BOGDAN FEDELES: Hi, welcome back. I am Dr. Bogdan Fedeles. Let's solve some more biochemistry problems. Today we're going to be talking about problem 3 of problem set 10. Now this is a problem about the spontaneity of gluconeogenesis. As you guys have already learned, gluconeogenesis is the process by which non-sugar precursors are used to produce glucose.

This problem should help you think of the following conundrum-- in a cell, when we have glucose we can make energy by doing glycolysis. But at the same time, the cell can take, for example, the endpoints of glycolysis such as pyruvate, or even further metabolites like amino acids, and use the pathway in reverse to make glucose. Now if one process is spontaneous in terms of thermodynamics, then how could we run the reaction in reverse?

So here we're going to be looking at a couple of steps of gluconeogenesis that are the exact reverse of the reactions that you've seen already in glycolysis. And then in the end, we're going to be talking about the whole pathway level and make some comments on the spontaneity of both glycolysis and gluconeogenesis.

Part A of the problem deals with the aldolase reaction, namely going from fructose 1,6 bisphosphate to glyceraldehyde 3-phosphate dihydroxyacetone phosphate. Now in gluconeogenesis, we will be going in reverse, starting with dihydroxyacetone phosphate and glyceraldehyde 3-phosphate to regenerate fructose 1,6 bisphosphate. Let's take a look at the reaction.

This is dihydroxyacetone phosphate. This is glyceraldehyde 3-phosphate or GAP. And these two come together in the aldolase reaction to form fructose 1,6 bisphosphate, or F 1,6 BP.

Now the Part 1 of the question asked us to write down the detailed mechanism for this reaction, running in reverse. So as you guys know, aldolase, specifically the Class II aldolases, use an active site lysine residue to covalently bind the substrates during the course of the reaction. So this reaction will proceed by dihydroxyacetone phosphate binding to the active site lysine of the aldolase.

Here we have the dihydroxyacetone phosphate and the lysine in the active site. So as a first step to the mechanism, the lysine is going to react as the carbonyl group in the dihydroxyacetone phosphate to form a Schiff's base, or an imine. As you can imagine, the reaction is going to be catalyzed by a general base, which is going to remove one of the protons from the lysine, which then becomes a good nucleophile and can attack the carbonyl. Which can then will be protonated by a general acid. So this way you obtain an intermediate as shown here, which can then lose the water molecule to form the Schiff base. Once again, we need a base that will take this proton and then the water will need another acid molecule to leave.

So what we have formed here, this is the protonated Schiff base, or what we call an iminium ion. And if you look at it from the point of view of where we started from, which was a carbonyl group, this is a version of an activated carbonyl. A carbonyl that would it's now poised to do chemistry. Once again, this is a iminium ion.

Now as we talked in the carbonyl video, iminium ions are activated carbonyls that can now undergo some of the carbonyl reactions with more ease. For example, this one is going to do an enolization. So we have highlighted here the alpha hydrogen next to the carbonyl group. So we're going to form the enol by removing this hydrogen, and the carbonyl with its positive charge on the nitrogen, so it's at a very good electron sync. So what we're forming here is an imine that's bound to a double bond. So this we're going to call an enamine.

So now this is the reactive version of the dihydroxyacetone phosphate. Now it's poised to react with the other partner in the reaction, GAP- glyceraldehyde 3-phosphate, which we've shown here. Now GAP is going to be the carbonyl component of this aldo reaction, while the enamine from the dihydroxyacetone phosphate is going to be the enolic component.

So the enol is going to be a very good nucleophile and is going to attack the carbonyl. The reaction is once again initiated by this amine group. The electrons move here and this attacks the carbonyl. And we can protonate it with a general acid.

So what we obtain here is now a molecule that has from two portions of three, is going to have six carbons. And if you look closely, this is really the fructose 1,6 bisphosphate bound to the active site of the enzyme as a Schiff space. So now all that needs to happen now is to hydrolyze the Schiff space and release our fructose 1,6 bisphosphate product.

So, we're going to need one water molecule. It's going to be activated by a general base. Then it is going to attack this imine and the electrons will move to the nitrogen. We get to this step and here we need a step in catalysis to regenerate the enzyme to its freed lysine form and then release the fructose 1,6 bisphosphate product of the reaction.

So once again, we started with dihydroxyacetone phosphate and GAP and you used this covalent catalysis where the substrates were bound in the active site of the enzyme to form our product, fructose 1,6 bisphosphate.

Now this, as we discussed in the carbonyl chemistry, is an example of direct adol reaction. And as the name of the enzyme suggests, aldolase this is the reaction going in that gluconeogenic mode.

Now in Part B of the question, we're going to look at another important reaction that can run both ways-- glycolysis and gluconeogenesis. That will be specifically going from glyceraldehyde 3-phosphate, or GAP, to 1,3 bisphosphoglycerate. This is the reaction catalyzed by GAP dehydrogenase.

Here is a representation of the reaction. We have glyceraldehyde 3-phosphate that is converted by GAP DH to the 1,3 bisphosphoglycerate. Now in the glycolysis step, we know we need one NAD equivalent, and one inorganic phosphate to go into the enzyme to be able to oxidize our aldehyde to an acidic anhydride in 1,3 bisphosphoglycerate.

Now in the gluconeogenesis, we're going to be going from right to left. We're starting with 1,3 bisphosphoglycerate We're going to need NADH and we're going to generate GAP and inorganic phosphate.

Now let's take a closer look at the mechanism of this reaction as it would run in gluconeogenesis. Here we have a representation of the active site of the GAPDH, where we highlighted the cysteine group, SH. And we also have a general base in the active site and we're going to call it B. And here is our starting material 1,3 bisphosphoglycerate.

Now if you remember how GAPDH works in the direct reaction in the glycolysis, we're going to have to form a covalent intermediate, in which the substrate is going to bind to the enzyme. That's going to form a thioester with that thiol group of the cysteine.

So this is what's going to happen here. So the base in the active site actually is going to assist the protonation of the cysteine. Then the thiol now is activated and can attack the

phosphoanhydride of the 1,3 biphosphoglycerate. And then the first step, it's just going to form a tetrahedral intermediate.

There you have it. Now we have a negative charge on the oxygen and we spelled out the phosphate with all the atoms here. As you know, this tetrahedral intermediate is now going to fall apart, releasing the best leaving group. In this case it is going to be the inorganic phosphate. So the electrons come down. They're going to be transferred onto the phosphate and presumably protonated.

So after the phosphate is released, now we have formed finally the thioester of our substrate in the active site of GAPDH. As you remember, this is a redox reaction so it involves the co-factor NAD. In this case, running the reaction from right to left, we're going to use NADH to reduce the bisphosphoglycerate to the aldehyde group.

So here is our representation of the NADH. As you remember, one of these hydrogens together with its electron pair, it's is to be donated as a hydride or H minus. So the reaction proceeds by rearranging the electrons on the pyradine ring of NAD and the H minus is going to be the nucleophile that's attacking now the thioester. And once again, I'm going to be forming a tetrahedral intermediate, which is shown here.

Now this hydride here is the one that came from NADH. And in the process the NADH co-factor is converted to NAD-plus. So now we can see we're just one step away. This is now like a hemiacetal like molecule. So it's one step away from forming glyceraldehyde 3-phosphate. All we need to do is release the enzyme in its original confirmation. So the electrons will flow now to reform the carbonyl, while the sulfur is going to get its electrons from the base and this regenerates the enzyme as we had it in the beginning. And we're releasing GAP, the product of the reaction.

So with this mechanistic insight, we have actually addressed how the GAPDH reaction runs in the gluconeogenic mode from bisphosphoglycerate to GAP. Now Part A of the problem also asked us to look up the free energy value for the aldolase reaction that is that delta G naught prime.

Now, if you're going to look in your favorite chemistry textbook, or I have here the Voet and Voet Third Edition, the book that we use in this course. Now you're going to find on page 511, you're going to see a table with all the free energies of glycolysis reactions. And for the

aldolase step, you're going to see that it's a positive free energy, that is the reaction is spontaneous in the reverse in the gluconeogenic direction. So now that may be a little surprising, but keep in mind that the reactions in these pathways are more often than not, governed by mass action, that is if we have an excess of the starting materials, the reaction would proceed towards the product, while if we have an excess of the products, the reaction will proceed backward towards the starting materials.

So both the aldolase reaction and the GAP dehydrogenase reactions are very susceptible to this mass action. So they will be controlled- the direction, the spontaneity of this reaction will be controlled by which of the starting materials or products are in excess.

Part C of this problem asked us to evaluate the spontaneity of gluconeogenesis at the level of the whole pathway. Now we know glycolysis is a spontaneous process. Not only when it goes from glucose to pyruvate, but also it generates some high energy intermediates like ATP in the process-- like we get two molecules of ATP per glucose. Now if we want to go backwards from pyruvate to glucose, in a gluconeogenesis pathway, can this process be spontaneous?

First let's look at glycolysis. Here is a schematic of a glycolysis. We're starting here from glucose and we're going to need a couple of molecules of ATP to activate it, to get to fructose 1,6 bisphosphate. And from there on, we're going to generate actually, two molecules of ATP at this step and then two molecules of ATP in the final pyruvate kinase step. And of course, we're also going to need an NAD- plus going to NADH here at the GAP step. Well, there's going to be two of these NAD-plus needed to oxidize GAP to 1,3 bisphosphoglycerate. And as we just said, even though we have some energy cost in early on, we're actually generating more ATP by the time we reach pyruvate so the pathway is in fact spontaneous.

Now what about gluconeogenesis. In gluconeogenesis, we are starting with pyruvate and we want to go back to glucose. Now if we were to reverse exactly every single step in glycolysis, that pathway is not going to be spontaneous. Step four in gluconeogenesis uses these alternate pathways in a couple of the steps in order to make the process spontaneous.

For example, going from pyruvate to phosphoenolpyruvate in gluconeogenesis is not a reverse of the pyruvate kinase reaction. It rather occurs in two steps.

So the first step we take pyruvate and convert it to oxaloacetate, using pyruvate carboxylase, so it's going to need a molecule of CO_2. And oxaloacetate has four carbons. And it's also going to need energy. So we're going to need a molecule of ATP going to ADP.

Now oxaloacetate can now be processed inside the mitochondria or it can be taken out of the mitochondria into the cytosol. And let's say that's the course of the reaction. Where it's going to find an enzyme called PEP carboxylic kinase, or PEPCK, that can take oxaloacetate to PEP. This enzyme once again requires energy. This time in the form of GTP going to GDP. And here we're going to lose that carboxyl group that we added on earlier.

Now while this might seem like a cumbersome way to reverse one reaction, this allows both the part of a kinase reaction and going from pyruvate going back to PEP, to be controlled in different ways and therefore allow both of these processes to happen spontaneously.

The rest of gluconeogenesis will have exactly the reverse of these steps in glycolsis. For example, PEP going to 2-phosphoglycerate. 2-Phosphoglycerate going to 3-phosphoglycerate. 3-phosphoglycerate going to 1,3 bisphosphoglycerate. Here, since in glycolysis we generated ATP here, we're going to need the ATP to come in and go to ADP in order to accomplish this step in gluconeogenesis. 1,3 bisphosphoglycerate going to GAP, this is the step we just discussed in Part 2 of the problem. As we said, we're going to need NADH going to NAD-plus. Then GAP and DHAP going to fructose 1,6 bisphosphate, this is the step we discussed in Part A of this problem, the reverse of the aldolase reaction.

Now going from fructose 1,6 bisphosphate to glucose, we're not going to do these kinase reactions in reverse, where we would be generating ATP and therefore that would be not spontaneous in the reverse direction. But rather, we're going to use alternate enzymes called phosphatases where we lose the phosphates without regenerating an ATP molecules.

So therefore, by using these two tricks we can go back to glucose without regenerating these ATP molecules, and therefore the pathway can be spontaneous. Now the bottom line here is that both glycolysis and gluconeogenesis are spontaneous, but gluconeogenesis uses most but not all of the glycolysis steps to run in reverse. And while glycolysis generates energy, we get a net of 2 molecules of ATP per molecule of glucose used, gluconeogenesis as you guys have seen here, actually uses energy to run spontaneously. That is, we need ATP molecules.

Now if you look at our diagram, we need ATP molecules to convert pyruvate to phosphoenolpyruvate, actually 2 of them. And then we're going to need more ATP here to form 1,3 bisphosphoglycerate. In addition to the redox, like NADH co-factor, to run the GAP dehydrogenase reaction in reverse.

So given these considerations, gluconeogenesis is in fact spontaneous, but it's going to cost us several ATP equivalents. While glycolysis is spontaneous and generates ATP.

Well, that solves problem 3 of problem set 10. Here, I hope you got a better understanding of why both glycolysis and gluconeogenesis are both spontaneous pathways inside the cell. Thank you.