BLOSSOMS MODULE
PROTEIN PURIFICATION

By
Natalie Kuldell, PhD
Department of Biological Engineering
Massachusetts Institute of Technology

Hello and welcome everyone. My name is Dr. Natalie Kuldell and I'm a faculty member in the Department of Biological Engineering here at the Massachusetts Institute of Technology in Cambridge, MA. I'm happy to have you hear today in my teaching laboratory and we'll talk about something that's common to all living cells, namely protein.

Now you may have heard about protein as something that's important for building muscles, something important for living cells, or even something that's built from a building block called amino acids, and all that is true. But today we'll be talking about something else, namely about how you might squeeze apart proteins inside a cell and separate them one from the other. And the reason that you might want to do that is because you might want to purify the proteins for food or for medicine or for materials.

So if you were to open up a cell and look inside you'd see that it's really crammed full of material, so full in fact that as biochemists or biological engineers you might need to take apart some of the things that are in the cells and separate the things you want from the things that you don't.

Before we start to talk about the techniques for doing that ~~squeezing~~ tweezing apart of materials, what I'd like you to do is take time in your classroom there and remember the kinds of things that are in the cells. I've started a chart for you and filled in one of the lines, but if you could take time there and talk about the materials in the cells that are started for you on this chart, and then join me here again we'll go over the table together.

Welcome back! I have filled out the chart and hopefully our answers agree reasonably well, so let's take a look. There are four molecules in the cell, four main, macro molecules. They include the nucleic acids which are the DNA and the RNA, the protein, we have the poly sacharides and also the lipids. All these macro molecules are made up of smaller building blocks and all play really critically important roles in the cell. For example the DNA and RNA are key players in the heredity of the cell, whereas the lipids make up the membranes that envelop the cell and hold it together. The poly sacharides provide energy and structure as well as among other roles in the cell. But of the four polymers that we've talked about, we're going to focus on the proteins today which are the major work horses in the cell. Proteins catalyze chemical reactions and let the cells do the job that they are actually supposed to be doing. When we eat foods that are rich in protein what we're doing is breaking down the proteins that are in the foods using the building blocks from those proteins to make new proteins that our cells need. For example those new proteins might be antibodies or hormones, enzymes or just important cellular structures.

So since we're all familiar with foods let's start by thinking about the kinds of foods that are very rich in protein. In this next slide you'll see that there are lots of foods that we think of as rich in protein. The beans for example, the meat, the eggs, the yogurt, all of these we think of as

protein rich foods. In fact though they have not only proteins but all the other macro molecules of the cell. So when we eat the beans that come from a living plant we're eating not just the proteins from that plant, but also the lipids and the DNA, the RNA and the poly saccharides.

So what if in fact what I wanted to do was study the proteins that are in those plant cells? There are not just proteins in those plant cells but many other things. So I will need a technique to separate the proteins from the other components of the cell and what I'd like to do next is show you a laboratory technique that separates proteins from DNA, lipids and poly saccharides. And that technique is called SDS-PAGE. It separates proteins based on their size and there are three main stages to running an SDS-PAGE experiment and we'll show you those now.

So if we wanted to compare the proteins that are in the lentils and the green peas we'd start by collecting some cells, some lentils and some green peas and to these cells we would add some dye. This dye also has some detergent that helps break the cells open. To further break the cells open we would boil the samples for a few minutes and finally move on to the next stage of the experiment where we would load a protein gel. The protein gel separates the proteins by size with the smallest proteins moving fastest through the gel and to the bottom using electro phoreseis_____. And in the last step we remove the gel from the electro_phoresis_____ chamber and mix it with the dye, ___Coomassie____ stain that will let us see where the proteins have moved through the gel.

And before I show you what the results of this kind of an experiment might be, I'd like you to take some time to make some predictions. I have three cartoons for possible outcomes from this kind of an experiment. In the first cartoon the two blue staining patterns look almost identical. For the sake of argument we'll call the one on the left the proteins from the lentils and the one on the right the proteins from the green peas. And in the first picture the protein pattern, which are the blue bands, look almost the same. In the second picture the protein pattern for the lentils has only a few proteins that were stained whereas the green peas there are many. And in the third picture it looks like there are many proteins for both kinds of peas but they're quite different from one another. So I'd like you to take some time and think about the kind of banding pattern you might expect for this type of an experiment and then come back and we'll talk about your prediction.

Hello again. I don't know which of the pictures you thought was most likely to be the result of our __Coomassie_____ staining experiment but in fact if you were here in my lab and ran this experiment, you would see that picture one is closest to the result you would find for the two protein profiles. And that's because most of the proteins that are in the lentils and in the green peas are the same. Most proteins in the cell have housekeeping functions that all cells have to carry out. So this is a little tricky then. What if you wanted to study something that was specialized, say the lentils and not in the green peas? And you wanted to find a protein and to study that protein just from lentils. To do that you would have to squeeze apart that specialized protein from all the general housekeeping proteins in that cell type. And the way we do that in the laboratory is to exploit some of the differences in different kinds of protein. So I have a cartoon here to remind you that proteins come in many shapes and sizes. Some are larger than others, some are negatively charged, some have molecules attached to them. And for protein purification techniques we can use these different properties of the proteins to separate them one from the other within a given cell type.

So we're going to try a very simple protein separation based on just three types of model proteins within a very simple cell. We're going to mix a very simple cell that's made from small

purple dots, from purple puffballs and from flat disks. And we'll make a very simple cell that has just three types of components in it. And over there I want you to think about ways that you might be able to separate those very small purple specks from the larger puffballs and the flat disks. And once you've done that, think of another way that you might separate the puffballs from the flat disks. And the only trick is you can't reach into this cup to pull one protein out from the other. Once you've come up with some strategies for separating this very simple mixture that represents proteins in a very simple cell, we'll come back here and we'll talk about ways we can do it in the laboratory.

Welcome back. I hope you've had some fun thinking of ways that you might separate the proteins that are in our very simple cell, the purple small dots from the puffballs from the flat disks. So if we want to separate the very small dots from the puffballs and the flat disks, one way that I thought of was to use the difference in size and use a sieve to separate the small dots from the larger fractions within the cell. And if we do that, it works reasonably successfully to separate the small purple dots from the puffballs and disks.

Then if what we wanted to do was separate the puffballs and the disks one from the other, I thought we could try to exploit the difference in their weight or their density by maybe adding some water. With the puffballs floating and the flat disks sinking. This would be, if we were separating proteins out from a cell , separation based on differences in density. There is another way to separate them out because I know something about those flat colorful disks, and that is that they're magnetic. So if we still had our very simple cell, a mixture of the small purple dots, the puffballs and the flat colorful disks, we could use a magnet to pull the small colorful disks out from the mixture and separate them that way. This would be akin to separating proteins based on their charge. So these are just some strategies that biochemists and bioengineers use to separate proteins that are within cells because cells are very complicated mixtures of proteins and sometimes you want to study just one type of the proteins within the cell rather than all of the proteins in the mixture.

So that's our quick tour of the proteins that are within the cell and different techniques we can use to separate them. I hope you've enjoyed learning about the techniques and the tools that we have for separating proteins and that you'll continue to think of interesting experiments that you might do with these tools and techniques.

Teacher Notes:

Hi! Welcome. I hope that the lesson on protein purification would be interesting for you and your students to work through. I appreciate that this lesson is not really a stand alone lesson and needs both a lot of basic understanding about the nature of cells and the kinds of things that are within cells. As well as some specialized things that you may or may not have at hand there. So what I'd like to do just briefly is talk about what I would recommend as background material for this kind of a lesson, as well as some modifications that you might make if you were interested in teaching this lesson but didn't have particulars of the equipment that we showed within the video.

So in terms of background I think it's important that the students have a very good understanding of what cells contain, the particulars of the molecules that make up the cells and their different functions. Very quickly we sort of review that there are monomers that make up polymers, that there are polymers that have very important roles within the cells. So we talk about nucleic acids being DNA and RNA and going into the functions of heredity, but that's not something we cover within the module. So if that's new information for the students, that should be covered and comfortable before trying to talk about just the proteins within the cell or you may run the risk of just tilting the cells and the student's understanding of cells to just protein, a bag of proteins.

Similarly the nature of proteins is also sort of presumed to have been understood by your students already. So things like proteins coming in different shapes and sizes and having different chemical properties like charge and density. That is something in designing this video that I've assumed you may have covered with your students already. And if not, you may want to cover with your students explicitly in advance of running this separation video.

In terms of the techniques that we show, I think it's very fun to think about ways that similar types of cells like the lentils and the peas are similar and ways that they are different, and really that that's an important question that biologists ask and bioengineers can make useful in different circumstances. So we chose plants and things that you might have readily available but if lentils and green peas aren't easily found, many similar types of cells could be compared in this sort of way.

In terms of running the gel and the very quick demonstration of the different stages of running a protein gel, that really was intended just to start a conversation. There is no way that I've shown you enough detail within the video for you to actually run a gel in the fashion that I've shown. I don't give the contents for example of the loading dye and there are certain safety issues that you might want to consider before you actually run the lab if you run a lab similar to this. I do think running a lab if you have that capacity would be very interesting and exciting to add to this video but it's certainly not necessary and there's certainly not enough information in the video to fully run the laboratory.

But I do hope that the stages of the SDS PAGE , the separation technique is clear from the video and if not, it's something that could be discussed further within the classroom. It could also be compared to other separation techniques, for instance _agarose gel electrophoresis____ which separates DNA by size and that might be an interesting add-on lesson to this lesson.

Finally, the last segment that we build our very simple cell made out of small purple dots, puffballs and flat metallic disks is something that I think is an effective demonstration of the different properties that we can exploit from proteins in order to separate proteins from a complex mixture. If you don't have exactly those materials there are lots of things that could be substituted. Anything that floats, for instance small pieces of styrofoam could be used in place of

the puffballs. Small bits of sugar could be used in place of the small purple dots. All sorts of things like that, so there is no particular magic to the three things that I mixed together to make my very simple cell. The only idea would be that you would want to mimic the kinds of things that you find as properties of the protein with the properties of the small object that you mixed together to form your very simple cell.

So I hope that this lesson provides a launching point for both a review of basic cellular structures and important macro molecules that you would find in the cell and also a way to advance the conversation into either techniques or technologies that you use to study cells or other kinds of experiments you might do on cells if you wanted to compare them one to the other. Proteins are just one way to compare cells. But I thought by focusing a lesson on proteins and protein purification it might be a way to launch into other discussions about different types of things you would try to study about cells and different separation techniques you might employ to further understand very complex mixtures.

So I hope it is an enjoyable and productive lesson for you and your students and thank you very much!

END OF LESSON