**Microscopes and Proteomic Techniques – Jan. 11**

Translational science: from bench to bedside

Looking closely and into cells

Phase/DIC -> changing optical path to see more \*resolution is extremely important

Techs: Electron Microscopy, Fractionation, Microarrays, Two Hybrid Analysis



**Fractionation**

- cell plasma membrane must first be “homogenized” by:

i) sonification: using sound to homogenize cells -> high frequency disrupts

 plasma membrane (doesn’t do anything to prs though)

ii) sheared: use stick to shear cells or use serrated glass

iii) pressure: French press -> increased pressure causes cell to explode

iv) detergents: poke little holes in bilayer and contents leak out i.e. Triton X-100

- then the contents are separated by Centrifugation

- once homogenized, often buffer is added as a “cushion” i.e. sucrose

**Centrifugation (p.392)**

1. Differential – crude separation

- spinning homogenate yields pellet and supernatant,

force needed to pellet things to bottom of tube

- increasing centrifugal force (gravity) to isolate organelles

based on mass

- heavy organelles i.e. nuclei pellet at low force of gravity (600 g)

- removing supernatant and a 1.5 x 104 g force to get mc and

lysosomes

- 3 x 105 g needed for ribosomes

1. Equilibrium Density-Gradient – finer, analytical separation,

-> need ultracentrifuge

- homogenate is applied to a gradient of sucrose

- separation based on density

- can put supernatant on top of sucrose gradient

 - sucrose gradient made by mixing diff concentrations of sucrose

- at high speed, organelles migrate to sucrose layer equal their

own density

- maintain stratified as long as you don’t shake it

- extraction: poke hole, use fraction collector and use spectrometer to know what density of sucrose

**Centrifuges**

 - smalls ones to get crude fractions

 i.e. bits of organelles/

 plasma membrane

 - gel electrophoresis is a good method to separate proteins from organelles but proteins are DENATURED

**Biologically-Active Protein Isolation by Column (Liquid) Chromatography used to get pure protein fraction**

- uses matrix of special beads; homogeneity = 100% pure

- isolation based on mass (size), charge, or ability to bind to other prs

Detergents and Solubilization

- ionic: SDS disrupts bilayer and attaches itself, disrupts ionic and H bonds -> denaturing pr

- non-ionic -> doesn’t denature protein but disrupts bilayer i.e. Triton X

3 Types: 1. Gel filtration -> isolation based on mass (size); based SOLELY on molecular mass

\*\*\* - molecular exclusion beads have pores of specific size

 - small prs get trapped in pores while prs larger than pores migrate faster

 - wash off (elute) and collect

 2. Ion-Exchange -> isolation based on charge

 - beads carry a specific charge

 - proteins are attracted or repelled by charge (repelled ones come out first)

 - elute (w. salt solution) bound proteins by changing charge

 3. Affinity -> isolation of proteins that **bind** to bead-linked antibody

 - buy antibody and attach it to bead (1 antibody specific to 1 protein)

 - proteins bind regardless of mass or charge

 - sample is washed, then proteins eluted by altering pH

 - cookie batter disease: hen produces antibacterial called avidin which binds to biotin (vitamin)

 - for kids, it will bind to biotin -> Avidin denatured when baked

Further purification of prs

- need purified pr cuz any contamination could kill you (testing for homogeneity)

- denatured -> non-functional

- Native/biologically active -> normal



- all polypeptides flooded with

same negative charge

- all negatively charged prs

move from cathode to anode

- separation based solely

on **mass**!

- compare unknowns

with standards

- all prs in stacking gel then

migrate into separating gel

- can see brom blue running

- once brom blue gets to

bottom, separate glass plates

**Visualization**

- dye physically binds to pr

-need chemical stain/dye to see it

- silver is 10 times more sensitive

- homogeneity would be indicated by a single band

- the prs here are not biologically active