Protein Standard Curve

Standard curves are used throughout biotechnology and biology. The idea involved using a series of standard solutions and then comparing them against some unknown solutions. Based on the comparison, you get a few intern values. This lab is perhaps the single most useful lab when it comes to understanding essential science skills. The idea that we will be looking at today is called spectrophotometric or color metric assay and what this does is that it revolves (00'30") around the observation that the more stuff there is in a solution, the more concentrated the solution is. As obvious as this might first sound, this is actually a rigorous concept in science. It is called Beer's Law and darker solution then contains more of a substance than lighter solutions. If we qualify this a little more specifically and try to work little more within the confines of science, we actually have a powerful tool that can be used in everything from checking DNA (01'00), RNA concentration, analytical labs looking at the concentration of the drugs in a sample taken from a suspect, looking at the amount of chemicals, in any chemical samples. You name it; the possibilities are virtually endless as far as this technology goes.

So what we are going to be doing today in lab is to create a standard curve and there are four major objectives we want to try to accomplish. First is that we will talk briefly about the Biuret test (01'30") and what this is. How does that allow us to visualize protein concentrations? Then we will learn about how to set up a standard curve (we will have to prepare the solutions) and some basic ideas of what this standard curve is. Then, we will learn how to use a spectrophotometer which is a device that allows us to look at wavelength of light and the absorption for a given wavelength of light. From all of this information, we will create a curve known as the standard curve, and use it to asses some unknown samples. (02'00") Also at the end, I will give you a couple of hints for time management to help you make sure the lab runs quickly. All right, let's go ahead and get things moving.

Basically, we are going to be using the Biuret test and the Biuret test is a throw back to a test you folks did about a couple of weeks ago in biological chemistry where you used a Biuret test to check for the presence of proteins. What we are going to be looking at for is a specific protein called BSA which is a short (02'30") for Bovine Serum Albumin. This protein is a common component of blood. It helps you buffer blood; maintain osmotic pressure and it's readily extracted from cow's blood (named bovine). So we will be using this protein as a

standard and when the protein reacts with the Biuret's reagent, it turns a dull dark purple. Now, this said then, the specific reaction focuses on the amino groups. Who said those functional groups were not good for you to remember (03'00")? So the amino group in proteins, more specifically, the amine which is what is left over after the amino acid forms a protein which reacts with a copper ion in the Biuret solution. So when it reacts, it goes from a light blue coloration to a dark purple coloration.

What is going to happen then is that you are going to set up a series of known concentrations for about 1 to 5 mg/ml of BSA and let them react (03'00") with the Biuret's reagent. As you might imagine, more concentrated sample should give you darker, more purple color.

Then what we are going to be doing is that we will go through and we will look at the theory a little bit more specifically. As I mentioned in the introduction, Beer's law is basically the idea that the darker something is, the more concentrated a sample is. Essentially increased concentration equals increased absorption. What we do is that (04'00") the spectrophotometer essentially allows us a way of really sort of standardizing this. Instead of having your friends sort of hold up the solution and you stare at it with him holding the flash light on the other side, we are a little more analytical in science. The spectrophotometer allows you for instance, to work with a specific

wavelength which allows you to look at particular colors and so with the purple wavelength of light that we are looking at, the 550 nm wavelength is actually the best to look at. (04'30")

Also then, we set up what is called the specific path length. Each time we do the experiment, we are looking through the same thickness of sample. As you might have imagined, if we just let that change, we would have a lot problems. Every time we look at the solution, it is exactly 1 cm wide and what is called the path length. A little later when you use the spectrophotometer, you will be using things called cuvettes and you will notice that they look like little test tubes, however, they are exactly 1 cm across which reflects the idea of working with the 1 cm pathway.

Basically then, you are going to set up a series of solutions. You will be given them to develop and I will give you a couple of hints on that in a few minutes. Then you will be putting them into the spectrophotometer. Now, when you work with the spectrophotometer, you want to make sure that you spend a few minutes learning the protocol. One of the most useful thing in lab is learning how to follow instructions and the technical staff will put together a one page what is called S.O.P. (05'30") This S.O.P. is a short for Standard Operating Protocol and walks you through the key steps for running a spectrophotometer and this is absolutely essential that you learn how

to read these procedures and learn them on your own. While your lab instructor and myself included will answer practically any questions you have, it is expected as a student of science that you will know how to read procedures and follow them with a great degree of detail.

This first page or rather one page S.O.P. is a really good start. Maybe have someone (06'00") in your group to look at that ahead of time but everyone in the group should understand it. Everyone in the group should know how to operate the spectrophotometer and understand the basic dials and what they are doing. Also you want to make sure that you zero and blank the spectrophotometer often. The reason for this is that as you blank the device, you are essentially telling the device what a zero concentration looks like. The device does not know this. It does not know that perhaps a light blue solution has no protein in it. You need to tell it this by zeroing (06'30") it and by doing that often, you help to avoid what is called drift. Drift is when the spectrophotometer's value change slightly. The spectrophotometer is an electric instrument. It is subject to temperature, electrical problems over time, and as it warms up, the values change very slightly. After most experiments, that is not a big issue but it is really a good idea to get in the habit of zeroing the device often and blanking the device often.

Now, once you have taken your samples (07'00") and that you have got your spectrophotometric data, you are going to create a graph and what the graph is going to be is that it is going to be a graph of absorption at 550 nm on the x-axis versus the concentration. Now, it will be from about 1 to 5 mg on the y-axis. Now you want to spread this out over the graph so that you use the entire graph. Do not cumb everything down to one corner because you are actually going to be using the graph a little bit later. Then what you are going to be doing is that you are going to go back after you have created the graph and repeat (07'30") the process for four unknown samples. At the front instructor's desk are four unknown samples of protein (A, B, C, and D) and you will then be doing exactly the same procedure. Then you will be able to use the standard curve you have just created to figure out what those unknowns are. The way that you would do that is that you will do a Biuret's development on the solution and you will get absorption. Let's say that (08'00") it has a certain value on the absorption axis, you will interpolate over. Basically, you just read up on the x-axis what the absorption is, you go up to the line you have just created from your standard, and over to your concentration. The notes and bibliography have a little bit more information on this if you are a little unclear on how to use the graph. That is basically the big point in the lab and you want to make sure that you are focusing on

those ideas as presented. However, I (08'30") am going to give you a couple of time management hints and couple of other ways to keep going through things.

First off, the solution volumes are very critical. Take very good care of how you measure out the solutions. You might want to aliquot the water first. Make sure that the right amount of water is in there, then the protein. Make sure that the right amount of protein and water is in there. That will give you the concentration. Then, you add the Biuret's. Now when you add the Biuret's, the clock is ticking. You have got 15 minutes to get them all developed. You then want to make sure that you are ready to add all of the right amount of Biuret (09'00") to each tube and each tube gets about 2 ml of Biuret's although your protocol will tell you very precisely what is the amount. So again, get all those tubes set up. You want to make sure that you work quickly. So while those tubes are incubating, do not sit there and waste time. Have someone in your group or maybe the whole group go over and start working with the spectrophotometer. Turn it on. See what the dials do. This spectrophotometer is an analog device, perhaps it is one of the simplest spectrophotometers we have. It is important that you learn how to use (09'30") this basic pieces of equipment. So again, take some time to look at this. Also then, everyone should make their graph and learn how to interpolate the

unknowns. Again, the graph making is essentially just graphing the concentration all on the y-axis, the absorption on the x-axis, and you will get a line. You will make what is called the best fit line. That is a line that approximates the dots which you will notice that the dots do not make a line but you can have a line that about best fits them then you will interpolate the unknowns.

Again (10'00"), the notes have a little more specific information but you will go across on the absorption axis. That is what the spectrophotometer gives you. Go up to the line you just created, the best fit line, and then go over to the left to the concentration. That will give you the color concentration of the unknowns. Since the entire class is working with the four same unknowns, you should have very clear values as to what they are. If you are plus or minus 85% (of #), that is a pretty reasonable accuracy. So check with your instructor, check with your fellow students. However, as you might have imagined (10'30"), this lab is a little bit behind the time as far as technology goes. In reality, we will probably use Excel to graph our data because the computer will create quicker, more accurate graph. There is a procedure for creating an Excel graph. I want to give you a couple of hints.

First off, realize that each version of Excel is a little different and also I want to remind you that what you are doing is what is called the

scatter plot. If you do the “Excel Wizard”, use the scatter plot (11’00”) to create the graph of the protein concentration versus absorption. Now, once you have got this graph and that you have an Excel spread sheet of it, the lab is essentially done. As you might have imagined, it is said that there is some ways in which we are going to improve this. For instance, there are current pieces of technology that will take your samples, read all ten of them including the unknowns at once, create the graph for you, plot the unknown on the graph, and tell you if there are statistically relevant, all thanks to computers (11’30”). For instance, this is exactly the lab we do in biotechnology. We take and do the exact same protein standard curve you did before using digital computer, digital spectrophotometer with an necessary computer to kick out a protein standard curve in about 30 seconds. So this is a very fast lab to show you how the technology is a basic idea but a lot of science is behind it, a lot of technology on top of it, and you can understand standard (12’00”) curves.

As I said before, this is perhaps the single most useful lab you will experience in cellular or molecular biology because it shows you a core technology that is used throughout science. One little quick safety note, when you are done with a all your samples, all your Biuret’s solutions need to go in the receptacle at the far side and make sure that you clean up your lab station so they look identical to the

way they were when you first came in. Remember that if the lab stations are clean, the technicians are happy. If the technicians are happy, the instructors are happy. If the instructors are happy, they tend to make happy exams. With that said, there is one last point I want to mention and that is again to drive home the point of everyone working with the technology: Do not watch somebody else's eyes on the spectrophotometer. You need to work with it directly and personally. If you understand how this analogue spectrophotometer works, you will be in a good position to understand (13'00") how the digital spectrophotometer works in biotechnology. This will then lead to what is called micro array which essentially are literally hundreds of little spectrophotometer grids side by side and this then helps understand automation. So a lot of this lab is an introductory lab. It is very strong in science and if you understand the basics of it, you are in a good position to understand emerging technologies that hopefully one day might get you a job! (13'30")