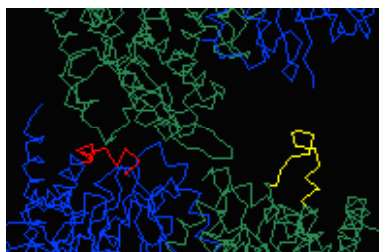
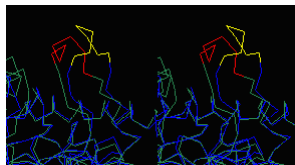
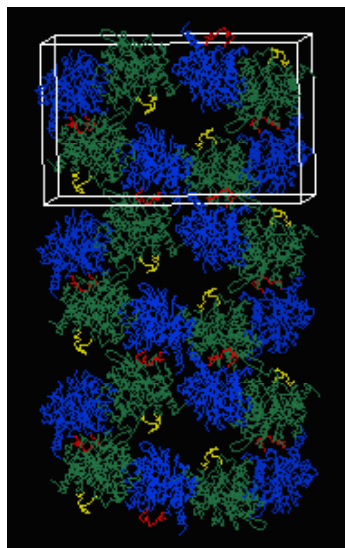
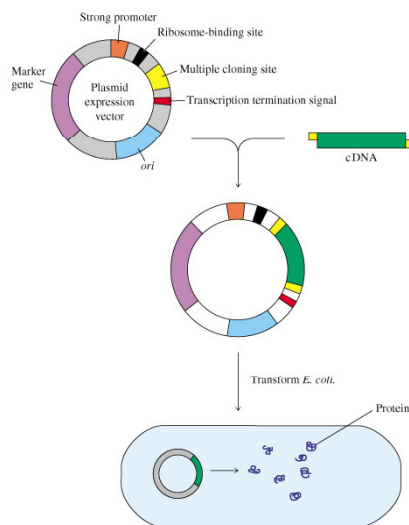


Class overheads for Protein Homogeneity, CHEM 645



<http://molvis.sdsc.edu/protexpl/xtlcon.htm>

Overview of Prokaryotic Expression



➤ Strong promoter – P_{lac}

➤ Ribosome binding – Shine-Dalgarno sequence
~ 7 b.p. before start codon:
AUG

➤ Multicloning site to put
your gene in with correct
frame and direction.

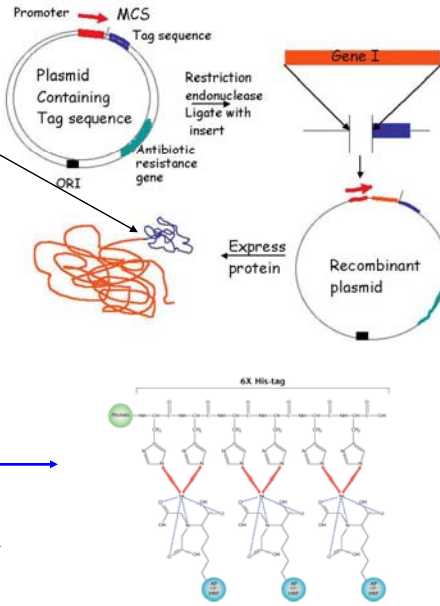
Class overheads for Protein Homogeneity, CHEM 645

Affinity Chromatography using fusion proteins

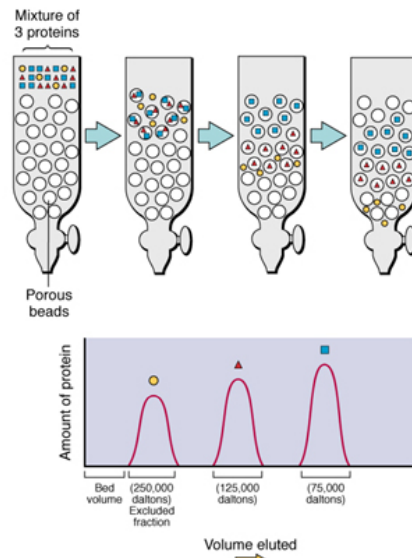
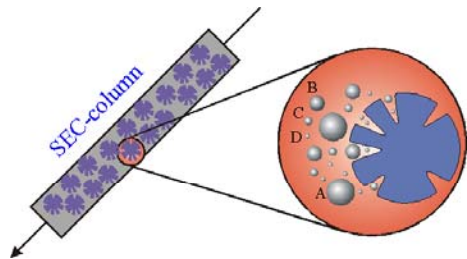
- Construct a fusion of affinity tag with your protein
- Add a protease cleavage site (thrombin)
- Express fusion protein
- Purify by affinity chromatography
- Cleave tag

Examples:

His-tag, →
 GST fusion,
 maltose binding protein fusion

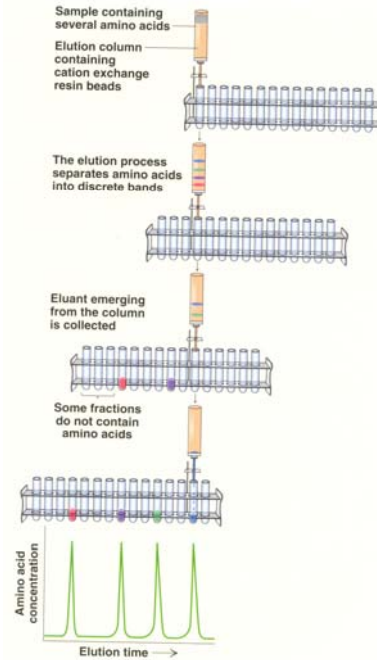
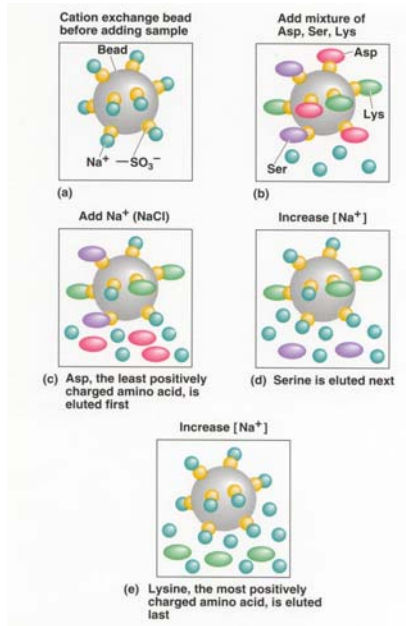


Gel Filtration (or size exclusion) Chromatography

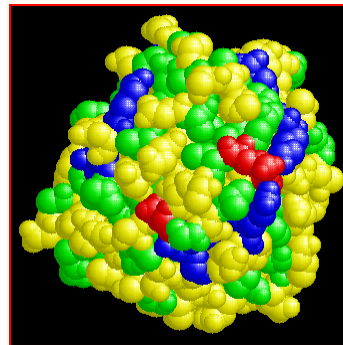
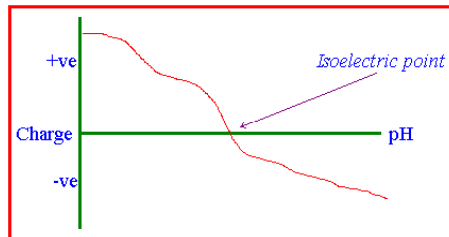


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Ion Exchange Chromatography

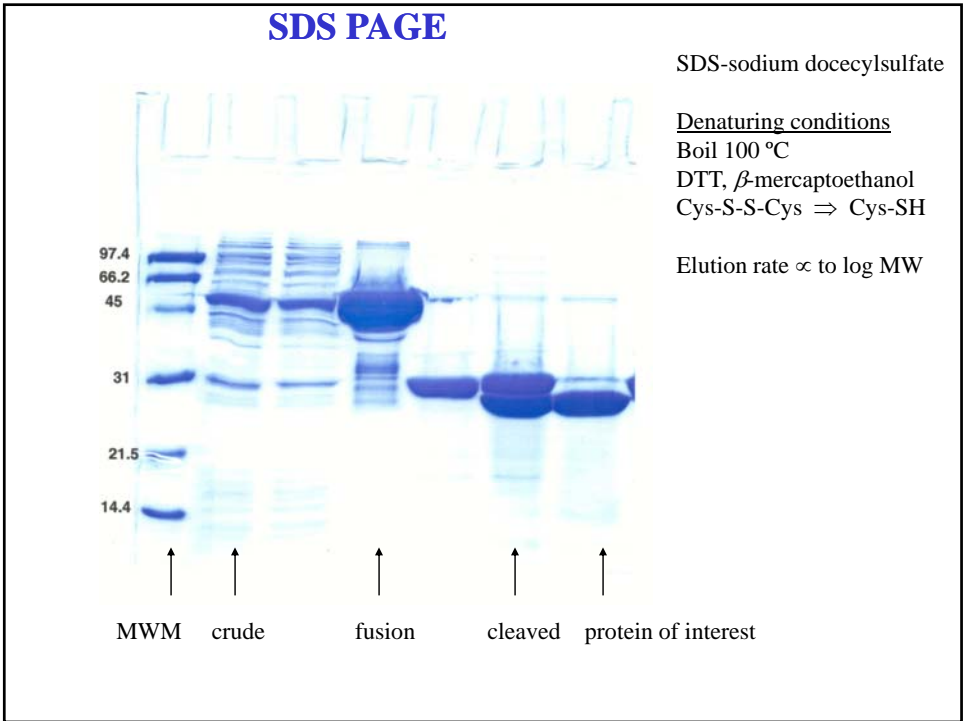
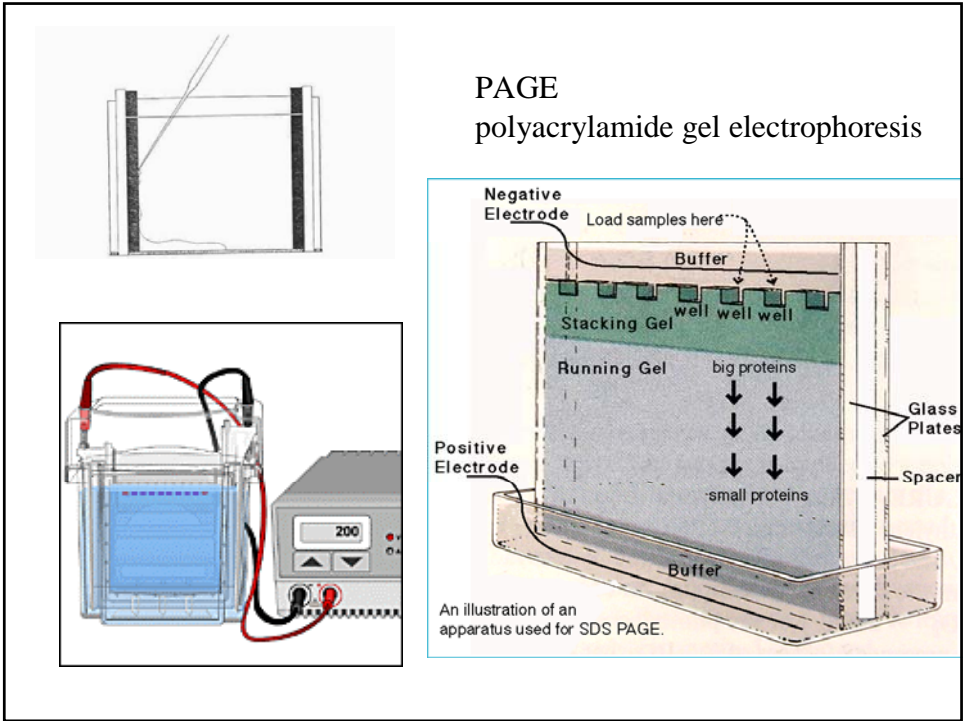


Protein's isoelectric point



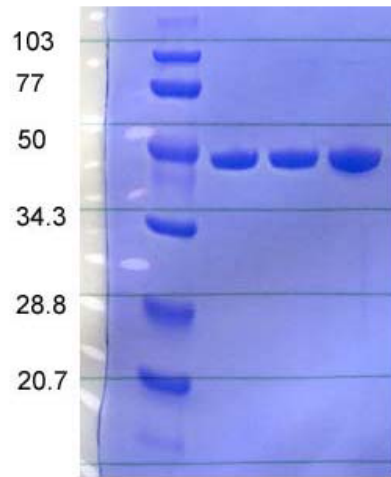
Blue – pos.
 Red – neg.
 Yellow - polar

<http://binfo.ym.edu.tw/bioflash/emboss/iep/iep.htm>

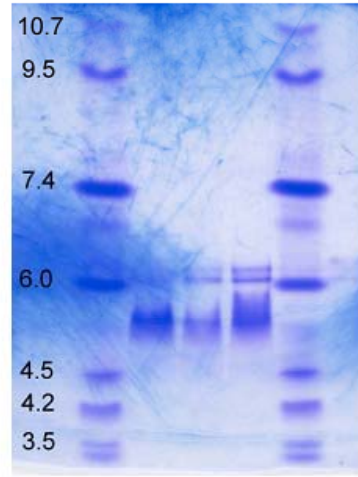


Don't ever be too sure that its pure enough!

SDS PAGE

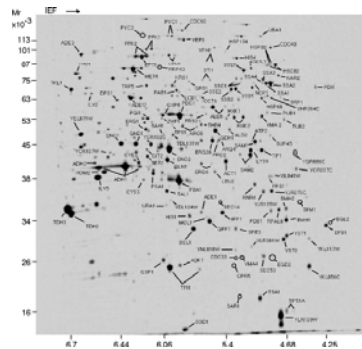
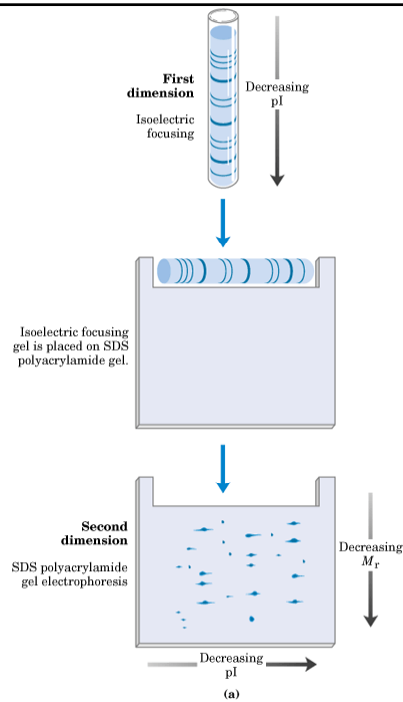


IEF



Plasma Platelet Activating Factor Acetylhydrolase gels from Bahnson lab

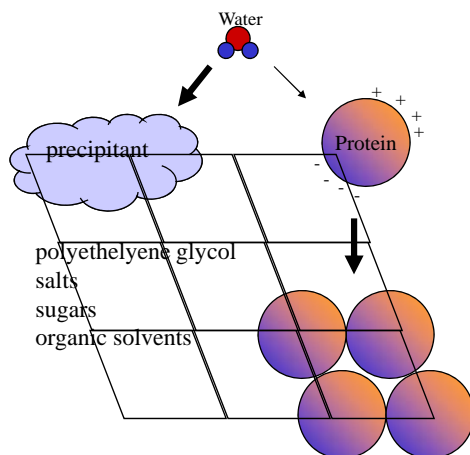
2D PAGE
IEF followed by SDS PAGE



Homogeneity / Heterogeneity

- A. Post translational modification – examples:
phosphorylation, glycosylation, myristoylation
- B. Chemical modifications – cysteine oxidation, Asn/Gln
hydrolysis
- C. Aggregation, unfolding
- D. Order / disorder
- E. Alternate Conformations – malate dehydrogenase loop

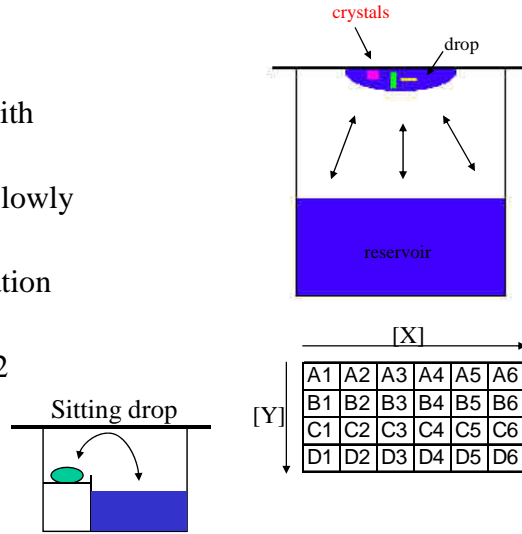
How Do Proteins Crystallize?



- For crystallization to occur it has to be thermodynamically favorable
- Precipitants remove available water forcing proteins to associate with each other
- Hopefully in a organized fashion

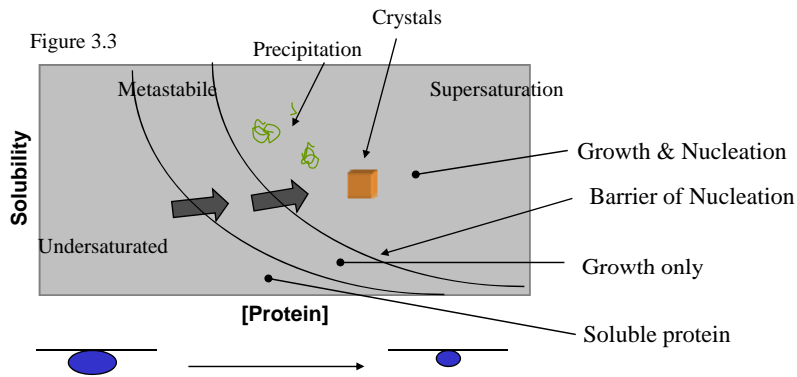
Growing Crystals: Hanging Drop Method

- Widely used
- Vapor diffusion
- Drop equalizes with reservoir
- Volume of drop slowly decreases
- Protein concentration slowly increases
- CMCC Figure 3.2



Phases of Proteins In Solution

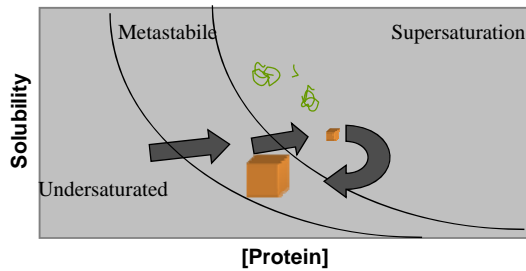
Not to be confused with phases of light



CMCC figure 3.3

Nucleation & Growth

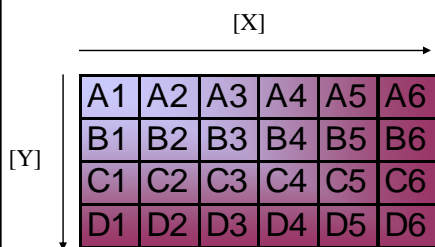
Figure 3.3 Phase diagram



Basic concept:

1. Concentrate solution enough so nucleation occurs in only a few cases
2. Initial growth pulls some protein out of solution
3. Reducing [protein] back into metastable range
4. Grow only a few large crystals

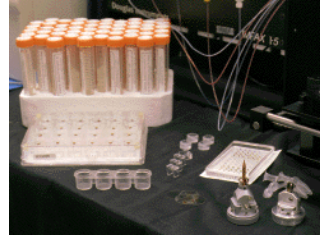
Optimize Crystal Growth



- The number of factors can be overwhelming
- Focus on those factors which most effect growth
- Set up arrays to vary two different conditions at once
- Cross your fingers

Crystal Screens

- Hampton Research screen tests a wide assortment of conditions of salts, buffers, pH's and additives
 - Best conditions from literature
- Often first hits with screens are small poor quality crystals
- Do not use the absence of crystals as a gauge of conditions rather use solubility



Factors Effecting Crystal Growth

***Most important**

- | | |
|-------------------------------------|-------------------------------------|
| • Ionic Strength* | • Vibrations |
| • Specific Ions (Ca ²⁺) | • Pressure |
| • Protein Concentration* | • Gravity |
| • Detergents | • Relative Proportion of Conditions |
| • Inorganic Precipitant | • Purity Of Protein* |
| • pH* | • Access to water* |
| • Temperature* | • Ligands |
| • Time | • Binding partners |
| • Monodispersion* | |

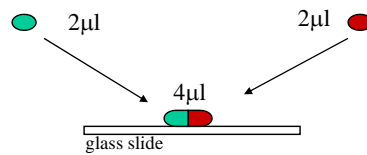
Lysozyme Demonstration

Buffer

30% w/v Polyethelene glycol 5000
1 M NaCl
50 mM NaAcetate pH 4.5

Lysozyme Protein

100mg/ml
50 mM Na Acetate pH 4.5



Should make large crystals in less than 15 minutes.
We will give it a shot up in 314 Drake Hall