7.02 Fall 2001 PBC Day 3 Recitation

Version 1.02

I. Today in Lab
   a. Equilibrate APTG column
   b. Load DEAE/AF load
   c. Run AF column
   d. Contemporaneously, equilibrate PD-10 column
   e. Qualitative assay on AF fraction, load on PD-10.
   f. Assays and calculation

II. Affinity Chromatography
   a. Separation based on affinity for substrate.
   b. What substrate would you want to use for β-gal?
      i. Analog of lactose—bound by β-gal.
      ii. *Shouldn't be cleaved*
   c. APTG (Draw) thio analog can t be cleaved by β -gal.
   d. Other considerations.
      i. Binding must be reversible
      ii. Specificity! Non-specific binding of analog may lead to high background. Other proteins that may bind?
         1. Galactose binding proteins transporters, storage proteins.
         2. Other enzymes envolved in sugar metabolism
         3. Proteins that bind β-gal! (This is sometimes useful!)
   e. Running the column
      i. Binding **What buffer is the protein in for binding?**
      ii. Washes. **Why?** *Remove stuff not sticking to APTG…*
      iii. Elution. Can be accomplished in 2 methods. **Which one do we use?**
         1. Competition. Knock-off by binding lactose analog in buffer to β-gal.
            a. Advantage won t cause activity loss via protein denaturation.
            b. Disadvantage analog will compete with reaction substrate. How to get rid of analog?
         2. Disrupting interactions by changes in salt, pH, etc.
            a. Advantage easy to switch back to normal via PD-10 column or dialysis.
            b. Disadvantage may cause protein denaturation and loss of activity.
         3. We use the disruption method. **What is so special about our elution buffer that disrupts the interaction?** *Answer: presence of borate ion and pH 10—destabilizes and partially unfolds β-gal.* **Do we use high pH to disrupt β-gal interaction anywhere else in this module?** *Answer—when stopping β-gal assays, use sodium carbonate, pH 12.*
      iv. **High salts/buffer pH are bad. How do we solve this? How did we get rid of unwanted salts before?** *Answer: run over another PD-10 column.*
   f. Applications of Affinity Chromatography. How can we use AF chromatography to isolate a protein with an unknown substrate? Make a fusion protein
      i. Insert gene encoding known protein in the open reading frame of the protein of interest.
      ii. When transcribed and translated, fusion protein composed of your protein plus a tag is formed.
      iii. Fusion protein can be isolated base on affinity of tag .
      iv. Tag can be removed once fusion protein is purified through use of proteases.
      v. Some common protein tags:
         1. GST (glutathione-S-transferase) binds glutathione. (Use glutathione affinity column to purify.)
         2. (His)6 Binds zinc.
         3. MPB (maltose binding protein) binds

III. Review of chromatography

Column Type	**Example used in Module**	**Separation based on:**	**Elution conditions**	**Elution order**
Gel Filtration	PD-10	size	Lots and lots of buffer	High MW-first. Low MW-last.
Ion Exchange	DEAE-cellulose	Charge (in this case, negative charge)	Compete of with ion of same charge	Un-charged or positive first, negatively charged last with increasing salt concentration.
Affinity	APTG-agarose	Affinity for substrate	Alter salt/pH, compete off with substrate	Low affinity first. High affinity elutes with altered buffer conditions.