
Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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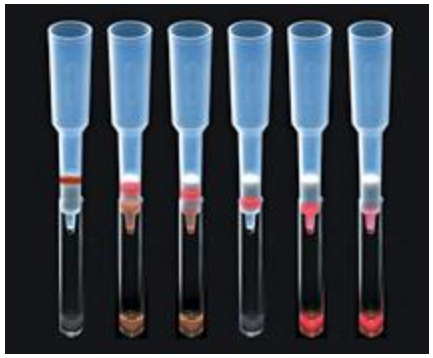
Size Exclusion Chromatography

Size Exclusion Chromatography

Instructors



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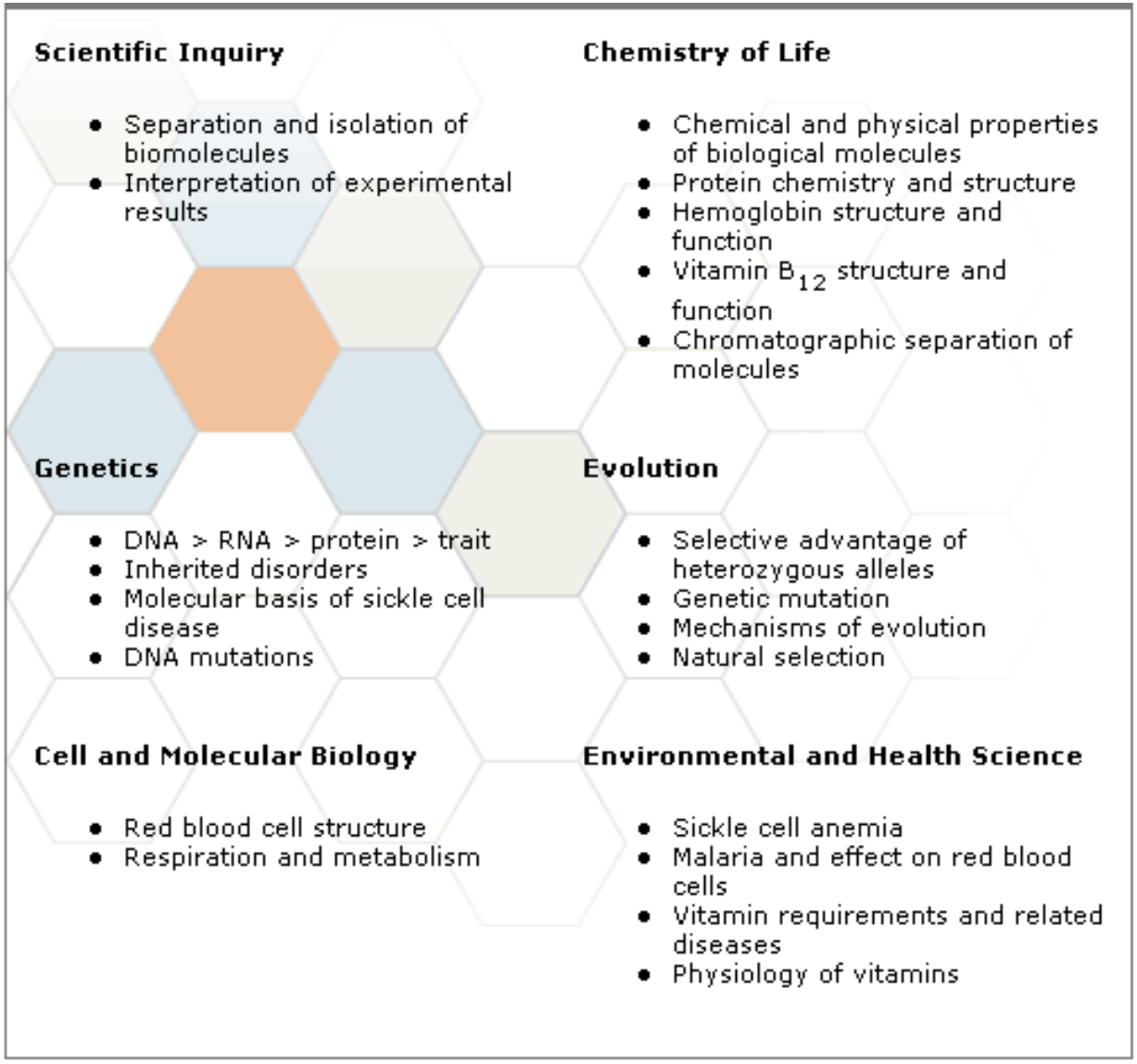
Why Teach

Size Exclusion Chromatography?

- Powerful teaching tool
- Laboratory extensions
- Real-world connections
- Link to careers and industry
- Standards based

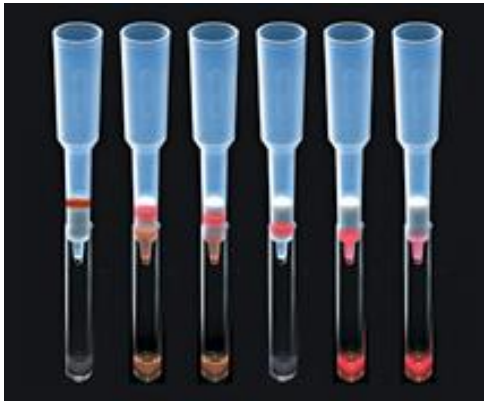

Scientific Inquiry
Separation and isolation of biomolecules
Interpretation of experimental results
Chemistry of Life
Chemical and physical properties of biological molecules
Protein chemistry and structure
Hemoglobin structure and function
Vitamin B_{12} structure and function
Chromatographic separation of molecules
Genetics
DNA > RNA > protein > trait
Inherited disorders
Molecular basis of sickle cell disease
DNA mutations
Evolution
Selective advantage of heterozygous alleles
Genetic mutation
Mechanisms of evolution
Natural selection
Cell and Molecular Biology
Red blood cell structure
Respiration and metabolism
Environmental and Health Science
Sickle cell anemia
Malaria and effect on red blood cells
Vitamin requirements and related diseases
Physiology of vitamins

Size Exclusion Chromatography Kit Advantages

- **Standards Based**
- **Can be used in Biology, Chemistry, or Physical Science**
- **Sufficient materials for 8 student work stations**
- **Easy preparation**
- **Easy visualization of separation**
- **Can be completed in one 45 minute lab session**
- **Study how the structure and biochemical properties of molecules are related to their separation**

Workshop Time Line

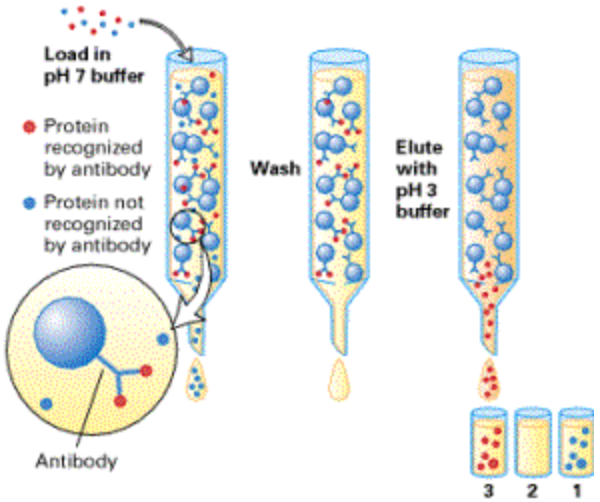
- **Introduction**
- **Comparison of different types of column chromatography**
- **Separation of a mixture of biomolecules by size exclusion chromatography**



Types of Column Chromatography

- Affinity
- Hydrophobic Interaction (HIC)
- Ion Exchange
  - Anion
  - Cation
- Gel Filtration or Size Exclusion (SEC)

Affinity Chromatography



- **Uses an affinity tag**
  - **allows molecules to bind to the column**
  - **specific to the tagged protein of interest**
  - **Examples: HIS-Tag, antibody, GST-Tag**
- **Proteins not bound pass through the column**
- **A buffer is used to elute the protein from the column**

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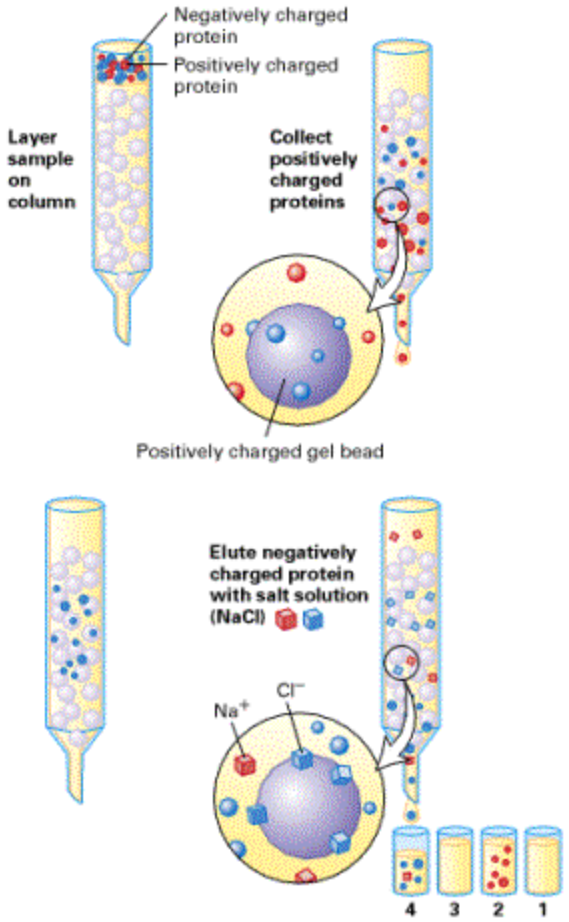
Ion Exchange Chromatography



- **Beads in the column are charged**

  Anion - positively (+) charged beads

  Cation- negatively (-) charged beads

- **Molecule to be purified will have the opposite charge from the beads in the column**

- **Molecules not binding to the beads pass through the column**

- **A counter-charged buffer is used to elute the molecule of interest**

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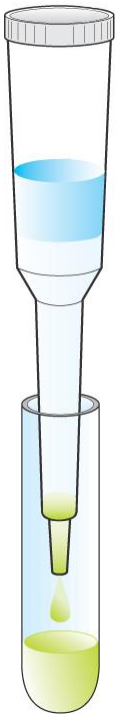
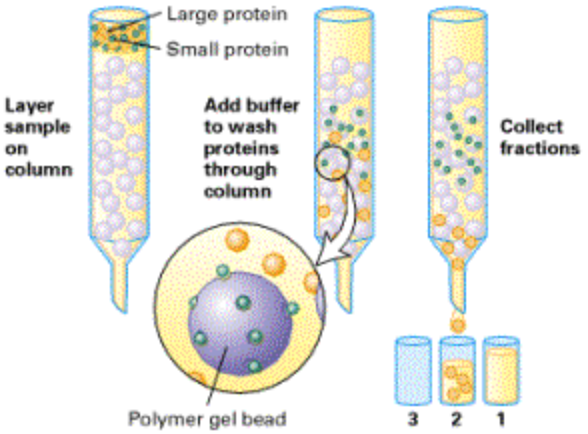
Hydrophobic Interaction Chromatography

HIC



- **Beads in the column are hydrophobic**
- **Column is treated with a high salt buffer**
- **Hydrophobic proteins bind to the beads**
- **A lower salt buffer elutes less hydrophobic proteins**
- **A no salt buffer elutes the protein of interest**

Size Exclusion Chromatography



- **Beads in column have tiny pores**
- **The mixture of molecules is added to the column**
- **Large molecules move through the column quickly traveling around the beads**
- **Smaller molecules move through the pores of the beads and take longer to pass through the column**

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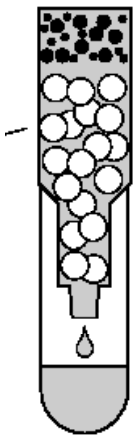
Principles of Size Exclusion Chromatography

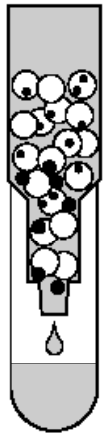
- **The mass of beads in the column is called the column bed**
- **Beads trap or sieve and filter molecules based on size**
- **The separation of molecules is called fractionation**
- **Size of pores in beads determines the exclusion limit** (what goes through the beads and what goes around the beads)
- **Molecules are dissolved in a buffer**

Principles of Size Exclusion Chromatography



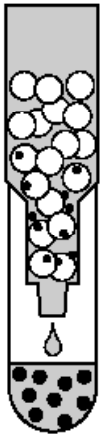
A mixture of large and small proteins is applied to a column of porous beads.

Fraction 1



As the buffer flows down the column, the small protein molecules penetrate into the beads and are slowed.

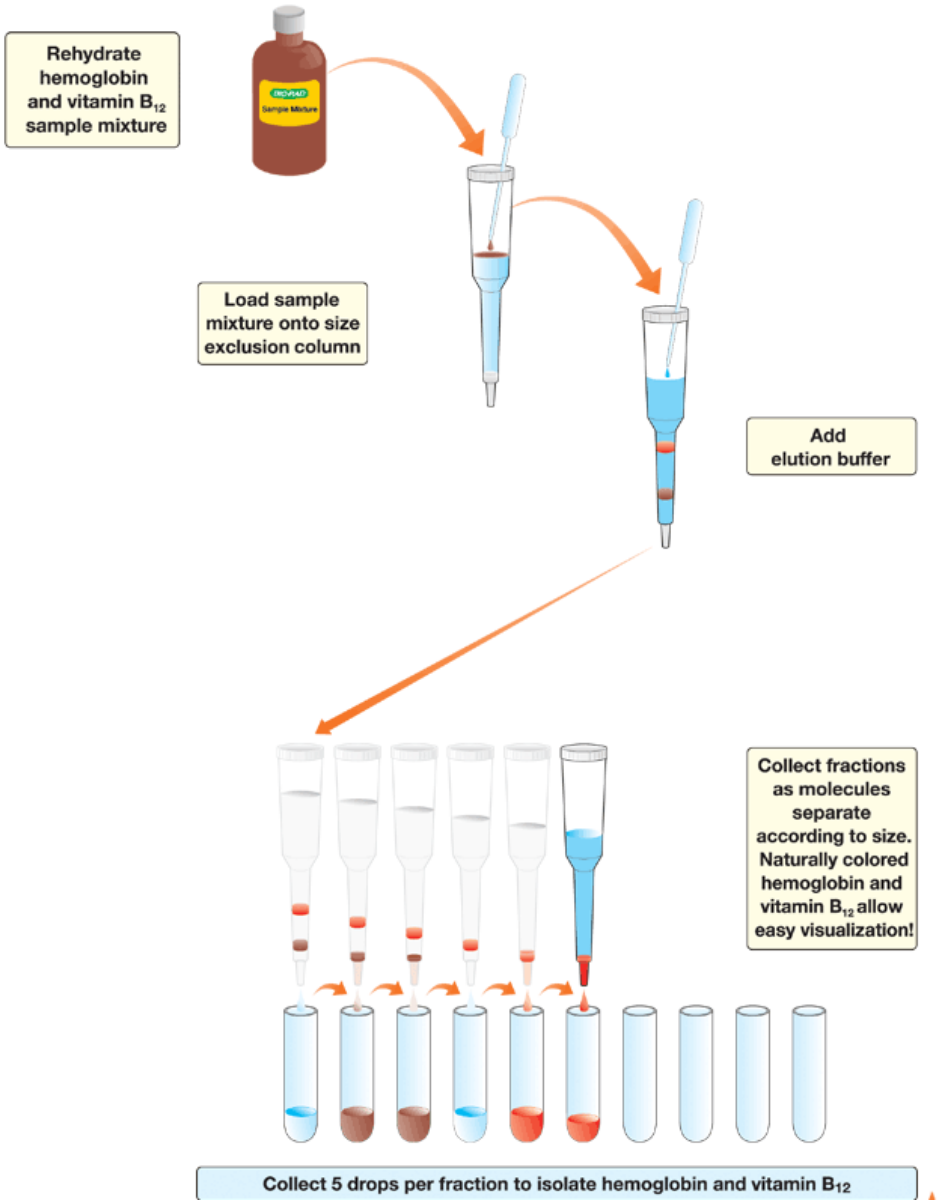
Fraction 2



The larger protein molecules emerge from the column first.

Fraction 3

Size Exclusion Chromatography Procedures Overview



Workstations

Student Workstation

Items	Number
Collection Tubes	12
Columns	1
Column end-caps	1
Pipet	1
Lab Marker	1
Test tube rack	1

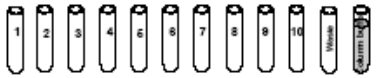
Common Workstation

Hemoglobin/Vitamin B mixture
Column Buffer

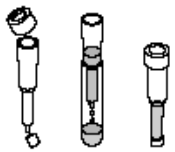
Laboratory Quick Guide

Laboratory Quick Guide
Size Exclusion Chromatography Kit

1. Obtain 12 collection tubes and label ten sequentially from 1 to 10. Label the tubes with your name and laboratory period. Label the final two tubes "Waste" and "Column Buffer". Using a clean pipette, transfer 4 ml of column buffer into the tube labeled "Column Buffer".

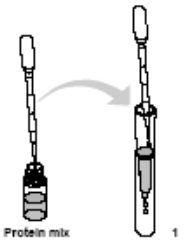


2. Remove the cap and snap off the end of the sizing column. Allow all of the buffer to drain into the waste tube. Observe the upper surface of the matrix and insure that all of the buffer has entered the column. Looking directly over and into the column, you should see the "grainy" appearance of the column matrix. Cap the bottom of the column.



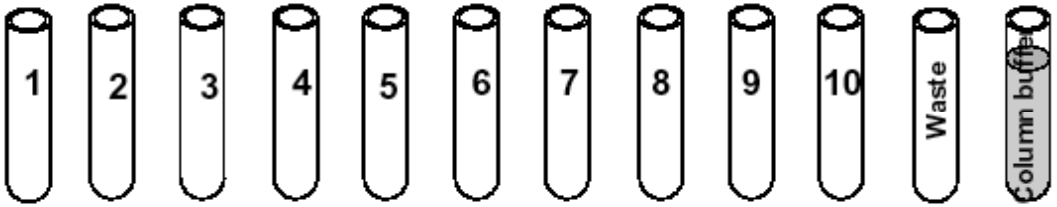
3. Carefully place the column onto tube 1. You are now ready to load (or the teacher may load) the protein sample onto the column.

4. When you are ready to load the protein mix, uncap the column. It is important to uncap the column only when you are ready to load your protein—you do not want your column to run dry. Using a pipette, add one drop of protein mix onto the top of the column bed (your teacher may do the loading for you). The pipette should be inserted into the column and the drop should be loaded just above the top of the column so that it minimally disturbs the column bed.



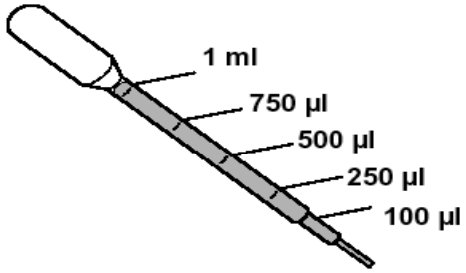
Step 1:
Label collection tubes

- **Label 10 collection tubes sequentially**
- **Label last 2 tubes “waste” and “column buffer”**



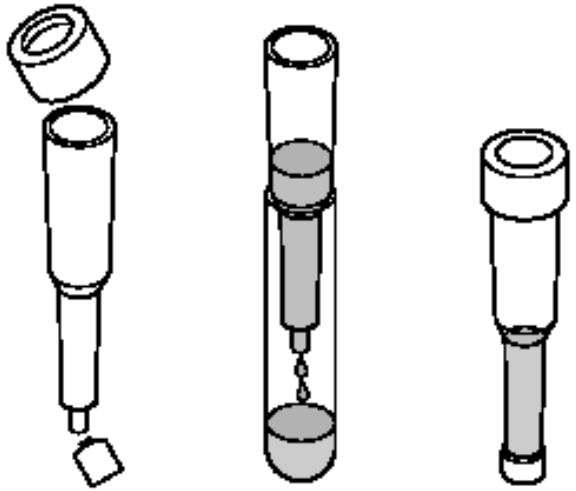
Step 2:
Column Buffer

- **Aliquot 4ml of Column buffer into the tube labeled column buffer**



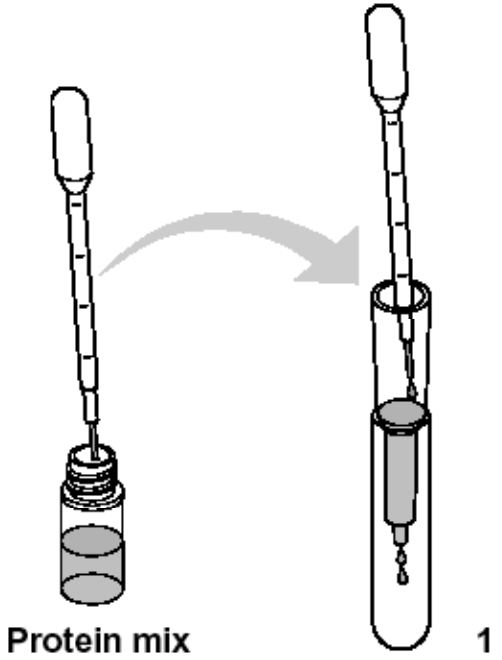
Step 3:
Prepare the Column

- **Remove the cap and snap off the end of the sizing column**
- **Allow all of the buffer to drain into the waste tube**
- **Cap the end of the column**

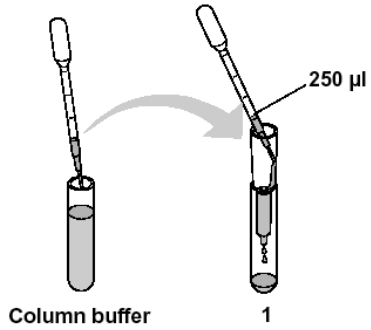


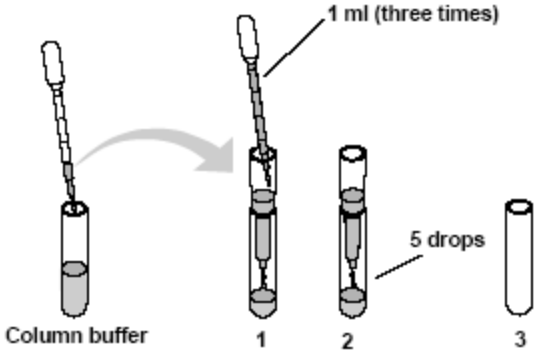
Step 4:
Add the protein mix to the column

- Place column in tube 1
- Add 1 drop of protein mix



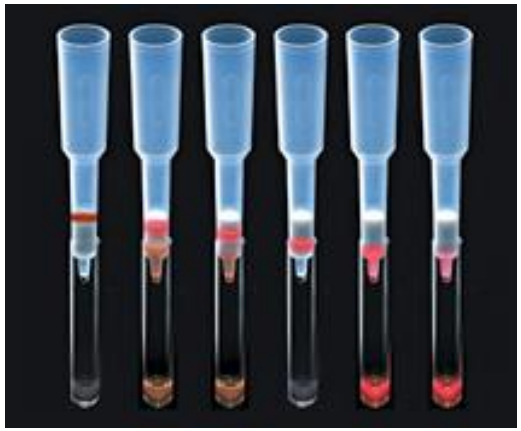
Step 5:
Add column buffer and collect fractions



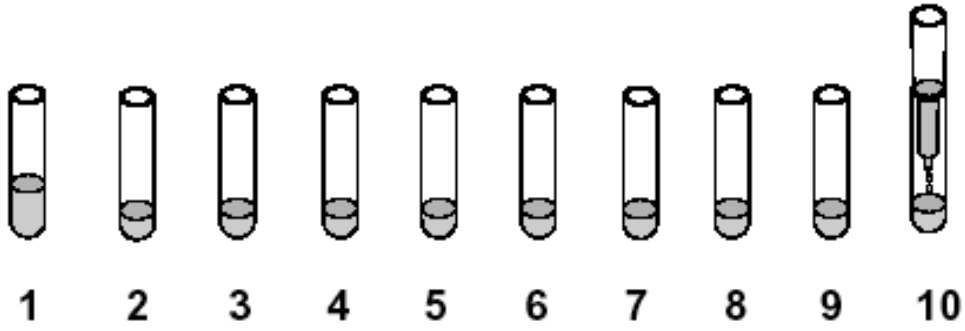


- **Carefully add 250ml of column buffer to the top of the column (2x) and begin to collect drops into tube 1 - Size separation will work best when the column is left undisturbed**
- **Carefully add 3ml of column buffer to the column**
- **Transfer column to tube 2 and begin fraction collection**
- **Collect 5 drops of buffer into tube 2 and transfer the column to tube 3**
- **Repeat the same collection procedure collecting 5 drops into each tube**
- **Collect 10 drops at tube 10**

Molecules of interest: Hemoglobin and Vitamin B12

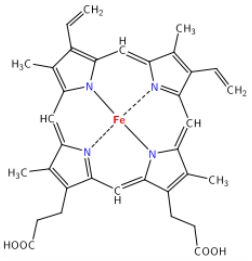


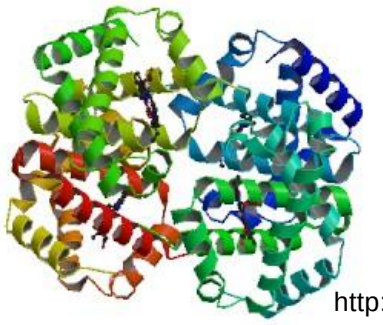
- Hemoglobin is brown and has a molecular weight of 65,000 daltons
- Vitamin B12 is pink and has a mass of 1,350 daltons
- The exclusion limit of the beads is 60,000 daltons: Hemoglobin will exit the column first, then Vitamin B12



Hemoglobin (Hb)

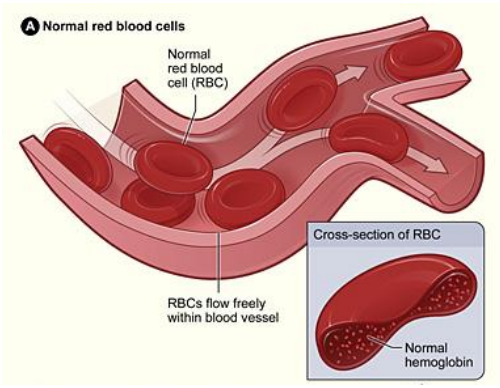
- **Metalloprotein**
- **Transports oxygen to the body**
- **Found in the red blood cells (RBC)**
- **Heme group contains an iron atom which is responsible oxygen binding**
- **Sickle Cell Anemia rises from a point mutation**

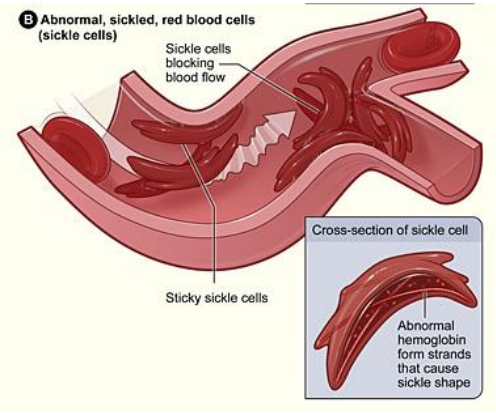




http://www.pdb.org

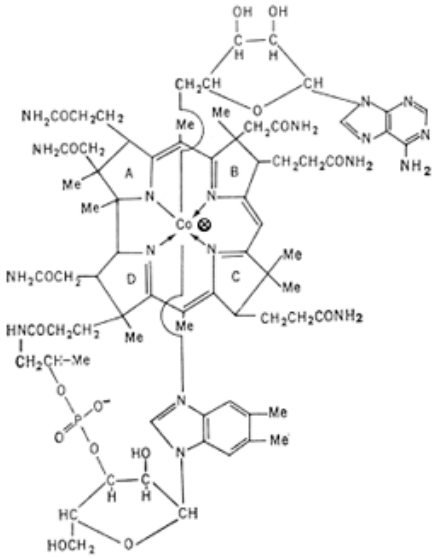
	Normal Cell	Sickle Cell
DNA:	**CCT GAG GAG**	**CCT GTG GAG**
Protein:	**Glu**	**Val**





www.nhlbi.nih.gov

Vitamin B12



http://history.nih.gov

- **Important for normal functioning of the brain and nervous system**
- **Involved in the metabolism of every cell in the body**
  - **fatty acid synthesis and energy production**
  - **DNA synthesis and regulation**
- **Cyanocobalamin**
  - Cobalt (Co) central metal ion
- **Synthesized in bacteria**
- **Coenzyme**

MUT: (Methylmalonyl-CoA mutase)
catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, a key molecule of the TCS Cycle

MTR: methyl transfer enzyme
(5-Methyltetrahydrofolate-homocysteine methyltransferase)
catalyzes the conversion homocysteine into methionine, an essential amino acid