

### How do cells generate ATP?

A variety of mechanisms have emerged over the 3.25 billion years of evolution to create ATP from ADP and AMP. The majority of these mechanisms are modifications on two themes: direct synthesis of ATP or indirect synthesis of ATP with two basic mechanisms known respectively as **substrate level phosphorylation (SLP)** and **oxidative phosphorylation**. Both mechanisms rely on biochemical reactions that transfer energy from some energy source to ADP or AMP to synthesize ATP. These topics are substantive, so they will be discussed in detail in the next few modules.

### Glycolysis: An overview

Organisms, whether unicellular or multicellular, need to find ways of getting at least two key things from their environment: (1) matter or raw materials for maintaining a cell and building new cells and (2) energy to help with the work of staying alive and reproducing. Energy and the raw materials may come from different places. For instance, organisms that primarily harvest energy from sunlight will get raw materials for building biomolecules from sources like CO<sub>2</sub>. By contrast, some organisms rely on red/ox reactions with small molecules and/or reduced metals for energy and get their raw materials for building biomolecules from compounds unconnected to the energy source. Meanwhile, some organisms (including ourselves), have evolved to get energy AND the raw materials for building and cellular maintenance from sometimes associated sources.

Glycolysis is the first **metabolic pathway** discussed in BIS2A; a metabolic pathway is a series of linked biochemical reactions. Because of its **ubiquity** in biology, we hypothesize that glycolysis was probably one of the earliest metabolic pathways to evolve (more on this later). Glycolysis is a ten-step metabolic pathway that is centered on the processing of glucose for both energy extraction from chemical fuel and for the processing of the carbons in glucose into various other biomolecules (some of which are key precursors of many much more complicated biomolecules). We will therefore

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examine our study of glycolysis using the precepts outlined in the energy challenge rubric that ask us to formally consider what happens to BOTH matter and energy in this multi-step process.

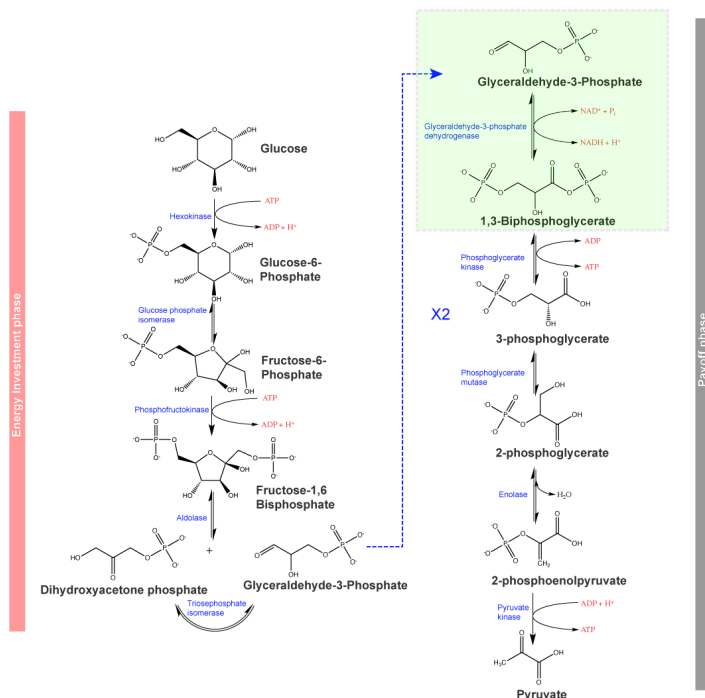
### The energy story and design challenge of glycolysis

Our investigation of glycolysis is a good opportunity to examine a biological process using both the energy story and the design challenge rubrics and perspectives.

The design challenge rubric will try to get you to think actively, and broadly and specifically, about why we are studying this pathway—what is so important about it? What "problems" does the evolution of a glycolytic pathway allow life to solve or overcome? We will also want to think about alternate ways to solve the same problems and why they may or may not have evolved. Later, we will examine a hypothesis for how this pathway—and other linked pathways—may have evolved, and thinking about alternative strategies for satisfying various constraints will come in handy then.

We ask you to think about glycolysis through the lens of an energy story in which you examine the 10-step process as a set of matter and energy inputs and outputs, a process with a beginning and an end. By taking this approach you will learn not only about glycolysis, but also some skills required to read and interpret other biochemical pathways.

So what is glycolysis? Let's find out.



**Figure 1.** The ten biochemical reactions of glycolysis are shown. Enzymes are labeled in blue. The structure of each sugar-derived compound is depicted as a molecular model; other reactants and

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products may be abbreviated (e.g., ATP, NAD<sup>+</sup>, etc.). The box surrounding the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase indicates that this reaction is of special interest in the course. Attribution: Marc T. Facciotti (original work)

**Table 1.** This table shows glycolytic enzymes and measurements of the energy at standard state ( $\Delta G^{\circ}/(\text{kJ/mol})$ ) compared with measurements taken from a living cell ( $\Delta G/(\text{kJ/mol})$ ). Under conditions of constant temperature and pressure, ( $\Delta G^{\circ}/(\text{kJ/mol})$ ), reactions will occur in the direction that leads to a decrease in the value of the Gibbs free energy. Cellular measurements of  $\Delta G$  can be dramatically different from  $\Delta G^{\circ}$  measurements because of cellular conditions, such as concentrations of relevant metabolites, etc. There are three large, negative  $\Delta G$  drops in the cell in the process of glycolysis. We consider these reactions irreversible and are often subject to regulation

Enzyme	Step	$\Delta G/(\text{kJ/mol})$	$\Delta G^{\circ}/(\text{kJ/mol})$
Hexokinase	1	-34	-16.7
Phosphoglucose isomerase	2	-2.9	1.67
Phosphofructokinase	3	-19	-14.2
Fructose-bisphosphate aldolase	4	-0.23	23.9
Triose phosphate isomerase	5	2.4	7.56
Glyceraldehyde 3-phosphate dehydrogenase	6	-1.29	6.30
Phosphoglycerate kinase	7	0.09	-18.9
Phosphoglycerate mutase	8	0.83	4.4
Enolase	9	1.1	1.8
Pyruvate kinase	10	-23.0	-31.7

Overall, the glycolytic pathway comprises 10 enzyme-catalyzed steps. The primary input into this pathway is a single molecule of glucose, though we discover that other molecules may enter this pathway at various steps. We will focus our attention on (1) consequences of the overall process, (2) several key reactions that

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highlight important types of biochemistry and biochemical principles we will want to carry forward to other contexts, and (3) alternative fates of the intermediates and products of this pathway.

Note for reference that glycolysis is an **anaerobic** process. There is no requirement for molecular oxygen in glycolysis - oxygen gas is not a reactant in any of the chemical reactions in glycolysis. Glycolysis occurs in the **cytosol or cytoplasm** of cells. For a short (three-minute) overview YouTube video of glycolysis, click [here](#).

### First half of glycolysis: energy investment phase

We typically refer the first few steps of glycolysis as an "energy investment phase" of the pathway. This, however, doesn't make much intuitive sense (in the framework of a design challenge; it's not clear what problem this energy investment solves) if one only looks at glycolysis as an "energy-producing" pathway and until these steps of glycolysis are put into a broader metabolic context. We'll try to build that story as we go, so for now just recall that we mentioned that some first steps are often associated with energy investment and ideas like "trapping" and "commitment" that are noted in the figure below.

### Step 1 of glycolysis:

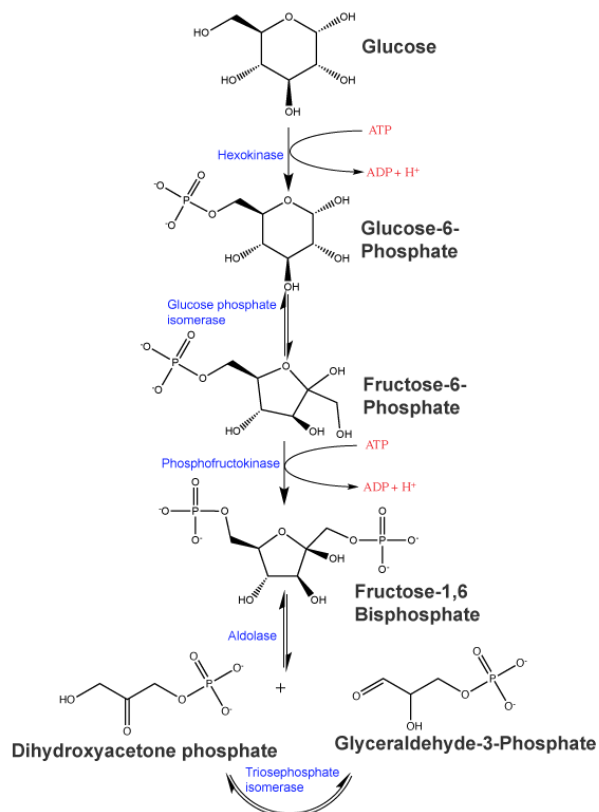
The first step in glycolysis, shown below in Figure 2, is glucose being catalyzed by hexokinase, an enzyme with broad specificity that catalyzes the phosphorylation of six-carbon sugars. Hexokinase catalyzes the phosphorylation of glucose, where glucose and ATP are substrates for the reaction, producing a molecule called glucose 6-phosphate and ADP as products.

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**Figure 2.** The first half of glycolysis is called the energy investment phase. In this phase, the cell spends two ATPs into the reactions. Attribution: Marc T. Facciotti (original work)

#### Note:

The paragraph above states that the enzyme hexokinase has "broad specificity." This means that it can catalyze reactions with different sugars, not just glucose. From a molecular perspective, can you explain why this might be the case? Does this challenge your conception of enzyme specificity? If you Google the term "enzyme promiscuity" (don't worry; it's safe for work), you will hopefully gain a broader appreciation for enzyme selectivity and activity.

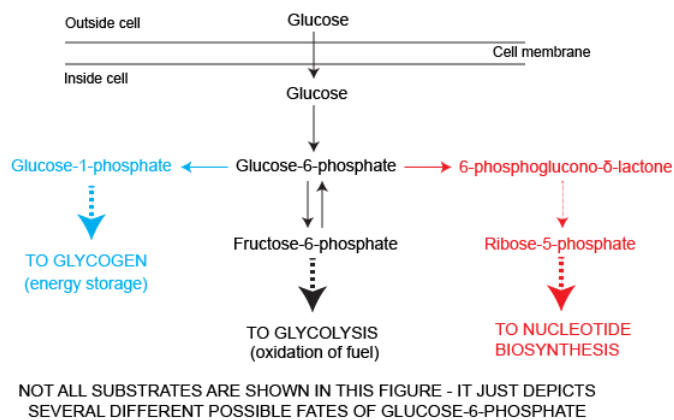
The conversion of glucose to the negatively charged glucose 6-phosphate significantly reduces the likelihood that the phosphorylated glucose leaves the cell by diffusion across the hydrophobic interior of the plasma membrane. It also "marks" the glucose in a way that tags it for several possible fates (see Figure 3).

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**Figure 3.** Note that this figure shows that glucose 6-phosphate can, depending on cellular conditions, be directed to multiple fates. While it is a component of the glycolytic pathway, it is not only involved in glycolysis but also in the storage of energy as glycogen (colored in cyan) and in the building of various other molecules like nucleotides (colored in red). Source: Marc T. Facciotti (original work)

As Figure 3 shows, glycolysis is but one fate for glucose 6-phosphate (G6P). Depending on cellular conditions, G6P may be diverted to the biosynthesis of glycogen (for energy storage), or it may be diverted into the pentose phosphate pathway for the biosynthesis of various biomolecules, including nucleotides. This means that G6P, while involved in the glycolytic pathway, is not solely tagged for oxidation at this phase. Perhaps showing the broader context that this molecule is involved in (in addition to the rationale that tagging glucose with a phosphate decreases the likelihood that it will leave the cell) helps to explain the seemingly contradictory (if you only consider glycolysis as an "energy-producing" process) reason for transferring energy from ATP onto glucose if it is only to be oxidized later—that is, glucose is not only used by the cell for harvesting energy and several other metabolic pathways depend on the transfer of the phosphate group.

### Step 2 of glycolysis:

In the second step of glycolysis, an **isomerase** catalyzes the conversion of glucose 6-phosphate into one of its isomers, fructose 6-phosphate. An **isomerase** is an enzyme that catalyzes the conversion of a molecule into one of its isomers.

### Step 3 of glycolysis:

The third step of glycolysis is the phosphorylation of fructose 6-phosphate, catalyzed by the enzyme phosphofructokinase. A second ATP molecule donates a phosphate to fructose 6-phosphate, producing fructose 1,6-bisphosphate and ADP as products. In this pathway, phosphofructokinase is a rate-limiting enzyme, and its activity is tightly regulated. It is **allosterically** activated by AMP when the concentration of AMP is high and when it is moderately allosterically inhibited by ATP at the same site. Citrate, a compound we'll discuss soon, also acts as a negative **allosteric** regulator of this

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enzyme. In this way, phosphofructokinase monitors or senses molecular indicators of the energy status of the cells and can in response act as a switch that turns on or off the flow of the substrate through the rest of the metabolic pathway depending on whether there is “sufficient” ATP in the system. The conversion of fructose 6-phosphate into fructose 1,6-bisphosphate is sometimes referred to as a commitment step by the cell to the oxidation of the molecule in the rest of the glycolytic pathway by creating a substrate for and helping to energetically drive the next highly endergonic (under standard conditions) step of the pathway.

#### Step 4 of glycolysis:

In the fourth step in glycolysis, an enzyme, fructose-bisphosphate aldolase, cleaves 1,6-bisphosphate into two three-carbon isomers: dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.

#### Second half: energy payoff phase

If viewed in the absence of other metabolic pathways, glycolysis has so far cost the cell two ATP molecules and produced two small, three-carbon sugar molecules: dihydroxyacetone phosphate (DAP) and glyceraldehyde 3-phosphate (G3P). When viewed in a broader context, this investment of energy to produce a variety of molecules that can be used in a variety of other pathways doesn't seem like such a bad investment.

Both DAP and G3P can proceed through the second half of glycolysis. We now examine these reactions.

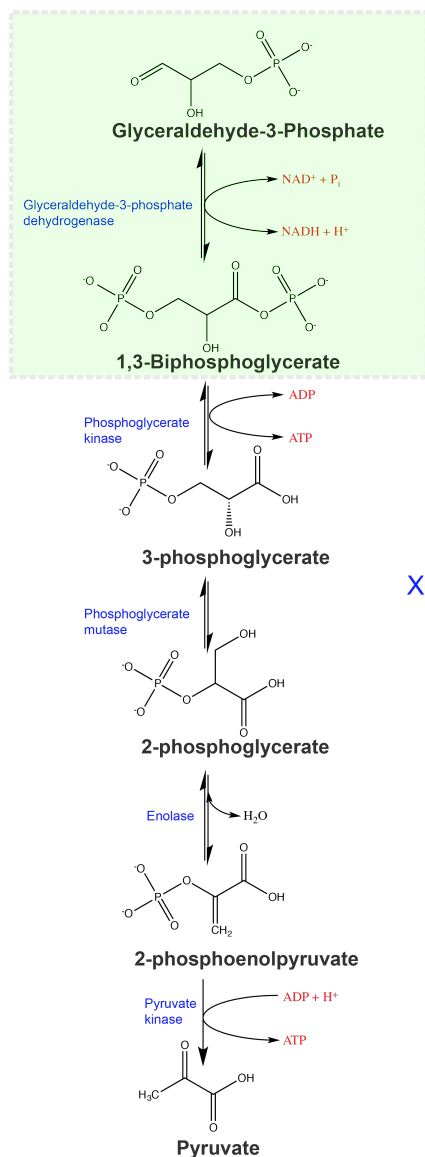
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**Figure 4.** The second half of glycolysis is called the energy payoff phase. In this phase, the cell gains two ATP and two NADH compounds. At the end of this phase, glucose has become partially oxidized to form pyruvate. Attribution: Marc T. Facciotti (original work).

#### Step 5 of glycolysis:

In the fifth step of glycolysis, an isomerase transforms the dihydroxyacetone phosphate into its isomer, glyceraldehyde 3-phosphate. The six-carbon glucose has therefore now been converted into two phosphorylated three-carbon molecules of G3P.

#### Step 6 of glycolysis:

The sixth step is key and one from which we can now leverage our understanding of the several chemical reactions that we've studied so far. If you're energy focused, this is finally a step of glycolysis where some reduced sugar becomes oxidized. The reaction is catalyzed by the enzyme glyceraldehyde 3-phosphate

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dehydrogenase. This enzyme catalyzes a multi-step reaction between three substrates—glyceraldehyde 3-phosphate, the cofactor  $\text{NAD}^+$ , and inorganic phosphate ( $\text{P}_i$ )—and produces three products: 1,3-bisphosphoglycerate,  $\text{NADH}$ , and  $\text{H}^+$ . One can think of this reaction as two reactions: (1) an oxidation/reduction reaction and (2) a condensation reaction in which an inorganic phosphate is transferred onto a molecule. Here, the red/ox reaction, a transfer of electrons off G3P and onto  $\text{NAD}^+$ , is exergonic, and the phosphate transfer is endergonic. The net standard free energy change hovers around zero—more on this later. The enzyme here acts as a molecular **coupling** agent to couple the energetics of the exergonic reaction to that of the endergonic reaction, thus driving both forward. This processes happens through a multi-step mechanism in the enzyme's active site and involves the chemical activity of a variety of functional groups.

It is important to note that this reaction depends upon the availability of the oxidized form of the electron carrier,  $\text{NAD}^+$ . If we consider that there is a limiting pool of  $\text{NAD}^+$ , we can then conclude that the reduced form of the carrier ( $\text{NADH}$ ) must continuously oxidize back into  $\text{NAD}^+$  to keep this step going. If  $\text{NAD}^+$  is not available, the second half of glycolysis slows down or stops.

#### Possible NB Discussion Point

Can you write an energy story for Step 6 of glycolysis (the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase)? When you discuss energy, just describe whether steps are exergonic or endergonic. As a group, try to build up an increasingly "expert" version that is complete, brief, and uses appropriate vocabulary. Amend one another's texts in a polite and constructive way.



#### Step 7 of glycolysis:

In the seventh step of glycolysis, catalyzed by phosphoglycerate kinase (an enzyme named for the reverse reaction), 1,3-bisphosphoglycerate transfers a phosphate to ADP, forming one molecule of ATP and a molecule of 3-phosphoglycerate. This reaction is exergonic and is also an example of substrate-level phosphorylation.

#### Step 8 of glycolysis:

In the eighth step, the remaining phosphate group in 3-phosphoglycerate moves from the third carbon to the second carbon, producing 2-phosphoglycerate (an isomer of 3-phosphoglycerate). The enzyme catalyzing this step is a mutase (isomerase).

#### Step 9 of glycolysis:

Enolase catalyzes the ninth step. This enzyme causes 2-phosphoglycerate to lose water from its structure; this is a dehydration reaction, resulting in the formation of a double bond

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that increases the potential energy in the remaining phosphate bond and produces phosphoenolpyruvate (PEP).

#### Step 10 of glycolysis:

The last step in glycolysis is catalyzed by the enzyme pyruvate kinase (the enzyme in this case is named for the reverse reaction of pyruvate's conversion into PEP) and results in the production of a second ATP molecule by substrate-level phosphorylation and the compound pyruvic acid (or its salt form, pyruvate). Many enzymes in enzymatic pathways are named for the reverse reactions, since the enzyme can catalyze both forward and reverse reactions (these may have been described initially by the reverse reaction that takes place in vitro, under non-physiological conditions).

#### Outcomes of glycolysis

Here are a couple of things to consider:

One of the clear outcomes of glycolysis is the biosynthesis of compounds that can enter into a variety of metabolic pathways. Likewise, compounds coming from other metabolic pathways can feed into glycolysis at various points. So, this pathway can be part of a central exchange for carbon flux within the cell.

If glycolysis runs long enough, the constant oxidation of glucose with  $\text{NAD}^+$  can leave the cell with a problem: how to regenerate  $\text{NAD}^+$  from the two molecules of NADH produced. If the cell does not regenerate  $\text{NAD}^+$ , nearly all the cell's  $\text{NAD}^+$  will transform into NADH. So how do cells regenerate  $\text{NAD}^+$ ?

Pyruvate is not completely oxidized; There is still some energy to extract. How might this happen? Also, what should the cell do with all of that NADH? Is there any energy there to extract?

#### Possible NB Discussion Point

To some, that glycolysis is such a complex, multi-step pathway may seem counter-intuitive: “*Why wouldn't evolution lead to a \*simpler\* way to extract energy from food since energy is an important requirement for life?*” Explain the necessity/advantage of having glucose get broken down in many steps.



#### Substrate-level phosphorylation (SLP)

The simplest route to synthesize ATP is substrate-level phosphorylation. ATP molecules are generated (that is, regenerated from ADP) because of a chemical reaction that occurs in catabolic pathways. A phosphate group is removed from an intermediate reactant in the pathway, and the free energy of the reaction is used to add the third phosphate to an available ADP molecule, producing ATP. This very direct method of phosphorylation is called *substrate-level phosphorylation (SLP)*. We can find SLP in a variety of catabolic reactions, most notably in two specific reactions in

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