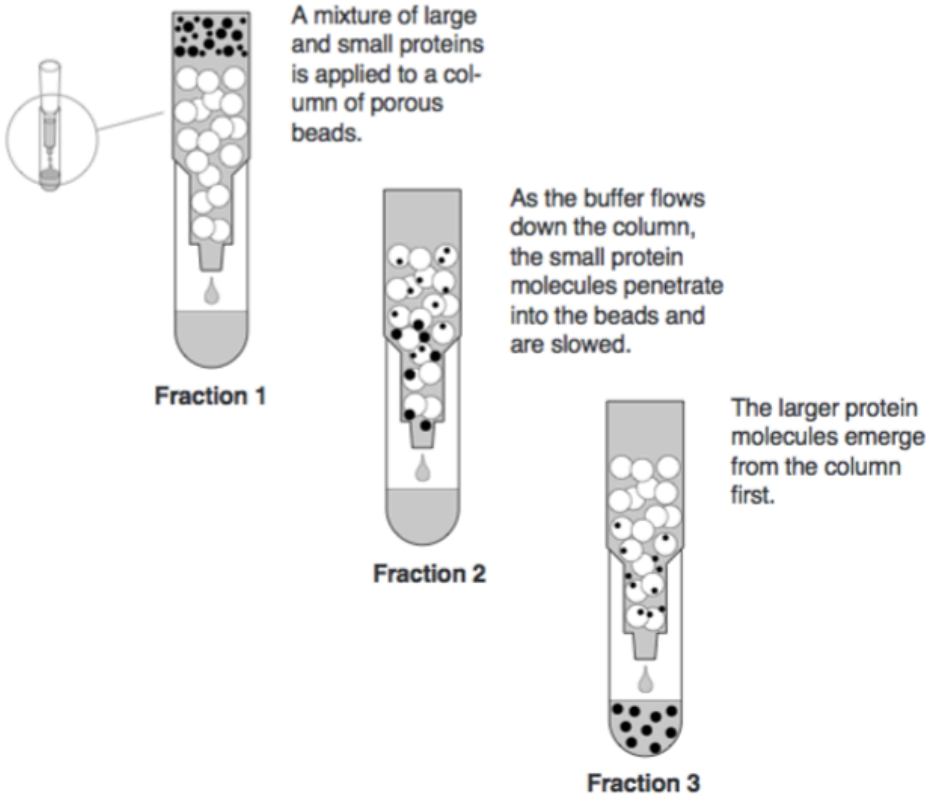
LAB: Size Exclusion Chromatography

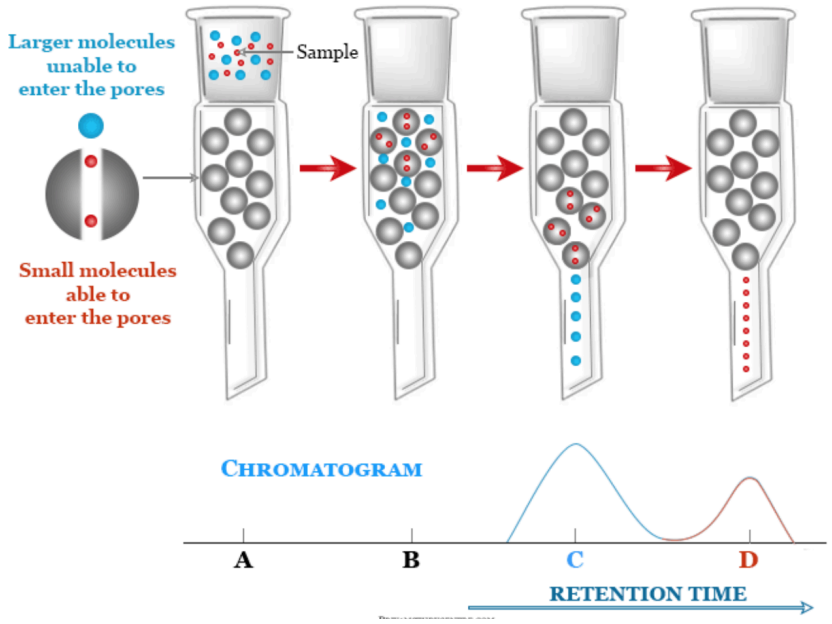
Background:

Hemoglobin and vitamin B12 are the two molecules which are being separated in this lab.

Hemoglobin, which is brown, has a molecular weight of 65,000 daltons (**big**) and is thus excluded from the column. Hemoglobin will **pass more quickly through the column** and appear in the early collection tubes.



Vitamin B12, which is pink, has a molecular weight of 1,350 daltons (not as big) and is thus fractionated by the column. The vitamin B12 molecules penetrate the pores of the beads, becoming temporarily trapped. As a result, they **pass much more slowly through the column** and appear in the later fractions.



Hemoglobin

Hemoglobin, a protein found in red blood cells, functions to transport oxygen to the tissues of the body. The hemoglobin used in this experiment is bovine hemoglobin. The use of bovine hemoglobin (rather than the human counterpart) avoids the potential health hazard presented when using human blood products.

Hemoglobin is made up of four polypeptides (small proteins) which associate to form a large, globular protein. Hemoglobin gets its name from the heme group, the iron-containing component of hemoglobin which physically binds oxygen. The iron-containing heme group is responsible for the red-brown color of hemoglobin.

Vitamin B12

Vitamin B12 is a vitamin that is essential to humans and other vertebrates.

Vitamin B12 is an essential cofactor of several biochemical reactions which occur in the human body. One function of vitamin B12 is the breakdown of fats. Sources rich in vitamin B12 include eggs, dairy products, and meats. Vitamin B12 is not found in plants and vegetable foods. Thus, people who have strict vegetarian diets are often deficient in vitamin B12, unless they take some supplementary vitamin. Vitamin B12 does a lot of things for your body. It helps make your DNA and your **red blood cells**, for example.

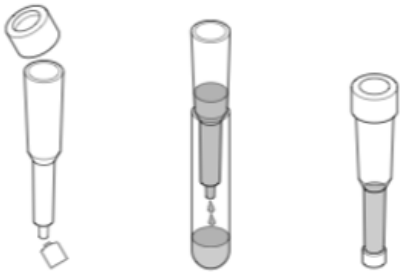
The challenge of consuming adequate amounts of vitamin B12 in food is finding the right foods that naturally provide this unique nutrient. Plants can't manufacture it. Animals can't either. Microorganisms – especially bacteria and fungi – are the only organisms definitively known to produce natural vitamin B12, which is why many foods that are high in B12, like some cereals and breads, come fortified with added synthetic B12.

For vegans and vegetarians, it's especially difficult to get enough B12 without supplementation.

According to VeganHealth.org, "unlike animals, most, if not all, plants have no B12 requirement for any function, and therefore have no active mechanisms to produce or store B12.

Laboratory Protocol (Methods)

1. **Remove the cap and snap off the end of the column. Drain** all of the buffer into a "waste" collection tube. Cap the bottom of the column with the column end cap.



2. Remove the end cap from the column. Gently place the column onto the collection tube (Do not jam the column tightly into the collection tubes-the column will not flow). You are now ready to load the protein sample onto the column.
Pipet 50µl of (Blood) protein mix just above the beads.

Blood 50µl top of the column

3. Allow the protein mix to enter the column bed.
Pipet 250 µl of column buffer to the top of the column. This is best done by pipetting just above the column bed. Begin to collect drops into the tube.

4. When all of the liquid has drained from the column, **Pipet 3 ml of column buffer to the top of the column**. This can be done by adding 1 ml from the p, 1,000 pipette three times.