ELIZABETH NOLAN: So we're going to spend the first few minutes finishing up our discussions of the evolved orthogonal ribosome called Ribo-X and experiments that were done to characterize it and determine whether it did a better job at allowing the tRNA to suppress the amber stop code. And then we'll be transitioning into module 2. So we'll be starting protein folding today and be spending the next couple of days on that area and thinking about some of the macromolecular machines and other proteins involved.

So just as a recap, where we left off last time with thinking about incorporating unnatural amino acids using a new ribosome. And so we discussed how an orthogonal ribosome was designed that binds to orthogonal mRNA. And then the idea was, can this orthogonal ribosome be improved, the immunogenicity and selection, to get a new orthogonal ribosome, where suppression of the amber stop codon by the tRNA is favored over termination by release factor 1?

So we talked about this issue with really factor 1 causing truncated protein phenotypes. And so where we left off was in a series of three types of experiments to look at how well this Ribo-X works. So we looked at protein yield to ask, is it translating polypeptide, as well as the starting orthogonal ribosome. And then the next experiment we were looking at, and where we left off with, was experiment 2, which was the question of amino acid incorporation.

And so what was done, just in recap, is that a GST maltose-binding protein, MBP, fusion protein was designed. And recall that this protein had a protease cleavage site and that the GST portion contained cysteine and MBP does not contain cysteine. So the idea was to use radio labeled cysteine as a probe for misincorporation of cysteine into maltose-binding protein.

So in terms of the actual experiment, what was done, imagine that we express this fusion. In the presence of the radio labelled cysteine, we use a protease. And in this case, it was thrombin. This protease gives us two fragments, GST plus MBP. And these two proteins have different size, so we can separate. So just doing SDS page.

And ask, where did the proteins migrate on the gel, and then where do we see radioactivity? And so the data are shown here that were reported by the authors. So what are we looking at? We're looking at, on the left, the Coomassie stain for total protein. And then on the right, we're looking at radioactivity here.

So what do we have here, in terms of the lanes? They've done some labeling to help us. So maltose-binding protein is running up here. GST is running down here. And we have four different conditions. What do we see? We have, in lane 1, Ribo-X is what's used.

In lane 2, we have a system where it's the initial orthogonal ribosome that was not involved. So that's our point of comparison. In lane 3, we have the native ribosome only. And in the 4, lane 4, a control for no gene. So what do we see?

AUDIENCE: All the ribosomes look like they're doing the same thing, except for when there's no gene.

ELIZABETH NOLAN: Right, no gene to translate. Yeah. So why do we come to that conclusion, they're all doing the same thing? What we see on the left here, in the Coomassie stain, is that in lanes 1 through 3, we see a band for MVP, and they're all similar, in terms of intensity. And likewise, for GST, we see a band, all similar intensity. So it looks like, in all cases, a similar amount of protein is being synthesized, and that this protease cleavage worked in a comparable manner.

AUDIENCE: Do you ever have different RNAs, depending on whether or not the orthogonal ribosome versus the native ribosome, and do you worry about how that might influence this experiment?

ELIZABETH NOLAN: Yeah, right. The native ribosome is not going to bind to the orthogonal mRNA, so you need to give a plasma that has a ribosome binding site compatible with the native ribosome. And yes, it's possible that native ribosome won't work as well. Or reverse. I'd say reverse is more likely, that one of these mutants won't work as well. But that doesn't appear to be the case here. So what does the gel on the right show? So where do we see radioactivity?

AUDIENCE: Mostly at GST, but then it looks like there's a lot of background.

ELIZABETH NOLAN: Yeah. Do we expect background? Where might the background come from? Right, so we see a strong band here, and this is at the same places where we see GST. So that's a good indication. We know that there's cysteine and GST, so we should see a band here. So what about background and what about MBP?

AUDIENCE: You're going to have some misappropriation in MBP. It's very much falsely compared with

GST. But a little band in MBP could be some amino acid misappropriation.

ELIZABETH NOLAN: OK, is that band MBP? Do we know that definitively in this gel?

AUDIENCE: Not necessarily because the Coomassie band's a little wider, so it can be a slightly smaller molecular weight then.

ELIZABETH NOLAN: Right. There's many species in these gels. It isn't just two bands, one for GST and one for MBP. So where did these other bands come from? Could it be from the initial protein purification and there's some contaminants? Could it be a result of the thrombin cleavage, and maybe thrombin cut at some places other than just this cleavage site?

So that's preferred, but maybe it can cut some other places. So there's other species in the gel. And cysteine comes up in other proteins, so it makes sense that there'd be some background to say this protein wasn't 99%, 100% pure, or maybe from cleavage there. In terms of this little band, is that MBP or not? A little hard to say. Where they placed the arrow indicates maybe not, right? But what could you do to find that out?

AUDIENCE: Western.

ELIZABETH NOLAN: Yeah. So maybe a Western Blot. If there's an antibody, you could run something less concentrated. You could do mass spec. There's options if you wanted to track that down. But the bottom line is if we compare the radioactivity here to what's seen here, there's much more with GST, which is what we would expect.

And so through some further analysis, they conclude the error frequency is less than 1 in 10 to the 3 for Ribo-X, at least for cysteine. So what's the limitation of this experiment, from the standpoint of misincorporation, which is what I asked you to think about in closing last time?

AUDIENCE: What you're testing for is cysteine in this incorporation.

ELIZABETH NOLAN: Only testing for cysteine in this incorporation. So we don't really know about other amino acids. So this is good news, but there's other information we're not getting from this experiment there. So is it possible that other amino acids are misincorporated? It would take some additional experiments to get at that.

So the last experiment we're going to look at is asking the question, does Ribo-X actually do a

better job than the progenitor or o-ribosome? So what we want to look is at the efficiency of suppression of the amber stop codon.

So last time, we talked about how one limitation of the Schultz method is that there's a truncated phenotype because RF one enters the A state, rather than the tRNA. So in order to look at this, they continued to use this type of fusion. But instead of having a protease cleavage site here, they stuck to the amber stop codon in between GST and MBP.

So they made two different plasmids, so GST, a plasma encoding, GST, the stop, and malE. So this is the gene named for MBP. So if this gets translated, the question is, do we get the GST-MBP fusion or GST? Because if the tRNA does its job, polypeptide synthesis will continue and we'll get the fusion protein. If release factor 1 ends up here at this position, we get termination and only GST.

And they also made an additional construct. So in addition to asking, what happens if there is a codon for one unnatural amino acid, what happens if there's two? So recall, with the Schultz method, what we saw last time is that when attempts were made to incorporate two unnatural amino acids in one polypeptide, the efficiency went down to below 1% there.

So what if two of these are here? Again, the question is, when these get translated, do we got GST-MBP or GST? And as we can see from the gel, we can differentiate pieces by size. OK.

So in addition to comparing these, what was done is in both sets of experiments, there was a comparison of the Schultz method we discussed first, and Ribo-X here. And just in terms of size, so GST-MBP is about 70 kilodaltons and GST is about 20 kilodaltons, 26. So easy to separate on the gel.

So what are the data? And what was the unnatural amino acid employed? So they ended up using an unnatural amino acid called BPA, which is a benzophenone, so a crosslinker. Here. And so I'm going to write up some details that came out from the gel on the board because there's a lot of things to navigate in that gel.

So effectively, what are the comparisons we want to make? So in lane 3, the method we have is the Schultz method. There is one codon for incorporating one unnatural amino acid. And what they see is they get about 24% efficiency of full length fusion protein. In lane 5, we have the Shultz method with two here, and we get about 1% efficiency

In lane 7, analyzing the orthogonal ribosome Ribo-X, and one. And what's seen is 64%

efficiency, so quite an improvement there. And then in lane 9, what we have is Ribo-X with two and an efficiency of 22% here. OK so here, we're looking at the wild type ribosome, and here, we're looking at the orthogonal ribosome, orthogonal mRNA with the two mutations we saw before.

So these values come up from quantification of the data here. So you can convince yourself by comparing the bands for GST, resulting from truncated phenotype translation termination and the bands for the fusion protein GST-MBP. So showing that there was successful suppression of the amber stop codon here.

So what are the major conclusions? The major conclusion is that, at least with this system, what we see is that Ribo-X has minimized this truncated peptide phenotype compared to the wild type ribosome, and that it's been possible to, basically, diverge the decoding properties of the orthogonal ribosome from the indigenous cellular machinery.

AUDIENCE: I'm trying to understand you right. So the percent here, that's the percent of the total expressed protein? Oh, so the other 99% would have been the GST only?

ELIZABETH NOLAN: Yeah. So here, for instance, if we take a look at lane-- let's compare lanes 3 and lane 5. So here's lane 3, and what do we see? So in here, we have incorporation of one natural amino acid. And we see that there is a band for the fusion protein and there's a band-- let me make sure I'm in the right lane, lane 3-- and a band for GST itself.

And the intensity of this band is greater than the intensity of that band, and you can imagine doing quantitation, whereas if we look at lane 5, where we're trying to incorporate two by this method, we see a band for GST. And what do we see up here? Very little. If we look at lane 7, we're seeing 64%. Lane 7 here, we see that we have this band for the GST-MBP fusion and a weaker band for GST alone there. So percent efficiency, percent of the total.

So there's other things happening in this field. So the Schultz method and these orthogonal ribosomes are two examples. One thing that came up after this work with Ribo-X was to design ribosomes that can use quadrupling codons, rather than triplets. So a lot of creativity and things to look up if you're curious.

But with that, we're going to close the translation module. We will not leave the ribosome. It will keep popping up throughout modules 2 and 3. But we're going to move into what happens to a polypeptide as it leaves the ribosome.

So how does it get its native fold? And so what happens to nascent polypeptides emerging from the ribosome, and how do polypeptides fold? And so there's reading posted for module 2 on Stellar and listed here, one required paper, which is a really wonderful review that came out about two years ago.

So let's think about folding. And as a point to thinking about that, let's think about our ribosome. And there's some emerging polypeptide chain, so the nascent polypeptide. So what happens to this polypeptide?

And the first thing to keep in mind is something we need to think about, is where is this polypeptide destined to go? Is this a polypeptide that will be in the cytoplasm? Is this a polypeptide that will become a membrane protein, or part of the secretory system? And so we can think about cytoplasmic protein versus membrane proteins, or a new karyote secretory here.

And so we're going to focus this module in terms of thinking about what's happening in the cytoplasm. We might touch upon this if there's time, but I think there won't be. So the cytoplasmic proteins are folded by chaperones that they can come into contact with as they're emerging from the ribosome, or also after the polypeptide is released.

AUDIENCE: Do extracellular matrix proteins fall into either of these categories?

ELIZABETH NOLAN: I actually don't know. Joanne, where do extracellular matrix proteins fall?

JOANNE: Well, they get made inside the cell.

ELIZABETH NOLAN: They're made inside the cell and then they have get shuttled.

JOANNE: So you should go talk to Matt Shoulders because if you look at collagen, that's exactly [laughter]

ELIZABETH NOLAN: OK, so these interact with a player called signal recognition particle, which allows for targeting to the membrane or endoplasmic reticulum in eukaryotes, and then folding can happen here. So we're going to be focused in the cytoplasm, just realize there's other machineries involved for membrane proteins.

So here's just another view of our ribosome. We saw this early on in the ribosome unit. And we want to think about this exit tunnel and the emerging polypeptide chain. So it's the 50S subunit. And as we discussed before, this exit tunnel is long and it's also quite narrow and it's lined by both ribosomes RNA and proteins.

And I know a few of you asked about the hydrophobic residues of proteins that line this tunnel after lecture 2. And the thing to keep in mind is that it's not all hydrophobic. There's also RNA there. There will also be other residues. And something just to think about, like can water molecules get in there, as well? So we see for instance, there's two proteins, L4 and L22, that line part of the tunnel. We have protein L23 at the exit. But there's also a lot of RNA there, so don't forget that.

So a question, just to address early on, does protein folding occur in the exit tunnel? And I'd say this has been a bit of a controversial question over the years and there've been camps arguing both possibilities, yes or no. I think the thing to keep in mind is that the dimensions are limited. And although we can imagine some confirmation of flexibility and dynamics in this exit tunnel, it can't undergo some tremendous change to, say, accommodate something like ubiquitin that you saw early on. That doesn't just make sense.

So is it possible for some alpha-helical fold to occur in this exit tunnel? Presumably. There is some work that indicates there's folding zones in the exit tunnel, so maybe some folding happens there. But really, the main conclusion is that most folding occurs outside of the ribosome and after the polypeptide emerges from the 50S here.

So if we're thinking about most folding of polypeptides as occurring in the cytoplasm for cytoplasmic proteins, what we need to think about is that environment. And we learned in the introductory lectures, or had a reminder, that the cellular environment is very crowded. So we have this issue of macromolecular crowding.

And in thinking about that, we need to ask the question, how does this emerging polypeptide fold to its native form in this type of environment? What type of machinery is there to help protect it? How is misfolding avoided and intermolecular molecular interactions that are non-productive avoided?

So where are we going to go in this module? We're going to look at protein folding from both the in vitro test tube perspectives and also from in the cell. And so in thinking about protein folding in vitro, we'll discuss some of the seminal study, so Anfinsen's hypothesis and folding of

ribonucelic A, Levinthal's paradox, which brings us to thinking about energy landscapes, and also touch upon some of the experimental methods that are employed.

And then in terms of machines, we'll think about, largely, post-translational protein folding in the cytoplasm, so GroEL, GroES, DnaK and J. We'll also talk about a protein called trigger factor that associates with the ribosome, and those nascent polypeptide chains. OK. So these machineries fold soluble proteins, not membrane proteins.

And I'd also like to point out, and again, we may or may not get to these systems, depending on time, but in addition to these chaperones and macromolecular machines involved in folding, there are classical enzymes that are really important. And these include enzymes that, say, isomerize prolene, also thyle oxidases and isomerases there.

So what are our questions for this module? So why and how are proteins folded? And in terms of how, in the lab versus in the cell, classical enzymes and micromolecular machines. What happens when proteins are misfolded? How does protein folding relate to disease? What methods are employed to study these phenomenon?

And for the case studies, we'll look at, in terms of cytoplasmic players, we want to understand, really, what are the structural properties of these different chaperones and their partners? How do their structures relate to function? How do they help peptides attain the native fold? And how good is our understanding of these systems? We'll see in the case of DnaK/J, it's actually pretty difficult to know what they're actually doing here. And really, what is the experimental basis for our understanding?

If we just take an overview of folding and misfolding-- and this is diagram for a eukaryotic cell and from many, many different types of studies-- what do we see? So we see that some sort of biomolecule called chaperone keeps coming up again and again. So these are proteins that assist with folding, or unfolding, disaggregation.

There's many possibilities for the trajectory of a protein here. So here, we see a nascent polypeptide emerging from the ribosome. And imagine that some folding intermediate is released. So this is not fully at the native fold, but it's somewhere along that pathway. What might happen? Right here, what we see is some chaperones allow this intermediate to form a native protein.

But look, there can also be unfolding, and this could work its way back. This native protein

could unfold to a misfolded state. We can think about remodeling, and maybe there's chaperones involved and taking this misfolded state back to an intermediate that's on a productive pathway. What happens here?

Maybe there's some trouble, and rather than reaching its native fold, this intermediate ends up aggregating. It forms some sort of protein aggregate, and maybe that can form oligomers, or some sort of amyloid fibril, like what we hear about with Alzheimer's disease. Here, we see there's chaperones that can be involved in having disaggregate activity, and they can help in breaking down these aggregates and getting back to some productive place here. OK.

So there's inherent complexity here and many players and relationships between protein misfolding and disease, just to be aware of. So we typically think about the protein fold providing function and protein misfolding can result in improper function. And there's many different types of improper function. It could be loss of function. It could be gain of function. It could be formation of some sort of aggregate that's deleterious to the cell for one reason or another.

And if we just take a look, in terms of human diseases that are associated with protein misfolding, what do we see? So there's examples out there, like Alzheimer's, Parkinson's, familial ALS, and mad cow. So Alzheimer's disease is associated with formation of Abeta plaques in the brain. In Parkinson's there's a peptide called alpha-synnuclein that aggregates in familial ALS, also called Lou Gehrig's disease.

There are single point mutations in an enzyme called superoxide dismutase that results in misfolding and some negative consequences there. And then misfolding of the prion protein. So a lot of these, in terms of neurological disorders. So in addition to fundamental studies, there's significant interest in understanding protein misfolding from the standpoint of disease and prevention.

And I'll just note, sometimes questions about natively unfolded proteins come up and those are outside of the scope of our discussions today. But be aware, there are proteins that are natively unfolded. You saw a little bit of that with some of the ribosome proteins that had those unfolded extensions going into the interior.

So in terms of thinking about protein folding in the test tube, where we're going to begin is with Anfinsen's hypothesis and his seminal experiment on protein folding. So Anfinsen is responsible for the thermodynamic hypothesis of protein folding. And he performed seminal

experiments on a protein, an enzyme, called ribonuclease A.

And so what Anfinsen hypothesized is that, in terms of a protein shape or fold, it's the primary sequence, so the sequence of amino acids, that dictates this final shape in aqueous solution. So whatever that primary sequence is, it dictates, basically, the array of possibilities and the thermodynamically most favorable result.

So what was the experiment Anfinsen did to probe this? What he did is look at denaturation and refolding of ribonuclease A. So this enzyme cleaves RNA single stranded. It's 124 amino acids in length. And in the native form, it contains four disulfide bonds. And since there's four disulfide bonds, there's eight cysteines in the primary sequence. So two cysteine side chains can come together to form a disulfide.

And so if we think about eight cysteines forming four disulfide bonds, there's many possibilities, in terms of how those cysteines are matched and the linkages. So different regioisomers, over 100 possible combinations of these eight cysteines to get four disulfides. And only one regioisomer, so one of these combinations, is the native form. So one out of over 100. So these native disulfide linkages that are formed indigenously are required for activity.

So what was Anfinsen's experiment? The experiment he did was to take native ribonuclease A, and I'm going to just sketch that. So imagine we have the four disulfides. So first, step 1, he reduced it. So he added a reducing agent to reduce these disulfides. We'll talk a little bit more what that might be in a minute.

And so the end result is, rather than having these disulfides, we have eight free cysteines. So free meaning not in a disulfide, indicated by SH. OK, so this is reduced. And so over the course of this, Anfinsen developed some assays to monitor for activity of this enzyme. And what was found is that there is a loss of activity.

Next step, add a denaturant. So a denaturant is some chemical, like urea or guanidinium, that is going to disrupt the fold of the protein. And in this case, he used urea. So as this is sketched, this is still folded, but the disulfides are gone. OK, so what's the result here? We get some unfolded polypeptide with the cysteine somewhere. So this is denatured, so we have no disulfides, no native fold, and inactive. It can't cleave the single-stranded RNA.

OK, so we've succeeded in destroying activity and destroying the fold of this protein. What did he do next? So the next step in this experiment was to ask, OK, if we start with this unfolded

polypeptide that's completely denatured and there's no disulfides, can it return to this native form by removal of the denaturant, and then allowing it to oxidize? So imagine here, we work backwards.

And step 3, remove the denaturant. So how that might be done, dialysis is a way to dialyze away the denaturant. And then what happens if we allow this to oxidize? So for instance, air oxidation.

So what he found is that in this order of steps, so the denaturant's removed and then the protein is allowed to oxidize, that greater than 90% of the enzymatic activity was restored. So you have this denatured polypeptide and dilute aqueous solution, and work your way back and you can restore this activity. You have a question?

AUDIENCE: The intermediate stuff, where it's reduced but not yet denatured, how do you confirm that the native fold is still the same or similar? And what were the results, in terms of activity, for that intermediate?

ELIZABETH NOLAN: Yeah. So how could we confirm if the fold is perturbed? What might be a method to do that?

AUDIENCE: Circular dichroism.

ELIZABETH NOLAN: Yeah, circular dichroism. So that's one possibility. Did he have that available? That's another question, but that will give you a readout on alpha helix C or beta sheet. That's one possibility. You can imagine other possibilities. Maybe it would run differently on some form of column there, as a possibility. There was a loss of activity here. Was it 100% or less than 100%? I'm not sure about that detail. Joanne?

JOANNE: I don't know. Are you sure they didn't denature before they reduced?

ELIZABETH NOLAN: I think he's done it other ways.

JOANNE: I mean, the protein with a lot of disulfides in it, they may not be accessible to reductant.

ELIZABETH NOLAN: I mean, often, you add them together to get here, right? And he did a lot of experiments, as well, with additives. And then definitely, in this direction, my understanding is he performed this both ways.

So effectively, if the denaturant's removed first and then it oxidizes, versus oxidizing it and then removing the denaturant, and when it was that later scenario, that the disulfides were allowed to form first, the end result was a sample that had negligible activity, less than 1%. And so from that you can imagine why because if this was allowed to oxidize, it's not pre-folded to allow the correct disulfides to form here.

AUDIENCE: So with those two steps, the polypeptide and the karyote enzyme just folded back up into its original state?

ELIZABETH NOLAN: Yeah, so isn't that incredible?

AUDIENCE: Yeah. How long did it take?

ELIZABETH NOLAN: For this case, I don't know. And depending on the polypeptide, it can vary from a short period of time. We have examples in my lab, where maybe in 30 minutes, you can see the properly folded form today, or even faster than that, depending. Like, seconds to days, depending on the protein and the size. Yeah, but that's what's really incredible about this experiment, just beyond the details of the ordering.

And what happened is the fact that he could take this 124 residue polypeptide that needs to have four specific disulfides, and just in dilute aqueous solution-- without any help, minus here-- it could come to its native fold. So this was support of this hypothesis, that the primary sequence of a polypeptide that can dictate shape.

And if these polypeptides are allowed to fold under dilute conditions, where intermolecular molecular interactions aren't a problem, they can achieve the thermodynamically most favorable result. And he did plenty of additional experiments, too, in terms of putting additives in and asking, how do these perturb the results?

So what did he actually have to say from his experiment? In his words, "the results suggest that the native molecule is the most stable configuration, thermodynamically speaking, and the major force in the correct pairing of sulfhydryl groups and disulfide linkages is the concerted interaction of psi chain functional groups distributed along the primary sequence." So this primary sequence dictates the array of possibilities.

So in thinking about that, that brings us to the paradox of Levinthal here. So he was thinking

about this problem of protein folding and just thought, well, imagine we have one polypeptide with 100 amino acids. So smaller than ribonuclease A. What if each amino acid had only two possible confirmations? What does that mean, in terms of possibilities? We have 2 to the 100th.

So if that polypeptide were to sample every possible confirmation during folding, taking just a picosecond per transition, the time required to fold the protein would be what? And based on his back of the envelope work here, it would be ridiculous, longer than the time of the universe. And that tells us that just can't be, in terms of how we think about this here. So each amino acid can't adopt its shape independently. That's just not working on a biological timescale.

So how do we think about this? We can use energy landscapes here. So thinking about tumbling through hills and valleys. And so basically, we can depict protein folding, and this is an example, say, in a test tube, where there's some ensemble of starting unfolded, or partially folded, structures, and these are of higher energy.

And there'll be some sort of stochastic search, and basically, these forms will give us ensemble of partially folded structures and, ultimately, converge to a native structure here that's of lower energy than that. So we have an ensemble of many denatured proteins that needs to make its way to the native form.

And just looking ahead a bit to our discussions of chaperones, these proteins that assist in folding, this is another view of an energy landscape, but it's taking chaperones into account. So we have energy here. And this depiction is from the assigned reading. It basically divides things up in terms of productive intramolecular contacts versus intermolecular contacts that lead to situations like oligomers and aggregates and fibrils.

So up here at high energy, we have unfolded, or partially folded, species. And what we see here-- bless you-- is that these chaperones are helping to allow these partially folded states to reach a native state by helping getting over these barriers. And the chaperones do not want to have the proteins going in this direction here to species that are potentially deleterious and result from intermolecular contact, so oligomers, fibrils, and aggregates here.

What are some methods, in terms of experimental methods, for folding? There are many that can be employed. So you just need to take studies by a case by case basis. Commonly used fluorescence, whether that be native emission from a protein. So if you imagine, you have, say, a tryptophan. Emission can vary, depending on where it is in a protein. Methods like

FRET. We just heard about circular dichroism, which tells us about secondary structure, NMR, FTIR, stopped-flow, and there's a large field in computation in theory, looking at protein folding as well.

What are some methods to denature a protein? So here, we saw urea used. There's many others, whether that be heat or pH. And denatured protein means unfolded protein, in the context of the lectures in this course. So often, studies in vitro start from using an unfolded protein sample, and then you look at how folding progresses.

What are some lessons from in vitro folding studies, just to keep in mind? 1, every protein's different. And even proteins that seem similar are very different. So maybe they have the same secondary or tertiary structure, some small peptide, but when you try to fold them, they may require different conditions.

There are multi-dimensional energy landscapes, like what we saw on the prior slides. You can often see intermediates along the folding process. And in dilute aqueous solution, as Anfinsen hypothesized, primary sequence dictates fold.

Just a note to anyone doing experimental work, why do we like to use the ice bucket in the cold room when working with proteins and enzymes? Many native proteins are only marginally stable under physiological conditions. So we can think about a delta G of denaturation per amino acid. So what this means is use your ice bucket in the cold room when working with your samples.

Just closing, thinking about protein folding in vitro versus in vivo. Do studies in vitro really enhance our understanding of what's happening in the cell? Just some observations to keep in mind. So on the benchtop, folding can occur over a tremendous timescale. From nanoseconds to hours, I have here. It can be days, depending on your peptide and conditions here.

The studies are generally performed in dilute buffer and in the absence of any additional protein. So you have some pure polypeptide that you want to fold or study and that's what you work with. And it's found that small proteins will often fold without assistance here. So they don't need helpers.

In the cell, how do we think about the rate of folding? So from one point of view, the rate of folding is limited by the rate of polypeptide biosynthesis and how quickly that polypeptide is emerging from the ribosome, if you're thinking about a nascent chain. And we can think about

the concentration of peptide coming off the ribosome, which is often quoted as low micromolar.

And as I mentioned earlier, we really need to keep in mind that this cellular environment is very crowded with many different biomolecules and players. And as a consequence of this crowding, there's many proteins that help in folding, especially the chaperones. We'll see that a number of these chaperones protect the polypeptide that needs to be folded from this environment here.

So a take home is that just spontaneous protein folding in the cell is error prone, if it were to happen, and that inter and intramolecular interactions are a big issue. And so these chaperones are available to help overcome these issues here. So where we'll begin on Monday is looking at trigger factor, GroEL and GroES and DnaK/J as an overview. And then we'll work our way through these different systems for protein folding in the cytoplasm.

[SIDE CONVERSATIONS]