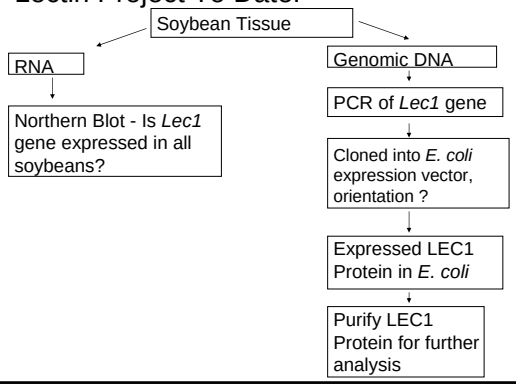
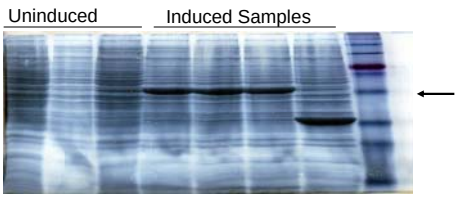
Purification of bioengineered proteins

CPSC 265
Week 12

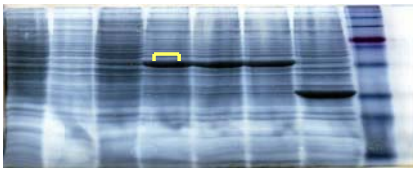
Lectin Project To Date:



Expression of LEC1 protein in *E. coli*



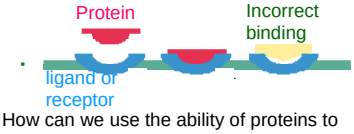
We want to work with pure LEC1 protein. How do we purify the LEC1 protein from all the other *E. coli* proteins?



It would be easier to “grab” the LEC1 protein out of solution if we had a specific handle, or “tag” on the protein.

Affinity chromotography takes advantage of special “tags” or interaction properties of proteins to allow for rapid purification from a protein extract.

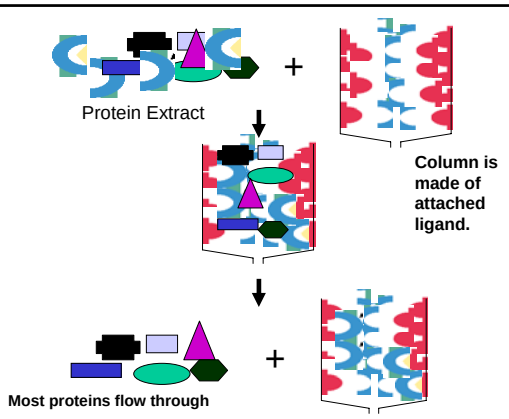
Lock and Key Model

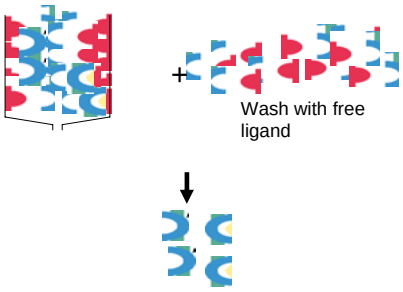


How can we use the ability of proteins to interact specifically with ligands to purify proteins of interest more easily?

Affinity Chromatography:

1. Attach the ligand to an insoluble matrix.
2. Add the protein extract.
3. Remove all proteins except the what is bound specifically to the ligand.
4. Then remove the bound protein from the ligand in a purified state!



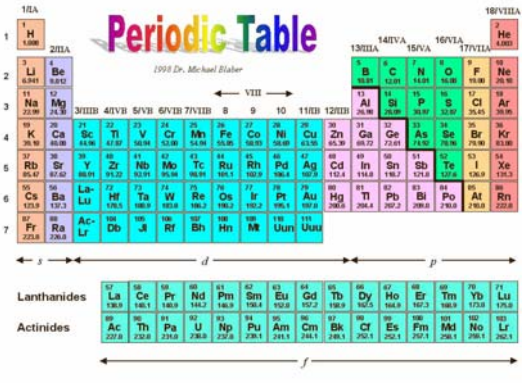


Examples of Affinity Chromatography

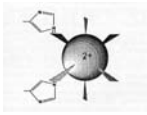
1. Antibody columns - for specific antigens
2. Cellulose columns - for cellulases
3. Starch columns - for amylases
4. DNA columns - for DNA binding proteins
5. Ligand columns - for specific receptors
6. Metal columns - for proteins that bind metal ions

IMAC, or Immobilized Metal ion Affinity Chromatography

IMAC takes advantage of the ability to histidine residues to interaction with various transition metal ions including Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}



Group 8 transition metal ions such as cobalt and nickel have six orbitals available for binding to histidine. But, you need six histidine residues close together.



When we expressed our lectin protein, we expressed it as a fusion protein, with extra amino acids.

What extra amino acids did we add to our LEC1 protein?

HP-Thioredoxin	EK Site	LEC1	V5	6X HIS

What is all this extra protein, and why do we need it?

1. HP-Thioredoxin: Thioredoxin increases the solubility and stability of foreign proteins in *E. coli*.

2. EK site: Amino acid sequence recognized by enterokinase (a protease). Allows for the removal of the thioredoxin part of the protein.

3. LEC1: the lectin protein we are interested in.

4. V5 epitope: An amino acid sequence recognized by the V5 antibody. Useful for western blot detection.

5. 6X His: Six histidine residues, which are used for affinity purification.

Is it a coincidence that we added all those extra amino acids?

We will use a metal affinity resin to purify our lectin protein with the 6xHis tag.

Metal Affinity Resin

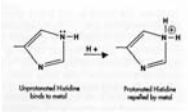
Protein with 6xHis Tag

Our resin uses cobalt, not nickel as the metal ion. This results in *quicker* purification and *cleaner* proteins.

We also used a "batch" protocol, rather than a column. Again, this is quicker and easier to do. (We don't have to wait for the liquid to drip through the column.)

Columns or Proteins can be eluted in 3 ways!

1. Lower the pH.



2. Add excess imidazole

3. Add EDTA to remove metal ion from purification resin.

The purified protein is now ready for further use, including western blotting (tonight and next week) and biochemical studies!