BOGDAN FEDELES: Hi, I'm Dr. Bogdan Fedeles. I'm a research associate at MIT doing research with Professor John Essigman. I love biochemistry, and I'm going to help you solve some problems today. Let's work together through question 5 or problem set 5.

This is an excellent question about sugar biochemistry. Specifically, we're going to find out how mannose an isomer of glucose gets to enter metabolism by being converted into fructose 6-phosphate.

Now mannose is a common sugar that's found in polysaccharides and glycoproteins. Here is the transformation that converts mannose, shown here, to mannose 6-phosphate in first step. And then in the second step, to fructose 6-phosphate. So our goal here will be to figure out the mechanism for these two transformations.

Let's take a look at the first transformation. We're converting mannose to mannose 6-phosphate. Now, as you guys have encountered in biochemistry, adding a phosphate group is a ubiquitous transformation and it's catalyzed by proteins called kinases.

Now, kinases typically use ATP, the energy currency of the cell, as a phosphate donor, and transfer the furthest most phosphate, so-called gamma phosphate, onto the substrate, and leaving behind ADP. A similar transformation will be happening here in part one of the problem, where mannose is going to be converted to mannose 6-phosphate by a kinase. And the source of the phosphate is going to be ATP, which, in the process, will yield ADP.

Now, in order for the kinase to work, to react with ATP, we also need magnesium. So a salt of magnesium with ATP is actually essential for this reaction to work. Now, let's take a look at the structure of ATP to gain a little bit more mechanistic insight.

As you know, ATP, or adenosine triphosphate, has these three phosphate groups attached to a 5-carbon sugar called ribose and a base, adenine, which we're not going to draw out here. Now, the phosphates in ATP are typically labeled alpha, the one closest to the sugar, beta,

and gamma. Now, in order for ATP to react, notice these four negative charges on the phosphates. They need to be neutralized in order for the molecule to become reactive.

So this is what magnesium is doing. So at least early on in the reaction, magnesium is going to neutralize two of these four negative charges, while the other two negative charges will be neutralized by positively charged amino acids in the active site of the kinase. Once all the charges are neutralized, then the phosphorus at the gamma position will become available to be attacked by a nucleophile. In our case, the 6 hydroxyl of mannose.

So therefore, the reaction proceeds as follows. A base in the active site will activate our hydroxyl, which then can attack the gamma position of the phosphate. And then the phosphate gets transferred to the mannose, and it's going to leave behind ADP. Now, notice during this reaction, magnesium, which was coordinating two of these negative charges, will probably move to coordinate these two charges or the newly formed charge here in ADP. And that would also help stabilize the ADP into the active site of the kinase. This mechanistic insight basically answers part one of the problem.

To talk about part two of the problem, we need to remember something fundamental about sugar biochemistry, namely that 5- and 6-carbon sugars exist in equilibrium between a linear form and cyclic forms. Now, sugars that you've encountered already, like glucose and fructose, they are in equilibrium between linear and cyclic forms. So let's take a look at them first.

This is glucose. As you know, it's an aldehyde that has hydroxyl group on all the other carbons in this stereochemistry. Now, this is a linear form of glucose, and as an aldehyde, it can react with good nucleophiles, like these hydroxyls, to form cyclic hemiacetals.

If the reaction occurs with a hydroxyl position four, we're going to close a five-membered ring hemiacetal, which is shown here. These kind of structures are called furanoses. So this is the glucofuranose.

Now if, instead, we react with a 5-carbon, then we're going to close a six-membered ring, which is called a pyranose. This is glucopyranose. Now, while the sugars are in equilibrium between the linear and the cyclic forms, the cyclic forms tend to be predominant. So in equilibrium, it will be primarily 99% cyclic form and only about 1% linear form. Nevertheless, the presence of the linear form allows the sugars to undergo some interesting transformations, one of which we are exploring in this question.

Similarly, here is fructose. Fructose has the carbonyl at the 2 position. It's a keto group, and then hydroxyl groups on all the other carbons. And just like glucose, it can form cyclic hemiacetals. So if the reaction happens between the carbonyl and the hydroxyl at position 5, it's going to close a five-member ring shown here. This is too a furanose. This will be fructofuranose.

If the reaction happens with the hydroxyl at the position 6, then we're going to be closing a six-member ring shown here. This is fructopyranose. Once again, just like for glucose, the equilibrium, it's strongly shifted towards the cyclic forms. Nevertheless, there is enough of the linear form to allow certain kind of chemistry to happen.

Now, going back to solving part two of our problem, that is converting from mannose 6-phosphate, which is a sugar with a six-member ring cyclic structure, to fructose 6-phosphate, a sugar with a five-member ring cyclic structure. Keeping in mind what we just discussed, that sugars, they're in equilibrium between these cyclic structures and their linear structures, linear forms, one good place to start to figure out how this transformation will happen is to write the linear forms of the two molecules here.

Now, for the mannose 6-phosphate, we notice we have a hemiacetal functionality at carbon 1, which is attached to both the hydroxyl group and another oxygen. So this carbon will form an aldehyde in the linear form. So let's write the linear form of mannose 6-phosphate.

All right, so notice at carbon 1, we're going to have an aldehyde. And then all the other carbons, we have a 6-carbon chain. Now, in terms of mechanism, this is just a reverse of the hemiacetal formation, and it's going to require a base to deprotonate the hydroxyl here in carbon 1. And then we're going to need to reprotonate at the hydroxyl in position 5 to form this hydroxyl here.

Now, something to keep in mind, though, the way this enzyme works is we don't really know if the enzyme is catalyzing this transformation as it's written here, or the enzyme is just binding the linear form that will be in equilibrium in solution of mannose 6-phosphate. Similarly, for fructose 6-phosphate, we can write its linear form. Notice, fructose 6-phosphate has a carbon too, it's a hemiketal functionality. This carbon is attached to both a hydroxyl and an oxygen. So that will open up to form a ketone.

All right, this is our fructose 6-phosphate in a linear form. And notice at 2 position, we have the

ketone. Now, it would be a good idea at this point to number the carbons to see what do we need to do, going from this linear form of mannose 6-phosphate to this linear form of fructose 6-phosphate.

So here we have carbons 6, 5, 4, 3, 2, 1. And we have carbons 6, 5, 4, 3, 2, 1. And if we contrast the two structures, we notice that carbons from 3 to 6 in both cases are, in fact, the same. And we even have the hydroxyls in the right stereochemical orientation, in the right stereochemistry. And all we need to do is move the carbonyl group from the 1 position here in mannose 6-phosphate to the 2 position in the fructose 6-phosphate.

So this transformation can be accomplished by an intermediate, which you have seen already in glycolysis, the intermediate that allows to go from glucose 6-phosphate into fructose 6-phosphate, which is a cis-enediol. So it's essentially enolization with the hydrogen on carbon 2. So if we were to form the enol here, which, as you know from the carbonyl video, so a base could deprotonate and allow the electrons to move and form another hydroxyl on the 1 position.

Because this is a cis-enediol, the two hydroxyl are going to end up on the same side of the double bond. Once again, let's number our carbons. 4, 3, 2, 1. And this is our cis-enediol. So we have two hydroxyls. They're both attached to a double bond. And cis means they're both on the same side of the molecule.

And this allows the enzyme to basically switch back and forth between which one of these hydroxyl becomes the carbonyl. If we go backwards, we have the carbonyl at the 1 position. However, if we go forward, we can move the carbonyl on the 2 position. So that's just the reverse of the enolization reaction in which we will deprotonate this hydroxyl and reprotonate at carbon 1 to take us forward to generate the 2-keto group, which is fructose 6-phosphate in the linear form.

Now from here, in the very last step, we just need to close the ring. So that's just a hemiketal formation. We have a base deprotonated hydroxyl at position 5. And this will attack the ketone to form the hemiketal group that we see in fructose 6-phosphate.

So now this is basically the curved arrow mechanism from going from mannose 6-phosphate to fructose 6-phosphate. Now, some key points to notice here is that this enolization reaction where the base removes this alpha hydrogen would not have been possible in the cyclic structures. If you look here, this hydrogen, the PKA is about 30.

Now, once we form the linear structure here, the PKA of this hydrogen, now it's about 18. So that's like 12 order of magnitude more acidic, and that allows the reaction to for this cis-enediol, which allows the formation of the fructose 6-phosphate. So the take home message here is that opening and closing the ring of the sugars allows a lot of interesting chemistry to happen.

Part three of this question asks us to comment on why, when going from mannose to fructose, we have to go through the mannose 6-phosphate intermediate. Now, this turns out to be a very common motif in carbohydrate chemistry, because adding a phosphate group is a way of regulation of which product is being formed. Taking a closer look at our transformation, we see that if we didn't have the phosphate group at the 6 position, this hydroxyl would be available and could potentially be involved in forming cyclic forms.

As we recall our discussion on fructose, whenever we have a 6-hydroxyl available, the fructose can form the fructopyranose form in addition to the fructofuranose form. So by blocking this position with a phosphate, we're limiting the reaction to produce only one product, namely the fructofuranose. Additionally, adding a phosphate group to a sugar imparts a negative charge, and that allows the sugar to be trapped inside the cell and allows metabolism to occur much more efficiently. This is something that you've already encountered in glycolysis, where glucose, in the first step, is first converted to glucose 6-phosphate.