ELIZABETH NOLAN: OK, so we're going to get started. And we're going to continue on with folding. So we had some introduction last time about this module and thinking about in vitro versus in vivo studies. And where we're going to move on today is discussing molecular chaperones. And effectively, there'll be three case studies over the next two to two and a half lectures-- so trigger factor, GroEL/GroES, and DnaK/DnaJ.

And so first is some background. We need to talk about what are molecular chaperones. And so effectively, these are proteins that influence protein folding within the cell. And they can do this by a variety of ways.

So they can help to prevent aggregation and intermolecular interactions between polypeptides. They can facilitate folding by limiting conformational space and preventing side reactions. An important point to keep in mind throughout this is that these chaperone proteins bind to proteins transiently here.

What are the types of processes they can assist in? A variety are listed here. And we see that it's quite broad. So they can help in de novo folding, so for instance, folding of a nascent polypeptide chain emerging from the ribosome. They can assist in refolding. So for instance, if proteins have unfolded or become aggregated because of stress, they can help here.

They can assist in the assembly of oligomeric proteins in protein transport, and they also assist in proteolytic degradation here. And so we can classify these chaperones into three main groups depending on how they act, and those are listed here.

So we can have holdases, foldases, and unfoldases. So something you might want to ask yourself as you see these different chaperone systems is to ask, what is the role? Is it one or multiple? So holdases help to stabilize non-native confirmations. So effectively, the chaperone will bind a polypeptide and a non-native confirmation and stabilize that for some period of time.

Foldases assist in folding of a polypeptide to its native state. And unfoldases, as the name

indicates, can help with unfolding proteins, so for instance, if a protein has misfolded and that needs to be undone, or maybe a protein needs to be extracted from some aggregate that's formed in in multiple proteins that's a problem.

And so we're going to think about the chaperones in the cytoplasm in two main groups for the ones that interact with newly synthesized polypeptides. So first, we can think about trigger factor, which is a chaperone that's associated with the ribosome, as we'll see. So trigger factor is involved in co-translational folding, meaning the polypeptide is still associated with the ribosome and de novo folding. And then we'll examine some downstream cytosolic chaperones. So these are chaperones that do not bind to ribosome-- GroEL/GroES, DnaK/DnaJ.

And just as a general overview of this molecular chaperone concept here-- so this is taken from the required reading-- effectively, what's shown in this scheme is a variety of different states a polypeptide can find itself in. So here we see a partially folded protein. This protein may form from an unfolded protein or maybe a native protein. We have an aggregate. And if we look down here, we're seeing the effect of some generalized chaperone.

OK, so one important point to make from this is that the chaperone's not part of this final structure. It's just helping the polypeptide get to its native state. OK, and we can think about different rate constants, whether it be for folding or aggregation, chaperone binding Kon, chaperone dissociation Koff. So for instance, if we look here, we have a partially folded polypeptide. And imagine the chaperone binds that. Or maybe the chaperone binds an unfolded polypeptide.

OK, it's going to act as a holdase or a foldase. And what can we see down here-- or an unfoldase-- what we see down here is an indication of an event that's driven by ATP hydrolysis. And so what we'll see and what's known is that many of these chaperones switch between low and high affinity states for some substrate polypeptide. And these low and high affinity states are somehow regulated by the ATP binding and hydrolysis.

So here, for instance, we see that step. And imagine a Koff getting us back into this direction here, right? So you can begin to ask yourself questions like, under what conditions and terms of these rate constants is folding efficient? When would a chaperone act as a holdase? When would aggregation occur? So aggregation would occur if this Kagg is much greater than, say, for instance, Kon here to work systematically through this scheme. And here are just some

points and words related to that scheme and things to think about from a broader picture.

So in terms of the systems we're going to examine in the cytoplasm, this is the overview slide. And where we're going to begin in this overview is with the ribosome. And we see that in red here we have a nascent polypeptide chain emerging.

OK, so what does this scheme indicate? What we see is that here is the player trigger factor, which is involved in co-translational folding. And we see that about 70% of nascent polypeptides interact with trigger factor. And these can arrive in a native conformation.

We see there's two other systems here. So on the right, we have GroES and GroEL. So GroEL provides post-translational folding. We look and see about 10% to 15% of peptides in the cell interact with GroEL/GroES. And as we'll see, it provides this folding chamber on a protected space.

We also see here that this system uses ATP. OK, and here we have another two players, DnaK and its co-chaperone DnaJ. And we see they're binding to some sort of polypeptide in a manner that's different than GroEL/GroES. OK, about 5% to 18% of polypeptides come into contact with these two players. We also see this system as ATP-dependent, and there's another player, GrpE, which we'll see is the nucleotide exchange factor needed here.

OK, so we see that maybe there's some crosstalk here. And here we have some needed native polypeptides. So just some things to keep in mind-- it's important to think about concentrations and some approximate concentrations are listed here. If we're thinking about the ribosome DnaK/DnaJ, GroEL, and trigger factor.

Just to note that many chaperones are also called Heat Shock Proteins, and this is because their expression increases with increased temperature or stress. So Hsp70, Hsp60, that's for heat shock protein. So where we're going to begin is with an overview of trigger factor. Yes?

AUDIENCE: I wanted to ask. What do you mean by native and this protein exist in several conformations in this slide?

ELIZABETH NOLAN: So proteins are dynamic, right? We know that. So native means a native fold, so a native state of this protein as opposed to the protein being unfolded if it's supposed to be globular or being some undesirable oligomer aggregate. So when this polypeptide comes off the ribosome, that's a linear sequence of amino acids. And it needs to adopt its appropriate conformation to do its job, OK? And as I said last time, we're not discussing natively unfolded proteins in the

context of this class.

AUDIENCE: So proteins can have many different native receptors in this slide?

ELIZABETH NOLAN: Yes, they're dynamic. But there is going to be, like if you need a beta sheet, a domain that has beta sheets, for instance, that needs to fold and form there. So we can discuss further if you have more questions about that. But think about ubiquitin, for instance, from recitation week one. That had a very defined shape, right? So it's native fold from looking at that PDB file.

AUDIENCE: OK, I think I just need to understand in that previous slide. When I talk about one protein here, right? 70% percent of the proteins--

ELIZABETH NOLAN: Yeah, this is thinking about all the proteins and all the peptides in the cell.

AUDIENCE: OK, I thought it was one type of protein.

ELIZABETH NOLAN: No, this is looking at proteins in broad terms. And so what we'll see as we move forward is that certain types of proteins interact with GroEL where others don't, right? Trigger factor interacts with many, many of them because it's associated with the ribosome, and the ribosome's synthesizing all polypeptide chains there.

OK, so we're going to start with trigger factor. And the first thing just to be aware of when thinking about trigger factor and where it acts-- so we saw it sitting on top of the exit tunnel of the 50S in the prior slide-- is that there is a lot of things happening near the exit tunnel of the ribosome. OK, so here we have our 70S ribosome. Here's the polypeptide coming out. A few proteins are indicated.

In addition to trigger factor, just be aware that there's other players here. OK, one of these is Signal Recognition Protocol, which I mentioned briefly last time. This is involved in delivering membrane proteins to their destination.

We also have enzymes that do work here, whether it's an enzyme for removing the N-terminal methionine, enzymes for deformulation of that N-terminal methionine, et cetera. So somehow, trigger factor needs to work in the presence of these other constituents there.

OK, so when we think about trigger factor, what you want to think about is a protein of about 50-kDa that's shaped like a dragon. OK, this is ATP-independent. So what I said earlier about

low and high affinity states and switching between these states being driven by ATP binding and hydrolysis, that does not apply for trigger factor. It's the exception in what will be presented in this course. And it's associated with the ribosome.

And what trigger factor does is it provides a folding cavity or cradle over the exit tunnel. And by doing so, it gives this emerging polypeptide a protected space to begin to fold, so reduction of intermolecular interactions. So if we take a look at trigger factor, one depiction is shown here. And as I said, think about a dragon. And it's actually described as having a head, arms, and a tail.

And so we can think about this also in terms of N and C-terminus. The region of trigger factor that interacts with the ribosome and binds the ribosome is down here in the tail region. And so what trigger factor does and what's indicated by the cartoon you've already seen is that it associates with the translating ribosome with a one-to-one stoichiometry.

So think about having one trigger factor over that exit tunnel here. And as you can see, these different domains also have some additional activities. And the main chaperone activity is attributed to the C-terminal region here I've color-coded.

So what are some characteristics of trigger factor? If we look at the surface and consider where different types of amino acids are found, so whether they have polar or non-polar residues, that's depicted here. OK, what do we see in this depiction? Where are these residues? Is a given type of residue clustered in any one spot?

Yeah, I see some heads shaking no. No, right. They're distributed all about. We see non-polar and polar residues distributed across the surface of trigger factor here. And so why might this be?

What's thought is that, effectively, trigger factor uses its entire inner cavity-- and you'll see how that forms in a minute-- for substrate accommodation. And you can imagine that all of these different polypeptides emerging from the ribosome have different amino acid compositions, right? So this allows it to be relatively versatile from that perspective.

If we take another look at structure and think about how trigger factor binds to the ribosome, what's found here, so we're looking at structures of trigger factor bound in orange and unbound in green to the ribosome. And the ribosome's omitted for clarity. There's a helix-loop-helix region that is involved in that interaction. And what's found is that when trigger factor is

bound, it's quite dynamic. And it can swivel around this ribosome binding site by about 10 degrees in every direction.

So why might that be important? One, this flexibility may allow it to accommodate many different polypeptides that are emerging from the ribosome. And it may also facilitate its coexistence with those other proteins and enzymes that are acting by the exit tunnel that we saw on the prior slide. OK, so here is just a model attempting to show the different ways and degrees to which trigger factor can move from various structural studies when attached to this ribosome here.

So this N-terminal domain, I have protein L23 listed here. It also contacts the 23S ribosomal RNA and the protein L29. And what's found is that some salt bridge interaction is important. And we'll see that in a moment.

Here, if we look at a depiction of trigger factor actually bound to the 50S, so here we have the 50S. Here's the exit tunnel. And here's our dragon-shaped molecule sitting. I like to say on top. I guess it's on bottom here. But anyhow, the polypeptide's coming out, and it has this cavity where it's protected from all of the other constituents in the cell.

And here's just a rotated view showing it on top-- so tail region, head, and arms. So we have this cradle over the exit tunnel. It's giving a protecting space for folding of that nascent polypeptide chain. If we just look at a little more detail here, what do we see?

So there's a salt bridge between an arginine residue, arginine 45 of trigger factor, and glutamate, glutamate 13 of the ribosomal L23 that forms a salt bridge. So in this depiction, we have trigger factor in red. We have L23 in green. OK, and so you need to be thinking about the amino acid side chains here, and that's something, too, Joanne and I want to stress a bit after recitation last week is, really, in this course, the importance of thinking back to the chemical structures and properties of the molecules that come up within this course-- so positive charge, negative charge, that interaction here for that.

So what happens when a polypeptide is emerging from the exit tunnel and it encounters trigger factor? There's many possibilities. And as I said, trigger factor is dynamic. And an interesting point about this protein is that trigger factor can differentiate between vacant and translating ribosomes,

OK, and so what's found from in vitro studies is that the binding affinity, which I'll describe as a

dissociation constant which is 1 over the Ka of trigger factor for the ribosome varies by several orders of magnitude depending on whether or not the ribosome's translating. So we have the Kd measured on the order of 1 to 2 micromolar if the ribosome is vacant, and a Kd of about 40 to 70 nanomolar for a translating ribosome. OK, and just in recitation 10, we'll talk about binding studies more. But just if needed for review, if we're thinking about A plus B going to AB, we have Kon and Koff. And Kd is Koff over Kon, and Kd is 1 over the Ka here.

So let's look at some aspects of a model for a trigger factor dynamics during translation. So as I said, it can differentiate the vacant and translating ribosomes. What's been found from in vitro studies is that the mean residence time on the ribosome is about 10 seconds. So what are the possibilities?

One, trigger factor can bind to a vacant ribosome, and that's shown here. And it can bind to a translating ribosome, and it does this with higher affinity, so greater Kon. So what happens after trigger factor binds to a translating ribosome?

What we see is that the nascent polypeptide chain is coming out. And in this cartoon, what's depicted is that it's beginning to fold in this protected region here made by the trigger factor cradle. And what we see from this point is that there's three possibilities.

So if we look first on the left, what happens? Trigger factor dissociated from that polypeptide that's emerging from the ribosome. So recall these chaperones bind and release the polypeptides. In this case, it's left. There's some folding that's happened, and this peptide is still associated with the ribosome.

So what might happen next? Maybe this polypeptide has the ability to reach its native state without the help of trigger factor anymore. So that's shown here. It's released, and it's folded. Maybe some other chaperones in the cytoplasm helped with that, but it's not shown here.

Alternatively, maybe trigger factor binds again. So maybe this is one domain, and then somewhere else, there's some other region that needs some help with folding. And we see that here. So it can bind and release the same polypeptide more than once.

What are our other options? So maybe trigger factor, after being here, remains bound to the ribosome, and the polypeptide is released. Or look what happens here. We have trigger factor bound. We see release of the polypeptide with trigger factor bound, or here we see that there's even two trigger factors bound to the same polypeptide emerging from the ribosome.

And just thinking about this from the perspective of the number of different polypeptides that are synthesized by an organism, all different lengths, all different levels of complexity, it's not too surprising that there's various possibilities here. So again, if you're presented with data, you need to ask, what does the data say? And what type of particular aspect of this model does it support? Yeah?

AUDIENCE: How often is the ribosome actually vacant?

ELIZABETH NOLAN: How often is the ribosome vacant? Yeah, I don't know how often the ribosome is vacant. So in vivo, in your test tube, you can completely control that, which is what's going to give some of these data here. Joanne, do you know? No. Yeah, anybody know? I don't know, right? So does it make sense to have many vacant ribosomes?

AUDIENCE: Are there more vacant ribosomes maybe like floating around than there are membranes bound [INAUDIBLE]?

ELIZABETH NOLAN: So I think that's a can of worms we're not going to go down right now in terms of where the ribosome is here. So let's look at a functional cycle. This is just another depiction of a potential functional cycle where we have the ribosome bound to mRNA. There's a nascent chain.

Here we see several trigger factors bound, and we see options. So either the native fold, or maybe there needs to be some work of downstream chaperones, right? And at some point, triggered factor will be dissociated, and it can come around and rebind again. So there is some evidence the formation of a trigger factor dimer when it is not with the ribosome. We don't need to worry about that detail too much for our thinking about what's happening here, because this is a one-to-one stoichiometry.

So how is trigger factor influencing the folding process? If we think about foldase, unfoldase, and holdase, so these cartoons show that some folding is happening in that cradle, especially the ones we saw before, right? And that's perfectly reasonable that somehow trigger factor is allowing or accelerating productive co-translational folding of that polypeptide. So from that perspective, it would be a foldase.

Is it possible that it's also a holdase? And could trigger factor, in certain cases, keep nascent chains unfolded? Maybe to help prevent premature folding that would be an error during polypeptide synthesis, that's another possibility. And they're not mutually exclusive, right? So again, it's a question of an individual system and looking at the data. So the behavior may

depend on the circumstance in the polypeptide chain. Rebecca?

AUDIENCE: I'm just curious. So when we're talking about it acting as a foldase, mechanistically, is the trigger factor physically interacting with and promoting a certain conformation? Or is it just providing a space where everything else is isolated? Or do we even know?

ELIZABETH NOLAN: Yeah, so do we even know? So this is something we'll talk more about in the context of the chamber GroEL. But what are the possibilities?

One is that trigger factor is just providing a safe place for this polypeptide to fold to its native conformation. Because recall last time, we discussed the primary sequence and how primary sequence can dictate the fold and what's thermodynamically most stable, right? But in the cell, the cell is very crowded, right? So trigger factor can protect this polypeptide from all those other constituents in the cell that might cause unwanted intermolecular interactions, for instance, and cause a different folding trajectory. Is it possible that the cavity wall of trigger factor could influence that energy landscape?

So that's the other aspect of your question. Is it an Anfinsen cage, so just allowing folding? Or is it actually affecting the landscape? I don't know if we're suggesting that it influences the landscape, the energy landscape. But that doesn't mean that literature is not out there for that. So I think of it typically as a cradle. And as I said, we'll come back to this idea with GroEL where there have been studies and people arguing one over the other.

OK, so with that, we're going to leave trigger factor and move to the macromolecular machine, GroEL/GroES. And so GroEL falls into a subset of chaperones that are called chaperonins. OK, and these are chaperones that are essential for viability in all tested cases. OK, so that tells you this machinery is really important for the cell and must be involved in folding of some important players here.

So in terms of GroEL/GroES, what do we have? I can describe this as bullet-shaped. a bullet-shaped folding machine. And so GroEL is the chaperone, and GroES is the co-chaperone. And they work together.

And so what we have if we draw this in cartoon form is we have GroES, and we can describe GroES as the lid of the folding chamber. And here we have GroEL. OK, and what GroEL is, this gives us cavities for folding.

OK, and we can think of it like a barrel. And as drawn, we see two pieces here. And as we'll look further, we'll see that these are two heptameric rings. The ring that has the lid attached is called the cis ring. Or sorry-- the chamber or heptamer with the lid attached is cis, and the one below is trans.

And this is huge. So this whole thing is on the order of 184 angstroms just to give some scale. So EL is the chaperonin, and ES is the co here. So what we'll do is look at the structural characteristics of GroEL and GroES individually and then think about function here.

So for GroEL, what we have are to have to heptameric rings. OK, and so if we look from the top here, what we have are the seven subunits arranged in this ring. OK, and what we see is that there's an inner cavity that's about 45 angstroms in diameter, OK? And each subunit is about 60 kilodaltons, which is why this is called Hsp70.

And so if we take a look in this structural depiction here, in the middle, this is the top view. OK, and the different subunits have been color-coded. They're all the same polypeptide. They're just differentiating them here so it's easy to see each one. And here's that inner cavity.

If we look at the side view again, we need to consider a little more detail. OK, so each one of these is a 7-mer. OK, and each subunit of GroEL three domains that are organized A, I, E-- so apical domain, intermediate domain, and equatorial domain. And then if we look at this bottom ring here, they're organized like that.

OK, so effectively, what we have is a back-to-back arrangement. OK, so we have 14 subunits and two back-to-back rings. And so if we take a look again at this depiction, what's been done is that in this top 7-mer ring, for one of the subunits, the three domains have been colored.

OK, so we see that the apical domain is an orange, the intermediate domain in yellow, and this equatorial domain in red shown here. And this is one isolated subunit again with this color-coding here. So what happens when the lid binds? So we're currently looking at this as just GroEL the to heptamers. But we need to begin to think about GroEL with its lid.

What happens when the lid binds is that there's a conformational change. OK, and so I'll just draw this, and then we'll look at the structure. OK, so imagine that this is one GroEL subunit of the 7-mer ring, OK? When GroES binds to that ring, what happens is that the GroEL subunits change from a closed conformation, which I'm kind of showing as closed, to something that's open. OK, so effectively, it's like opening up a hinge.

OK, and so the consequence of this is that when the lid binds to this cis cavity, the size of the central cavity expands dramatically. So it basically doubles. And that's something that's not clearly indicated here. So we can modify the cartoon.

OK, so let's take a look and then talk about why that's important. So here are two depictions where we have GroEL/GroES, and we can look at a GroEL subunit that does not have GroES bound, so with the trans ring. Or we can look at a GroEL subunit where the GroES lid is bound, so in the cis.

OK, and so this is actual structural depiction of what I tried to indicate on the board here where we have closed and open. And so this opening is making this cis ring much larger in terms of its central cavity, OK? So these are major conformational changes and details of which are described here.

But effectively, the two points to keep in mind is, one, that diameter and size of this central cavity doubles. And we can think about why that might be important in terms of accommodating a larger polypeptide as we get towards the functional cycle of this. And also, what we'll see as we move forward is that the distribution of hydrophobic and hydrophilic residues on the interior of this cavity changes dramatically when GroES binds here.

So briefly, to look at GroES, what does that look like from a structural perspective? So GroES is also a heptamer. OK, each subunit is only about 10 kilodaltons. It's about 30 angstroms in height and about 75 angstroms across here. And what we see if we look at the structure of an individual GroES, so again, here, what we see is that there is a beta sheet region. And there is this region here that's described as a mobile loop. And if you look, the beta sheet region's on top, and this mobile loop is down where it binds to GroEL.

OK, so effectively, when GroES docks onto GroEL, these mobile loops bind to hydrophobic peptide binding pockets that are on the top of this heptamer there. OK, here's another depiction. So you're seeing the beta sheet parts on top, and here are the mobile loops that can bind to peptide binding grooves of GroEL.

AUDIENCE: So does that mean the inner cavity of the cis part of GroEL is always open I guess after GroES binds? And trans is always closed? Because it looks like just from the way we've drawn it. Does GroES bind to the other side also?

ELIZABETH Yeah, so right, this is how we've drawn it. We've drawn it like a bullet. And so does GroES bind

NOLAN: to the other side? And how do these two chambers function? OK, and as we move forward getting to the functional cycle, what we'll see is that both rings are functional, but they're functional at different points in the cycle.

OK, so GroES, yes, can bind to either one, but it's this bullet type of shape that is considered to be functional. So you might ask, what about a football? If we stick another GroES on the bottom, we get a football-shaped species. And there are some in vitro studies that show a formation of a football with two GroEL rings and two GroES rings, but those are found at very high ATP concentrations. And so it's thought that they may not be significant, that they're a transient species effectively of unknown significance that at least in the test tube, you can form at very high ATP. OK, yeah?

AUDIENCE: And then is the cis and trans, it's not predefined, just it depends on wherever the GroES makes it?

ELIZABETH NOLAN: Right. It's going to depend on wherever the GroES is. OK, and what we'll see as we move forward is we need to think about also how ATP binds. And ATP binding will also happen in one or the other, depending at the point in the functional cycle here, OK? So we just want to get the structural aspects under control before we look at the functional cycle.

So this is one last slide on the structure. And so I find this to be a really beautiful machine. Here we have the bullet-shaped two GroELs and one GroES. And here we have the different domains colored.

And here what we have is a cutaway view to look at the interior of the chambers. And so we have the cis chamber on top, the trans chamber on bottom. And in the color-coding here for this cross-section, what we have in yellow are hydrophobic residues, and in cyan, hydrophilic residues. OK, and so what's important to do is take a look at the cis chamber and the trans chamber and ask, what's going on in the interior? And why might that be important? So what do we see comparing the distribution of yellow and cyan, or hydrophobic and hydrophilic? Lindsay?

AUDIENCE: It's much more hydrophobic in the trans chamber.

ELIZABETH NOLAN: Yeah, right. The trans chamber is much more hydrophobic in terms of that lining than the cis. So the cis chamber, as we'll see in a minute, is where the polypeptide will be folding. So a polypeptide will end up in the cis chamber, and the lid will be on top. So why might this be an

important feature-- not only that we need this cavity size to grow, but we need a change in the lining to be more hydrophilic?

AUDIENCE: Because if it's assisting folding, it's likely that the hydrophobic residues are more likely to be buried in the center the protein in the polypeptide. So you'd want to facilitate favoring the hydrophilic residues to be interacting on the outside of the protein?

ELIZABETH NOLAN: Yeah, so often, that's right to think about. Where do we find different types of residues, say, in a protein with a complex fold? And typically, we think about hydrophobic residues on the interior and hydrophilic residues on the exterior. So for instance, there is a model of folding called hydrophobic collapse. And effectively, you have hydrophobic interactions, and then the rest of folding occurs, right? So you'd imagine there's a benefit to having a hydrophilic exterior if you want the exterior of the protein to be hydrophilic here.

So what is the functional cycle? And in thinking about this, we need to think about ATPs and ATP hydrolysis. And what you'll find as you read is that often the model is drawn a bit differently depending on the paper you read. And that's because they're just some uncertainties out there. So don't get hung up on that. I have two different examples within the lecture slides here.

OK, but if we just think about the functional cycle-- and I'll just draw a little bit, and then we'll go to the board. So imagine we have one GroEL here, OK? And as drawn here, there's no GroES. There's no peptide, and there's no ATP. So imagine some peptide comes in that needs to be folded by this machinery and ATP.

OK, and these end up inside of the chamber. And then we can have our lid come in. OK, so now this polypeptide is in this protected cavity, and ATP can bind in the equatorial domain of GroEL. So each GroEL monomer will bind one ATP. So there's seven ATP bound in one heptamer if it's in the ATP-bound form, OK?

And so let's take a look in a little more detail. So what do we see? And the thing to keep in mind, as I said before, is that both chambers are active and functional. They're just functional at different points within this overall cycle.

OK, so if we begin here, what do we see? This top GroEL heptamer has no cap. What we see here is that we have the bottom GroEL bound to ADP and GroES. Some unfolded polypeptide comes along. It binds, so maybe there's some hydrophobic interaction between the top of

GroEL and some region of this polypeptide.

What do we see happening? The ATPs come in, so I indicated them together, there's some timing where there's questions. These ADPs from the bottom chamber are ejected. We see ATP binding, so there's seven-- one per subunit. The polypeptide binds, and here comes GroES.

OK, and so once this polypeptide is encapsulated in this chamber, there's some residency time. And this is often quoted on the order of 10 seconds. Also note here. Look what happened at the bottom ring. GroES got ejected. OK, so with GroES binding here, there was ejection of GroES from the bottom and loss of these ADPs. OK, there's ATP hydrolysis during this time. The polypeptide is trying to find its fold.

And then look what happens here. We see GroES coming into the bottom. Again, we have release of ADPs, release of GroES, and this polypeptide kicked out, which may or may not be in its native fold, OK? If we take a look showing this as a complete cycle here-- and again, I said before there can be some differences from depiction to depiction-- but here, we are seeing GroEL. We have the top one and the bottom heptamer.

Here's some polypeptide that needs to be folded. It's initially grabbed by the top part of GroEL. ATP comes in. We have this ATP-bound form. Here comes GroES. The polypeptide gets pushed into this chamber, and now it's closed. We have ATPase activity, so ATP hydrolysis to give the ADP-bound form.

OK, and then what happens here? OK, what we're seeing now, this bottom ring is becoming functional. ATP binds another polypeptide. OK, and then we have release of GroES in the polypeptide from the top chamber. OK, and then you can flip this and work around the cycle again.

OK, so this is a case where we can think about the affinities of the ATP and the ADP-bound forms of GroEL and what that means GroES binding here, OK? And so the ADP-bound form of GroEL has a lower affinity for GroES than the ATP-bound form here for that. So each GroEL heptamer acts as a single functional unit, and both rings are active as shown here but in different points of the cycle. OK, and so the thinking is that ATP binding and hydrolysis drives uni-directional progression through this cycle.

With that said, there's a lot of questions as to how. So what is it about this ATP binding and

hydrolysis event that allows this work to happen? That's a question that I see as still pretty open.

And so I'll close with that here now. I suggest to review this cycle before next time. And what we'll address on Wednesday is experiments that have been done to sort out what are the polypeptide substrates for GroEL/GroES. So we know they must be some important players given that these are essential for viability. What are they? And how is that determined?