## Overview and Considerations:

This is a useful technique for immobilizing proteins to beads. This technique relies on your protein having free amines. If you are coupling peptides, a resin that contains a linker may be more appropriate.

We have two types of CNBr activated Sepharose: 4B is appropriate for protein-protein interactions, whereas 6B is more appropriate for enriching organelles/cells.

**Ligand Preparation:** Ligands to be coupled should be dissolved in, or dialyzed into, coupling buffer. Note, ideal coupling is obtained if the ligand concentration is between 5-10mg/ml, though decent coupling is achieved at >2mg/ml. (We almost always use 2mg/ml)

**Capacity:**

As the column couples to amino groups, capacity is dependent on the number of amino groups in your protein of choice. For chymotrypsin, the capcity is reported to be 20-60mg/ml. In addition, capacity will be affected by coupling efficiency. I usually assume the capacity is 5mg/ml coupled.

## Reagents:

1. Buffers:
2. Coupling buffer:
   * 200mM Sodium Bicarbonate, 500mM NaCl, pH8.3 (note: bicarbondate buffer goes off quickly, as such should be made within 1 day of coupling).
3. 1mM HCl: Used to activate the resin. Should be ice-cold.
4. Blocking buffer:
   * Sepharose 4B: 1M ethanolamine, pH8.0
   * Sepharose 6MB::= 200mM Glycine in 200mM Sodium Bicarbonate, 500mM NaCl, pH8.3 (After dissolving the glycine in coupling buffer, adjust the pH back to 8.3)
5. Low pH wash buffer: 50mM Sodium Acetate, 500mM NaCl, pH 4.0 (**5 column volumes**)
6. High pH wash buffer: 50mM Tris-HCl ,500mM NaCl, pH8.0 (**5 column volumes**)
7. Storage buffer: PBS, 0.01% Sodium Azide

## Protocol:

1. Perform a protein estimation on your samples, determine how many mg/ml as well as the volume of your samples.
   * Take 100ul of sample and dilute it to 1ml with coupling buffer. Measure the A280. Do not discard this sample.
2. Determine the amount of CNBr-activated sepharose you need. Remember that 1g swells to ~3.5mls of resin.
3. Swell resin for 30min on ice, in 1mM HCL.
   * Use **5 column volumes** of HCL per ml of resin.
   * Thus, is you resuspend 1g, you will use 17.5 mls.
4. Wash column extensively with 1mM HCL to remove impurities (**20 column volumes**)
   * Typically we do this in a Biorad Econocolumn
5. Add ligand. Incubate with mixing at **room temperature** for **90minutes**, or **overnight** at **4oC**.
6. Drain ligand and measure A280.
   * Measure start A280, which you diluted 1/10, again and determine coupling efficiency.
   * If the coupling efficiency is less than 90% you can incubate the samples longer.
   * If no additional binding is observed in 1h, you have saturated the column.
7. Block the column with **5 column volumes** of 1M Ethanolamine, pH8.0. Incubate at **room temperature for 2-4h** with mixing.
8. Wash the resin with **3 cycles** of a low/high pH washes
   * Low pH: 50mM Sodium Acetate, 500mM NaCl, pH 4.0 (**5 column volumes**)
   * High pH: 50mM Tris-HCl ,500mM NaCl, pH8.0 (**5 column volumes**)
9. Store columns in PBS, 0.05% Sodium Azide at 4oC.