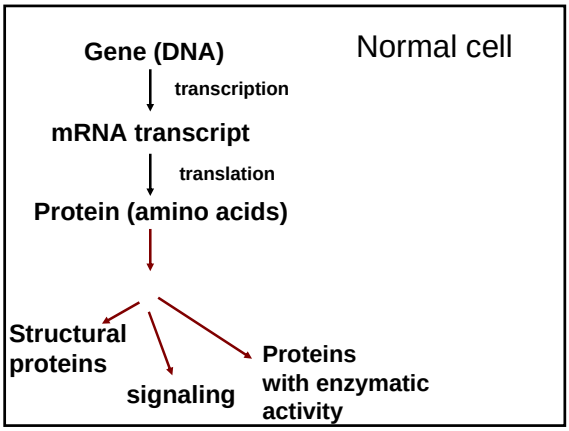
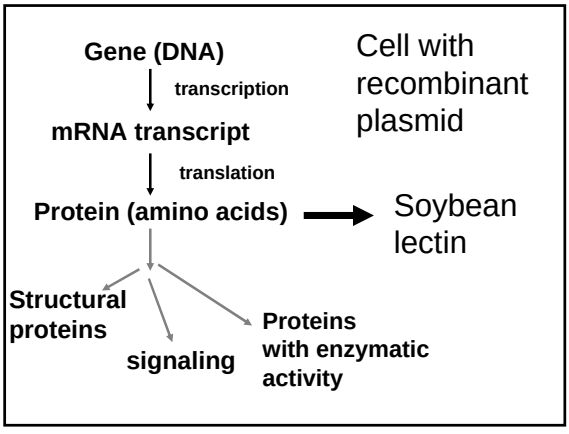
Expression and analysis of recombinant proteins in E. coli

Class 11
CPSC265



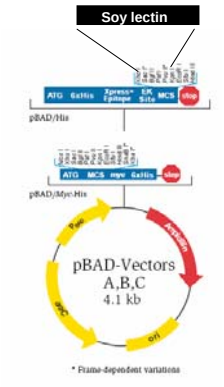


Inducible gene expression

- So that we don't stop the cells growing (or kill them) we like to grow the cells without our protein being expressed, then switch it on when there are plenty of cells
- We do this by manipulating the *transcription* of the mRNA for the protein from our plasmid

Inducible gene expression (cont)

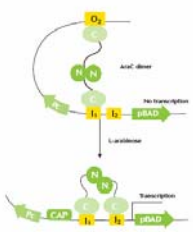
- E. coli naturally keeps some genes turned off. For example, it turns on the genes needed to metabolize galactose, or arabinose, only when these sugars are present.
- By cloning the *promoter* for the *operon* containing the arabinose or galactose genes in front of our gene in the plasmid, we can keep it turned off until we are ready. Then we add the sugar, and the gene is turned on.



pBAD uses the arabinose promoter

Regulation of pBAD

Figure 1 - Regulation of the *araBAD* promoter

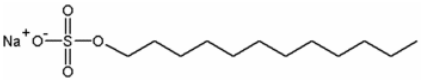


pBAD is regulated by the product of the *araC* gene (N), a transcriptional regulator that forms a complex with L-arabinose. In the absence of arabinose, the AraC dimer contacts the O2 and I1 half sites of the *araBAD* operon, forming a 210 bp DNA loop (Figure 1).

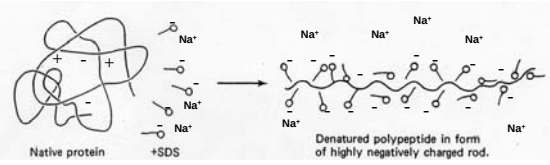
What I did already this week

- Grew a liquid culture E. coli containing the pBAD plasmid with soybean lectin gene.
- When the E. coli cells were abundant, but still growing rapidly, (OD 0.5) I added arabinose to 0.2% concentration
- I incubated the “induced” cells for 4 hours

Denaturing SDS protein gels



anions of SDS bind to the peptide chain at a ratio of one SDS anion for every two amino acids

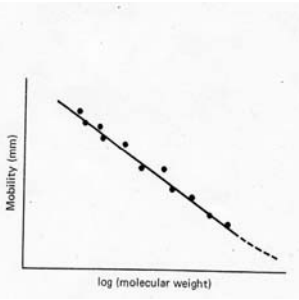


In an SDS gel, migration is proportional to molecular weight

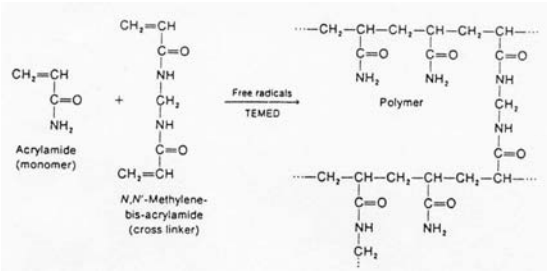
One negative for every 2 a.a.s massively outweighs the native charge of the protein

Otherwise proteins would not behave like this.

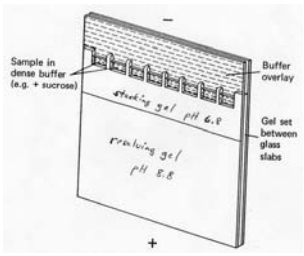
They would migrate towards either electrode, dependent on pH, composition and structure

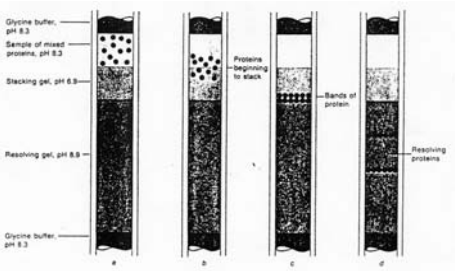


Using acrylamide, we can make a polymer full of hydrophilic pores. The size of the pores is proportional to the % of acrylamide



Laemmli (discontinuos) SDS protein gels





Stacking gel concentrates proteins into narrow, well-defined bands. Resolving or separating gel resolves them by molecular size.

Coomassie staining

- You can view the proteins in the gel by staining with coomassie blue
- This is a dye that binds all proteins regardless of their amino acid makeup
- Fortunately it is bright blue – no UV required

What you will do today

- Take culture and spin down
- Extract, denature and reduce disulfide bridges of all proteins
- These are done simultaneously by boiling in SDS + beta mercaptoethanol

Today (cont.)

- Separate the proteins by size using denaturing, discontinuous SDS electrophoresis
- Look for the induced protein using coomassie blue staining