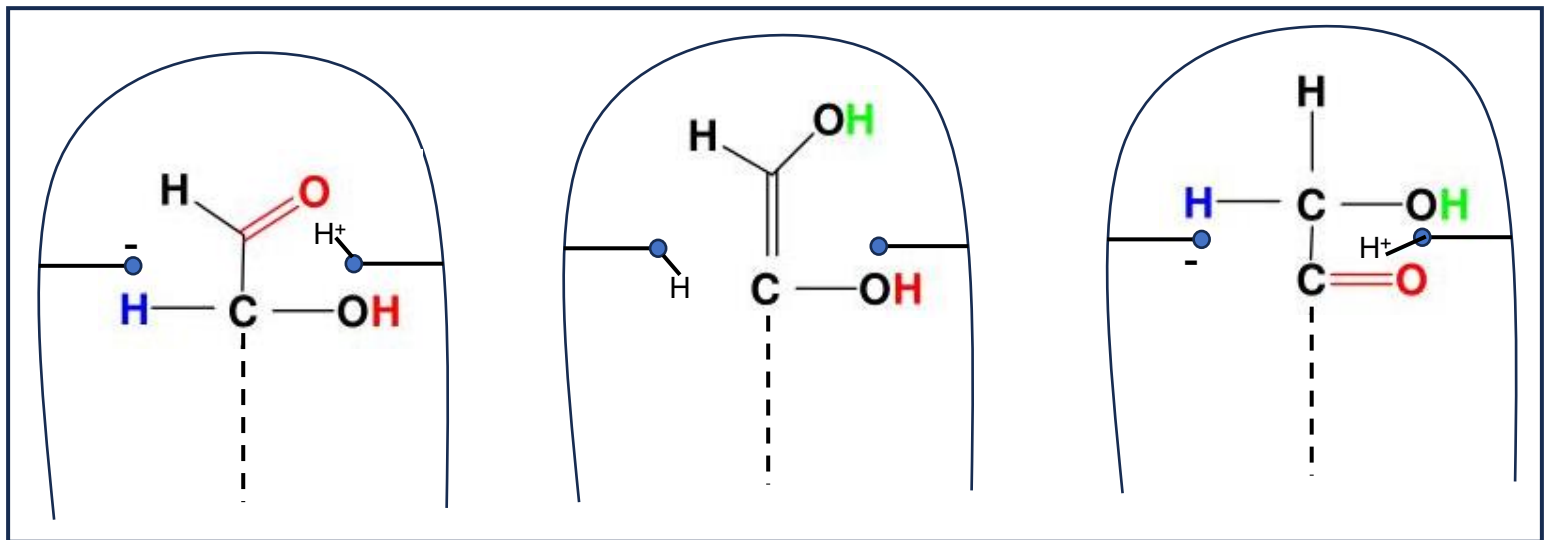
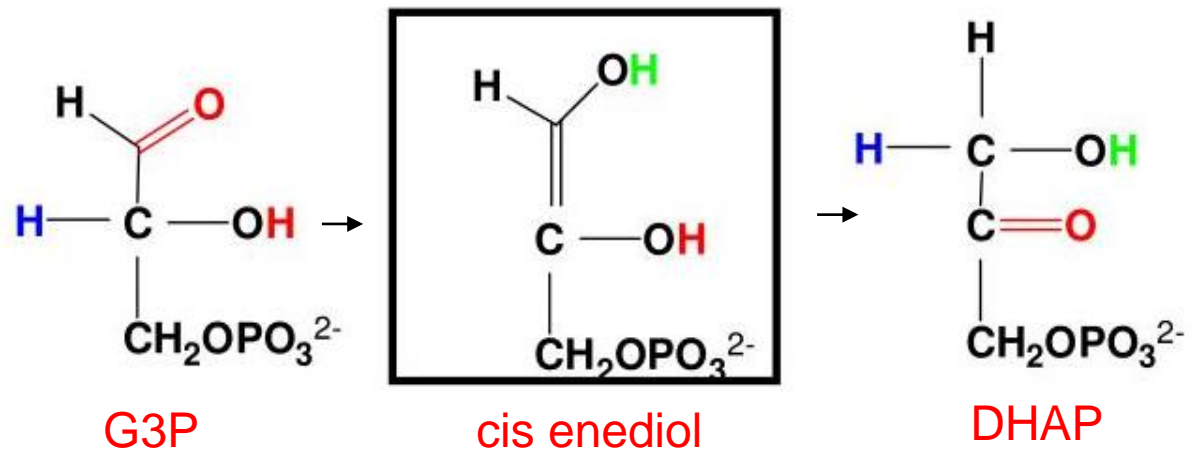


# Biochemical Pathways

# Remember TIM?...

- It took one particular molecule called G3P, and it changed it into another molecule, DHAP.
- And it did that by stabilizing the transition state / intermediate,
- And by preventing other reactions from going on that would destroy our happy little transition state molecule.

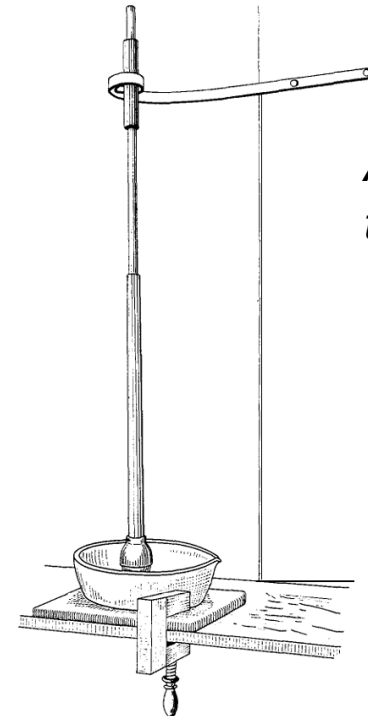


# There is more...

- Well, that's just one chemical transformation.
- That alone is not enough to explain what Buchner found in that experiment we talked about in the second lecture.
- What Buchner found, remember, was that if you took a glass of fruit juice and you put it out, it would ferment.
- You would get sugar, carbon dioxide, and ethanol.
- Well, there's a long and complicated pathway to go from carbon dioxide and ethanol and that's what we're going to talk about today,
- **Biochemical pathways** that string together transformations.

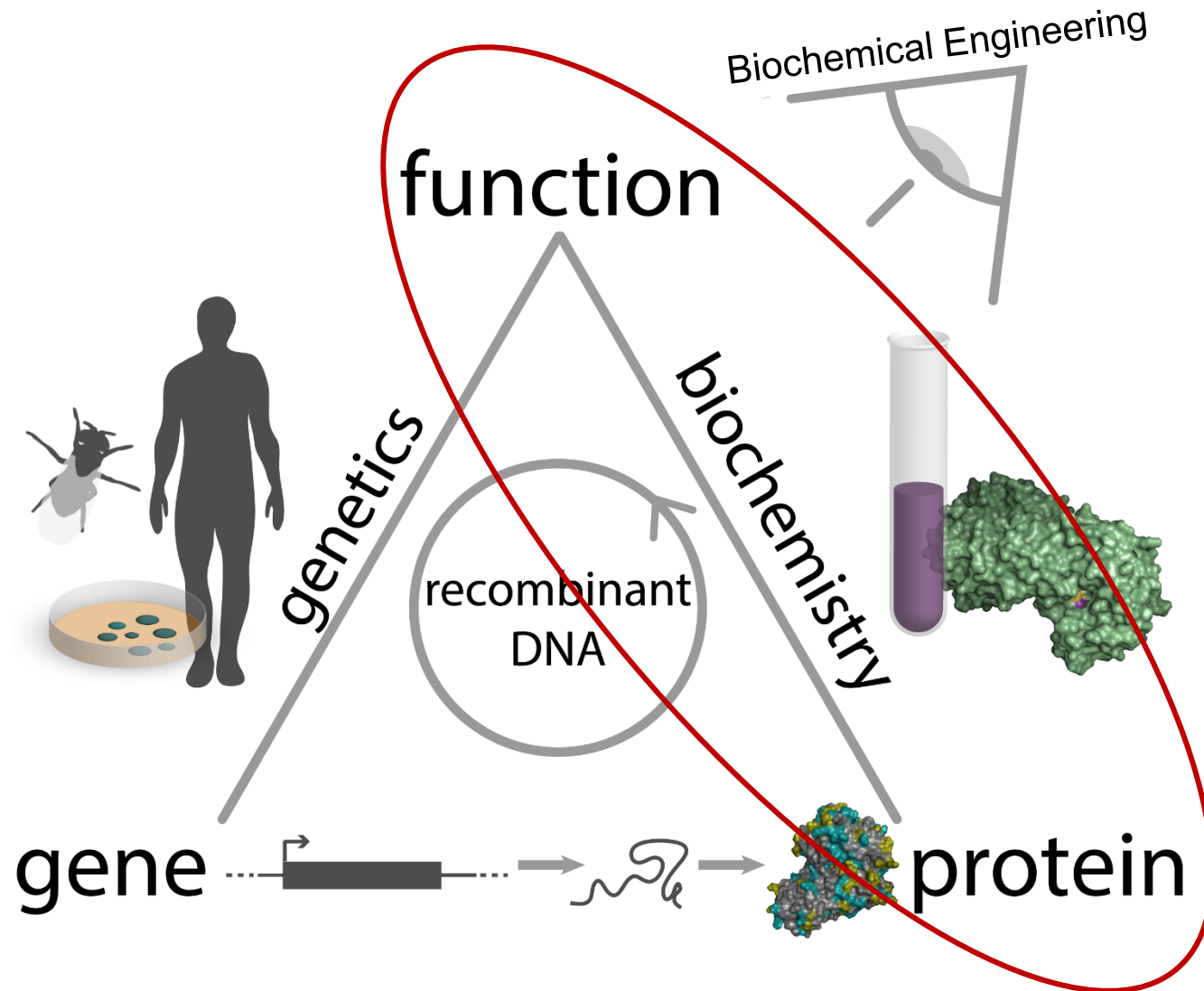


**Eduard Buchner**  
(1860-1917)  
1897 found fermentation in  
broken yeast cells  
1907 Nobel Prize in Chemistry



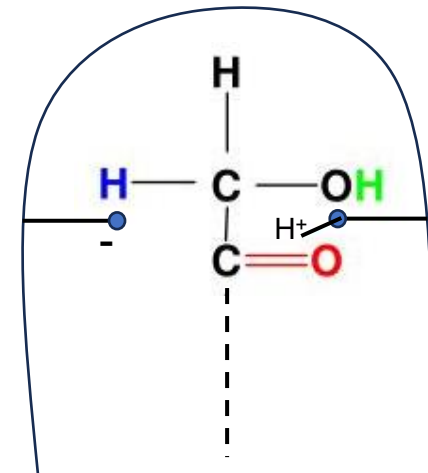
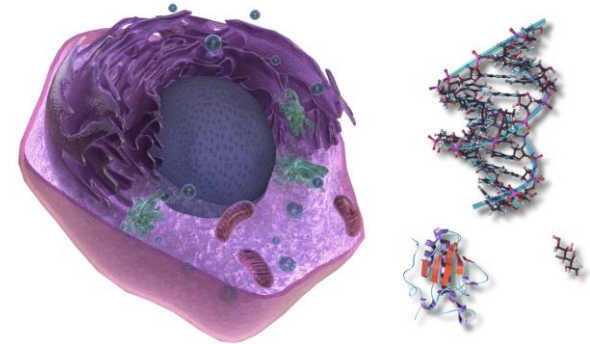
*Amazing  
transformations*

# Secret of Life



# We know....

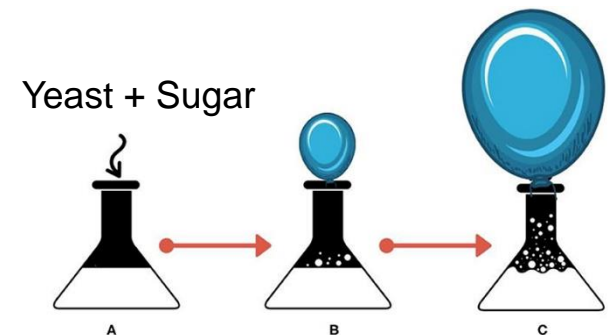
- So we've got protein structure under control.
- We've got the amino acids.
- We understand how their various different chemical properties control the shapes of proteins.
- We've talked about our enzyme--  
our enzyme triose phosphate  
isomerase, TIM
- And we talked about how TIM has an active site, and how that active site stabilizes that transition state to help us go from G3P to DHAP.



G3P → DHAP

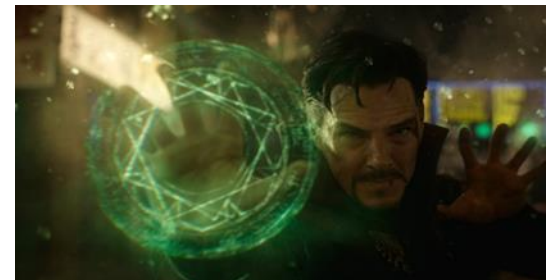
# But...

- But what does that have to do with how we go from our glass of fruit juice,
  - which has yeast,
  - and six carbon sugar.Let's say it has glucose or some other six carbon sugar.
- The six carbon sugar-- remember,  $C_6H_{12}O_6$ --, We call a six carbon sugar a hexose.
- And it's going to ferment  
 $C_6H_{12}O_6 \rightarrow CO_2 + \text{ethanol}$ .



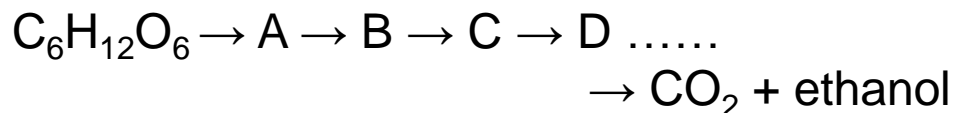
How does it do that?

Does it do that in one magic step?



# No, there is no magic

- It turns out it does it via a pathway of steps
- It passes through steps



- That's the simple aspect of pathway.
- And what we want to do is understand the logic of pathways. How do pathways work?
- We understood the simple aspect of pathways, but now **we really want to understand the logic of pathways**

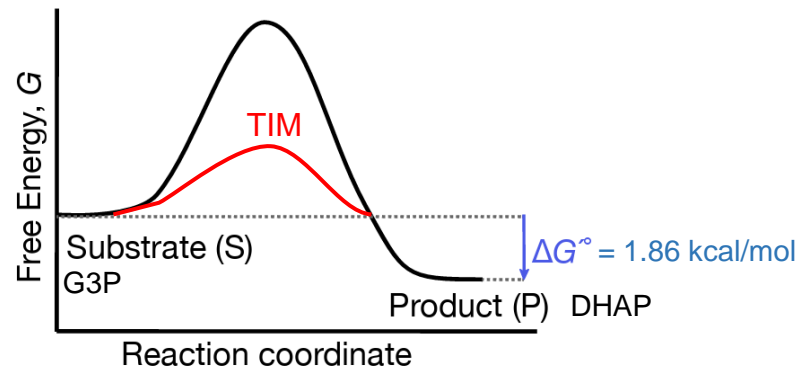
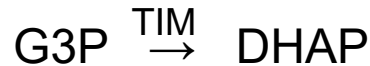


# Energetics of ~~Pathways~~ Reaction

Let's just start simply with the energetics of a single reaction in a long pathway



# TIM, again...!



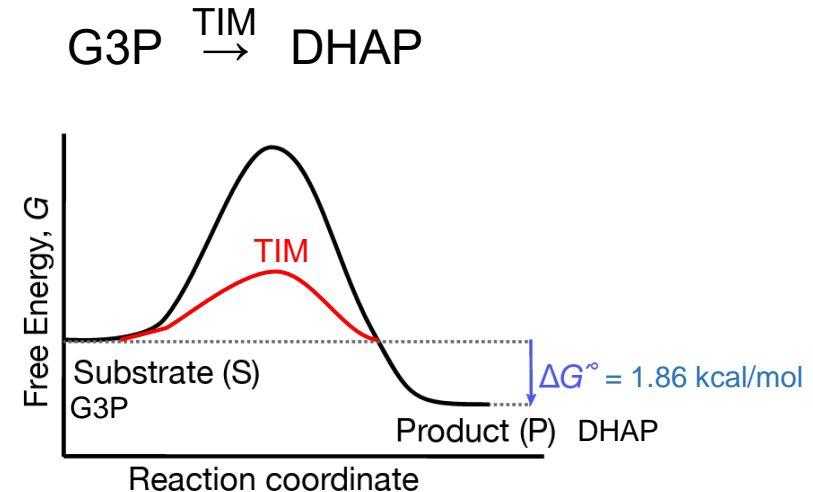
- We talked about that big activation barrier to get to DHAP and how triose phosphate isomerase lowered that activation barrier.
- But the point was there was a difference in the energy states and it was -1.86, so DHAP was more favorable than G3P.

*Does that mean that if you have G3P, all of it will get converted to DHAP?  
What do you think?*

# It is not like falling down the stairs!

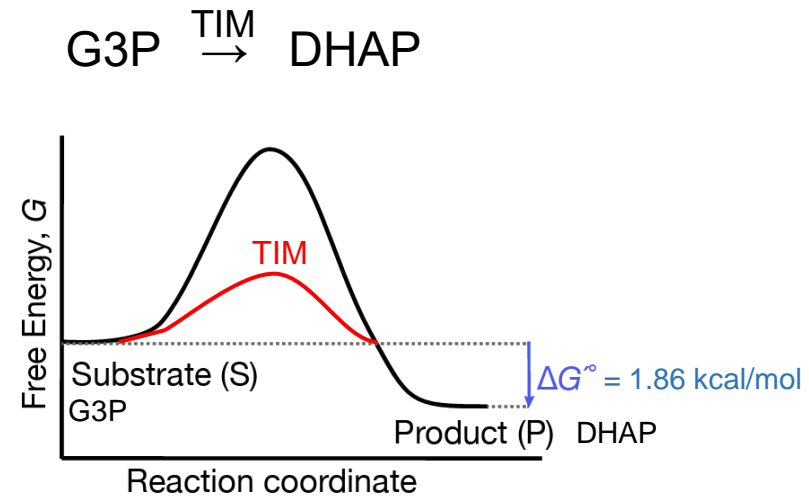
So according to  $\Delta G'^{\circ}$

- DHAP is favorable to a lower energy state.
- And it makes enormous sense that if that's a lower energy state, all of it is going to go to the lower energy state. That's the obvious thing.
- It's like falling down the stairs, right?
- The problem is although that makes enormous sense, it's wrong.
- It's just not the way it works.
- But the way chemistry really works is- it involves these equilibria.



# How the chemistry really works!

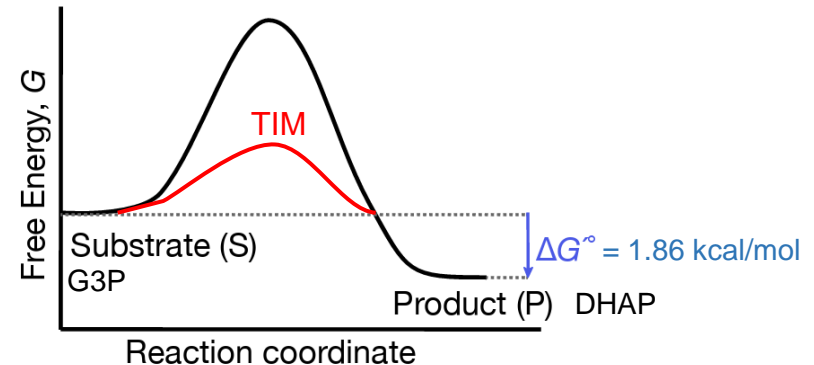
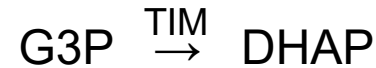
- If all of the molecules end up over there in DHAP, that's a very uneven, unlikely distribution and we have this problem of entropy.
- Entropy says we can't have all those molecules on one side.
- Then there's a statistical distribution, and it turns out that what happens depends on both the free energies but also concentrations.



# The energetic consideration

- If you had a tube that was only DHAP, the more favored state, triose phosphate isomerase, TIM would run some of that reaction backward, some.
- Now you wouldn't get all G3P.
- In fact, you'd only get about 5% that would get converted to G3P.
- But that's the important thing to think about is there's an *energetic consideration* and what's called *entropic consideration*.
- And it has to do with those concentrations.

*And so we have to actually understand how does that work?*



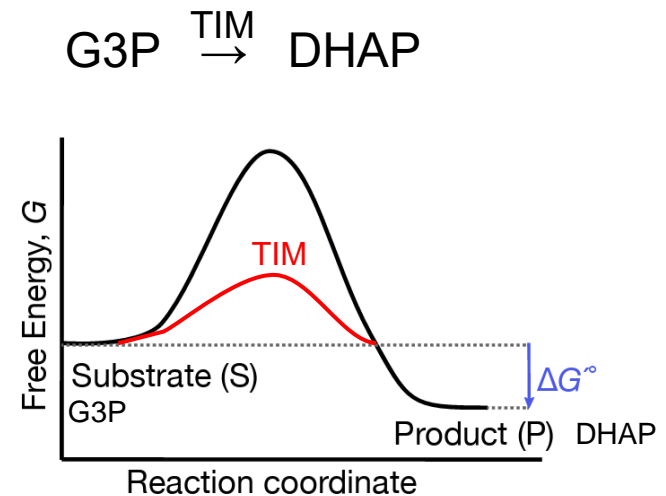
# How to include the energetic consideration

- Well, the way it works, the way you know which way a reaction is going to run,

whether all of G3P will get converted to all of DHAP or,

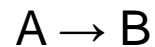
DHAP will get converted 5% back to G3P depends on this number,  $\Delta G$ ,

It is  $\Delta G'^{\circ}$  plus a term that has to do with relative concentrations



# The reaction energetics

So for a reaction

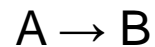


$$\Delta G = \Delta G'^{\circ} + RT \ln ([B] / [A])$$

If $\Delta G < 0$	$\Rightarrow$	Reaction runs forward/favorable
If $\Delta G > 0$	$\Rightarrow$	Reaction runs backward/unfavorable
	$\Rightarrow$	Unfavorable when you balance out the considerations of energy and concentrations

# Lets try this out in practice

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([B] / [A])$$

What if  $[A] = [B]$ ?

$$\Delta G = \Delta G'^{\circ}$$

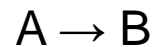
And it just is all determined by  $\Delta G'^{\circ}$

So  $\Delta G'^{\circ}$  is really telling you the way the reaction will run if the concentrations are equal.

And when they're equal, that tells us it's going to go from G3P to DHAP.

# Lets try this out in practice

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([B] / [A])$$

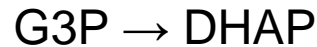
What if  $[A] \lllll [B]$ ?

- What will  $([B] / [A])$  ratio be?: A big number.
- And what's the log of a big number?: A big number.
- And when I add this big number to a negative number, it could be positive.
- So if there's enough B around, then  $\Delta G$  will be positive and the reaction will go the other way.



# Example...

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([\text{DHAP}] / [\text{G3P}])$$

What if  $[\text{DHAP}] / [\text{G3P}] = 30 : 1$ , we need to calculate  $\Delta G$

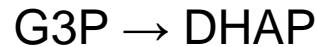
We know that  $\Delta G'^{\circ} = -1.86$

What about  $RT$  ?

- Now,  $RT$  depends on the temperature.
- So do we have to know what temperature our reaction is going on at?
- It turns out we don't need to know, because it's the temperature - Kelvin,  
and in Kelvin you'll live your life at essentially the same Kelvin in biology.
- Even if you're running a fever or something like that or if your yeast is growing at a slightly lower temperature,  
for all practical purposes for all of biology,  $RT$  equals 0.6 kcal/mol.

# Example...

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([\text{DHAP}] / [\text{G3P}])$$

What if  $[\text{DHAP}] / [\text{G3P}] = 30 : 1$ , we need to calculate  $\Delta G$

We know that  $\Delta G'^{\circ} = -1.86$ ,  $RT = 0.6$

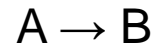
Plugging in the equation,

$$\Delta G > 0$$

- So that tells you it's a positive number, and that tells you when there's 30 times as much DHAP, the reaction will actually be generating G3P.
- It's a little counterintuitive.
- Anything sensible you'd think would all go to the low energy state, but it doesn't.
- It bounces back and forth due to this entropy consideration

# Equilibrium...

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([B] / [A])$$

If  $\Delta G < 0$   $\Rightarrow$  Reaction runs forward/favorable

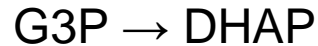
If  $\Delta G > 0$   $\Rightarrow$  Reaction runs backward/unfavorable  
 $\Rightarrow$  Unfavorable when you balance out the considerations of energy and concentrations

*What if  $\Delta G = 0$ ?: Does the reaction run forward or backward?*

*Neither. It's at equilibrium.*

# Concentrations at equilibrium...

So for a reaction



$$\Delta G = \Delta G'^{\circ} + RT \ln ([\text{DHAP}] / [\text{G3P}])$$

And so if you set that equal to 0, you could solve for the concentration at equilibrium.

The equilibrium concentration for our reaction there is about 1 G3P to 22 DHAPs.

That's where the reaction is balanced perfectly at equilibrium.

What does that mean? – There is no reaction going on, meaning there is no molecule moving left to the right and right to the left?

Or are they moving in equal amounts?

# What does equilibrium mean?

- They're moving in equal amounts because,  
Each molecule doesn't know what the other molecules are doing.
- It's a mass action kind of thing.
- Every molecule is doing its thing.
- It bounces into triose phosphate isomerase, it converts to the other thing.
- But the thing is when there's a lots of DHAPs bouncing into triose phosphate isomerase and converting back the other way.
- And when there are a lot of G3Ps, they're bouncing.
- Every molecule's behaving independently, and what's really happening is we're counting out the dynamic equilibrium, not a static equilibrium.
- It's not all the molecules decide we voted which we're going to be and we stay that.
- They're constantly moving back and forth.
- And this is the dynamic equilibrium that's obtained..

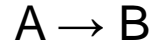


# How do enzymes affect the energetics?

- How does the enzyme change  $\Delta G'^{\circ}$ ?- It doesn't.
- The enzyme actually had no effect on these energetics.
- It had no effect on whether the reaction is running forward or backward.  
because that enzyme is just a catalyst.
- It's not changed, it doesn't do anything, it doesn't change the energy states.
- What does it do?
- It helps speed up that reaction, because in the absence of that enzyme TIM we'd have to wait for it to get over the energy barrier.
- All TIM does is speeds it up, and that's a really important observation.
- The enzyme doesn't change a thing other than the speed with which this is all going and that other thing that it protects that intermediate sometimes from a side reaction.
- But it's all about speed.
- As we talked about, the enzyme speeds it up merely by a factor of  $10^{10}$ , and the  $10^{10}$  was the difference between one second and three centuries.
- But if you don't care, if you're very patient, it's quite immaterial.
- I'm not that patient.
- Life isn't that patient, and that's why the evolution of enzymes was so very important.

# The challenge for life...!

- Suppose I have a reaction that a cell would like to carry out



- And suppose with the concentrations that would normally be there,

It's an unfavorable reaction.

- What are we going to do about unfavorable reaction?
- $\Delta G > 0$ ; The reaction doesn't want to flow that way.
- But maybe to build something, to create a molecule, we need it to flow in that direction.
- How are we going to persuade this reaction to go forward even though it is unfavorable?

**That's a challenge for life.**



# What do we do?

- What do we do? How do we make this happen?
- How about using an enzyme?
- Will that help?
- No, we know that an enzyme is not going to make any difference
- The enzyme is a catalyst.
- All the catalysts do is speed things up.
- They can't change what happens.
- They can urge the reaction on, but they can't actually do anything more than that.
- How about temperature change?
- Will that help?
- The problem is, that has serious other consequences for you.
- I could try to lower and raise your temperature, but that would be a bad thing for you!
- I got a lot of different reactions in the cells.
- How about we use body temperature!



# Trick 1

- Suppose I have a reaction that a cell would like to carry out.  
 $A \rightarrow B$ .
- And suppose that the concentrations that would normally be there, it's an unfavorable reaction.
- What are we going to do about unhappy reaction?
- Couple this unhappy reaction to a happy reaction



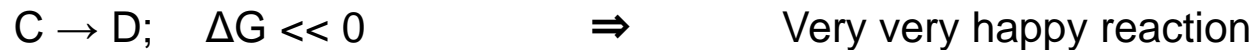
# Trick 1 : Coupling

- Couple this unhappy reaction to a happy reaction

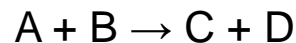
- We have an unhappy reaction



- Suppose we have:



- Suppose we have an enzyme that carries out both the reactions



- So what is the  $\Delta G$  ? Sum of both  $\Delta G$ s ! ;  $\Delta G < 0$
- The reaction becomes favourable!



# Trick 1 : pretty neat trick!

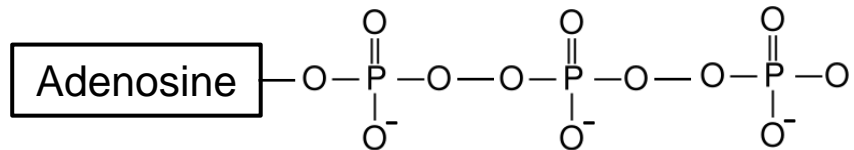
- In other words, if I can get something done that is unfavorable
- by coupling it to something that's very favorable
- and they take place at the same time
- in the same active site of that one enzyme

**that suddenly becomes a favorable reaction.**



- That's a pretty neat trick!
- But that means I need some favorable reaction, some reaction that has a really good negative  $\Delta G$
- Any reaction comes to mind? Anything we talked about?

# Any high energy molecule come to mind?

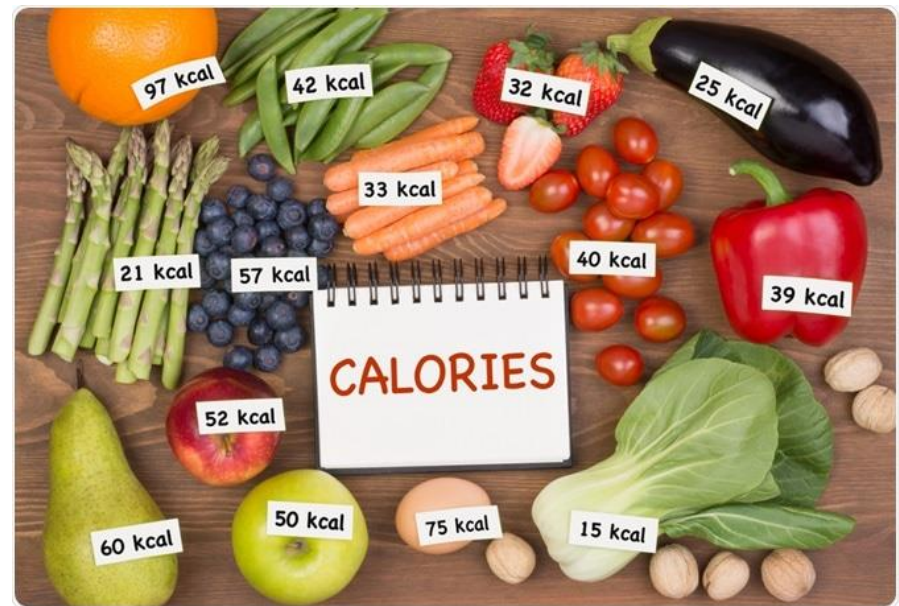


What do negatives do?

Repel each other!

It's a great way to store energy!

If these bonds break,  
it releases energy.



When cells need energy,  
it resorts to **Adenosine Tri Phosphate (ATP)**

# ATP is the energy currency

## Money Conversion

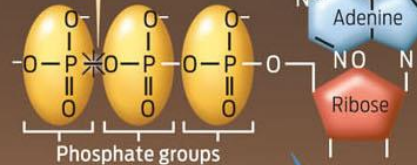


Money in a form that can be used to make purchases.



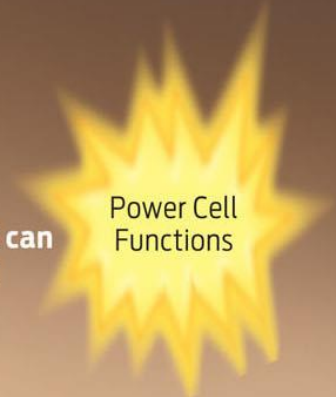
## Dietary Energy Conversion

Adenosine triphosphate is a nucleotide that stores chemical energy in the bonds between its phosphate groups. Breaking these bonds releases energy.



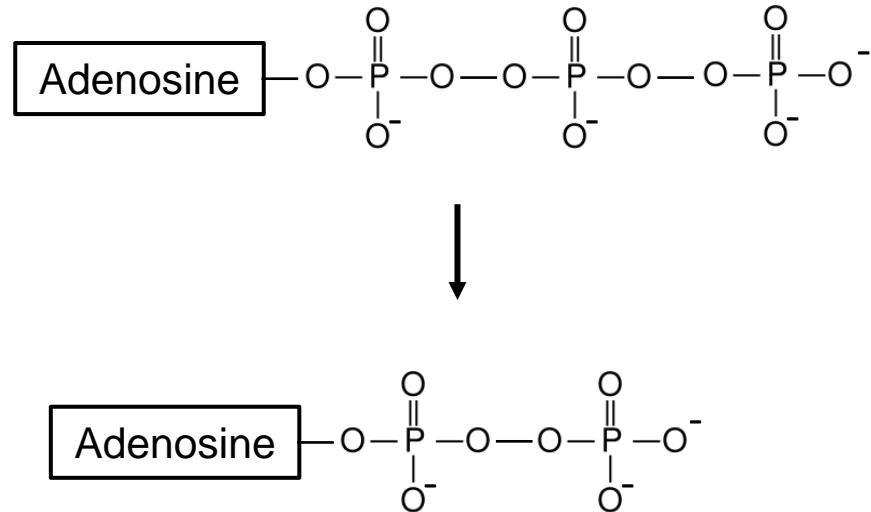
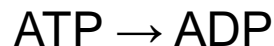
ATP

Energy in a form that can be used to power cell functions.

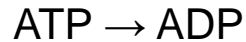


# ATP energy

- We said that this adenosine triphosphate was a high energy storage molecule.
- All these negatives were together.
- And if we were to cleave one phosphate off and convert ATP to ADP.



# ATP to the rescue!



$$\Delta G'^{\circ} = -7.3 \text{ kcal/mol}$$

- That turns out that is really good.
- It's a nice trick,
- It happens to be the **fundamental trick used all over to drive reactions**
- **Burn an ATP.**
- Hydrolyze that bond there.
- Do that, and **you can drive reactions that otherwise wouldn't go forward**
- This is direct coupling



## Trick 2: Indirect coupling

- In trick 1, we've directly coupled two reactions going on at exactly the same time, directly coupled reactions.
- But I can also do a cute trick of indirectly coupling two reactions!
- Now, We have an unhappy reaction
$$A \rightarrow B; \quad \Delta G > 0 \quad \Rightarrow \quad \text{Unhappy reaction}$$
- What would happen if I can change that concentration of B to zero?



## Trick 2: What if there is no B?

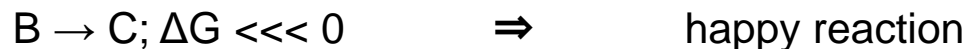
- Now, We have an unhappy reaction



- It's going to forward, right?
- Because if the concentration of B is zero, then As will be bumping into and converting to Bs.
- There are no Bs bumping into it and converting back to As, so it's going to go forward.
- So all I gotta do is get rid of the Bs.
- How am I going to get rid of the Bs?
- Use another reaction.
- Maybe,  $B \rightarrow C; \Delta G \lll 0 \quad \Rightarrow \quad \text{Very happy reaction}$
- And we're going to force that reaction forward.
- We're going to force the  $A \rightarrow B$  forward by pulling the B down.
- And so I've indirectly coupled both the reactions.

## Trick 2: What if there is no B?

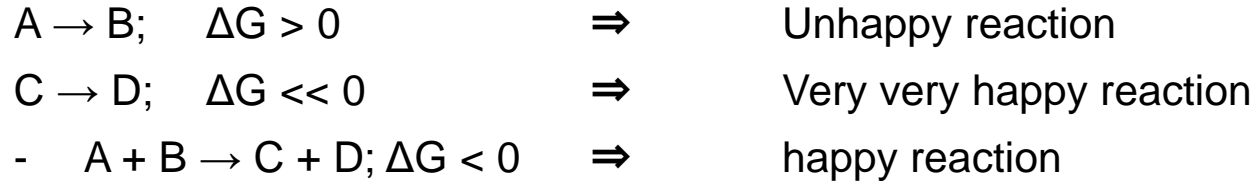
- Now, We have an unhappy reaction



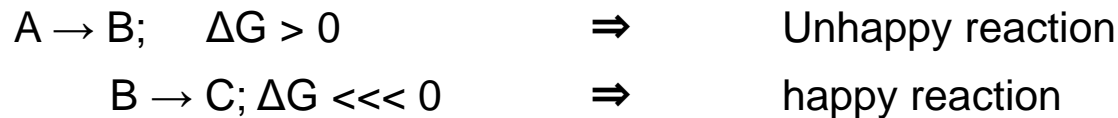
- And I can force it down the A-B pathway even if this is energetically unfavorable on its face.
- If the C-D is energetically favorable, I can pull it down.
- In fact, that'll work for quite a number of steps.
- If I'm pulling hard enough, I can force things down the pathway.
- That's why I dwelled on the logic of this, because once you realize that it has to do with those ratios, I can work my magic on these pathways by pulling with favorable reactions.
- So I need some favorable reactions.

# Trick 1 or Trick 2?

Trick 1:



Trick 2:



- If our end goal is to produce B: we use trick 1 or ~~trick 2~~?
- If we have a pathway, we're in the business of fermenting our sugar, and we have to encounter one unfavorable step, but the next step is favorable: we use ~~trick 1~~ or trick 2?

# Question?

Under standard biological conditions, the reaction  $C \rightarrow D$  is unfavorable. Select all of the possible mechanisms that are used within a cell to drive the reaction forward.

- ☐ a specific enzyme catalyzes the  $C \rightarrow D$  reaction
- ☐ the  $C \rightarrow D$  reaction is coupled to the hydrolysis of ATP by an enzyme
- ☐ D is consumed in a subsequent reaction that is very favorable

# Glycolysis: a Pathway to break down sugar

*We know energetics, logical tricks for pathway  
Now we start with actual pathways in the body*

# Glycolysis

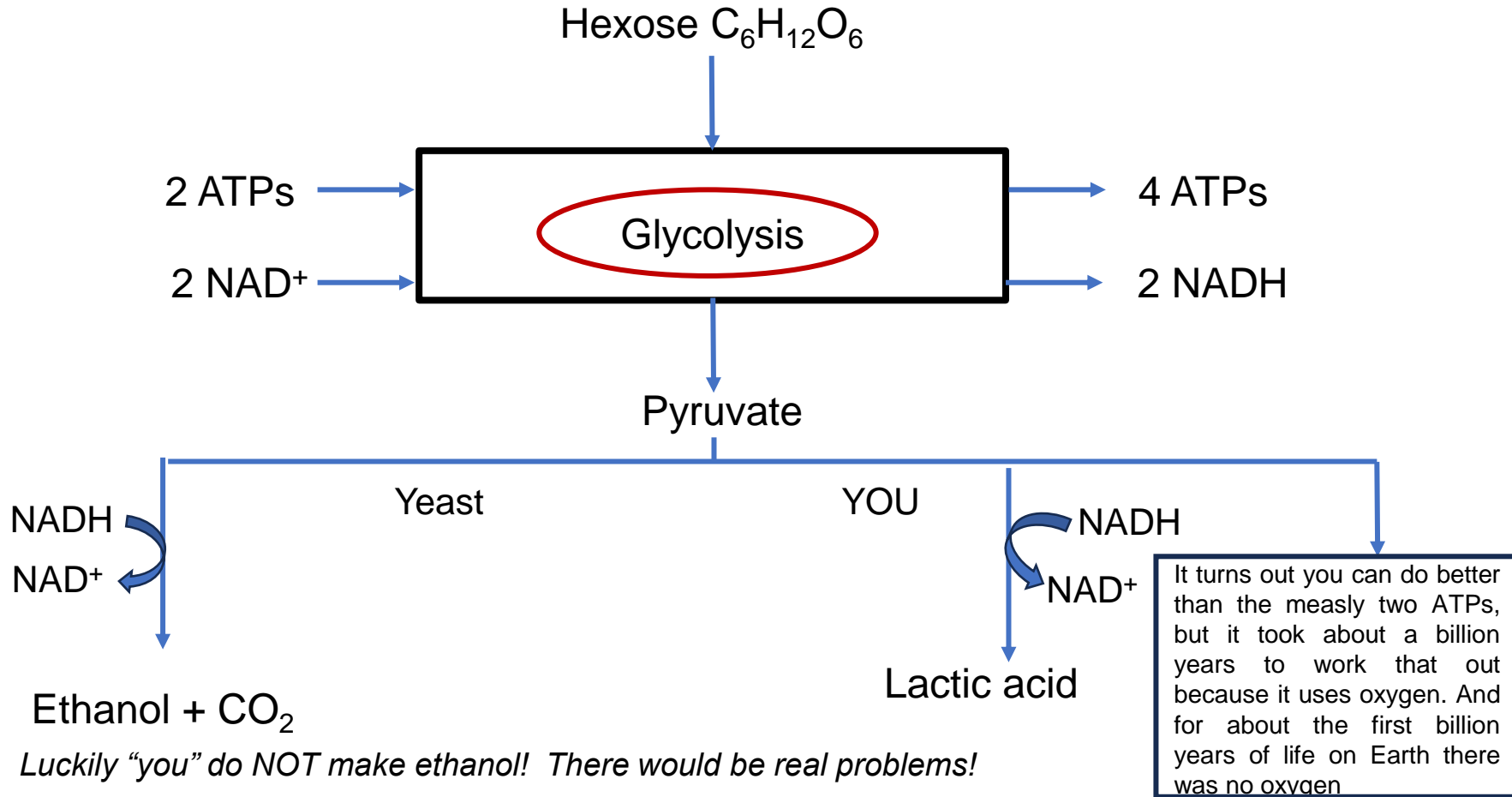
Glyco + lysis : Sugar breaking (or lysing)

So a good nickname for a “sweet” daughter that loves to “break”

Glycolysis



# Breaking sugar in living organisms



*Luckily "you" do NOT make ethanol! There would be real problems!*

Why you or yeast bother doing this at all?

To produce energy - ATP

Does yeast make carbon dioxide and ethanol for the enjoyment of humans?

The whole point of breaking down sugars is when you break bonds, you liberate energy from the bonds. You use the energy from the bonds to put them into ATP, which you use for other reactions.

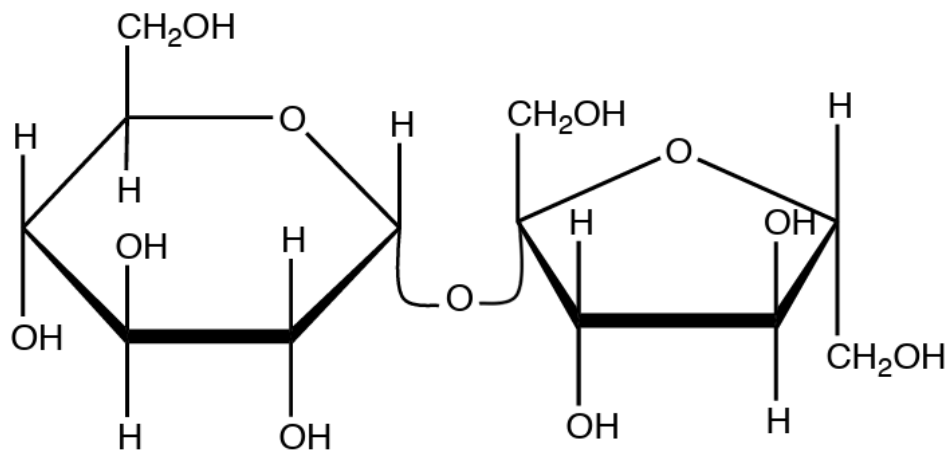
The entire point of this whole fermentation thing that Buchner and everybody studied is to make ATP.

# Wait a minute... breaking down sugars?

Shouldn't we be worried that about our sugar in the kitchen getting exploded or broken down?



## Sucrose



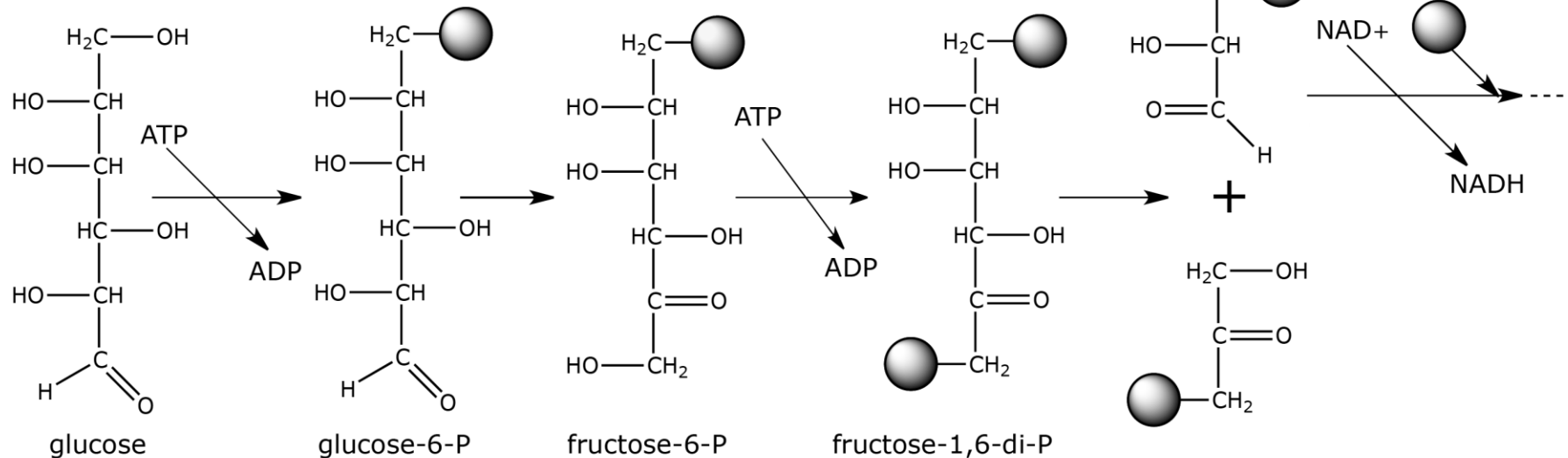
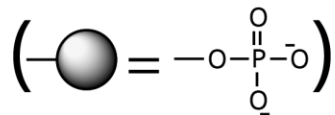
$\alpha$ -D-Glucose

$\beta$ -D-Fructose

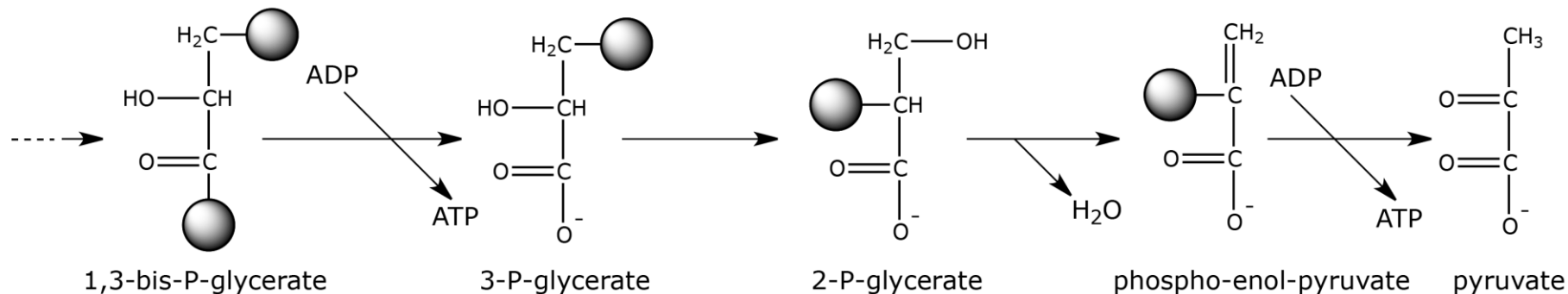
It will take 3-4 centuries to do so!



# Glycolysis

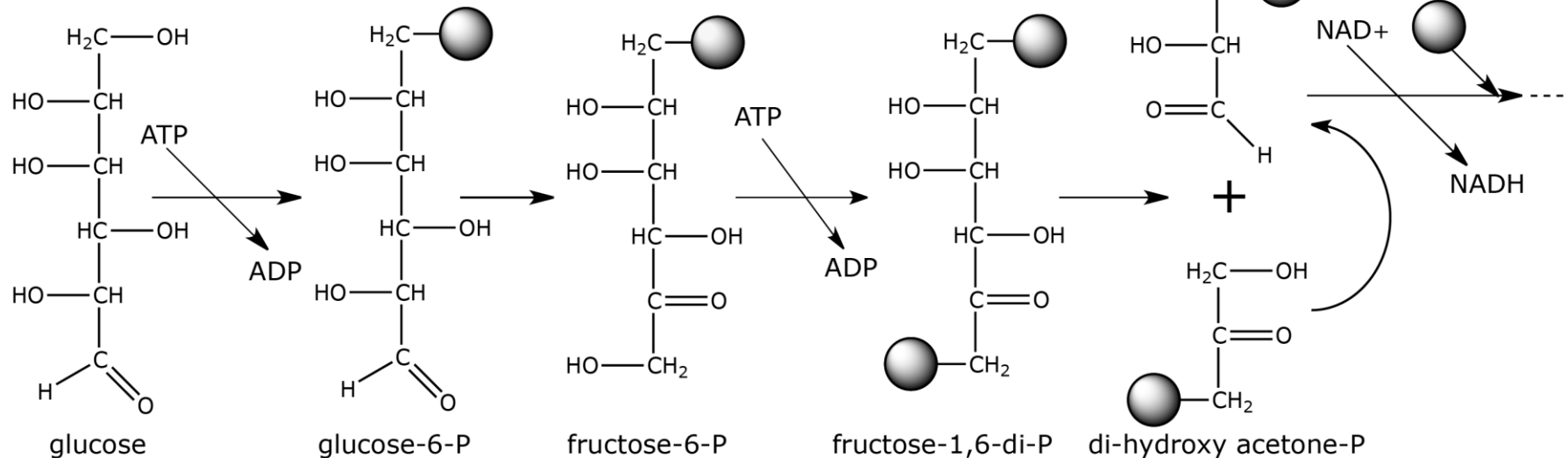
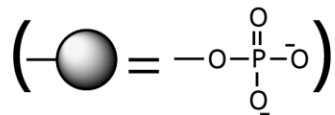


$\Delta G_0'$ :	-4.0	+0.4	-3.4	+5.7	+1.8	+1.5
$\Delta G$ :	-8.0	-0.6	-5.3	-0.3	~0.0	-0.4

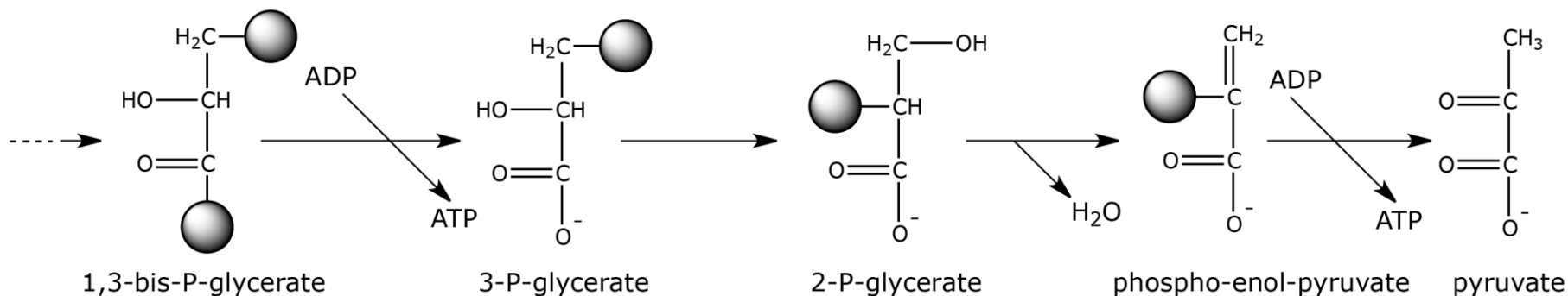


$\Delta G_0'$ :	-4.5	+1.1	+0.4	-7.5
$\Delta G$ :	~0.0	~0.0	-0.8	-4.0

# Glycolysis



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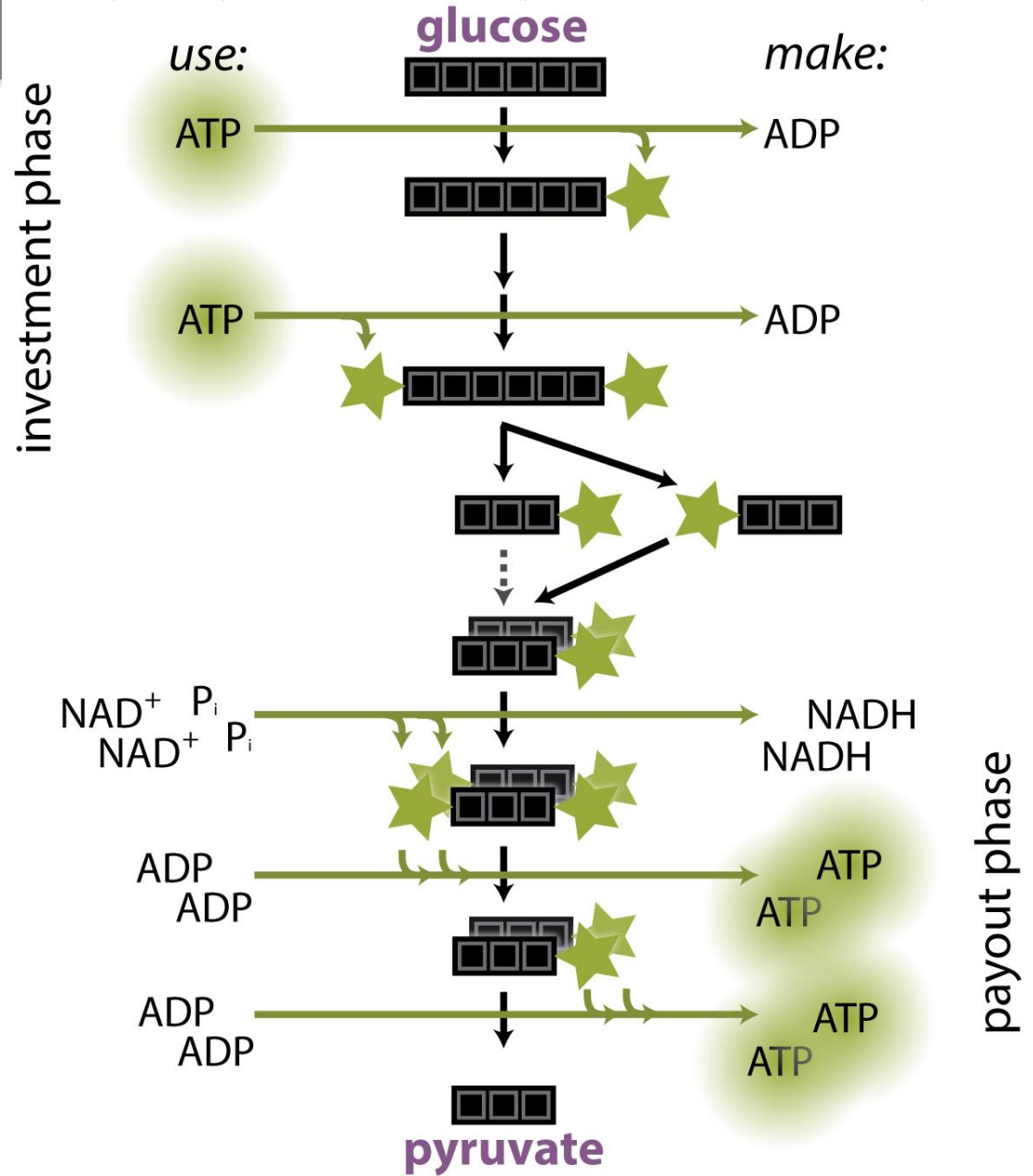
$\Delta G_0'$ :	-4.5	+1.1	+0.4	-7.5
$\Delta G$ :	~0.0	~0.0	-0.8	-4.0

# This is how glycolysis work!

- There's an investment phase, where you attach these phosphates,
- you build up these sugars, you break them apart, you send them down,
- you make the molecules more and more unfavorable, being clever at every stage to either couple it to a favorable reaction or suck it down the pathway by coupling it directly or indirectly to a favorable reaction.
- And at the end of day, in order to get two extra ATPs, we had to actually use both of those three-carbon structures, and that's what triose phosphate isomerase did for us.
- TIM saves the day!

# Glycolysis

## glycolysis: an enzymatic pathway



# Glycolysis

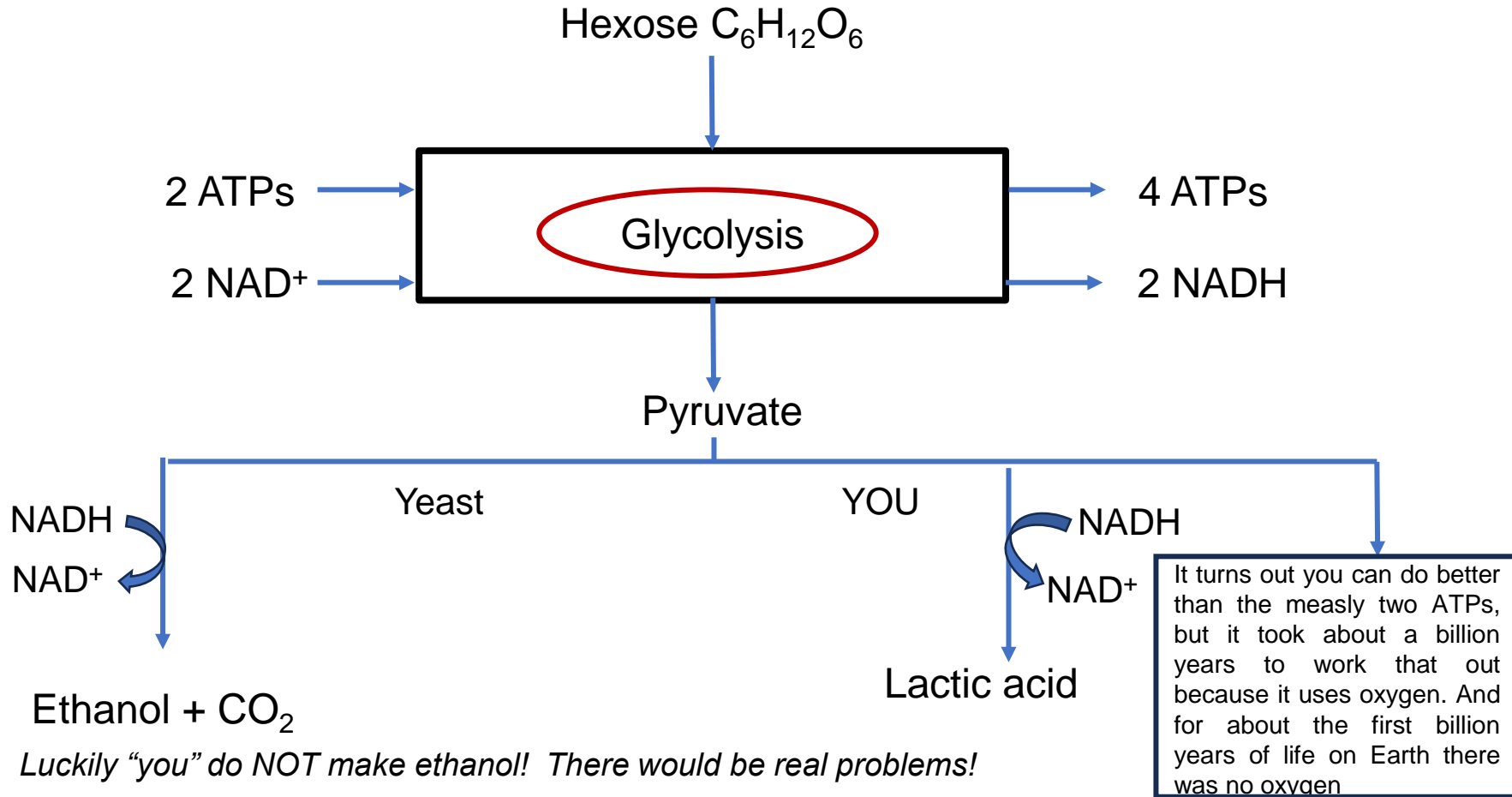
Glyco + lysis : Sugar breaking (or lysing)

So a good nickname for a “sweet” daughter that loves to “break”

Glycolysis



# Breaking sugar in living organisms



*Luckily "you" do NOT make ethanol! There would be real problems!*

Why you or yeast bother doing this at all?

To produce energy - ATP

Does yeast make carbon dioxide and ethanol for the enjoyment of humans?

The whole point of breaking down sugars is when you break bonds, you liberate energy from the bonds. You use the energy from the bonds to put them into ATP, which you use for other reactions.

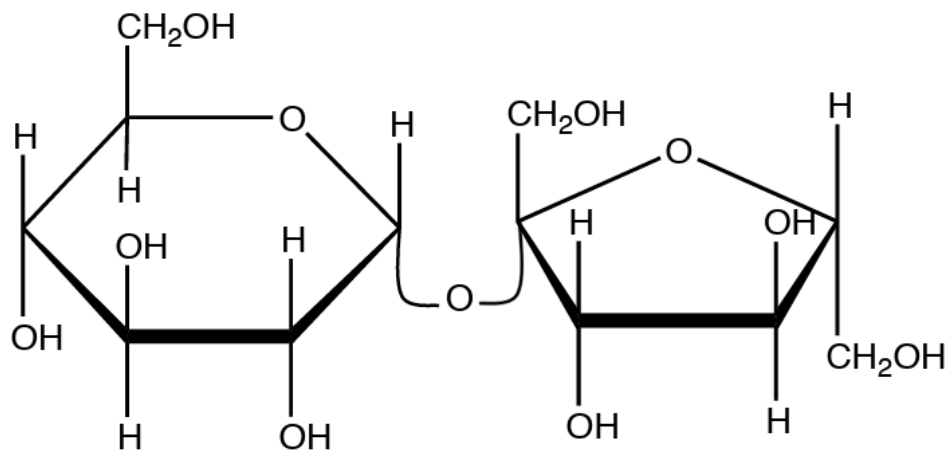
The entire point of this whole fermentation thing that Buchner and everybody studied is to make ATP.

# Wait a minute... breaking down sugars?

Shouldn't we be worried that about our sugar in the kitchen getting exploded or broken down?



## Sucrose

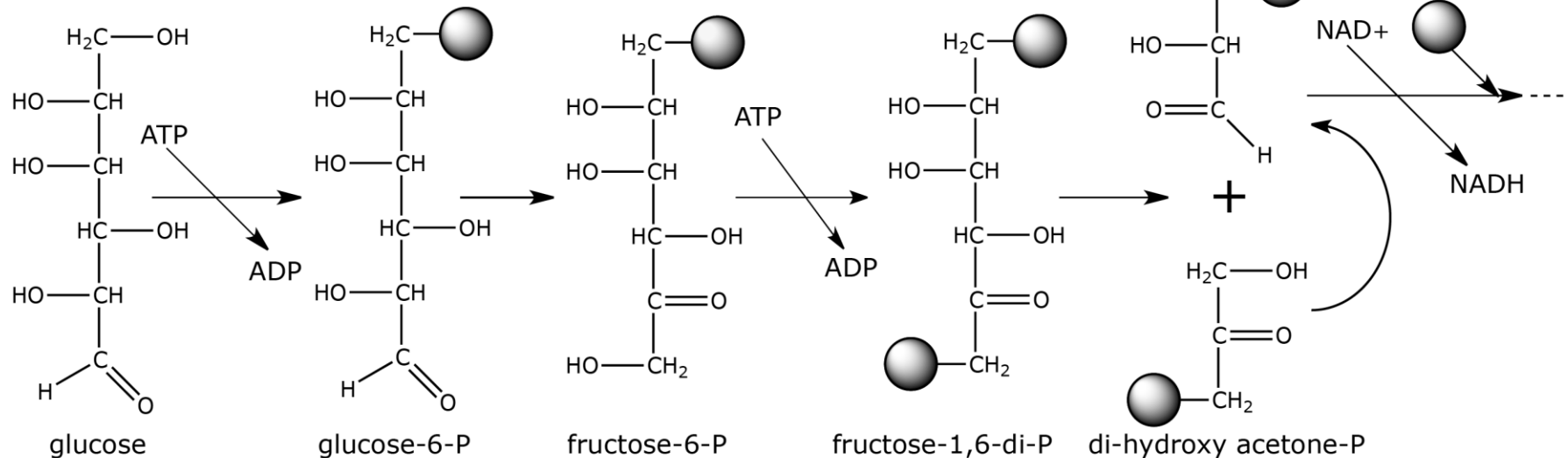
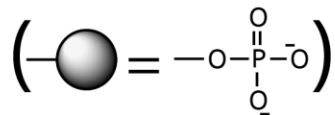


$\alpha$ -D-Glucose

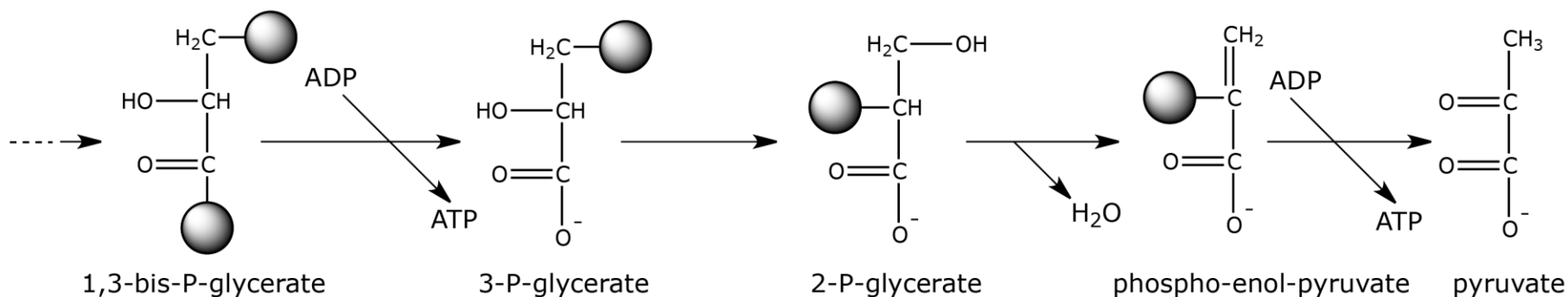
$\beta$ -D-Fructose

It will take 3-4 centuries to do so!

# Glycolysis



$\Delta G_0'$ :	-4.0	+0.4	-3.4	+5.7	+1.8	+1.5
$\Delta G$ :	-8.0	-0.6	-5.3	-0.3	~0.0	-0.4



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$\Delta G$ :	~0.0	~0.0	-0.8	-4.0

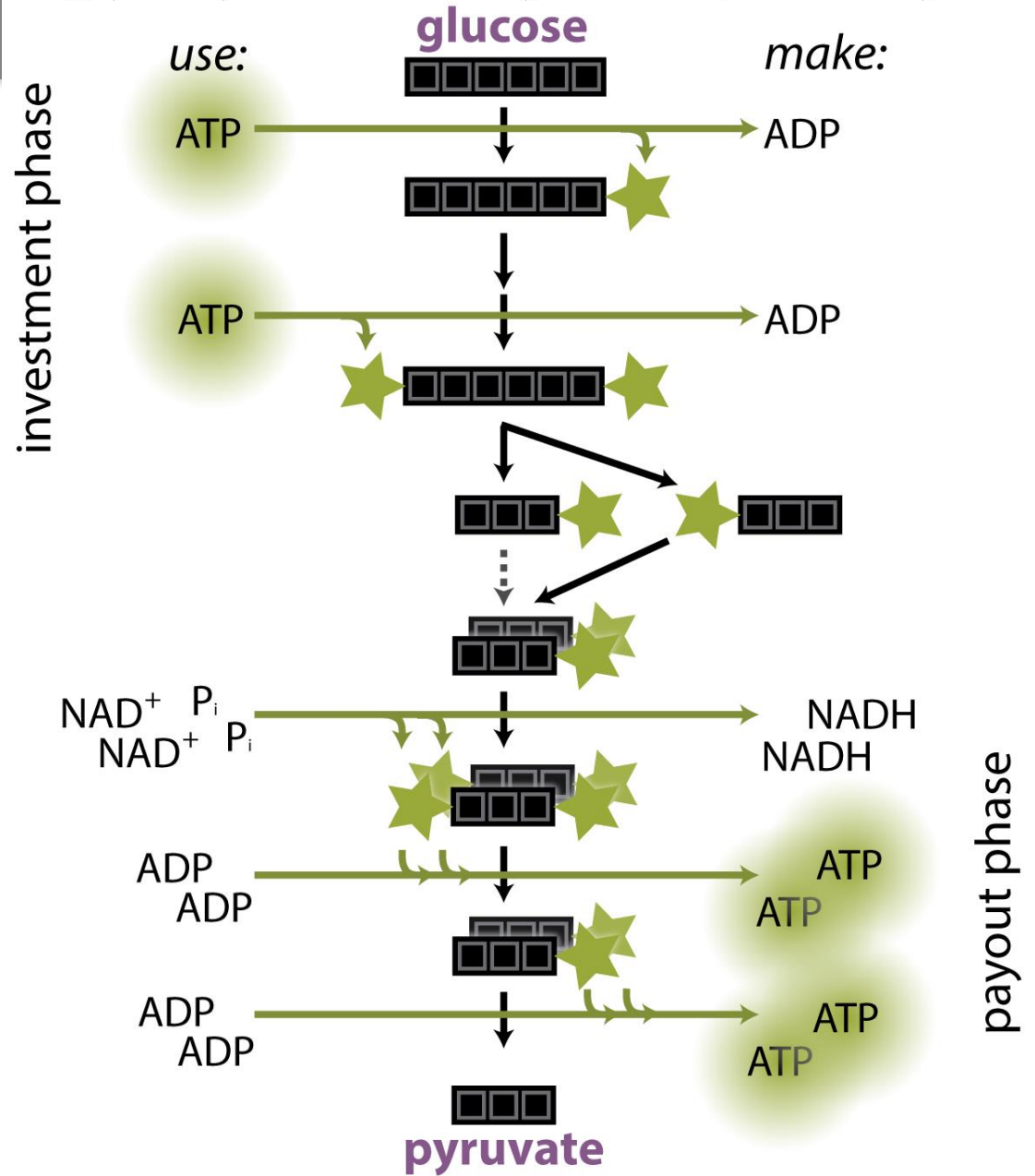


# This is how glycolysis work!

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# Glycolysis

## glycolysis: an enzymatic pathway

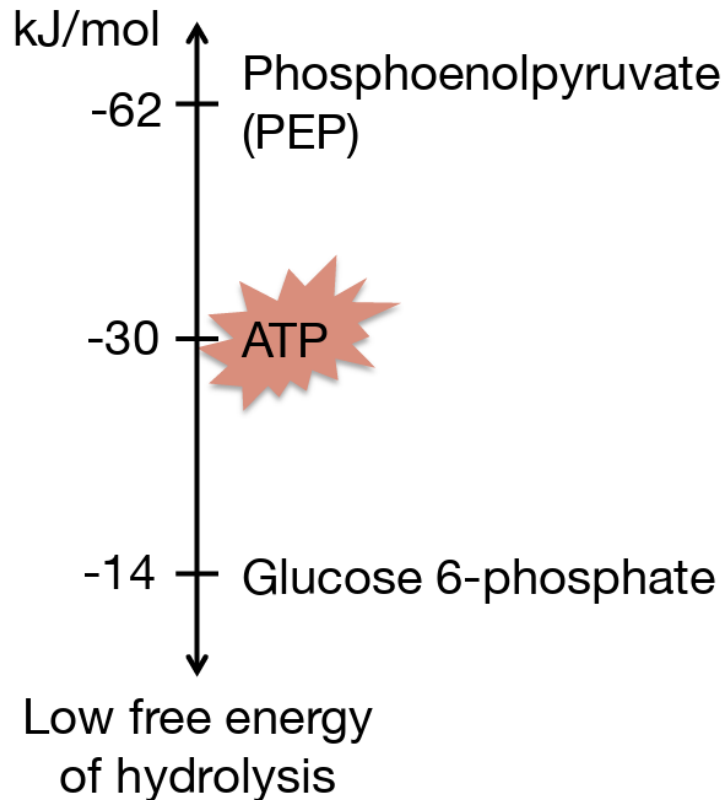


# A bit about ATP!

- You probably know that ATP is the main source of energy that cells use to do work.
- Because the hydrolysis of ATP is a source of energy that drives forward unfavorable reactions.
- However there is a common misconception.
- Some people say that the hydrolysis of ATP is the most exergonic reaction in a cell.
- If that was true, of course, ATP could drive many, many endergonic reactions.
- But there will be no reaction exergonic enough for a molecule to transfer its phosphate to ADP to regenerate ATP.
- So very quickly, the cell will run out of ATP.
- So to better understand the central role of ATP, we will use few examples with intermediates of glycolysis.

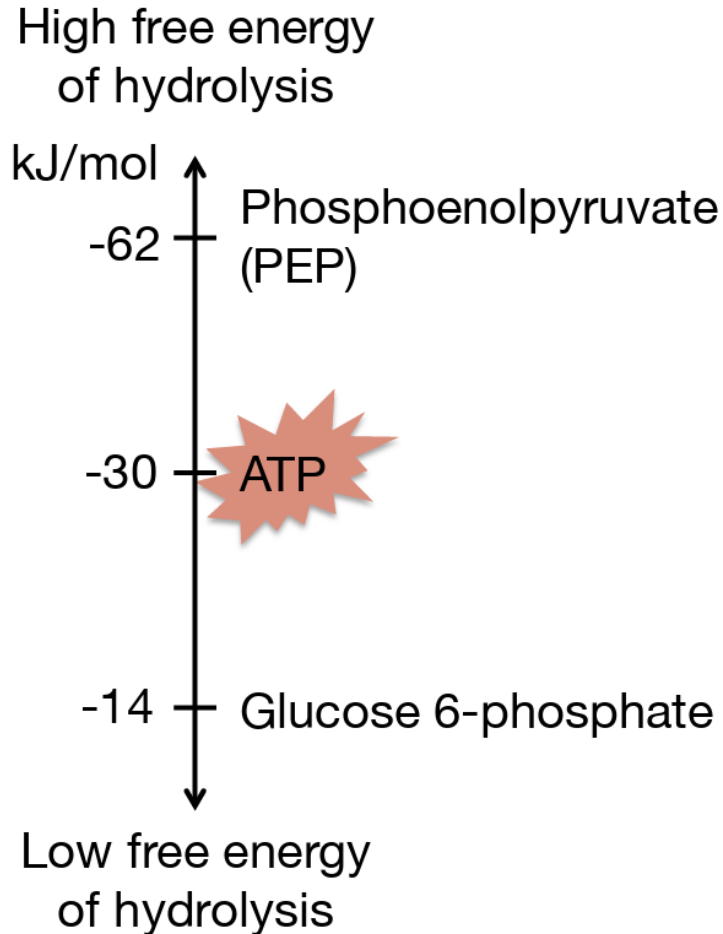
# A bit about ATP!

High free energy  
of hydrolysis



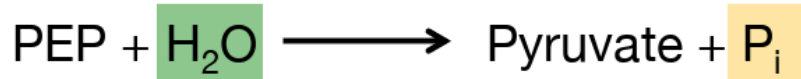
- So in this graph, we have positioned the hydrolysis potential of different molecules
- starting with phosphoenolpyruvate with a very high free energy of hydrolysis with a  $\Delta G$  of -60 kJ/mole.
- And at the bottom of the graph, we have glucose 6-phosphate that has a lower free energy of hydrolysis, -14 kJ/mole.
- And in between, we have ATP with -30 kJ/mole.
- So this intermediate place of ATP makes the synthesis of ATP from ADP possible when it's coupled with a reaction that is more exergonic than the synthesis of ATP, for example, the hydrolysis of phosphoenolpyruvate during glycolysis.

# A bit about ATP!

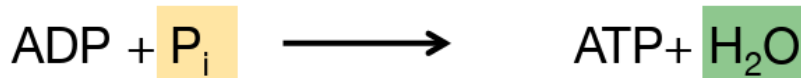


- In contrast, many endergonic reactions can be coupled with the hydrolysis of ATP that will provide enough energy to drive this unfavorable reaction forward.
- For example, it will be the case of the phosphorylation of glucose into glucose-6 phosphate.
- Let's take few examples to really understand the central role of ATP.

# Synthesis of ATP!

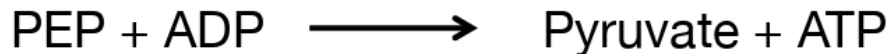


$$\Delta G^\circ = -62 \text{ kJ/mol}$$



$$\Delta G^\circ = +30 \text{ kJ/mol}$$

common reactants

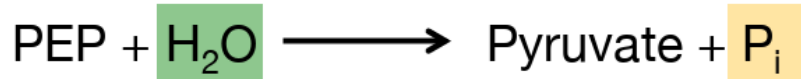


$$\Delta G^\circ = -32 \text{ kJ/mol}$$

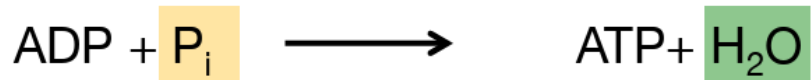
“coupling”

- So let's take a first example where phosphoenolpyruvate is hydrolyzed
- This reaction is highly exergonic.
- The change of free energy is very negative.
- In contrast, the synthesis of ATP from ADP plus inorganic phosphate is unfavorable because its change of free energy is positive, 30 kJ/mole.
- However, if we combine these two reactions into a single reaction these two reactions share a common reactant, so that's why we can combine them.
- And the change of free energy for this combined reaction is negative,
- Therefore, this combined reaction is thermodynamically favorable

# Coupling requirement

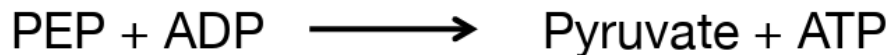


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common reactants



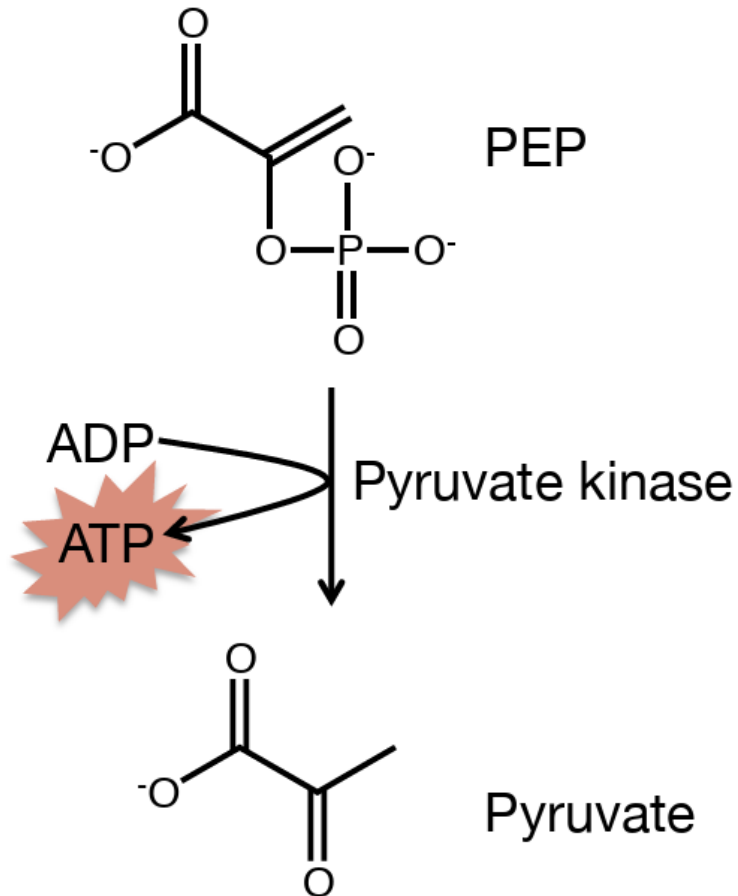
$$\Delta G^\circ = -32 \text{ kJ/mol}$$

“coupling”

Most commonly, a coupling is achieved when the combined reactions share

- a common reactant,
- they have a net change of free energy negative,
- and they are catalyzed by the same enzyme.

# Synthesis of ATP example!

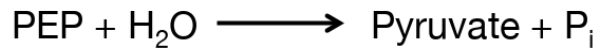


So for example, the coupling between

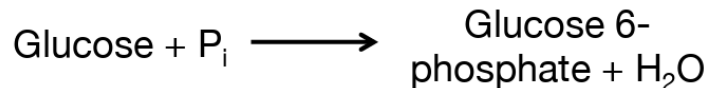
- the dephosphorylation of phosphoenolpyruvate and
  - the synthesis of ATP is catalyzed by pyruvate kinase
- by one single enzyme.



# No coupling!



$$\Delta G^\circ = -62 \text{ kJ/mol}$$

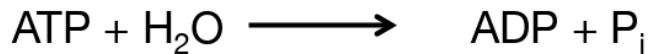


$$\Delta G^\circ = +14 \text{ kJ/mol}$$

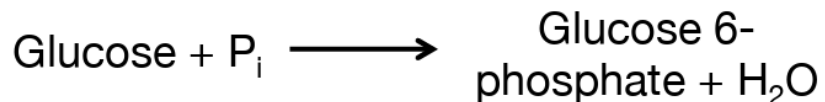
No coupling

- Having a shared reactant and a sum of free energy that is negative does not ensure coupling
- For example, starting again with the hydrolysis of phosphoenolpyruvate
- and now introducing a new reaction, the phosphorylation of glucose into glucose 6-phosphate.
- These two reactions share a common reactant, the inorganic phosphate.
- The sum of the change of free energy is also negative.
- **However, the cell does not contain any enzyme that can catalyze both of these reactions.**
- As a consequence, these two reactions are not coupled.

# Another example: Central role of ATP!

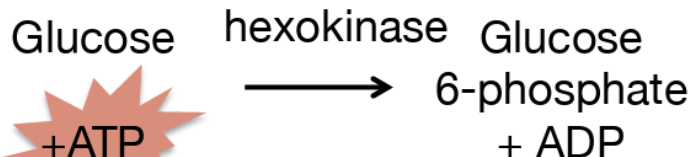


$$\Delta G^\circ = -30 \text{ kJ/mol}$$



$$\Delta G^\circ = +14 \text{ kJ/mol}$$

Coupled reaction:



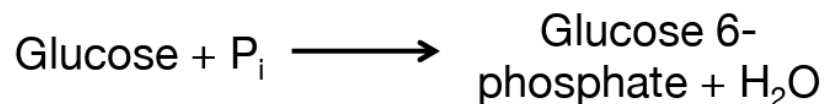
$$\Delta G^\circ = -16 \text{ kJ/mol}$$

- Let's take a final example to once again emphasize the central role of ATP.
- The hydrolysis of ATP into ADP and inorganic phosphate is exergonic with a change of free energy of -30 kJ/mole.
- The phosphorylation of glucose into glucose 6-phosphate is endergonic with a change of free energy of 14 kJ/mole.
- These two reactions can be combined, because they share a common reactant.
- In this case, the change of free energy of the combined reaction will be negative.
- And an enzyme called hexokinase catalyzes both of these reactions.
- So the two reactions are coupled.

# Central role of ATP!

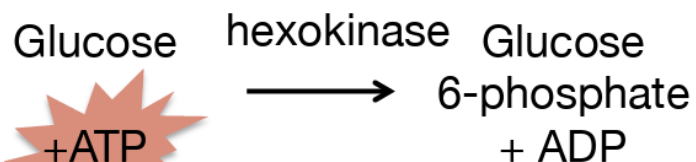


$$\Delta G^\circ = -30 \text{ kJ/mol}$$



$$\Delta G^\circ = +14 \text{ kJ/mol}$$

Coupled reaction:



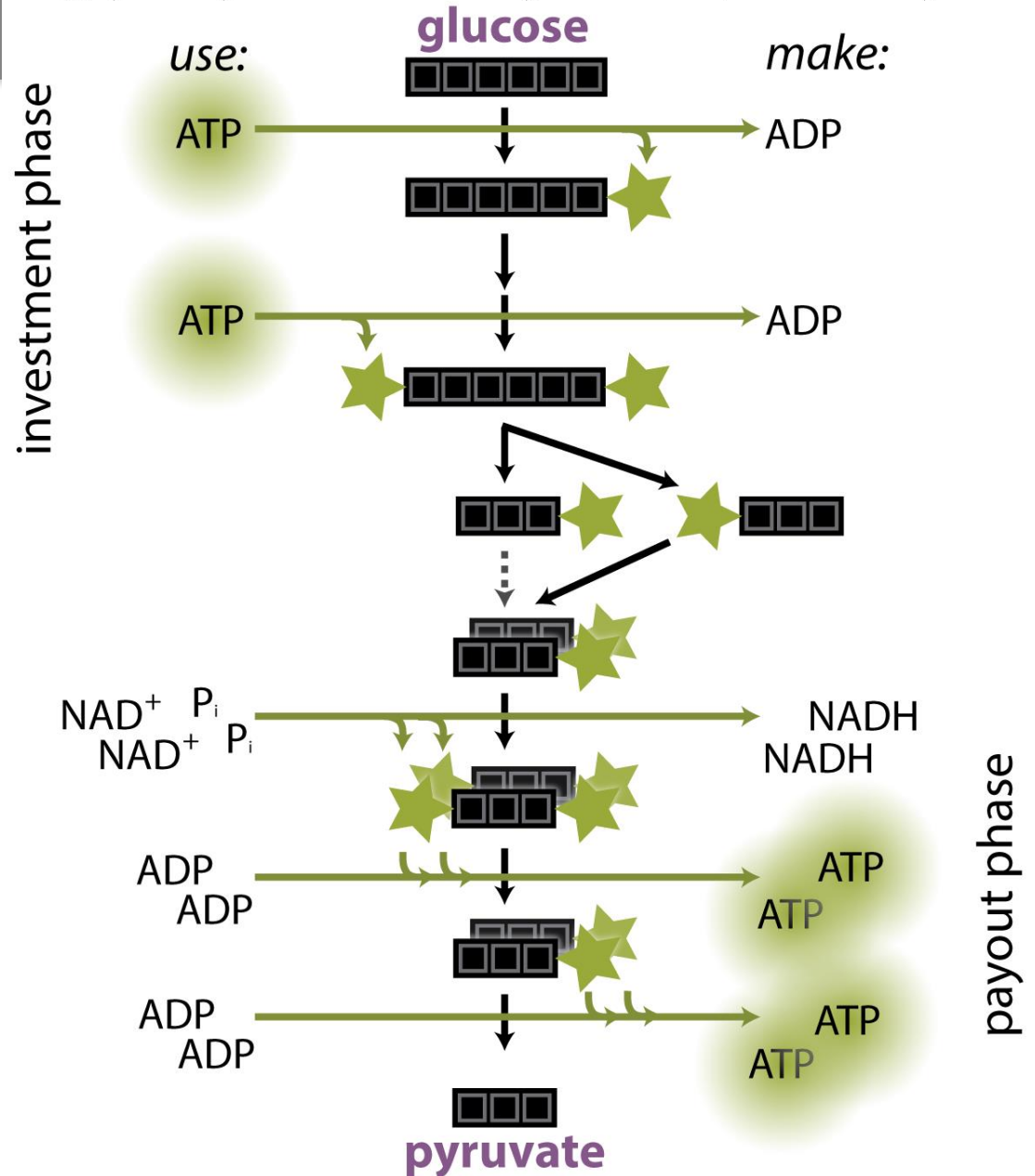
$$\Delta G^\circ = -16 \text{ kJ/mol}$$

- Many unfavorable reactions are coupled with ATP hydrolysis.
- **That is why the core of the cellular metabolism is devoted to the regeneration of ATP.**

# Glycolysis

- It all works beautifully.
- You're getting sugar, you're producing ATP.
- What if you don't need so much ATP?
- What if you don't have so much sugar?
- Should we just have this pathway be running that way all the time or would you like it to be have regulators or dials on it, to be a little adjustable?

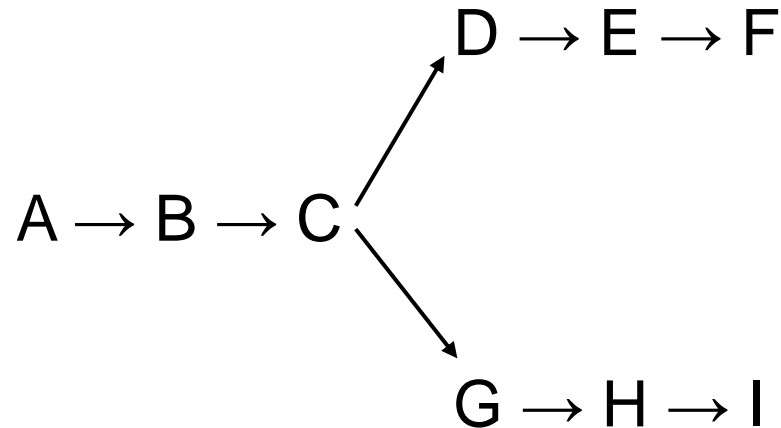
## glycolysis: an enzymatic pathway



# Regulation of pathways

So let's take a look at how we can regulate this nice pathway.

# Let us consider a random reaction!

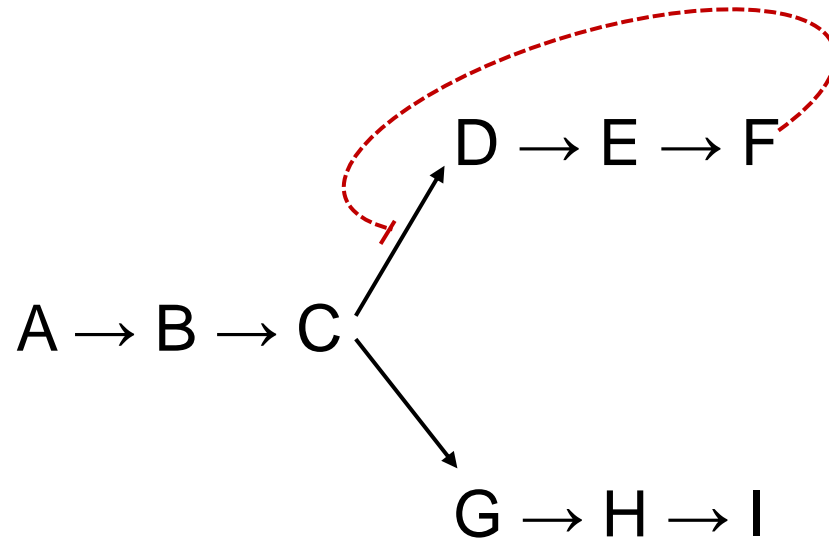


Suppose we have too much F.

The cell says- I've got too much F. I'm trying to balance things out. Maybe F is ATP, maybe F is something else-- I've got too much of it-- yet this pathway's just churning out more of it. That's not good-- it's a waste.

**I don't want that much more F. What do you want to do?**

# Which dial should we turn down?

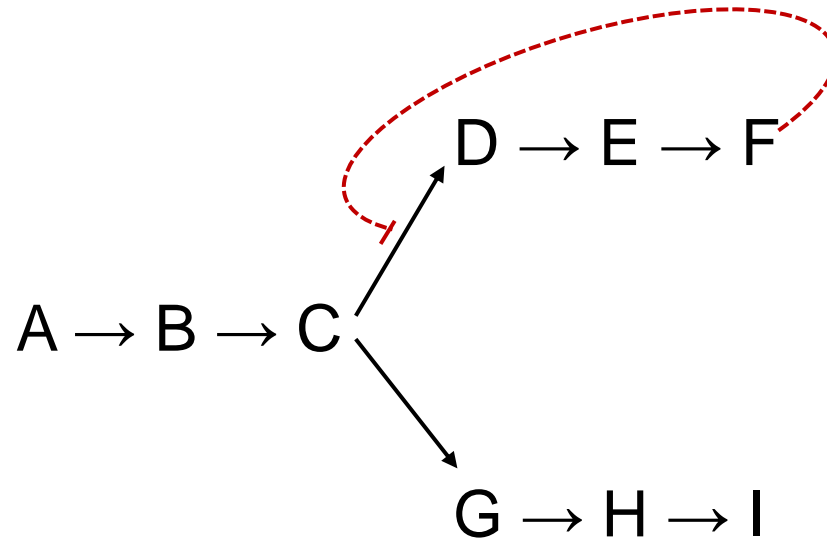


What does this arrow indicate? – **Rate**

What controls that rate? – **Enzymes**



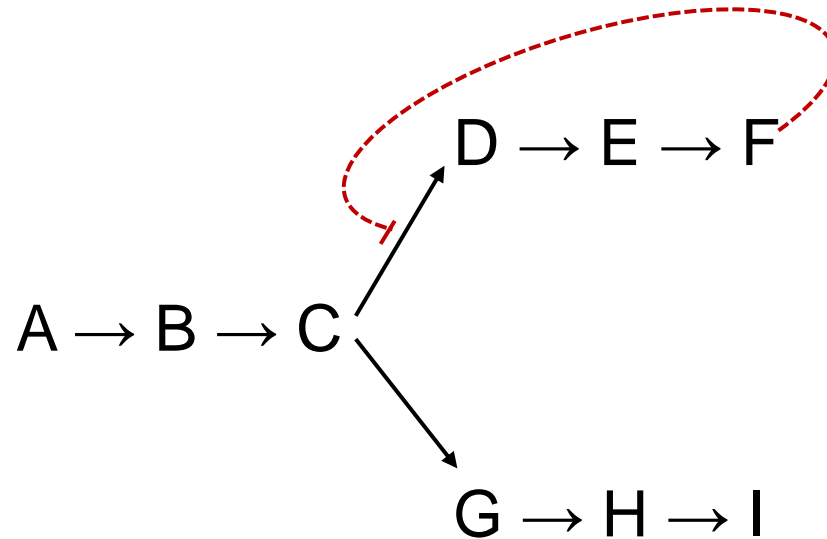
# How to turn down the dial?



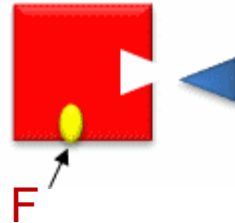
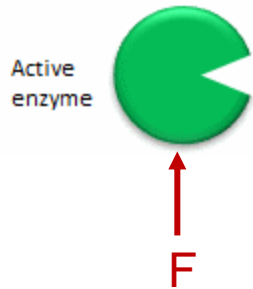
- Enzymes are good at controlling rates.
- We can really slow things down if we could just persuade that enzyme to-  
“please stop working or work a little less well!”
- How's it going to know there's too much F and what would it do in response to it?



# How to turn down the dial?



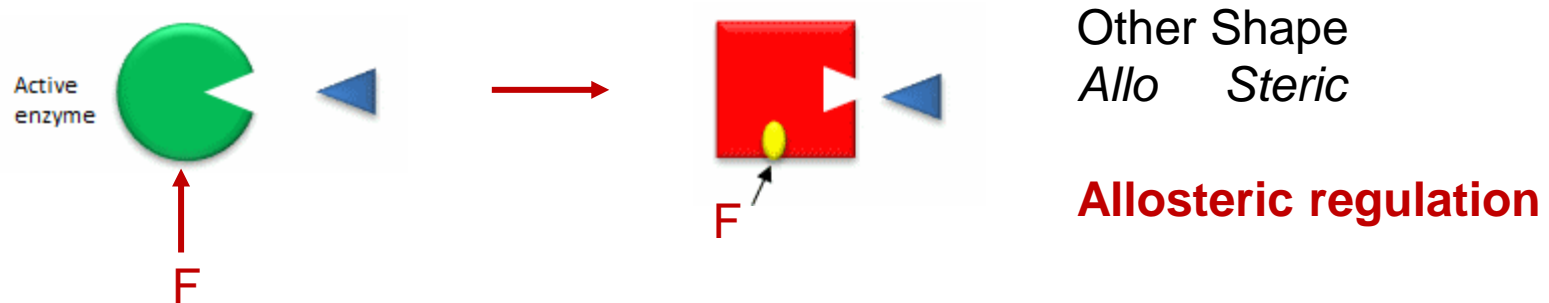
How's it going to know there's too much F and what would it do in response to it?



Other Shape  
*Allo* *Steric*

**Allosteric regulation**

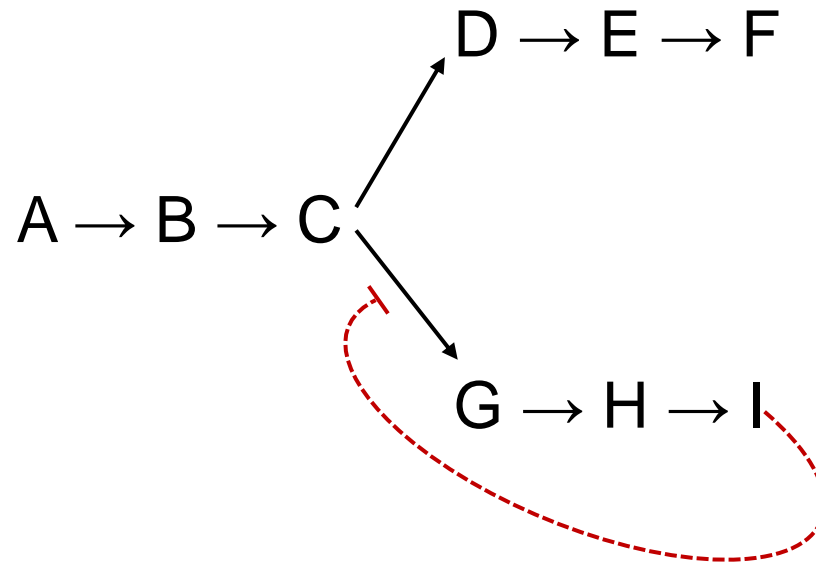
# That is a pretty good trick!



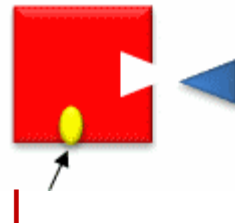
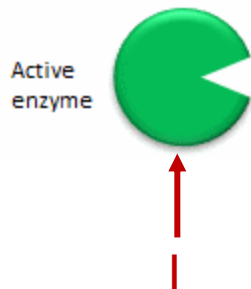
- And we were wondering, actually, weren't we, about this big protein with all these amino acids and all these things and what'd you need all that for?
- Well, some of that might be there because F can bind to it
- and then transmit the binding of F into its shape shifting and **affect the active site**.
- That's a pretty good trick.

We call it **Feedback Inhibition**

# What if I have too much I ?



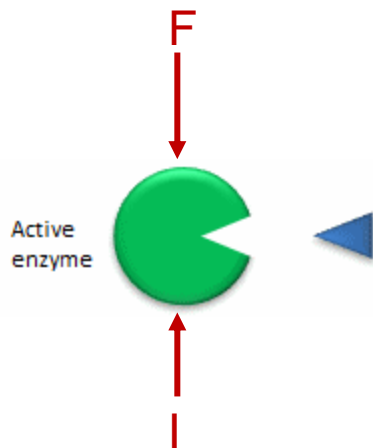
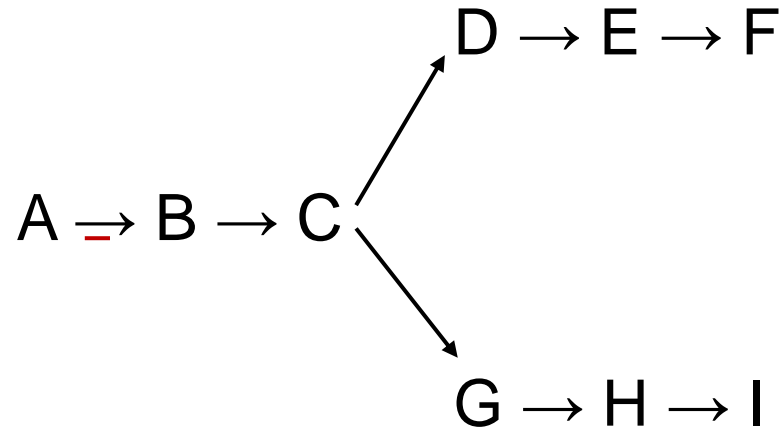
Same deal!



Other Shape  
*Allo* *Steric*

**Allosteric regulation**

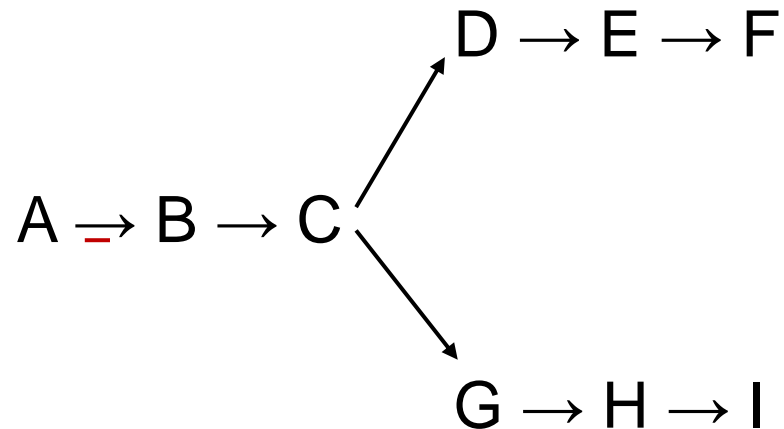
# What if I have too much F and I ?



Then maybe, it has active sites for both F and I

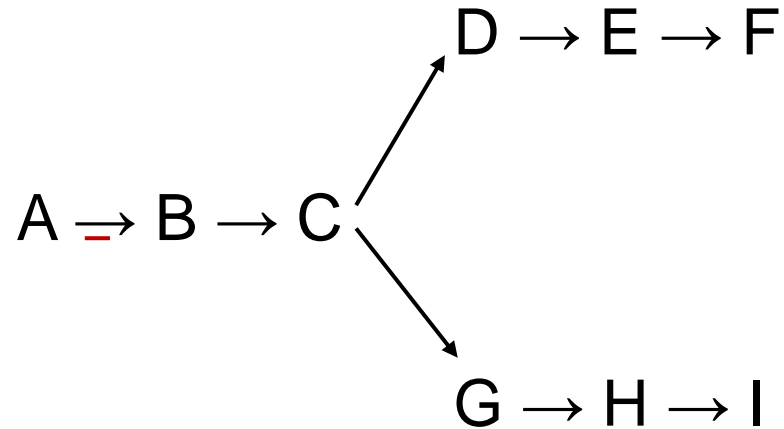
**Allosteric regulation**

# What if it is the other way around?!



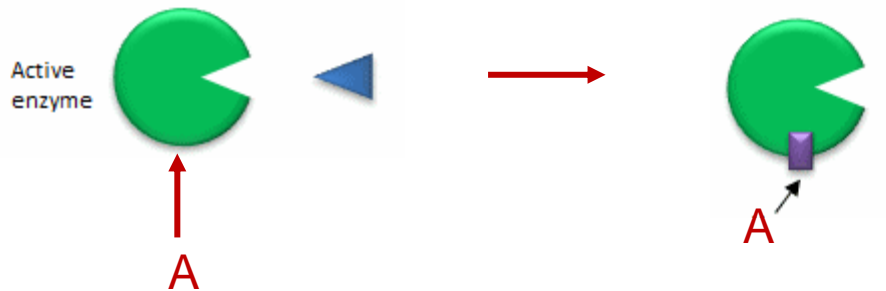
- Now suppose it's not that I have too much product at the end of the reaction, but I have too much reactant at the beginning of the reaction.
- I've got too much glucose and I haven't made enough ATP.- It's building up
- I've got a lot of A. I want to get this reaction to go faster.
- I can't change the delta Gs, but I want this reaction to go faster.
- **How am I going to get it to go faster?**

# How am I going to get it to go faster?



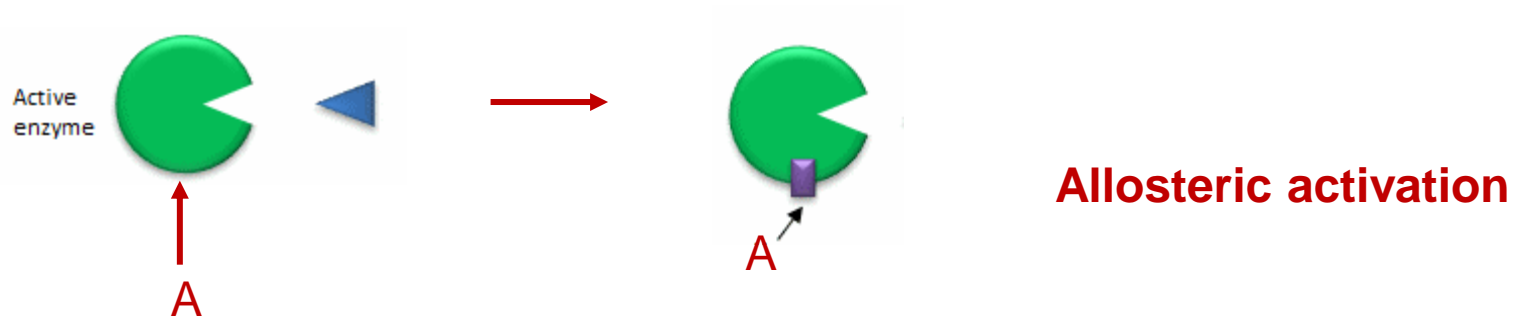
Why can't I run allosteric regulation in the activation direction also?

Maybe when As bind to a downstream enzyme, it activates the enzyme and makes it better.- **So I can run that both ways.**



**Allosteric activation**

# How am I going to get it to go faster?



It's allosterically changing to improve binding

Or we can say is that we could allosterically change to improve our enzyme speed as well.

We call it **Feedforward Activation**

# It happens all over!

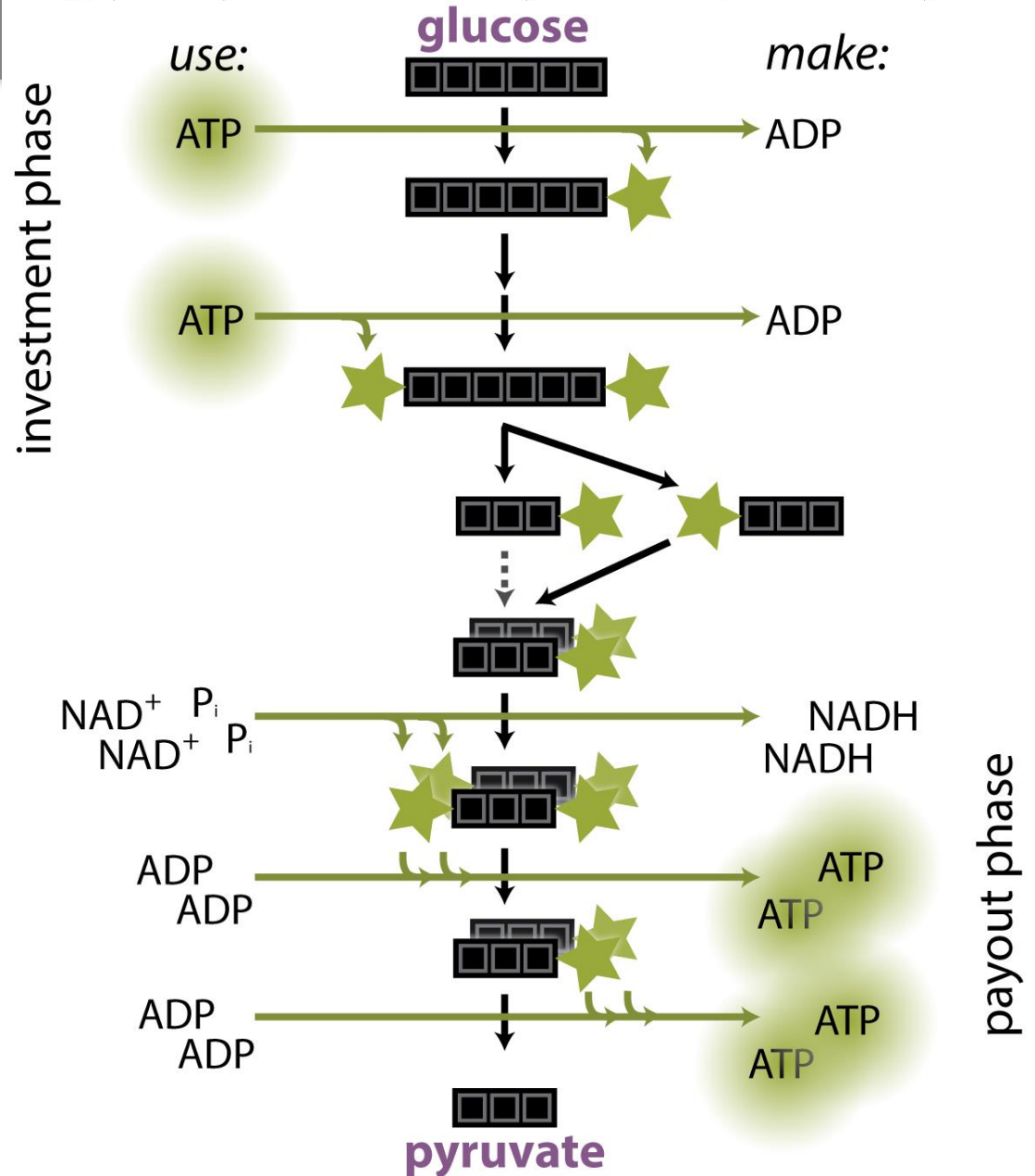
- **Feedback inhibition** and **Feedforward activation** happen all over the place.
- Probably when this reaction first evolved in evolution there, long, long time ago, it didn't have all those nice tweaks.
- But over time, as organisms competed with each other, those who evolved cute tricks like being able to do feedback inhibition or feedforward activation outcompeted their friends and all of these cool little tricks were layered on top and on top and on top.



# Glycolysis

- So we actually see this happening in glycolysis.
- First step, glucose to glucose-6-phosphate.
- That's an expensive step, isn't it?
- I'm spending an ATP.
- Well, suppose I have a lot of glucose-6-phosphate around.
- Would I want to do that?

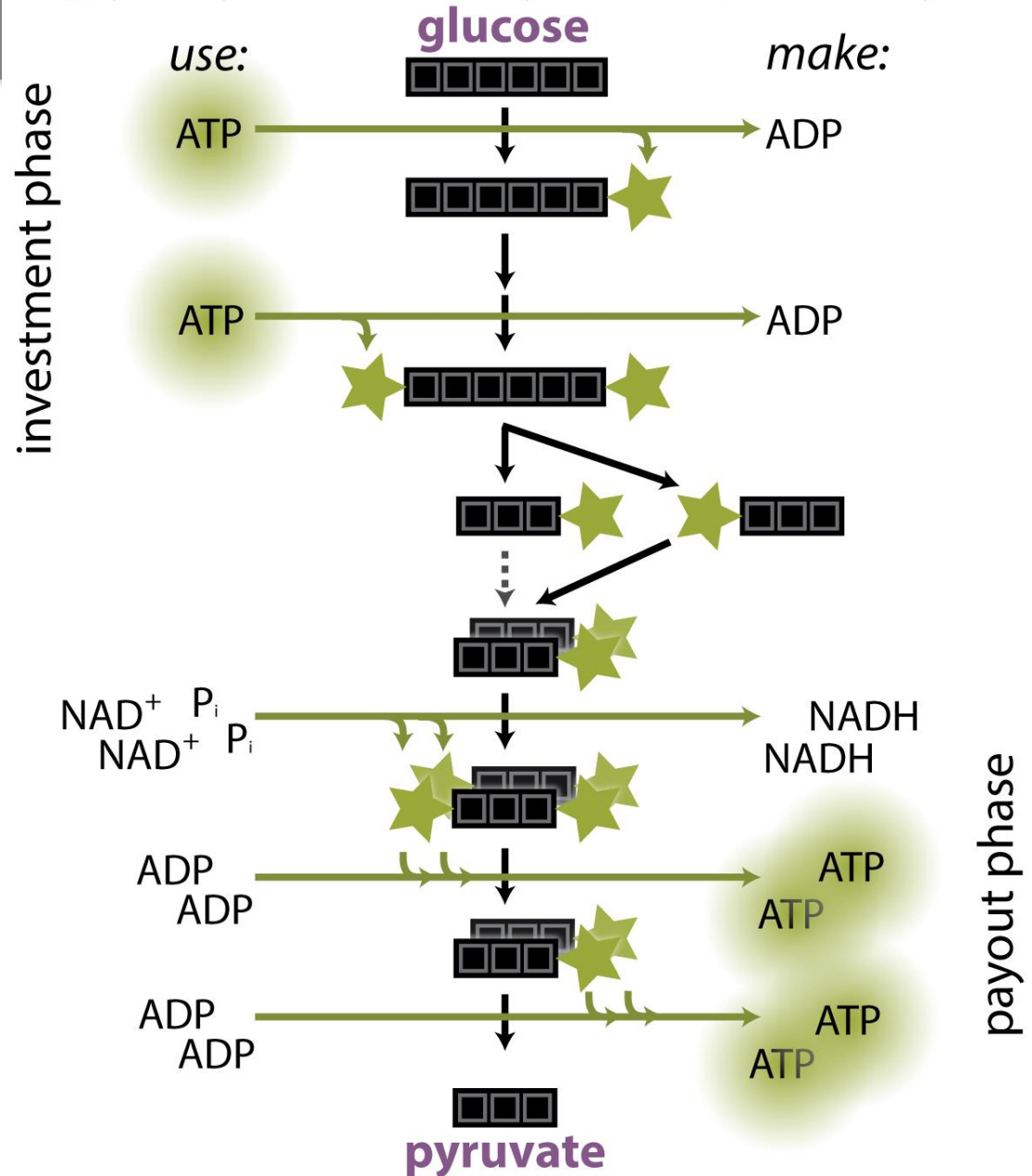
## glycolysis: an enzymatic pathway



# Glycolysis

- It turns out glucose-6-phosphate itself can inhibit the enzyme that makes glucose-6-phosphate.
- Pretty cool.
- That enzyme's called hexokinase, because kinase means puts a phosphate group on.
- And so the product actually inhibits the enzyme and says, if I've got too much product, please don't bother making any more.

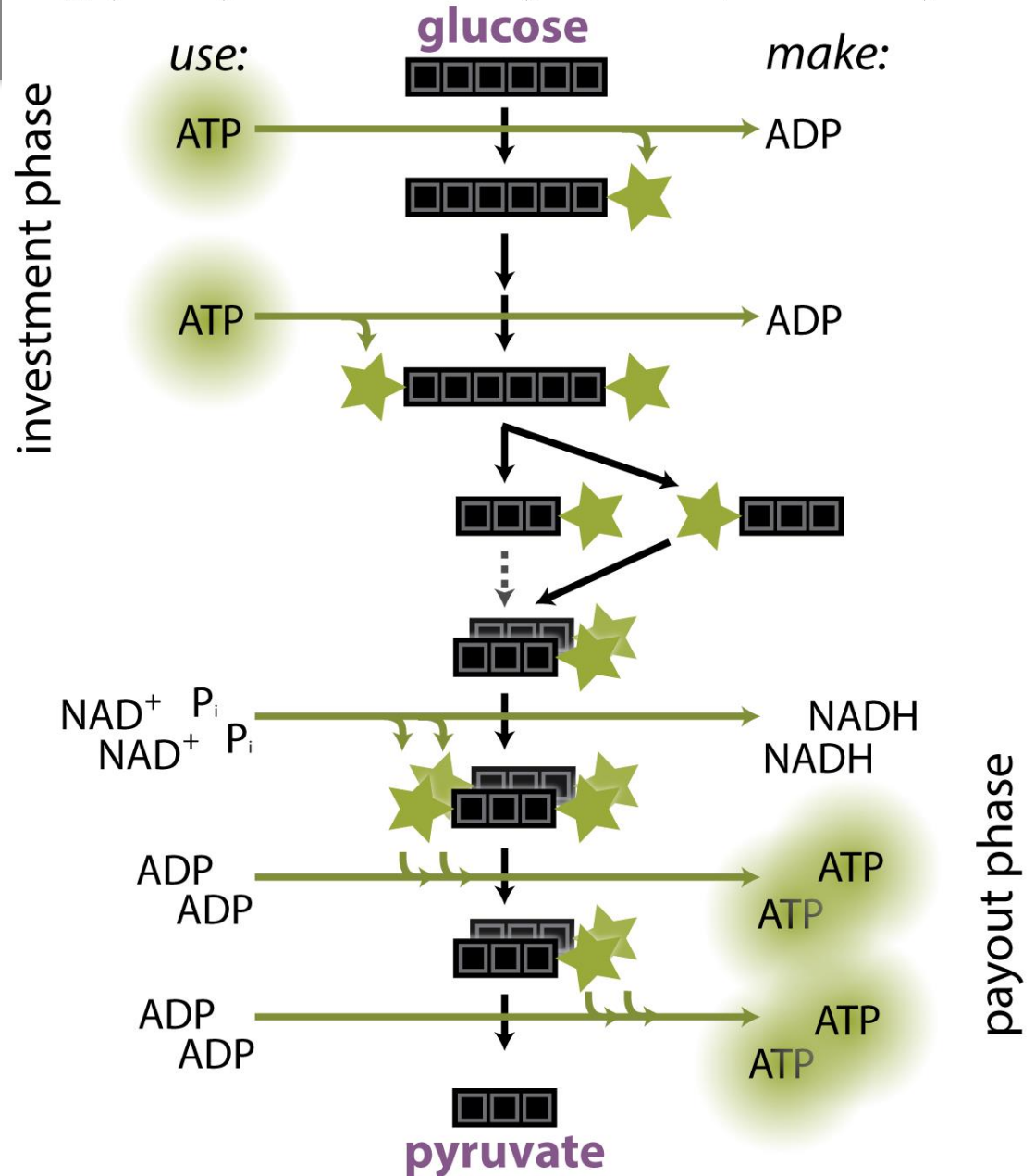
## glycolysis: an enzymatic pathway



# Glycolysis

- Now there are some other things here.
- Some of these other things here can be inhibited by too much ATP.
- I can inhibit them, because there's an ATP binding site.

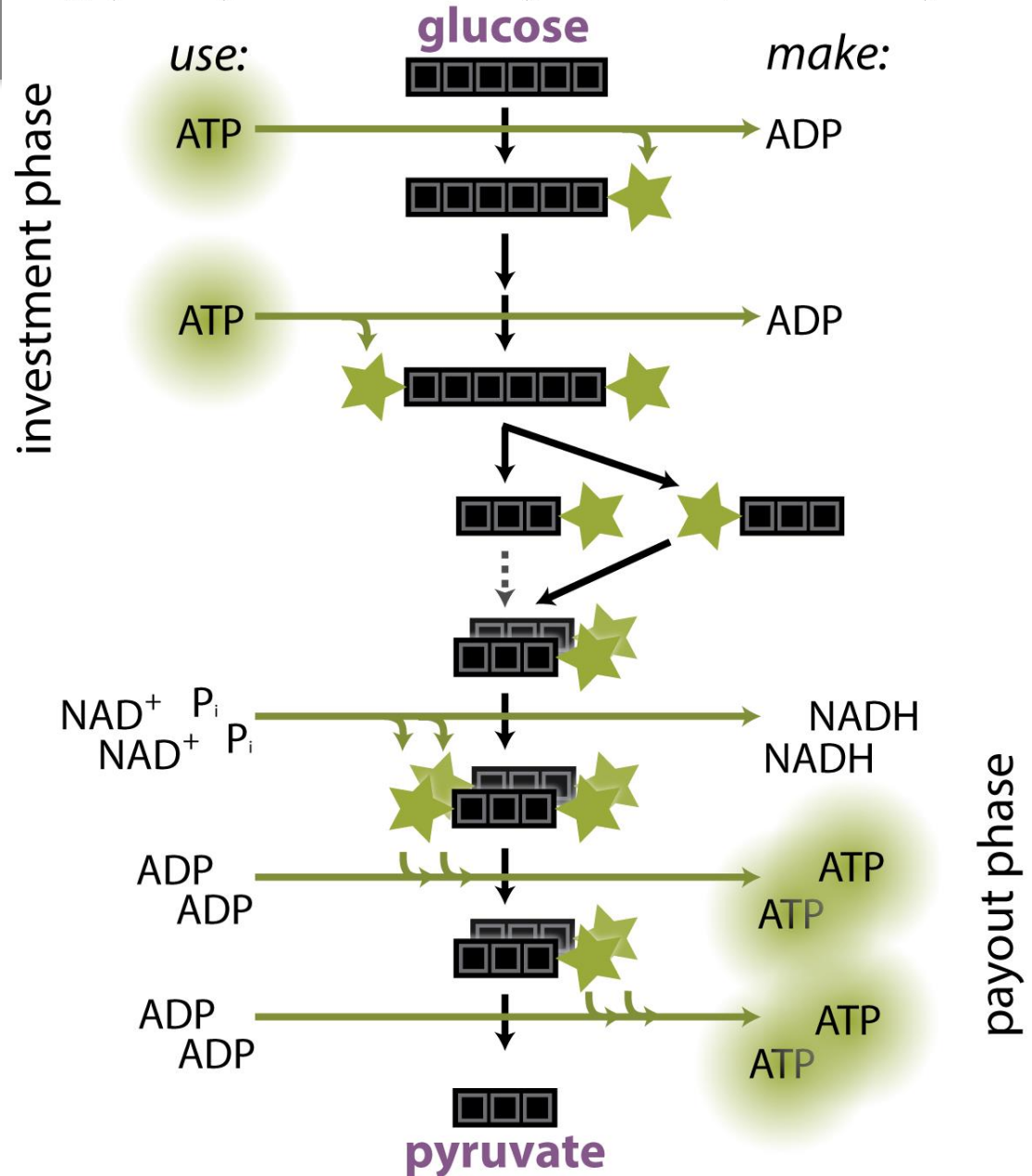
## glycolysis: an enzymatic pathway



# Glycolysis

- If I've got too little ATP, then there's too much ADP, or actually, often the molecule that has just one phosphate, adenosine monophosphate, and those guys can come back and they can activate.
- And in fact, this is an incredibly important and an expensive pathway.
- I'm spending ATPs and it's highly regulated along the way.
- So that's what happens there.

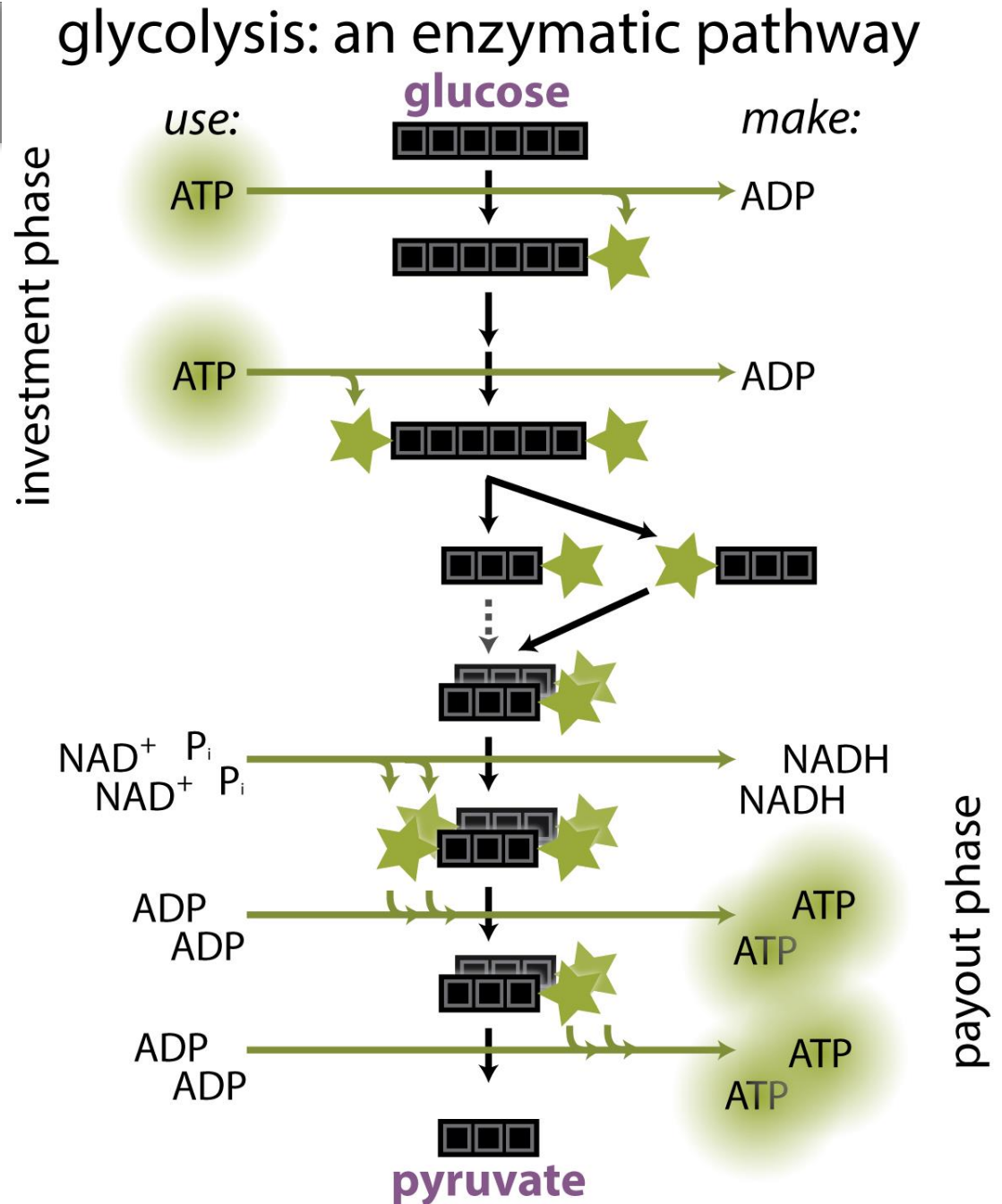
## glycolysis: an enzymatic pathway



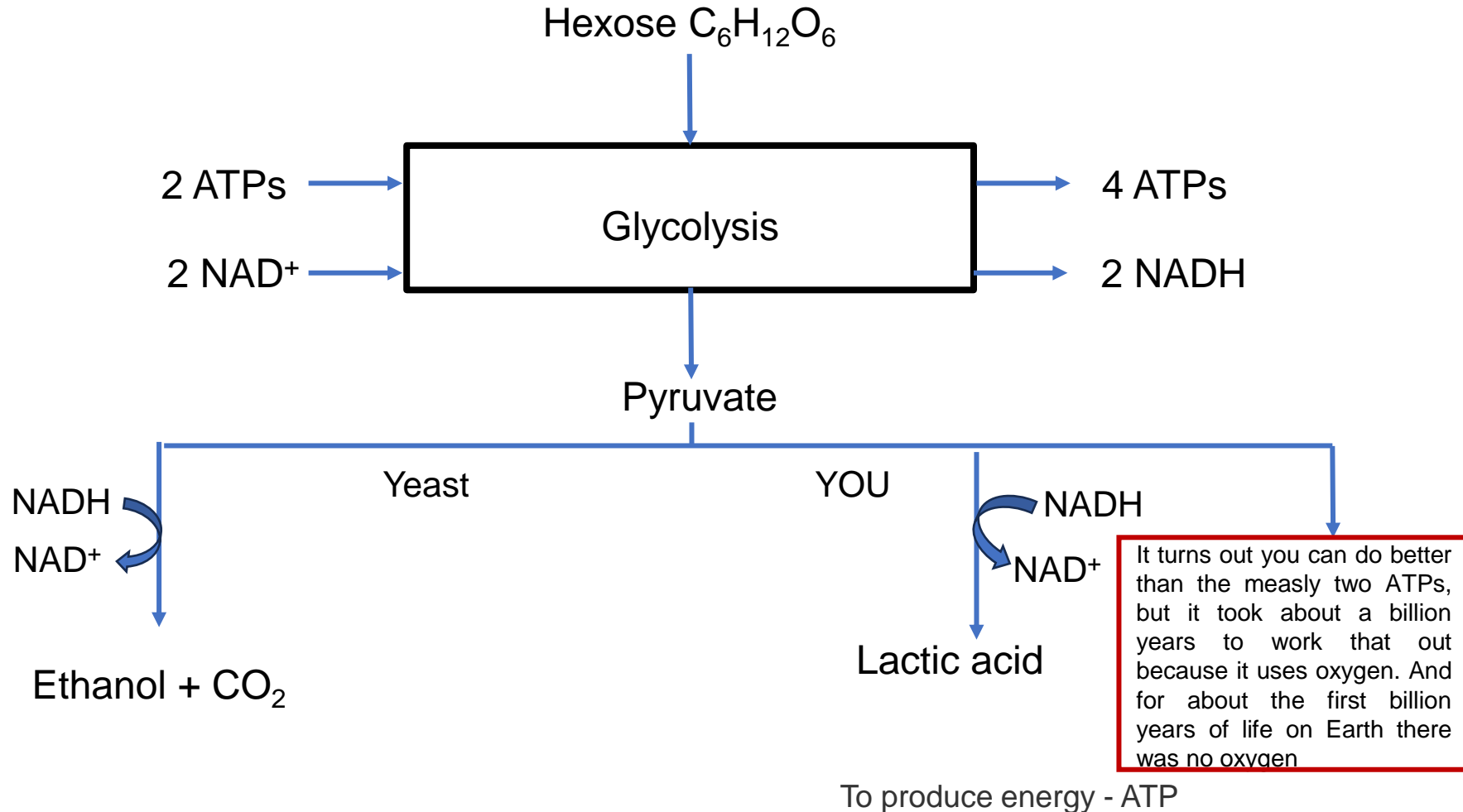
# Glycolysis

- There's actually a lot of a lot of fancy tricks that I won't get into, and not all the regulation for most pathways is even fully understood
- because they're still probably lots of aspects we don't fully know.

Now- Glycolysis is just a very simple pathway.



# I told you there is something else we can do!

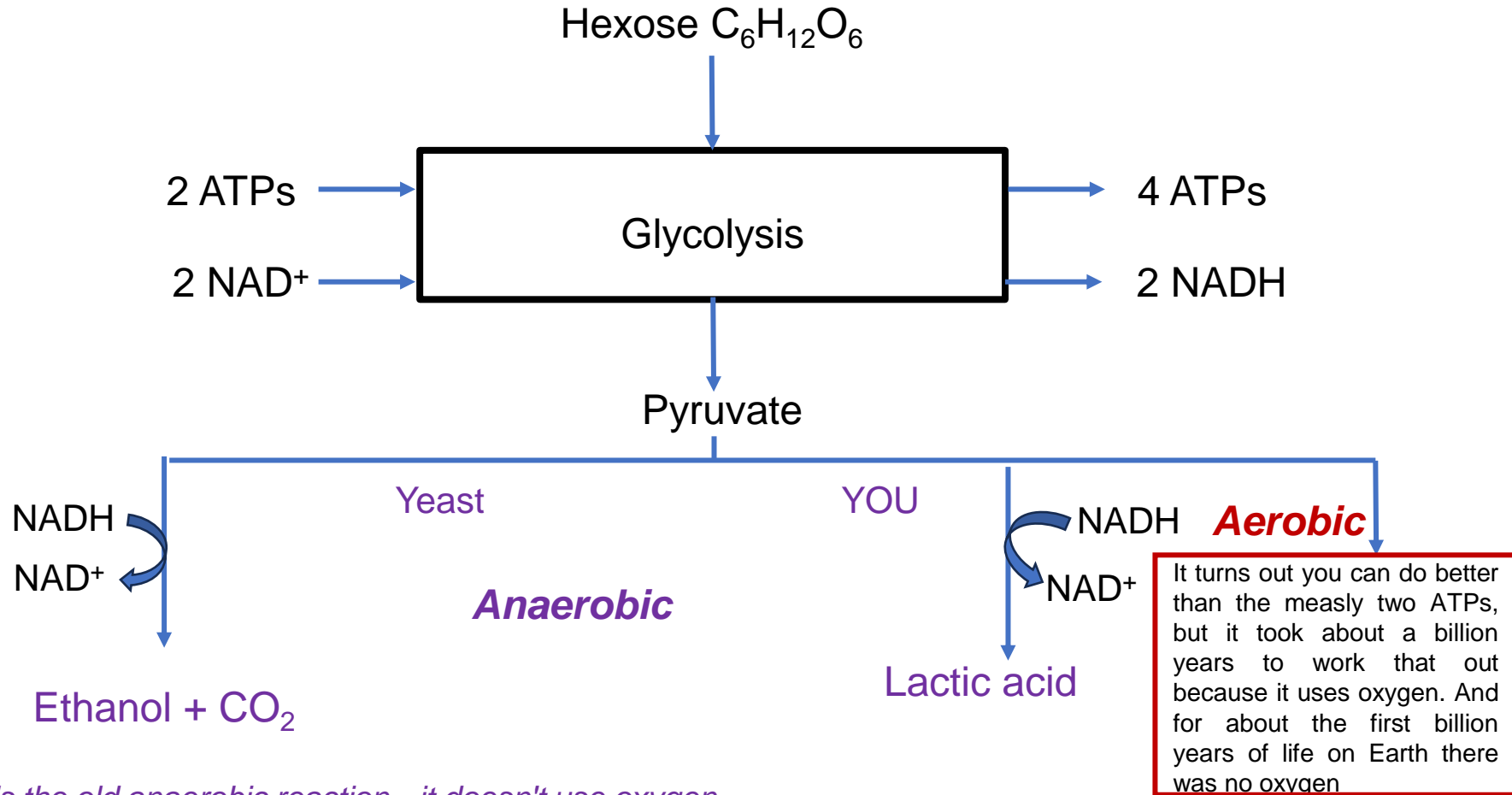


*Now, I told you there was something else you could do here, too.*

*We will talk about this- something else!*

# Cellular respiration and fermentation

# Aerobic and anaerobic process



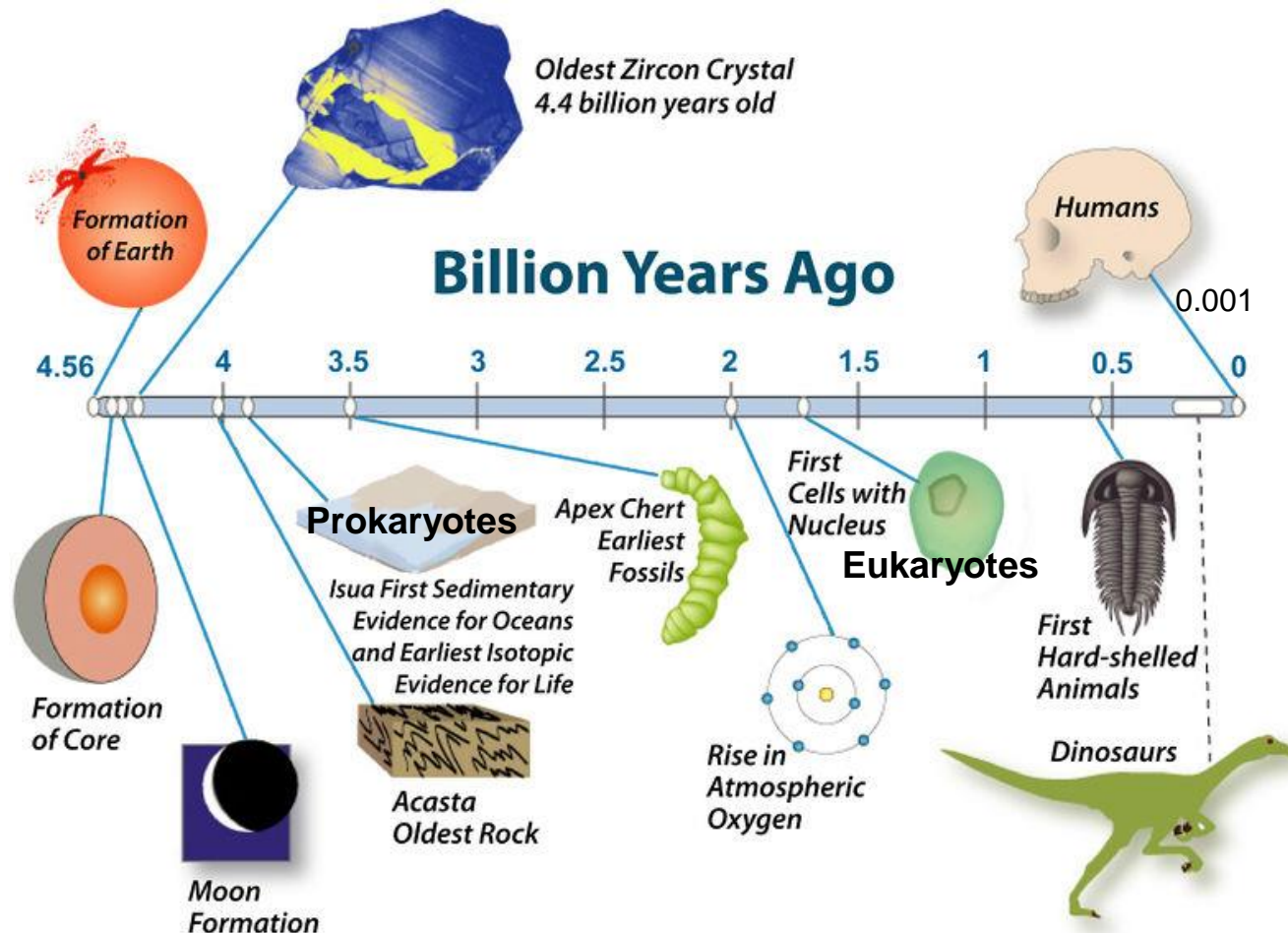
*It's the old anaerobic reaction-- it doesn't use oxygen.*

*The aerobic reaction-- it uses oxygen.*

*It took awhile to develop, as I indicated- The first life had this 3.5 billion years ago. It took another billion years to develop this very complex process here, that goes on mostly in this organelle called the mitochondrion.*

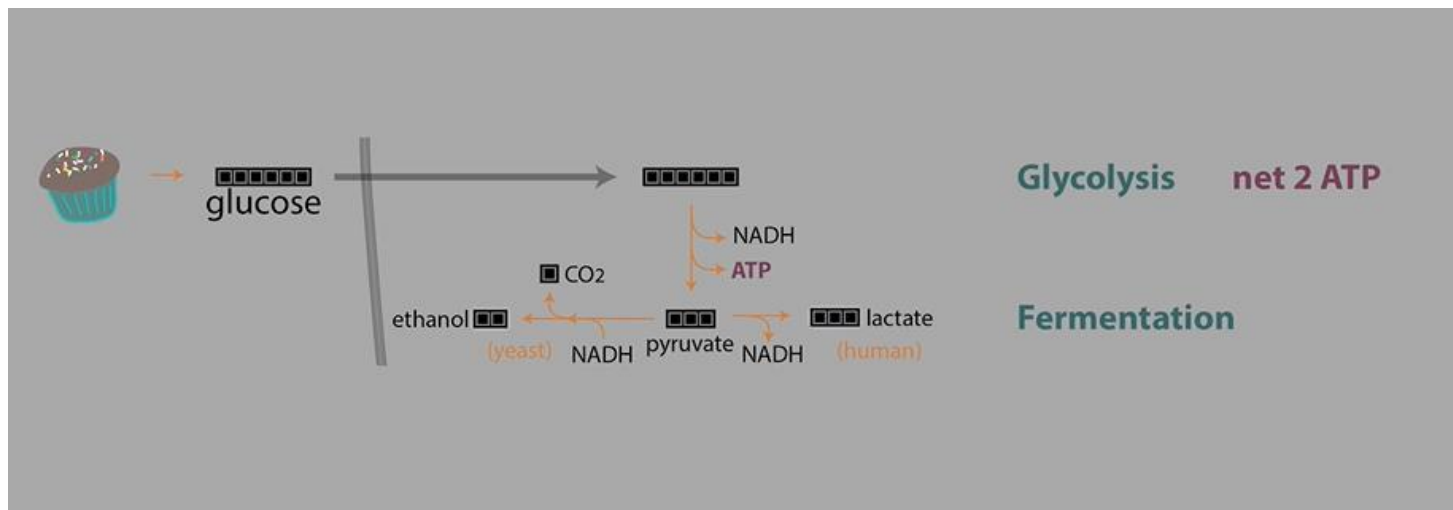


# Evolution of life- some important dates



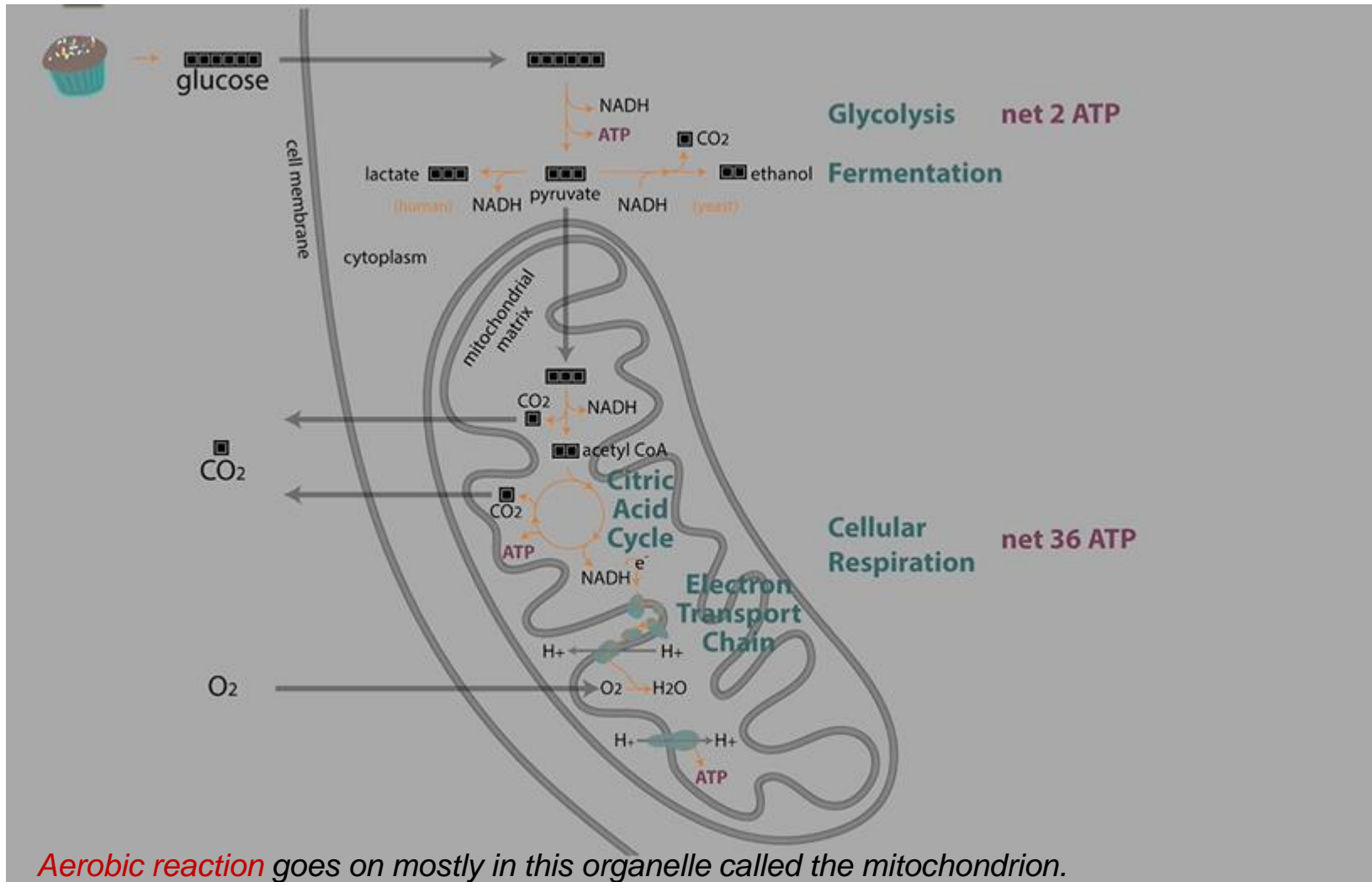
0.00000007 Billion Years Ago- IIT Kharagpur was founded!

# What is happening in our body!



- The cupcake is actually broken down elsewhere
- Glucose comes into the cell here.
- We have glycolysis, we've got our pyruvate.
- I haven't gone into this last step of either in yeast, the pyruvates making alcohol
- in you, it's making lactic acid.

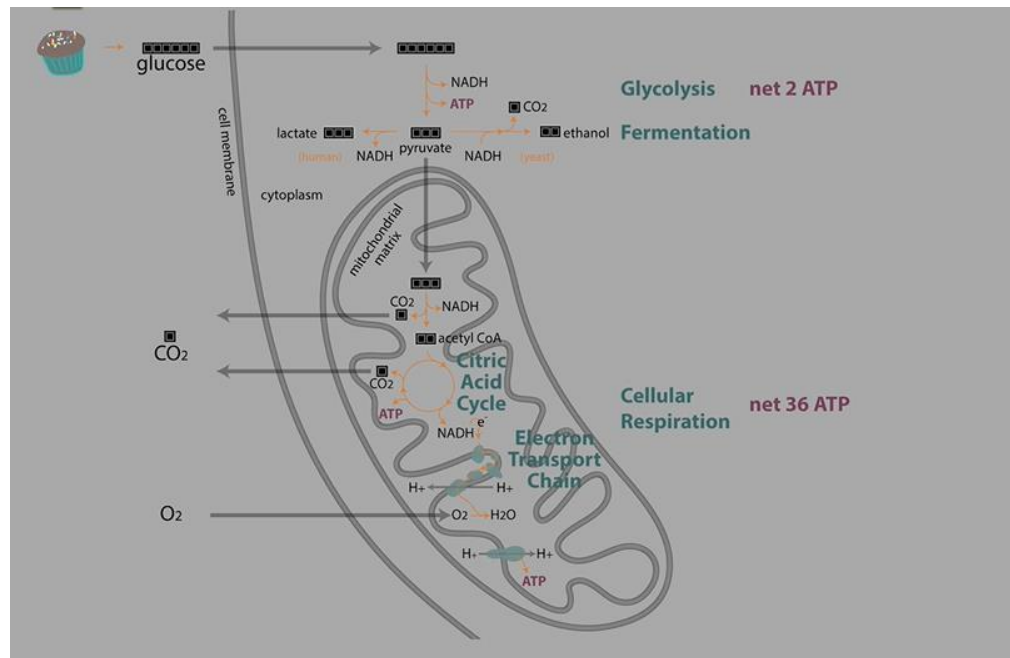
# What is happening in our body!



**Aerobic reaction** goes on mostly in this organelle called the mitochondrion.

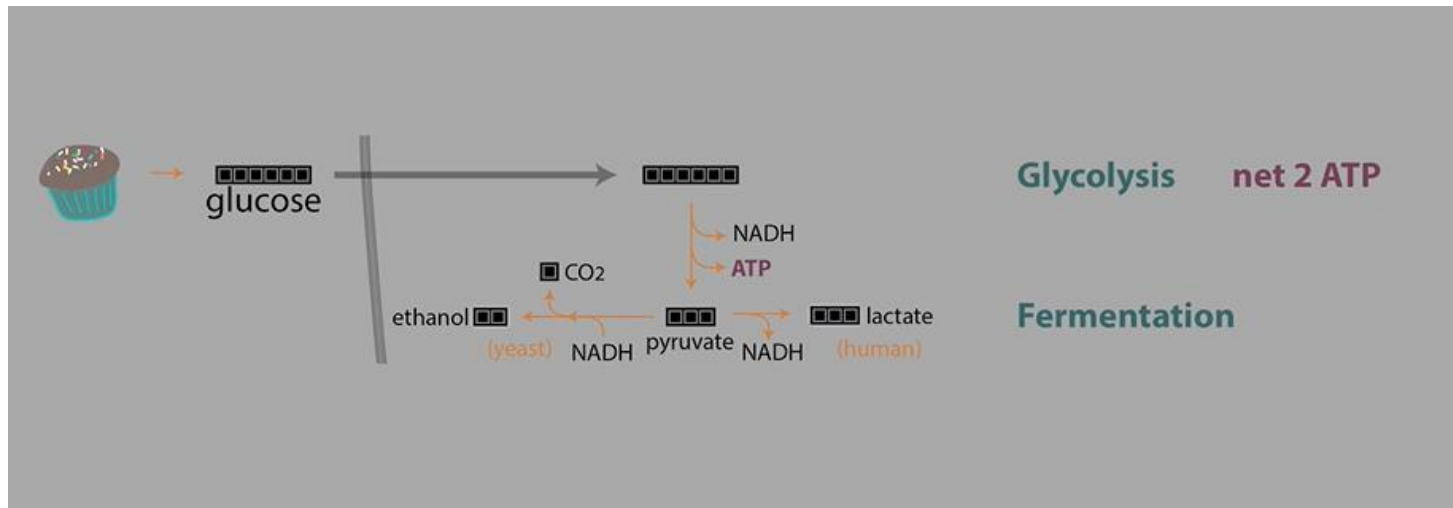
And what you get is instead of measly breaking it down into pyruvate, you break it all the way down into the  $\text{CO}_2$  plus water. **You get 36 ATPs instead of two ATPs.**

# What is happening in our body!



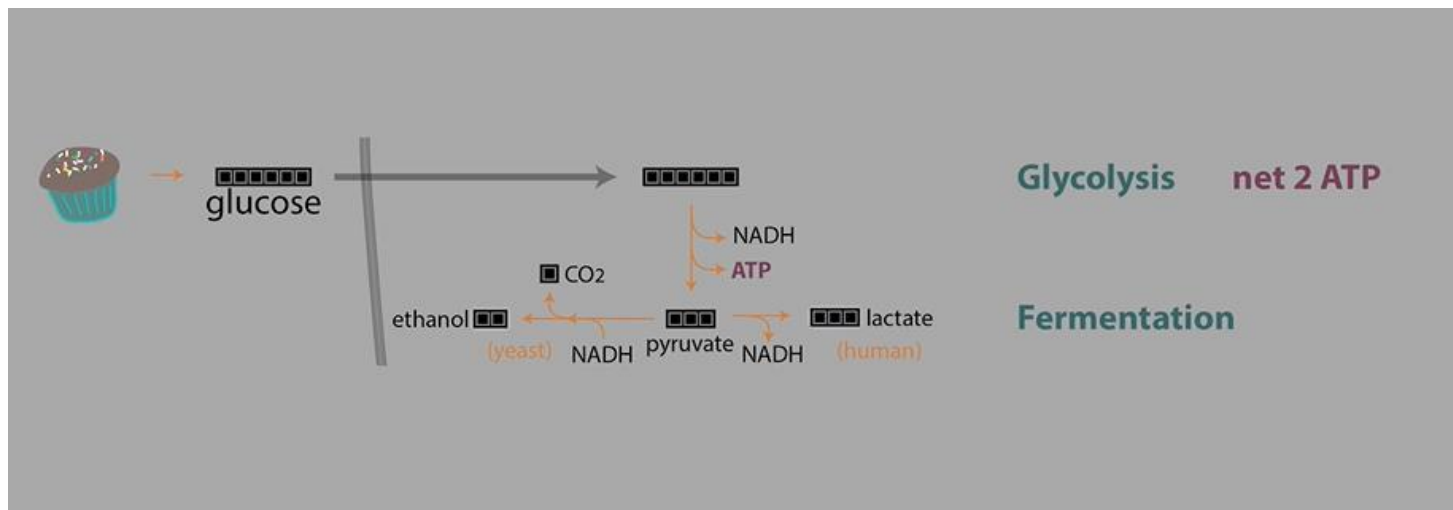
You can imagine that when organisms figured out how to do this, they'd much prefer to take a sugar and break down all those bonds and get 36 ATPs rather than just two ATPs, and so would you.

# So when am I building up lactic acid?



- When do you find that you have a lot of lactic acid?
- When you exercise a lot, in your muscle that's lactic acid building up.
- Why is that happening? – lack of oxygen
- The issue is why this is happening is because you're carrying out glycolysis, which is an anaerobic reaction.
- It's the old anaerobic reaction-- it doesn't use oxygen.

# What is happening in our body!

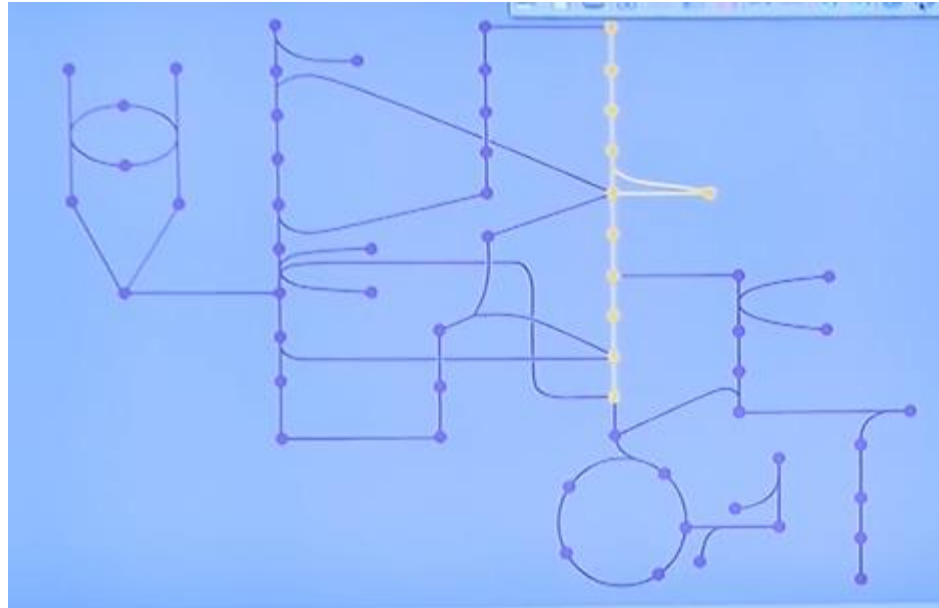


- Why am I doing glycolysis and sending it over to make lactate instead of doing cellular respiration there and using that pathway there?— lack of oxygen
- Because I'm not getting enough oxygen to my muscles,  
so now I'm feeling the burn there that acid is building up!
- When you feel the burn, you should now be thinking about you're using that ancient glycolysis pathway instead of that incredibly efficient cellular respiration pathway.

Glycolysis is not the only pathway!

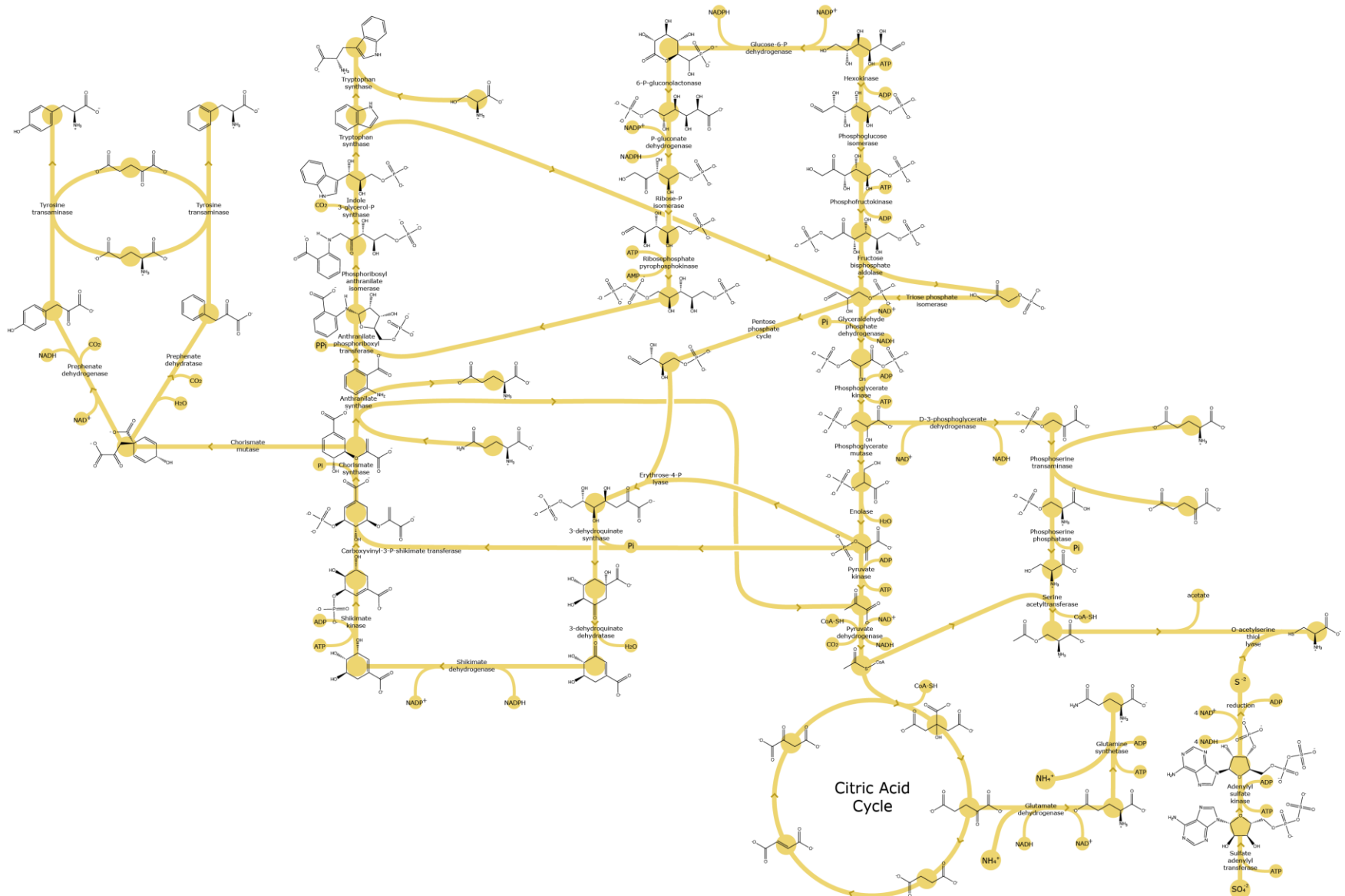
It is a part of the bigger picture!

# Glycolysis is just one pathway!

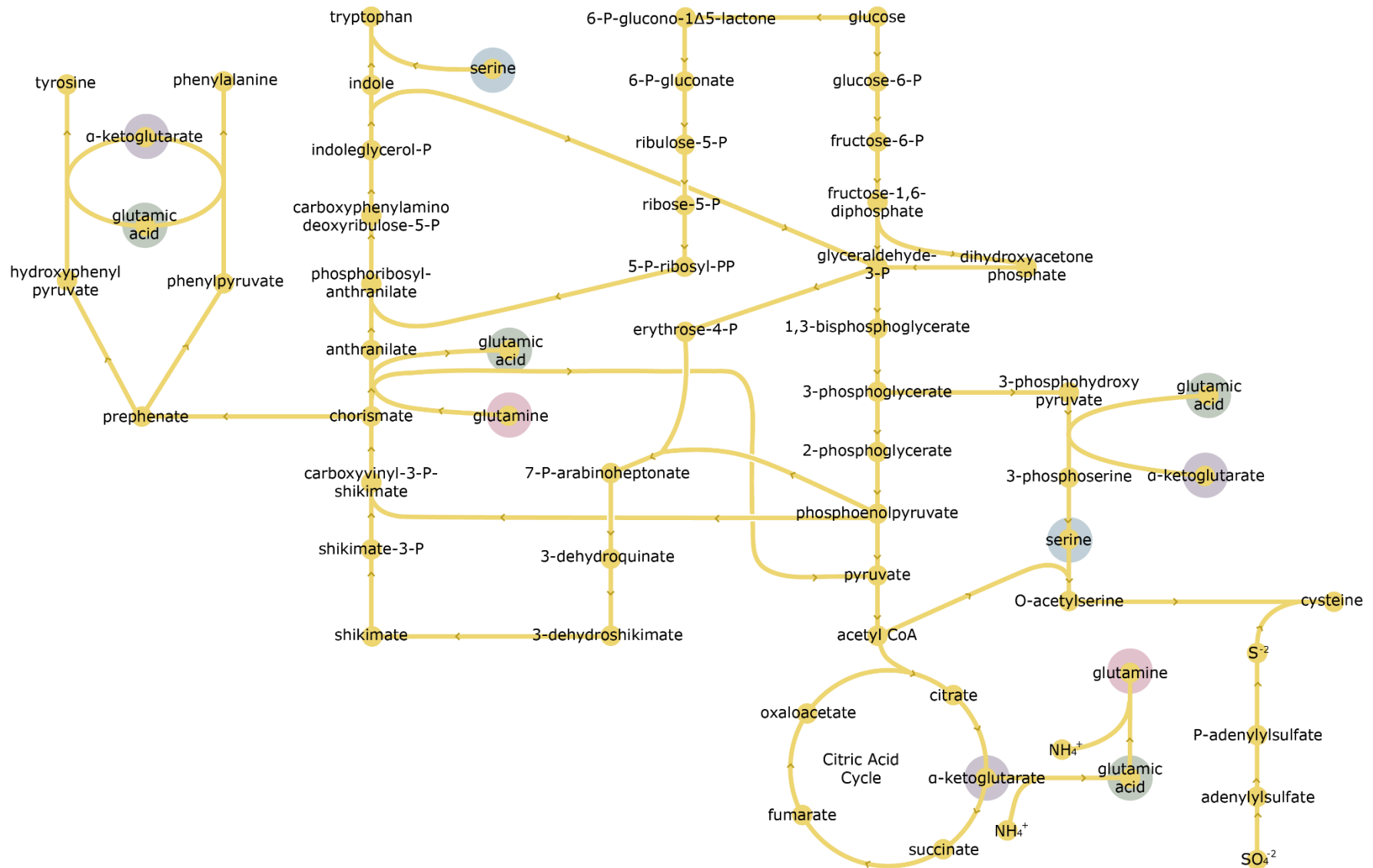




# Glycolysis is just one pathway!



# Glycolysis is just one pathway!

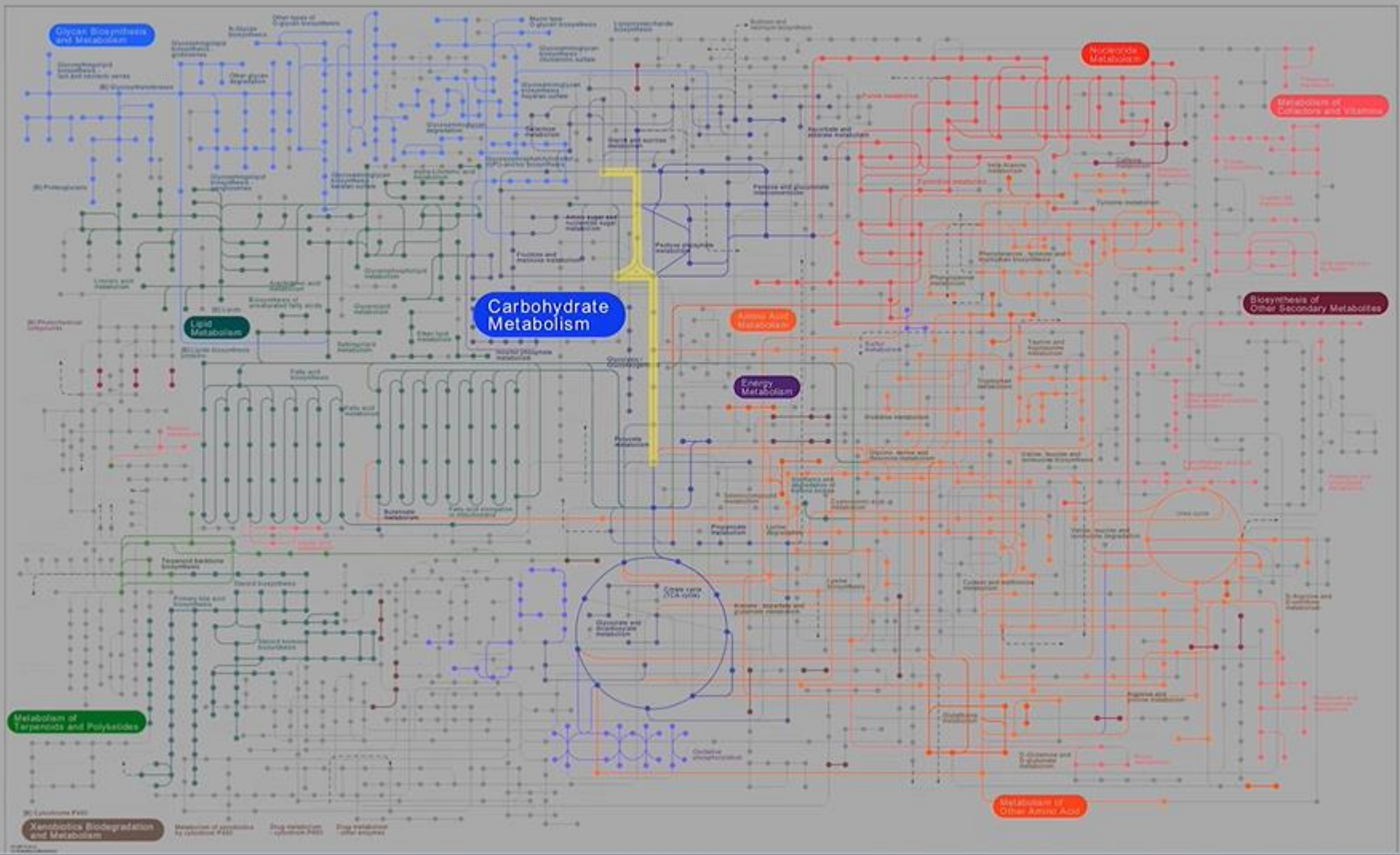


# What is happening in our body!

- You make these amino acids.
- So we have the breakdown of sugar-- that's one of the things pathways do, it breaks down sugar to make energy stored in ATP.
- But you can also take these pathways and use it to create molecules, to do the biosynthesis of molecules like, for example, amino acids, which are then going to go on to make your proteins.
- So you're going to break things down into small molecular entities and you're going to build them back up, and these pathways are involved in both breaking down and building up products.
- Do you think this is the complete picture?

Here is a slightly more complete picture!

This is slightly a complete picture!



*Definitely complicated than a Delhi metro map!*

# It starts getting complicated!

- As you can imagine, it starts getting complicated.
- Trying to understand exactly who's being sucked through where and when,
- what's being regulated back under what circumstances and
- which products are feedforwarding and which products are feedbacking
- and how the concentrations all depend under which circumstances,

is really interesting.

It's really, really interesting to be able to work all of that out.

