ELIZABETH NOLAN: Welcome to the class. We're going to discuss the themes that are going to basically permeate every topic and module we'll talk about here. And one of the central themes of this class is that we're interested in studying the cellular processes of life at a molecular level, right? So as biochemists and chemists, we're interested in this level of understanding. And what we see here is a cartoon depiction of the cell. And we see that there's many types of biomolecules in this environment.

So what are our core themes for this year? First, we believe that life must be studied on a molecular level to truly understand it. And so we need to think about the cellular environment, both on a macroscopic scale, and on the molecular level. And this environment is complex, and it always needs to be considered, right? So as experimentalists in biochemistry, often we're doing experiments in aqueous buffer with proteins or some other biomolecule. How does that relate to a context like this one here where the environment is very different and much more complex?

Something we'll see, especially in the first half, the first four modules of this course, is that in cells, complex processes are carried out by macromolecular machines and elaborate systems. And these systems are fascinating. You'll see that we know a lot, but as we learn more, there's more and more questions that come up, and more questions we need to address with that. In addition to these macromolecular machines, some additional themes for this course involve homeostasis and signaling. And these will be especially emphasized in the second half of the course when Professor Stubbe takes over there. So how do we think about homeostasis and signaling in these contexts?

Something that will come up again and again is how, basically, understanding cellular processes at a molecular level, or the molecular features, can help explain mechanisms of human disease, as well as therapeutics. So an example we'll see in the early part of this lecture involves the ribosome. So many antibiotics target the ribosome. And by understanding

ribosome structure and function, we can understand how these small molecule therapeutics work. Another example involves the proteasome which we'll hear about in the second half of the course. So there's therapeutics that target the proteasome, for instance, for cancer. And cholesterol biosynthesis will come up, and how does our understanding of cholesterol biosynthesis lead to ways to treat coronary disease?

Something that JoAnne and I really like to think about day-to-day and convey to you in this course is the importance of experimental design, and choice of methods. So as scientists and experimentalists, how do we think about designing an experiment, because that design is really critical to the outcome, and what we can make of the data? And so throughout lectures and recitations, things to keep in mind, and that we'll reiterate, are that all techniques have inherent strengths and limitations. And so it's something we all need to keep in mind when we analyze data and think about how an experiment was done.

And these systems we're going to look at in 5.08 are very complex. And what that means is that many different types of experimental method are needed in order to answer complex-- and sometimes not so complex-- questions. So one method alone just often isn't enough. We need insights from many different techniques and types of expertise. And so we look forward to informing you about different types of methods-- whether they be established and quite old or new-- that are important today.

And as I alluded to before, something we have to keep in mind when doing biochemistry in the lab is that the test tube is very different from the cell. These environments are vastly different, and so we always need to think about how to relate data back to a cellular or physiological context. If you measure a dissociation constant of one micromolar, what does that mean in a cell versus one picomolar, for instance. Another point to make is that the hypothesis is a moving target. So we have the hypothesis, experiments are designed to test this hypothesis, and there's some outcome. Maybe that supports the hypothesis, maybe not. Or maybe there's some new insight from a related field that really changes how we think about something.

So in many cases we're integrating data and insights that are quite new, and Professor Stubbe and I won't have all of the answers. And so that type of uncertainty is something that we aim for you all to gain some level of comfort with. So there's many complexities in primary data, often uncertainties. And that's just an aspect of this course. And scientists, it's something we grapple with every day in our own work. So we're introducing that to you here.

And along those lines, just keep in mind, we know so much. And I think it's amazing, and-- if I step back and think about this for some of the systems we'll see-- actually overwhelming. And it's really due to dedicated efforts of many, many people over many, many years. But with that said, there are so many remaining unanswered questions, and we hope that you'll find inspiration in some of these questions as looking forward within this field. There.

OK, so what about the cell and macromolecular crowding? Just to emphasize this point a bit more, here we have an E. coli. OK, so E. coli are laboratory workhorses for biochemists. They're fascinating, I love E. coli. But I just show you this simple E. coli cartoon and this depiction here to emphasize how crowded the cellular environment is. So we have an equal E. coli of about two microns long, and maybe half a micron wide, a volume of about a femtolitre.

And if we think about the E. coli genome for a minute, it encodes about 4,000 proteins. That's a lot of proteins. And if we think about one E. coli cell of this small size, can just ask a simple question, how many ribosomes are there? So we all know the ribosomes are needed for polypeptide biosynthesis. How many ribosomes are packaged in one E. coli? Any guess? So, 10, 100, a million.

AUDIENCE: Order of 1,000?

ELIZABETH NOLAN: Pardon?

AUDIENCE: Order of like, 1,000?

ELIZABETH NOLAN: Yeah, let's say 1,000 times 15 or 20. So there's about 15,000 to 20,000 ribosomes in one E. coli cell. And as we'll see in Friday's lecture, the ribosome is very large. How did they all fit? And there's not only the ribosomes, but there's many, many other players, just as noted here in this cartoon. So you can think about what does that mean in terms of concentrations. We'll bring up concentrations of biomolecules in the cell throughout this course, and what does it mean having them packaged together so much here?

So, very different than the test tube. Our goals, some of which I think have been communicated by me so far. But just to emphasize, we're interested in these macromolecular machines and chemical processes responsible for life. We hope by the end of this course, everyone gains an appreciation for the complexity of life, and our current understanding of the topics we present to you this spring. There's close links between basic fundamental research

and medicine, and technology development as well.

Understanding the experimental basis for understanding, methods and hypotheses. And what we think is something that we hope to achieve, and that you can bring to other places after this course is really to be able to knowledgeably and critically evaluate methods and results, especially primary data. And we also hope that we convince you that biological chemistry is really thought provoking and fun, and hope you all think that right now as well.

So what are the actual topics we're going to cover? We organized this course into modules, and these modules are listed here. And different modules will have different numbers of lectures dedicated to them. But where we'll go between now and spring break-- I'll present to you during these weeks-- is that we're going to focus on the lifecycle of a protein for the first three modules. And many of you are familiar with aspects of this. We're going to present these topics, I think, a bit differently than what you've seen before. Again, very much from the standpoint of experimental methods and hypothesis testing.

So we'll cover protein synthesis, doing a careful case study of the ribosome. We'll continue with protein folding. So asking the question, after the ribosome synthesizes a polypeptide chain, how does that polypeptide assemble into its native form? What happens when proteins are misfolded? And then we'll move into protein degradation, and we'll look at proteases and machines that are involved in proteolytic degradation.

And where we'll close the first half is with module four, which is on synthases, or often called assembly-line enzymology. And this is a different type of template-driven polymerization that's involved in the synthesis of natural products. And then after spring break, Professor Stubbe will take over, and the focus will be on cellular processes that involve homeostasis, metabolism, and signaling.

And so these topics will involve cholesterol biosynthesis, and a type of molecule called terpene. And so a third way to make a carbon-carbon bond will be introduced in this section. So you've heard about Claisen and Aldol condensations in prior biochemistry courses, this will be another route. And then, we both love metals and biology, so there's a whole field of bioinorganic chemistry, and it will be introduced to you here with iron homeostasis as a case study.

And moving from here, and something quite related, involves reactive oxygen species. So I'm sure you've all heard about these somewhere, maybe in the news, maybe from your lab work.

What are these reactive oxygen species? Are they all reactive? What kind of chemistry do they do in a cell? How do we study that here? And then, of course, we'll close with a section, a module on nucleotide and deoxynucleotide metabolism-- excuse me-- as well as regulation. And then an integration of course concepts. So we have a lot of exciting topics and exciting things to tell you about.

In terms of level of understanding for this course, as I said, many of these systems are complex. We're going to look at huge macromolecular machines, and multi-step processes. This is a biochemistry course, and we are interested in molecular level, in addition to this big picture. And so things to keep in mind when thinking about structure. You need to think about the amino acids, and please review these if you're a bit rusty. So to know the side chains, PKAs, et cetera, that's all important to have in mind.

What are the protein folds? What are the arrangements of these macromolecular assemblies, and how do we study that? In terms of reactivity, we'll see bond-breaking and bond-forming reactions. So again, we need to think about things like PKAs, nucleophiles, and electrophiles. If you need to brush up, organic chemistry textbook or biochemistry textbook is a good place to go. And then something to keep in mind is dynamics. So the macromolecular structures and enzymes and proteins we'll look at are dynamic. Often we only have a static picture or some number of static pictures. But there's conformational change, transient binding occurs, and we always need to think about kinetics. So these are things just to keep in mind when you're reading and questioning to yourself about any given system here.

So what about experimental methods? This is just another topic to go over in this course overview. So there's many methods that come up in 5.08. And we don't expect that you have knowledge of any or all of these at the stage of starting the course. The difficulty that comes up is that we can't introduce all of these methods to you at once in a level of detail that's needed for everything we do.

OK, so what will happen is that if methods come up in problem sets that haven't yet been addressed, we'll give you enough background information in the problems that material, such that you can think about the questions and answer them. And we'll let you know when a method comes up. You know, you'll hear this in recitation x, or we'll talk about it more in class.

So right now, what I'd like to do is just go over a few of the methods that you're going to see multiple times. And the thing to keep in mind is that the context in which these methods are

being used may differ, but the underlying principles are the same. And we choose methods that are being used today, and are important. Some of these were developed decades ago, some of these are very, very new, and hot off the press. So if it's an older paper, please don't brush it off as, like, oh, this is old. And so, you know, it's not new. We're all really excited by technology and everything here, but many of these older methods are robust, and used all the time here.

So what are some methods and tools that we'll have under our belt? The first to point out are methods involved in macromolecular structure. So we care a lot about structure, because we need structural understanding to be able to comprehend how these systems work. And so one method we'll see a lot-- and you'll discuss in recitation this week-- is x-ray crystallography. And in addition, a method that will come up quite a bit-- and we'll see both of these in the initial discussions of the ribosome-- is electron microscopy.

And another method to be aware of-- and if you're curious, talk to your TA, Shiva-- is NMR. OK, so NMR has a lot of applications here within biological chemistry, but we won't discuss that. What can go along with methods is bioinformatics. So how many of you have used BLAST? How many of you know what BLAST stands for?

AUDIENCE: Basic Local Alignment Search Tool.

ELIZABETH NOLAN: Yeah, Basic Local Alignment Search Tool. So what does this let you do? It lets you find regions of similarity between sequences, whether that's amino acid sequence, a nucleotide sequence. And you can use that information to make hypotheses and design experiments there. So that will come up.

I have additional methods and possibilities. What about fluorescence? So how many of you have done an experiment that involves fluorescence, either in lab, or in your research? How many, did that involve a fluorescent protein? What about a small molecule? Yeah. That's fluorescent. So have you thought about why the protein was used, versus maybe why a small molecule, and what are inherent strengths and limitations or one or the other, depending what you want to do?

So fluorescence is used in many, many different contexts. We can think about proteins like green fluorescent protein, we can think about using small molecules. And we like fluorescence because it allows us to see. We can get visual information. And so, where fluorescence will first come up in this class is with the ribosome. And in recitation week two, there'll be some

discussion about using small molecule fluorophores to label tRNAs, and using fluorescence as a readout of steps in the translation process. And there's a lot of considerations and caveats to that.

Do we have a pizza delivery? Thank goodness no. Often in this class, we get pizza deliveries for someone else. I didn't know if that's already starting. Yeah, yeah. We'll also see GFP being used in the proteasome section for degradation. So a folded protein has fluorescence, a degraded protein does not. What about kinetics? So what different types of kinetic studies can be done? So what do we all hear about in introductory biochemistry class? Pardon?

AUDIENCE: [INAUDIBLE].

ELIZABETH NOLAN: Yeah, steady state kinetics, right? Turnover. So we have steady state, which I encourage you to review Michaelis-Mentin Kinetics here. And you'll also be introduced in the first weeks of this course, and especially recitation three-- so recitation two is going to build up to this-- pre-steady state kinetics. So here, you've heard about this in 5.07 or another course, introductory course. And we're looking at multiple turnover of an enzyme.

And these experiments are set up with an excess of substrate, right, in order to afford conditions that allow multiple turnover. So there's formation of an enzyme substrate complex, and then there's product formation. So review as needed. So what about pre-steady state kinetics? How many of you are familiar with this method? Not so much. So what does the name suggest? Pardon?

JOANNE STUBBE: So I'm deaf, you have to speak louder.

ELIZABETH NOLAN: Yeah, we're both deaf.

JOANNE STUBBE: I'm really deaf. So if you want to say something, so I can hear it. Speak up.

AUDIENCE: Yeah, maybe observing single molecule by some spectroscopy.

ELIZABETH NOLAN: Yeah, a single turnover, maybe, I think is what. If we're having multiple turnovers here in the steady state, right? If we're before the steady state, what does that mean, right? It means we're in the initial, really initial part of this reaction, where we're looking at a single turnover here. And how would you do this?

Basically, you look with subs having limiting substrate rather than excess substrate. And this is just to give a little prelude in terms of thinking about experimental design. So here, look at the first moments of a reaction. So what type of time scale is that?

AUDIENCE: Small.

ELIZABETH NOLAN: Yeah, small. Maybe a millisecond time scale, compared to a timescale of seconds or minutes. So what does that mean? It means you need some different experimental setup. You can't do pre-steady state kinetics in the way we've done steady state kinetics, say in a lab class for instance. So you need a special apparatus.

And what does it let you see? Here you're looking at multiple turnover, products forming. You know, here in the early stages, what can you see? Maybe intermediate formation. And why might that be important for thinking about mechanism? So those will come up in the first weeks of recitation.

Another topic that will come up, and is something that you always need to think about, and relates to integrity of materials, is that of purification. So how are proteins purified. For studying the ribosome, how do we get ribosomes that are pure and are correct? Or what if you'd like to use a mutant ribosome? How does that get generated? So here, you can talk about ribosome or protein purification.

And so, I'll present to you on ribosomes and mutant ribosomes in week four of recitation. And this topic more generally of proteins will come up in passing again and again. So how many of you have purified a protein? Many. How many of you used an affinity tag? So are they the answer to all problems?

No. They can be a huge help, but they can also be problematic in one way or another, right? So with the ribosome we'll look at a case where there was really some elegant work done using an affinity tag approach to allow researchers to obtain new ribosomes. We'll also, though, talk about the limitations of that type of methodology, and the things you need to think about if you're doing protein biochemistry, and how a tag may affect your experiments and data there.

In addition, to think about is assay development, and analytical methods. And so there will be many different types of assays that are presented in this course. And something just to think about-- how do you develop the right assay, and what are all the considerations? How do you

know your assay is a good one for the question you want to address there?

This is actually really complicated. And so there'll be some case studies that come up in the course, but just more broadly to think about. So often in lab classes, you may have an assay, but you might not be aware of all of the considerations that went into actually developing that assay such that it works. And then there's the analytical methods that are used, either for analyzing assay data or other data. And again, these have strengths and limitations.

Just some that will come up, to present western blots and immunoprecipitation. So these methods involve antibodies, and so we need to think about the antibodies themselves here. Radioactivity. OK, how does this work? Why do biochemists like to use radioactivity and assay development? And how to think about this productively and correctly. So should you be afraid of iron-55, yes or no? How does that exposure compare to being in an airplane, for instance.

Seriously, because there's a lot of fear associated with radioactivity that may or may not be well-founded, depending on what you're doing. And so this gives us a lot of sensitivity. And JoAnne will talk in week two of recitation about radioactivity, and designing experiments that use this as a read out. What else?

So affinity measurements. OK, so dissociation constants, or affinity constants, how are these measured? When reading the literature, is the value a good one, or a not-so-good one, and how can you make that distinction? Mass spec and proteomics. So these will be in the later half of the class-- I believe recitations 11 and 12-- and many others. And we're introducing CRISPR this year, in the context of the cholesterol unit as well. So as I said, we can't take care of all of these methods immediately. We'll let you know when they're coming up, when you need to know more details about them as we go through the course here for that.

So we can get started. And in the last few minutes, what I'll do is just give you a brief overview of the macromolecular machines we'll look at through modules one through three. And basically, what is the big picture? And then we're going to break that down into looking at individual components. So if we think about the lifecycle of a protein, basically, we'll fast forward to having mRNA from transcription of the genetic code. And then we have the macromolecular machine, the ribosome that allows for translation of this method message to give us a polypeptide chain.

So some linear sequence of amino acids. And then what happens? We need to get from a polypeptide chain to some functional unit. And so there's a whole number of interesting

players that are involved in protein folding. So we have folding, which is enabled by chaperones, is what we call these proteins that facilitate folding. And that's going to give us some structure that has some function here. And this protein has some lifetime in the cell.

So at some time, for some reason, it will be time for this protein to get degraded. In which case, we need machinery that will facilitate the process to break down this folded protein into smaller fragments-- whether that be individual amino acids, or short polypeptide chains of seven to eight amino acids. So from here, we have degradation to give us small fragments. And the players here are proteases and chambers of doom, one of which is the proteasome.

And actually, I forgot to mention there will be a second guest lecturer in recitation this year, Reuben Saunders, who is a senior, and does research in the Sauer Lab on one of these chambers of doom, called ClpXP. And so he'll present on single molecule methods, and fluorescence methods to study how this degradation chamber works. So that will be really exciting. He was a student in our course two years ago.

So let's just take a look. We have the ribosome here. What are the structural features of this macromolecular machine, and how does it do its job? We'll look at a number of seminal studies that were done. And it is truly fascinating and incredible. What about protein folding? So look at this macromolecular machine here, GroEL, GroES, look at how big this is. So how does this chaperone allow some nascent polypeptide that's unfolded or partially folded to obtain its native structure? And there is many details in this depiction here that probably aren't apparent yet. But by the time we're done with module two, it will be there.

Protein degradation. So here is just a cartoon-type depiction of a chamber of doom and its accessory protein from E. coli, ClpZ, ClpP. So look, we have a folded protein here, it's a beta barrel, our friend GFP that emits green light. And somehow, this protein gets threaded through ClpX, enters this chamber-- which has multiple protease active sites-- and that protein gets all degraded. So how does this work? How did ClpX and P work together to allow degradation of this condemned protein?

And then finally, where I'll close is on something I think a little bit different for most everyone, and it's a type of template-driven polymerization involved in the synthesis of small molecules like penicillins and erythromycins. So these are antibiotics. So how do we get at molecules like these from simple amino acid precursors, or precursors like those you've seen in fatty acid biosynthesis here? And often, these are described as assembly lines.

And something we'll just need to keep in mind in this unit is, are these proteins really acting like an assembly line, or is this just a way to help us think about the templates and what's going on here? So that's where we'll close. OK, so with that I'll finish up, and on Friday we'll begin with looking at the structure of the prokaryotic ribosome.