

## 3.1: Protein Purification

### Assays, Specific Activity, Initial Fractionation

A successful protein purification procedure can be nothing short of amazing. Whether you are starting off with a recombinant protein which is produced in *E. coli*, or trying to isolate a protein from some mammalian tissue, you are typically starting with gram quantities of a complex mixture of protein, nucleic acids, polysaccharide, etc. from which you may have to extract milligram (or microgram!) quantities of desired protein at high purity, and hopefully with high yield.

The first step in any purification is the development of a **specific assay** for the protein of interest. The specific assay can be based upon some *unique characteristic* of the protein of interest

- Enzymatic activity
- Immunological activity
- Physical characteristics (e.g. molecular mass, spectroscopic properties, etc.)
- Biological activity
- Ideally, an assay should be
  - **Specific** (you don't want a false positive)
  - **rapid** (you don't want to wait a week for the results)
  - **sensitive** (you don't want to consume all your sample in order to assay it)
  - **quantitative** (you need an accurate way to measure the quantity of your protein at each step in the purification)

Protein purification can be thought of as a series of fractionation steps designed so that:

- The protein of interest is found almost exclusively in one fraction (and with good yield)
- A significant amount of the contaminants can be found in a different fraction

During purification you will need to monitor several parameters, including:

1. **Total sample volume**
2. **Total sample protein** (can be estimated by  $A_{280}$ ;  $1.4 \sim 1.0$  mg/ml)
3. **Units of activity of desired protein** (based on specific assay)

This basic information will allow you to keep track of the following information during each step of purification:

1. **% yield** for each purification step
2. **Specific activity** of the desired protein (units/mg total protein)
3. **Purification enhancement** of each step (e.g. "3.5x purification")

In designing a purification scheme you typically have to balance **purification** with **yield**.

- For example, it may be relatively straightforward to obtain 90% pure material with good yield.
- However, it may be difficult to improve that purity an additional few percentile with good yield.
- The planned application of the purified protein determines the target purity.
- If the protein is to be used to determine amino acid sequence information, maybe 90% is acceptable. However, if the material is to be used in clinical trials, 99.99+% may be the target purity.

### Initial steps in purification

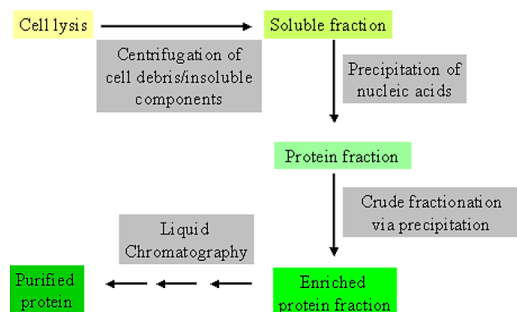


Figure 3.1.1: Purification steps

It is extremely helpful to have some information not only on the general physical and chemical characteristics of the protein you are trying to purify, but also on the *contaminating* components. For example, many *E. coli* proteins are generally *low molecular weight* (<50,000 Da) and somewhat *acidic* in isoelectric point. Usually the initial steps in purification make use of general physical and/or chemical differences between soluble proteins and other cell components. Soluble proteins can be separated from general cellular debris, and intact cells, by *centrifugation*. Thus, cells are physically disrupted (via homogenization or a cell press) to allow release of cell contents. This is then followed by centrifugation to separate generally soluble components from those which are insoluble.

## Monitoring the Purification Process

- There are several criteria. One criteria is that we cannot improve upon the *specific activity* of our sample. This value refers to the *functional activity of our sample in relationship to the total protein concentration of the sample*.
- In the initial stages of purification this value will be low (not much activity in relationship to the total amount of protein).
- This value will increase after each purification step as we *remove* other proteins from the sample.
- At some point the specific activity will *plateau*, and by definition, *if it is pure we cannot increase the specific activity*.
- There may be a published value for the specific activity which we can compare ours to.

Also, each step of the purification should be monitored by gel electrophoresis.

The following chart represents the typical data one would monitor during a purification:

**Table 3.1:** Monitoring the Purification Process

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	% Yield
Crude cell lysate	5500	6600	1.2		
30-70% Ammonium sulfate cut	1020	5910	5.8	4.8	89.5
DEAE Sephadex pool	187	5070	27.1	4.7	85.8
CM Sephadex pool	102	4420	43.3	1.6	87.2
Phenyl Sepharose pool	56	3930	70.2	1.6	88.9
Gel Filtration pool	32	2970	92.8	1.3	75.6
Affinity resin type #1 pool	5.8	2520	434.5	4.7	84.8
Affinity resin type #2 pool	5.3	2390	450.9	1.0	94.8
Total purification	376				

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	% Yield
Total yield (%)	36				

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