Sheet ○Slides

Subject :	purification
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Number :	8

Extracting Pure Proteins from Cells

Review:
We have cells, and we are looking to purify a certain protein of interest; in a mechanism called **protein purification**
To do that, first we need the cell content and the medium to be homogeneous by degrading the cell membrane and breaking up cells, in a process called (Homogenization)

Ways of achieving homogenization:
A. Potter–Elvejhem homogenizer (grinding)
B. Sonication
C. Repeated Freezing and thawing
D. Detergent

Now we have a homogenous mixture containing Proteins, Organelles and some parts of the plasma membrane.

The second step after homogenization is (Differential centrifugation)

Centrifugation (rotation) leads to sedimentation (precipitation)

Particles of different densities or shapes will sediment at different rates, depending on:
A. Density: the particles with higher densities will come out of the solution faster (direct correlation)

B. Mass: higher mass will make sedimentation faster (direct correlation)

C. Shape: larger shape of particles will increase the resistance of the solution and that will decrease the sedimentation rate (inverse correlation)

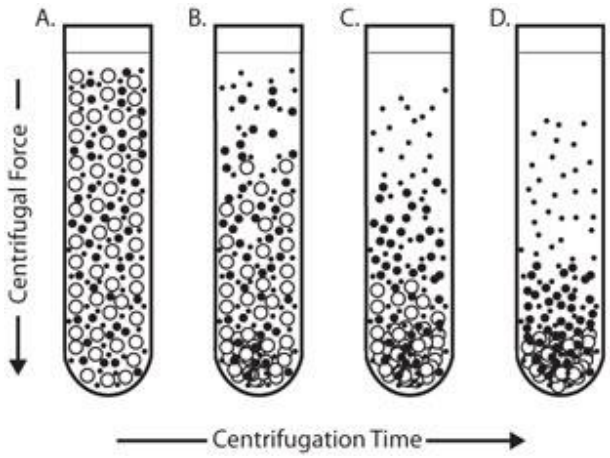

figure 1

D. Density of the solution: if the solution's density is high then sedimentation won't be high (inverse correlation)

So, sedimentation rate differs between particles. The sedimentation of each particle is constant and can be defined as a sedimentation coefficient

[Sedimentation coefficient = (10^{-13} s) = 1 Svedberg]

Example: If we applied 800g force of centrifugation to a solution, the whole unbroken cells will precipitate, and by increasing the force to 20,000g mitochondria will precipitate, and by the force of 150,000g ribosomes which produce proteins will come out of the solution.
And because proteins are much smaller we use a process called Ultra Centrifugation to make proteins come out. (check figure 2)

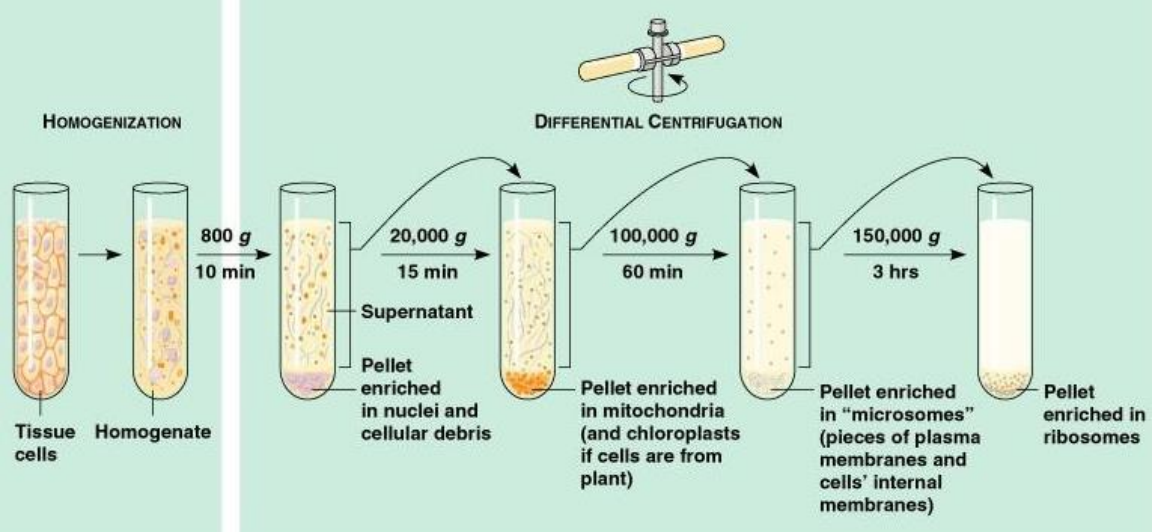

figure 2

Now we have a mixture of proteins, but we need one protein of interest, so proteins can be purified depending on the differences between them like Solubility, Size, Charge and specific binding affinity.

Mechanisms of purifying proteins:

Salting in & Salting out

Pure protein's solubility in pure water is relatively low.
By adding salt to the water, a complete ionization will occur, which will increase the electricity of the solution by increasing the partial charges on different atoms, so, the polarity of water will increase, and the binding between water and proteins (H Bonds) is increasing. So, the solubility of protein in water will increase. (Salting in)

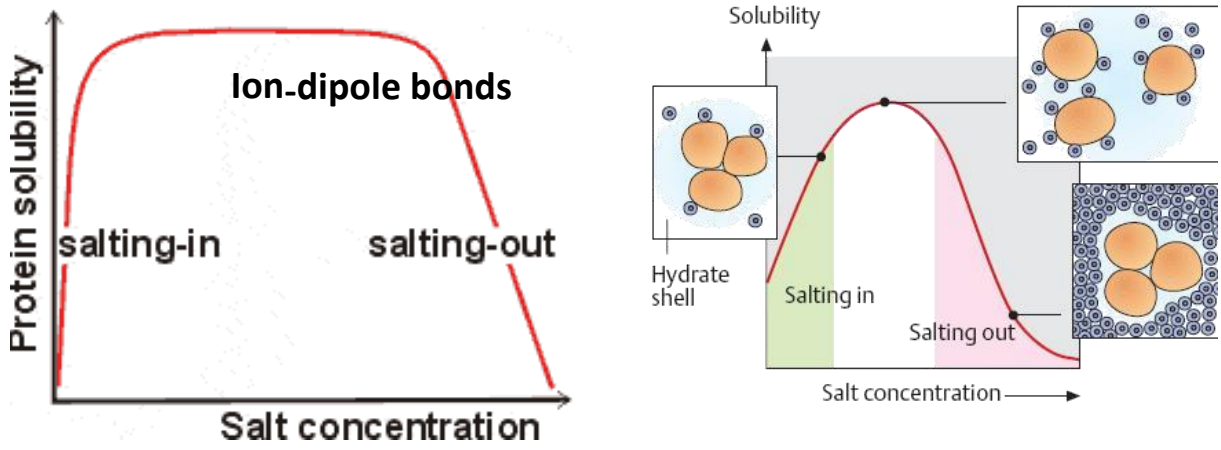

figure 3

After a while, adding too much salt will cause hydration shells around the salt's ions, and a competition on the water molecules will start between the protein & the salt, and water molecules will be attracted to salt ions more than the attraction to proteins. After a short time, all the protein will precipitate out of the solution, starting from the least soluble to the most soluble. (Salting out) (check figure 3)

The results of this technique are crude (not pure), but it's a good idea to start with this technique.

This method is mainly depending on the solubility of proteins in water, as well as ammonium sulfate is the most common reagent to use at this step.

Dialysis

This mechanism is all about putting you sample in a bags called dialysis bags, which consist of sheet designed to have pores with a certain size according to your need. We put the bags in water, diffusion happens for proteins with a molecular weight that allows them to fit in the pores, other proteins which can't pass by the pores are going to stay inside. (check figure 4)

Example: you have a protein with a MW of 30k Daltons, you have to choose a dialysis bag with pores less than 30k Daltons, 25k Daltons for example.

Notice that all the proteins with MW more than 25k Daltons will stay inside the bag. So the results of this procedure are crude.

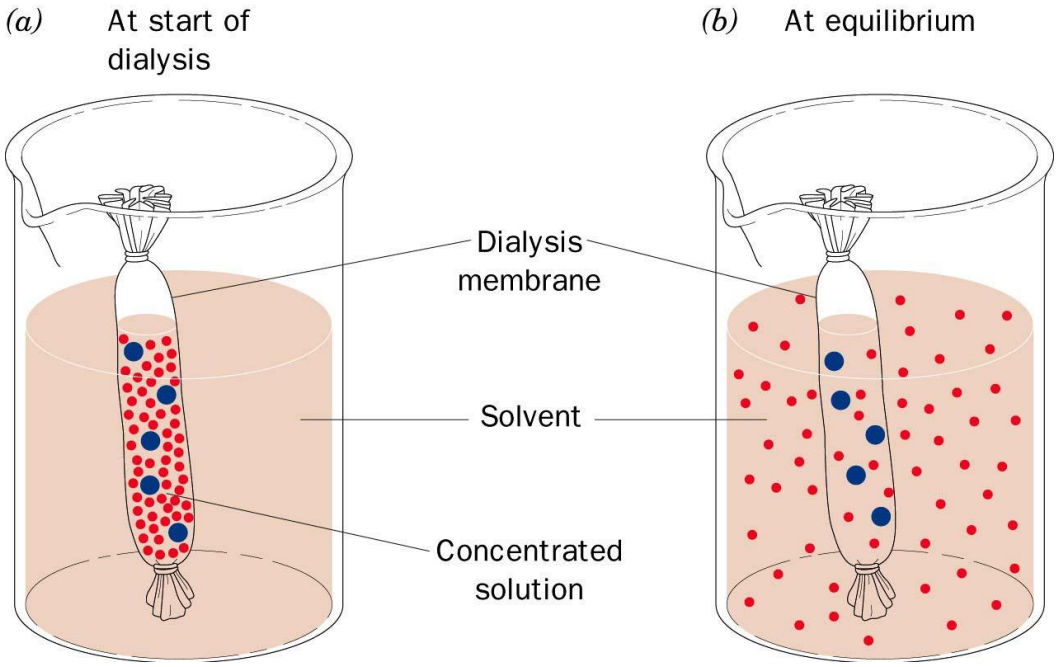

figure 4

Chromatography

It is a procedure of purification of any materials mainly protein ,based on the fact that different compounds can distribute themselves to varying extends between different phases, or separable portions of matter, Using colours (in the past) & wave lengths (now)

Column Chromatography:
A column containing jelly-like material which has pores that the liquid can pass through it. We use this mechanism in purification of protein by putting the solution containing different types of protein on the column. Proteins differ in the size and shape so they pass through the column in different rates (speeds). So, we Track our protein (certain colour) till it goes out of the column and collect it. (check figure 5)

Not all proteins have visible colour. so, now the column Is connected to sensor that can look for a visible or invisible range (infrared, UV) according to the wave length of the passing protein, and connected to a computer that tells you which protein is coming out now exactly, and you start collecting it out.

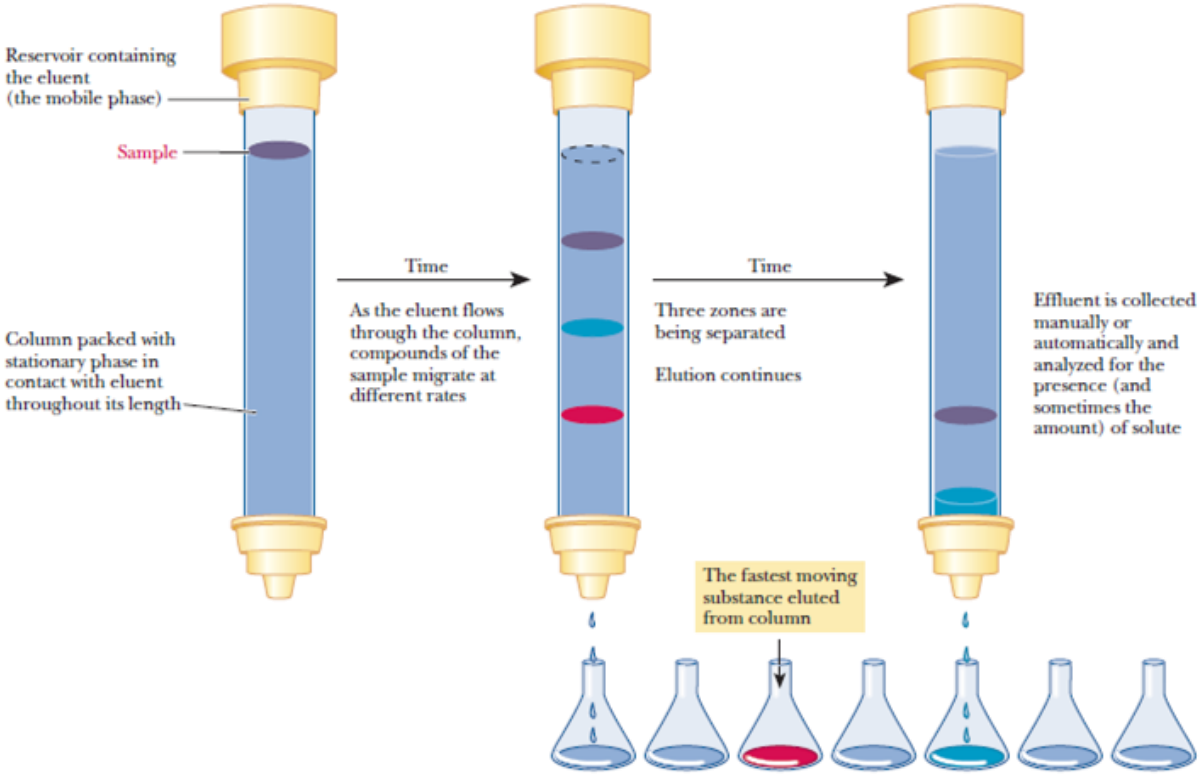

figure 5

In any chromatography procedure we have 2 phases:
1. Stationary phase: the fixed material within the column that you design to attach to a protein of interest which act as a sieve.

2. Mobile phase: the protein of interest in the sample which pass through the column, this phase flows over the stationary material and carries the sample to be separated along with it.

Note: The components of the sample interact with the stationary phase. Some components interact relatively strongly with the stationary phase and are therefore carried along more slowly by the mobile phase than those that interact less strongly. The different speeds of the components are the basis of the separation.

- Size-exclusion chromatography (Gel-filtration chromatography)
  It also called Molecular-sieve chromatography, separate molecules on the basis of size, making it a useful way to sort proteins of varied molecular weights.

The stationary phase (jelly-like material) has a specific pore size, It is like a group of lines crossing each other with pores within them, when you put your sample on the column, the first protein to come out is the protein with the larger MW because it won't stuck on the lines and will pass easily, and none of it will stay on the lines, and protein with smaller MW will come out much more slower, because a lot of it will stay on the lines. (check figures 6 & 7)

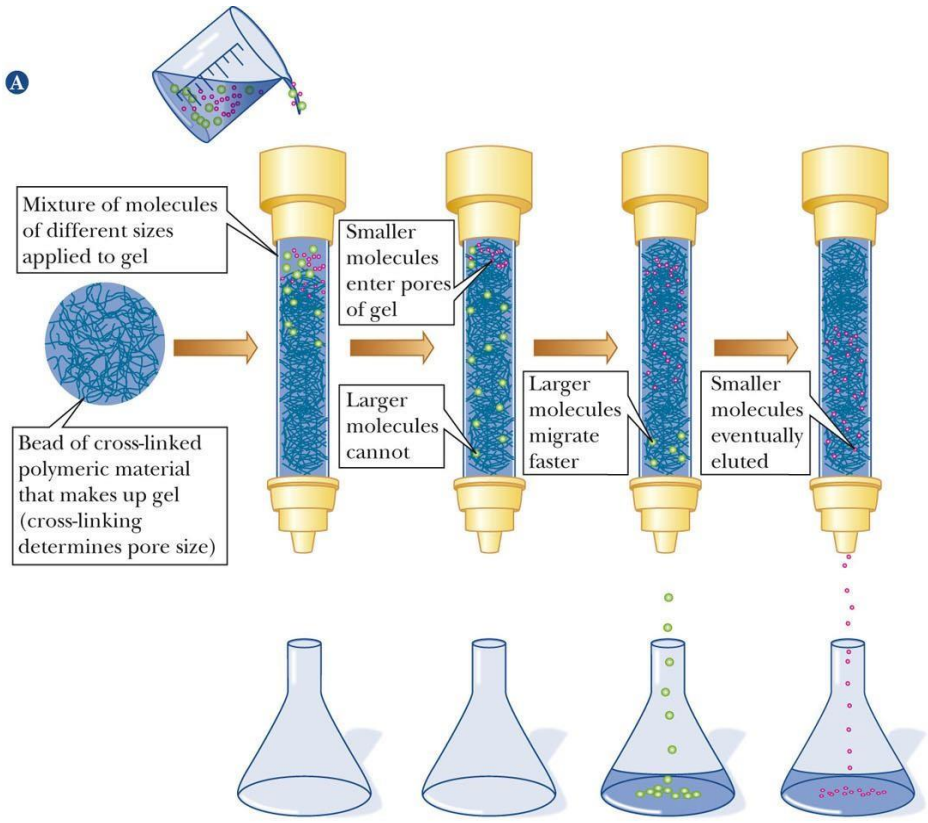

figure 6

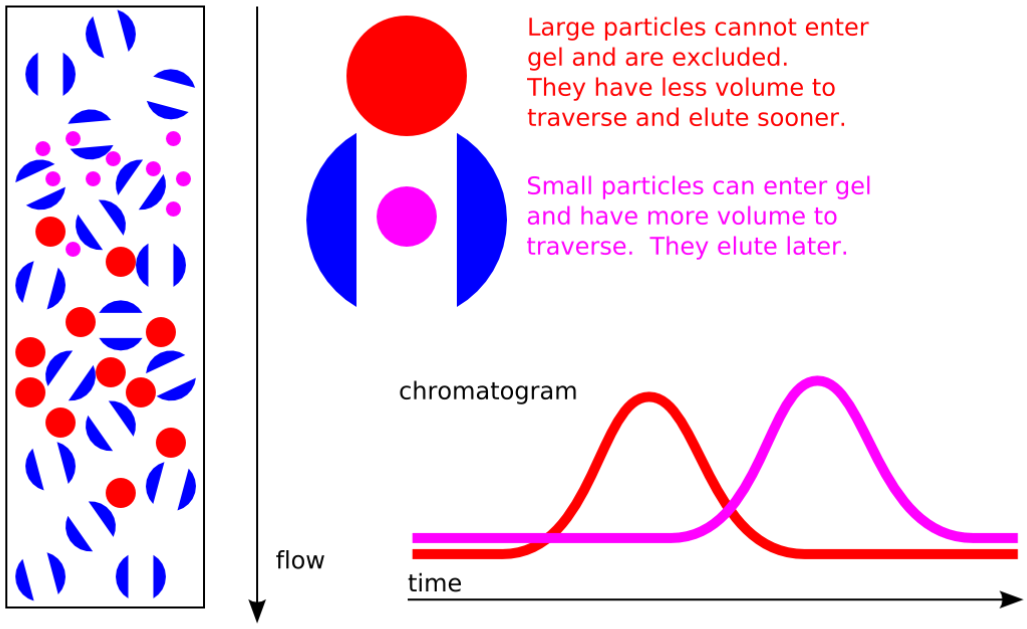

figure 7

The results of this technique is better than dialysis, but you will get some protein with the same MW, so it is not pure.

The material used in the Gel consist of one of two kinds of polymers; the 1st is a carb. polymer (ex. dextran or agarose), The 2nd is based on polyacrylamide. But in general you use the column with the Molecular size that you want.

- Ion-exchange chromatography

This technique mainly depends on the total charge of the protein, if I need a protein with a total positive charge, I will choose a material within the column that have a negative charge. And if the needed protein has a total negative charge, I will use a positive material within the column.

Mainly, we are dealing with 2 main materials in columns: (check figure 8)
weakly acidic (-): CM cellulose
weakly basic (+): DEAE cellulose

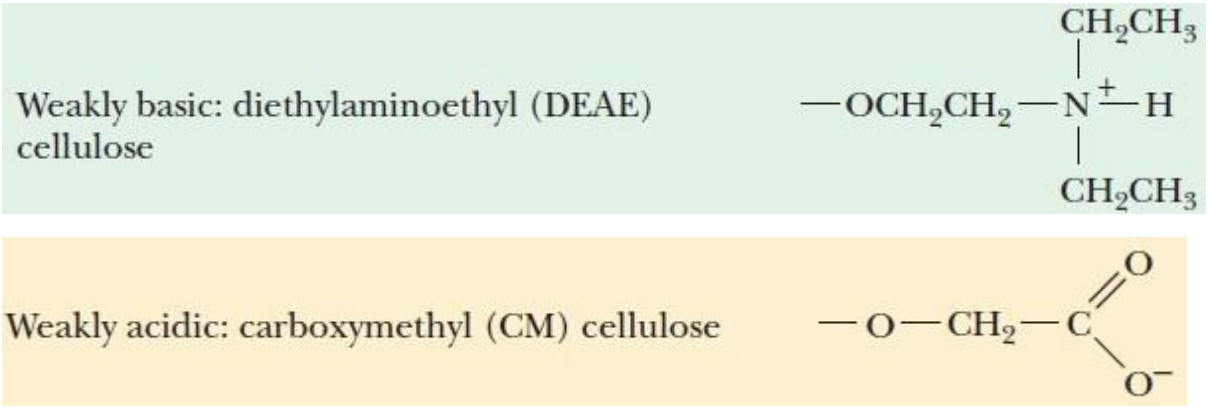

figure 8

When you put your sample on the top of the column it will go down and all the proteins with total negative charge will bind (in the case of DEAE cellulose), or all the proteins with the total positive charge will bind (in the case of CM cellulose).
Now we have a column full with our protein, how could we take the protein out?
By Adding salt! After adding salt, a complete ionization will occur and the salt will take the place of protein so all the protein will precipitate, starting from the least charged protein to the protein with the biggest charge, and we can collect it easily. (check figure 9)

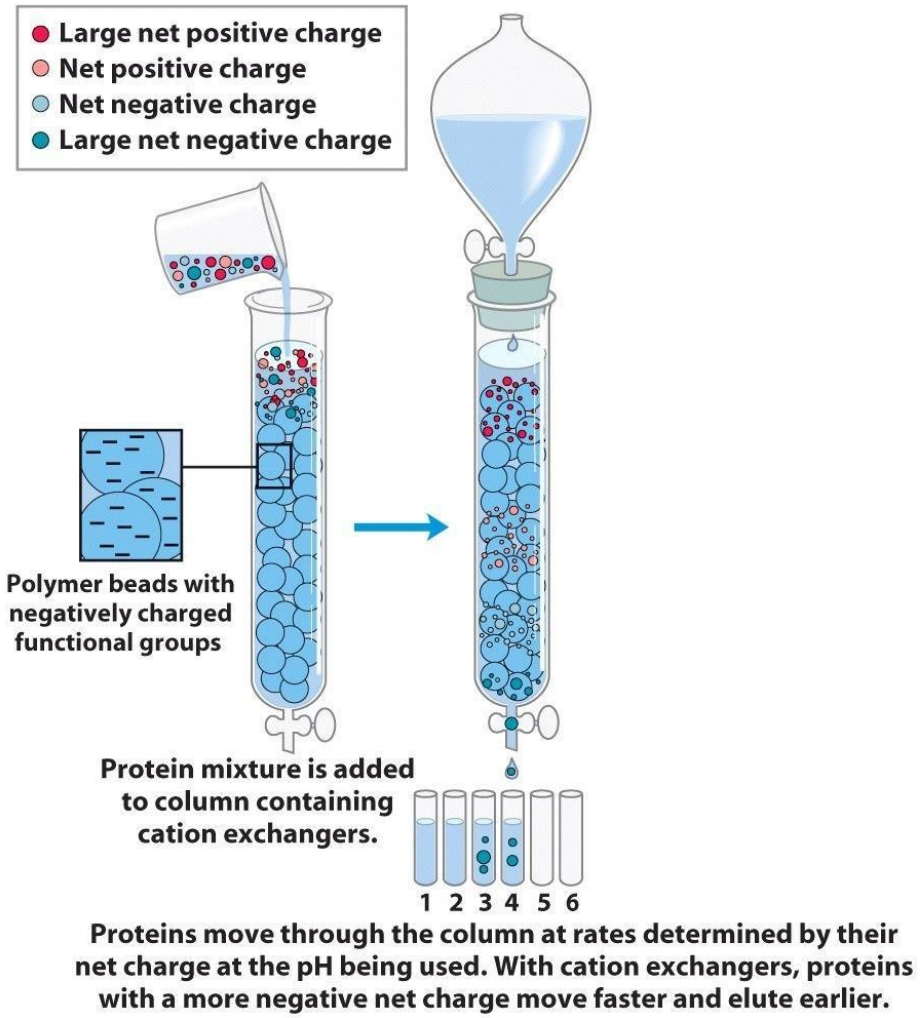

figure 9

Sorry for any mistake
Good Luck Doctors :D