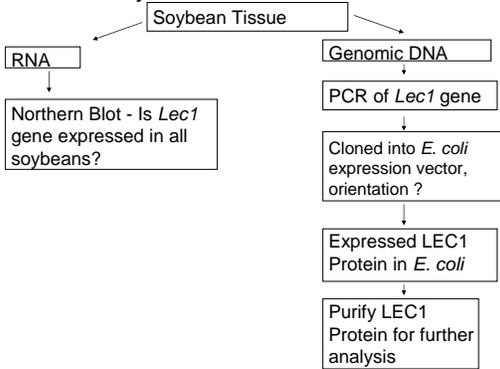


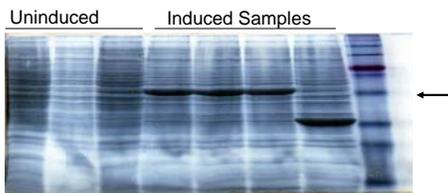
Purification of bioengineered proteins

CPSC 265
Week 12

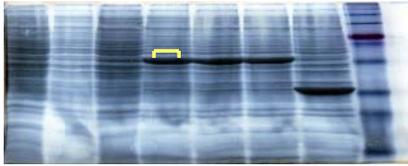
Lectin Project To Date:



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

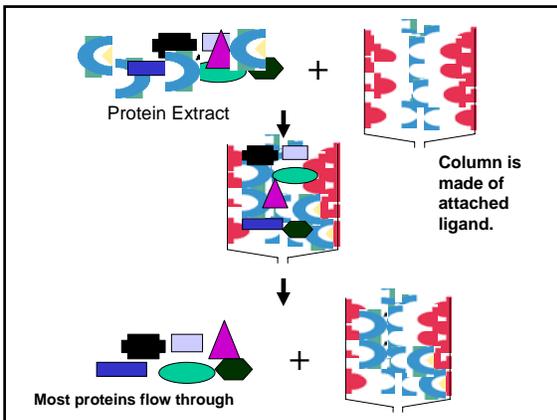
Lock and Key Model

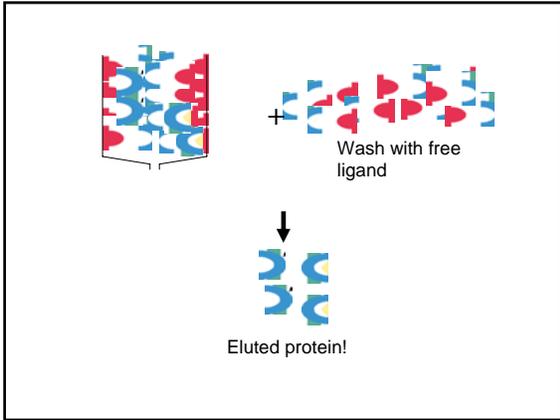


How can we use the ability of proteins to interact specifically with ligands to purify proteins of interest more easily?

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

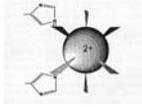
IMAC takes advantage of the ability to histidine residues to interaction with various transition metal ions including Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+}

1908 Dr. Michael Eilsher

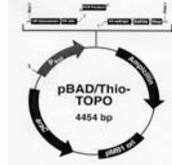
Periodic Table

1 1H 1.008	2011A																2 2He 4.002
3 3Li 6.941	4 4Be 9.012	VIII										5 5B 10.81	6 6C 12.01	7 7N 14.01	8 8O 16.00	9 9F 18.99	10 10Ne 20.18
11 11Na 22.99	12 12Mg 24.31	VIII										13 13Al 26.98	14 14Si 28.09	15 15P 30.97	16 16S 32.07	17 17Cl 35.45	18 18Ar 39.95
19 19K 39.10	20 20Ca 40.08	21 21Sc 44.96	22 22Ti 47.88	23 23V 50.94	24 24Cr 52.00	25 25Mn 54.94	26 26Fe 55.85	27 27Co 58.93	28 28Ni 58.69	29 29Cu 63.55	30 30Zn 65.39	31 31Ga 69.72	32 32Ge 72.64	33 33As 74.92	34 34Se 78.96	35 35Br 79.90	36 36Kr 83.80
37 37Rb 85.47	38 38Sr 87.62	39 39Y 88.91	40 40Zr 91.22	41 41Nb 92.91	42 42Mo 95.94	43 43Tc 98.91	44 44Ru 101.1	45 45Rh 102.9	46 46Pd 106.4	47 47Ag 107.9	48 48Cd 112.4	49 49In 114.8	50 50Sn 118.7	51 51Sb 121.8	52 52Te 127.6	53 53I 126.9	54 54Xe 131.3
55 55Cs 132.9	56 56Ba 137.3	57 57La 138.9	58 58Ce 140.1	59 59Pr 140.9	60 60Nd 145.0	61 61Pm 144.9	62 62Sm 150.4	63 63Eu 152.0	64 64Gd 157.3	65 65Tb 158.9	66 66Dy 162.5	67 67Ho 164.9	68 68Er 167.3	69 69Tm 168.9	70 70Yb 173.0	71 71Lu 175.0	
73 73Fr 223.0	74 74Ra 226.0	75 75Ac 227.0	76 76Th 232.0	77 77Pa 231.0	78 78U 238.0	79 79Np 237.0	80 80Pu 239.0	81 81Am 243.1	82 82Cm 247.1	83 83Bk 247.1	84 84Cf 251.1	85 85Es 252.1	86 86Fm 257.1	87 87Md 258.1	88 88No 259.1	89 89Lr 262.1	
← s		d										p					
Lanthanides																	
Actinides																	
		f															

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?

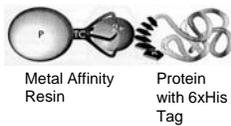


What is all this extra protein, and why do we need it?

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.

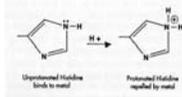


Our resin uses cobalt, not nickel as the metal ion. This results in *quicker* purification and *cleaner* proteins.

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!
