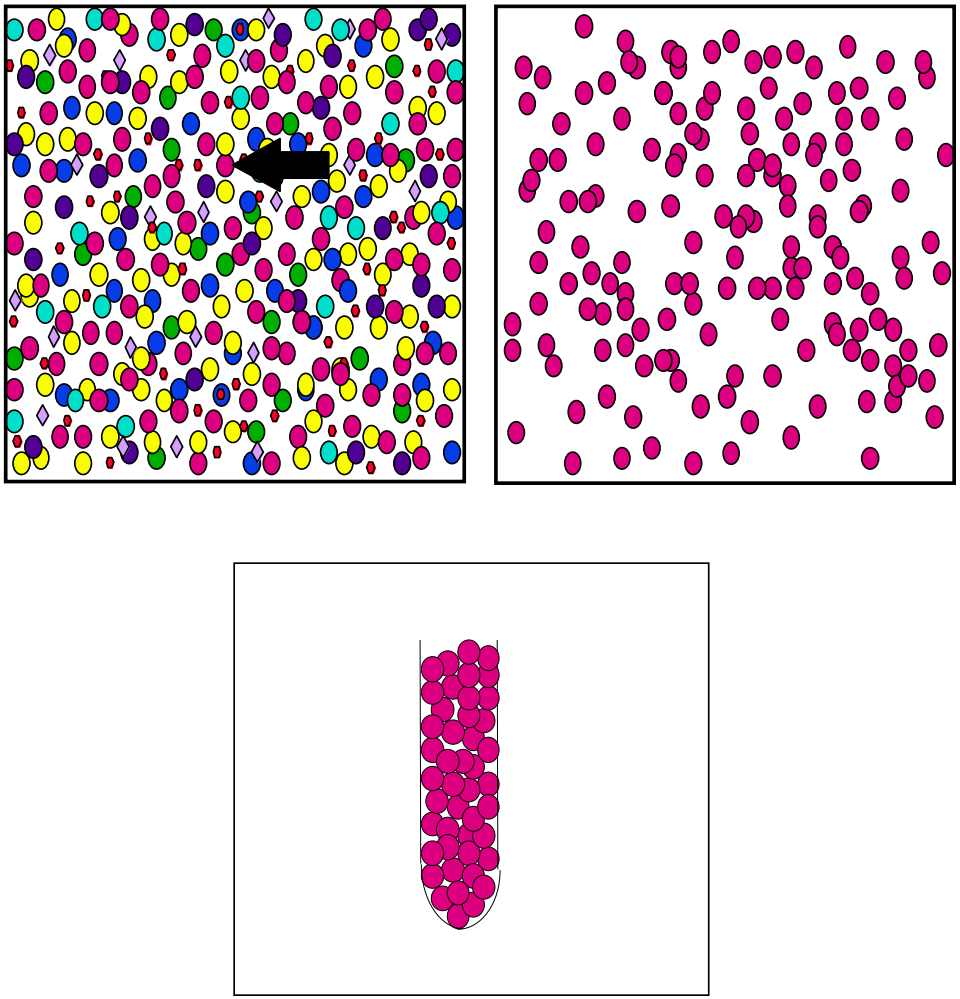
Protein purification

Low abundance



Protein purification

High abundance



Methods for protein purification

3. Make an extract from source

- almost always want to keep it very cold (just above freezing)
- gentle breaking
- in some cases can obtain big purification in one step by separating cellular compartments (e.g., purify nuclei from cytoplasm before extracting nuclear proteins)

4. Begin separating components

- remove nucleic acids, polysaccharides, cell membrane debris
- ammonium sulfate precipitations, other crude fractionations (pH or other salt precipitations, antibody clearing, "autolysis")
- these crude steps are often needed to avoid ruining or overloading chromatography agents

Methods for protein purification

5. Fractionate by chromatography

- several steps are almost always needed
- need to assay for amount and purity at each step
- need a way to decide when you're finished

Separation principles

Sizing

- principle is based on exclusion of larger molecules from pores in resin; smaller molecules require longer times of transit
- sizing resins = "gel filtration"
- different matrices have different size ranges
- examples: Sephadex, Sephacryl, Ultrogel, some HPLC resins

Ion exchange

- separate on the basis of net charge
- wash and elute with higher salt concentrations
- determine highest [salt] that your protein binds to the resin, and use this to load or to pre-wash before eluting your protein
- examples: DEAE cellulose, phosphocellulose, some HPLC resins

Affinity chromatography

- separate based on binding to residues specific or semi-specific to the protein you are purifying
- easy to overload with non-specific proteins
- examples: sequence-specific DNA sites, antibody resin

Purification table

Purification of DNA

- DNA
  - many types you might want to purify (genomic, plasmid, viral, particular segment of genome, some linear, some circular)
  - pretty hardy, so fairly harsh methods can be used
  - specificity of desired segment is relatively easy to assess

- Assays for purification of DNA molecules
  - functional assays
    - complementation in a microorganism or tissue culture cell (usually must be mutant, or missing)
    - Inhibition of function in a microorganism or tissue culture cell
  - hybridization
    - must already know something about the DNA sequence
    - blots, in situ hybridization, microarray hybridization
  - PCR
    - especially helpful for gene families, related sequences
  - DNA sequencing
    - particularly possible now because of huge databases from genome sequencing projects

Purification of RNA

- **Assays**
  - why? mRNA purification or enrichment, snp's, enzymatic RNAs, structural RNAs
  - not as hardy as DNA, although easier to work with than proteins if special care is taken to avoid RNases
  - slight chemical difference between RNA and DNA makes biochemical properties quite different (don't heat with Mg+2)
  - most RNAs are single stranded, but with much secondary (double stranded) character

- **Assays for purification of RNA molecules**
  - cDNA and EST libraries
    - screen by PCR, hybridization, direct sequencing
  - gel assays (Northern blotting)
  - *in situ* hybridization
  - functional assays
    - microinjection
    - inhibition

Food for Thought

What are the pitfalls/caveats of purification approaches?