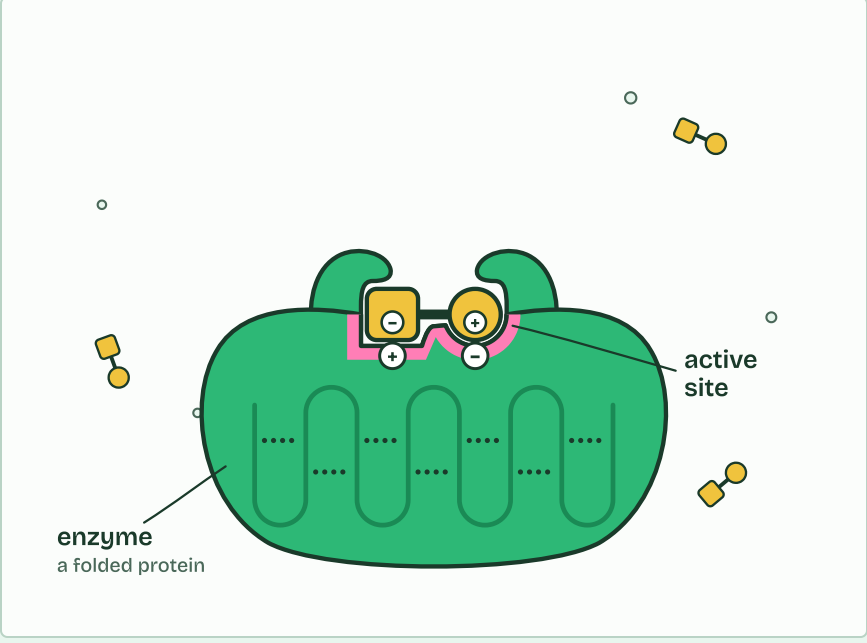
REVISION GUIDE

How *enzymes* run a cell

Enzymes and cellular energy

In a test tube, some of the reactions inside you would take years. In a cell they are over in a thousandth of a second. This is how enzymes do it, what slows them down, and where the energy for life comes from.



8 chapters · a dry lab

The interactive guide, with every animation:

biologybybradford.com/guides/ap-biology-unit-3-enzymes

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By the end you can

- [ ] Explain how the shape and charge of an active site make an enzyme specific to its substrate 3.1.A
- [ ] Explain how an enzyme speeds up a reaction by lowering its activation energy, and why it leaves ΔG unchanged 3.1.A
- [ ] Explain how heat and extreme pH change an enzyme's structure, and when that change can be undone 3.2.A
- [ ] Predict how temperature, pH, substrate and product concentrations and inhibitors change the rate of a reaction 3.2.B
- [ ] Describe why a living cell needs a constant input of energy, and how ATP couples an uphill reaction to a downhill one 3.3.A
- [ ] Explain why energy pathways run in small steps, and what the same pathways in every domain of life tell us 3.3.B

Tick each one when you can do it without the book.

3.1.A ENZYME STRUCTURE

01 The *active site* fits one substrate

An **enzyme** is a biological catalyst: a protein that makes one reaction go faster and is not used up by it. One molecule of catalase, the enzyme in this guide's dry lab, breaks down millions of hydrogen peroxide molecules every second, for as long as its shape lasts.

That shape is everything. An enzyme is a chain of hundreds of amino acids folded into a precise 3D shape, held there by hydrogen bonds and ionic bonds between R groups. A small pocket in the fold, lined by just a few of those amino acids, is the **active site**. The molecule the enzyme works on is its **substrate**.

A substrate binds only if two things match: its **shape** fits the site, and its **charges** are opposite to the charged R groups lining it. That double check is why an enzyme is **specific**. As the substrate settles in, the site shifts to grip it more tightly, which strains the bond that is about to break: **induced fit**.

KEY POINTS

- Enzyme names usually end in *-ase* and name the substrate: lactase splits lactose, lipase splits lipids.
- Shape and charge both have to match, or there is no binding and no reaction.
- The enzyme lets go of the products unchanged and binds the next substrate.

KEY TERMS

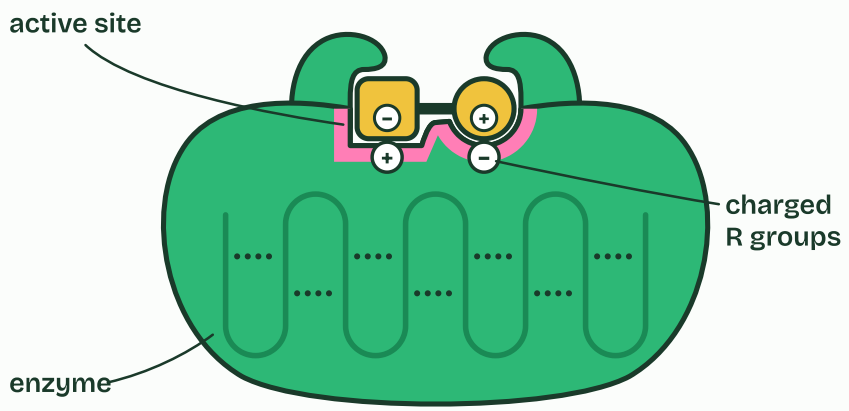
enzyme
A protein that speeds up one chemical reaction in a cell and comes out of it unchanged, ready to work again.

active site
The pocket on an enzyme where the substrate binds and the reaction happens. Its shape and its charges match the substrate.

substrate
The molecule an enzyme acts on. It binds in the active site and is turned into products.

specific
Acting on one substrate, or a few very similar ones, because only they fit the active site.

induced fit
The change in shape of an active site as the substrate binds. The site closes around the substrate and strains its bonds.



Flexible loops Two loops of the chain beside the active site. As the substrate settles in they swing in and close round it, which strains the bond that is about to break: induced fit.

Active site The pocket where the substrate binds and reacts, lined by just a few of the enzyme's amino acids. Its shape, and the charges of the R groups lining it, match one substrate.

Substrate Its square half fits the square end of the site and its round half the round end, and each of its charges sits over an opposite charge. It binds, the loops close round it, and the bond in its middle is strained.

Wrong shape Its charges are right, but its round half is far too wide for the round end of the site. It cannot get close enough for the charges to pull it in.

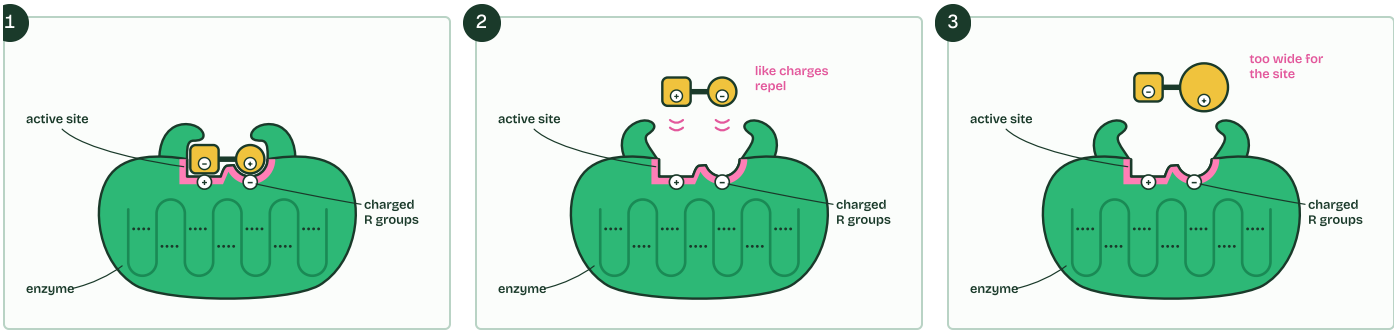
The folded chain An enzyme is a protein: a chain of amino acids folded into one precise 3D shape. Hydrogen bonds and ionic bonds between R groups (the dashed lines) hold the fold, and the fold makes the active site. Break enough of them and the site loses its shape.

Charged R groups Side chains of two amino acids lining the site: one positive, one negative. The substrate carries the opposite charges in the same places, so it is pulled in and held.

A look-alike The same shape as the substrate, but its charges are the other way round. Each one faces a like charge in the site, and like charges repel.

SWITCH IT

3 SETTINGS



Substrate **Look-alike** **Wrong shape**

WATCH OUT

The substrate and the active site are not "the same shape". They are complementary: the site is the mold and the substrate fills it, as a hand fills a glove. And the site is not a rigid lock. It moves as the substrate binds.

EXAMPLE

Lactase splits lactose, which is glucose joined to galactose. Sucrose is glucose joined to fructose: exactly the same atoms as lactose, arranged differently. Lactase leaves it alone: the site grips the galactose half, and sucrose has none.

PREDICT

A molecule has exactly the substrate's shape, but its two charges are the other way round. What happens at the active site?

(A) It binds, because its shape fits the site

(B) It binds, but is broken down more slowly

(C) It is pushed away, because like charges repel

(D) It binds and changes the enzyme's shape for good

Decide before you look. Answer on page 30.

3.1.A ENZYME CATALYSIS

02 Enzymes lower the *activation energy*

Breaking a bond always takes energy in; forming a bond releases energy. So even a reaction that releases energy overall must first be pushed over a hump, while old bonds strain and start to break. That push is the **activation energy**. At body temperature very few collisions carry enough of it, so most reactions in a cell would crawl.

An enzyme gives the reaction a lower route. Its active site holds the substrate in exactly the right position, induced fit bends the bond that has to break, and charged R groups pull electrons the right way. Less energy is needed, so far more molecules get over the top each second.

What the enzyme cannot change is where the reaction starts and where it ends. The free energy of the reactants and of the products, and so the difference between them, **ΔG**, are the same with or without it. It changes the route, not the destination, and it speeds the reverse reaction just as much.

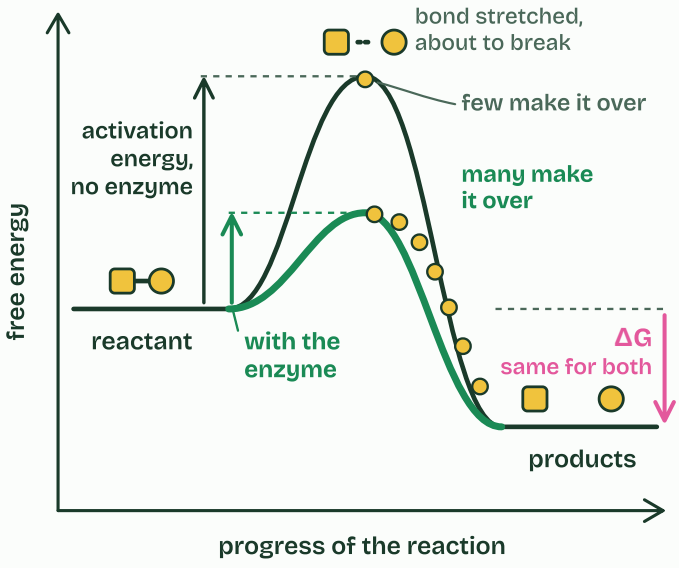
KEY POINTS

- The enzyme lowers the activation energy. It supplies no energy of its own.
- ΔG does not change, so an enzyme cannot make an uphill reaction go by itself.

KEY TERMS

activation energy
The energy reactants must take in before a reaction can start: enough to strain and begin to break their bonds.

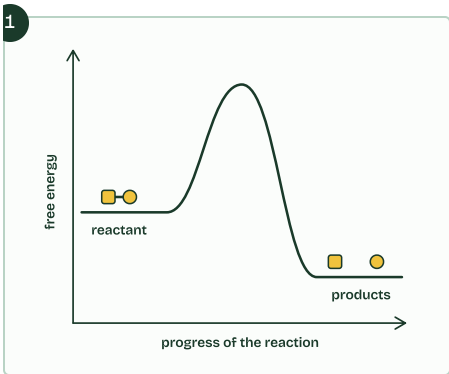
ΔG
The change in free energy in a reaction. Negative when free energy is released (exergonic), positive when it must be put in (endergonic).



STEP BY STEP

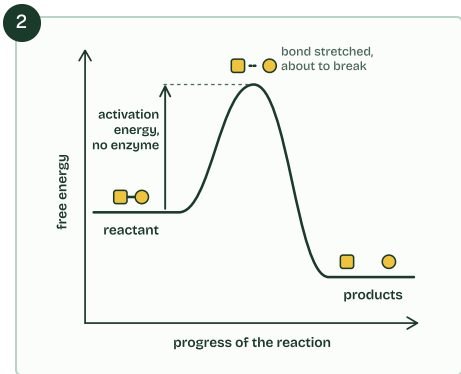
5 STEPS

1



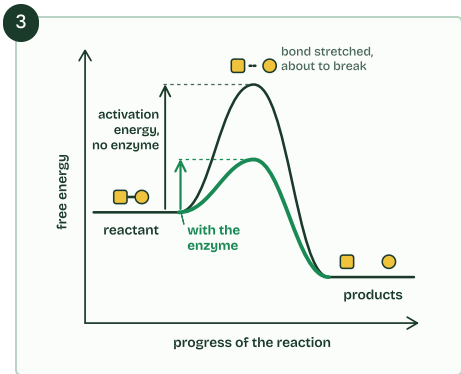
The reactant must pass over a **hump** before it can become products. Here the products end up lower: this reaction releases energy.

2



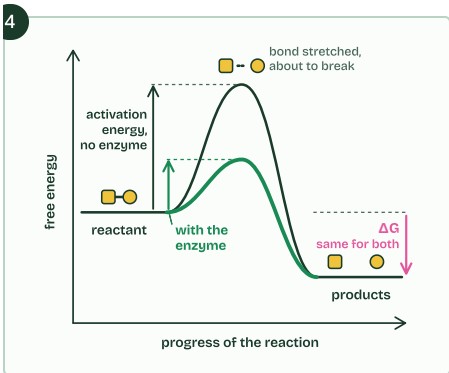
At the top of the hump the bond is stretched and about to break. Getting there takes the **activation energy**, and at 37 °C few collisions have that much.

3



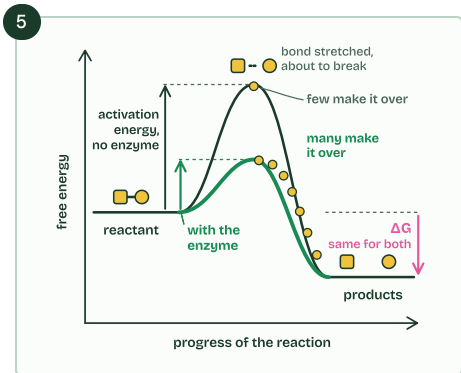
With the enzyme the route is **lower**: the active site holds the substrate in place and strains its bond, so less energy is needed.

4



The start and the finish have not moved, so **ΔG is the same** with or without the enzyme. The enzyme changes the route, never the destination.

5



A lower peak means **many more** molecules have enough energy to cross it each second, so the reaction runs far faster.

WATCH OUT

"Breaking bonds releases energy" is wrong, and it costs marks every year. Breaking a bond always takes energy in. Energy is released when new, more stable bonds form, and the enzyme only lowers the cost of getting started.

EXAMPLE

One step in making the nucleotides of DNA and RNA has, with no enzyme, a half-life of about 78 million years. Its enzyme, orotidine phosphate decarboxylase, gets each molecule through in about a fortieth of a second: a speed-up of around 10^{17}.

PREDICT

An enzyme is added to a reaction with ΔG = −20 kJ/mol. What is ΔG now?

(A) More negative, so more energy is released

(B) −20 kJ/mol: the enzyme does not change it

(C) Less negative, as the enzyme absorbs energy

(D) Zero, because the enzyme brings it to balance

Decide before you look. Answer on page 30.

03

3.2.B SUBSTRATE AND ENZYME CONCENTRATION

More substrate, more collisions, up to a limit

Enzyme and substrate molecules jostle about at random. A reaction can only happen when a substrate meets a free active site the right way round, so the rate depends on how often that happens.

Add more substrate and those meetings happen more often, so the rate climbs. But each enzyme molecule can only work so fast. Once there is so much substrate that every active site is refilled the moment it empties, the enzyme is **saturated** and the rate levels off at its maximum. Past that point, only more enzyme raises it.

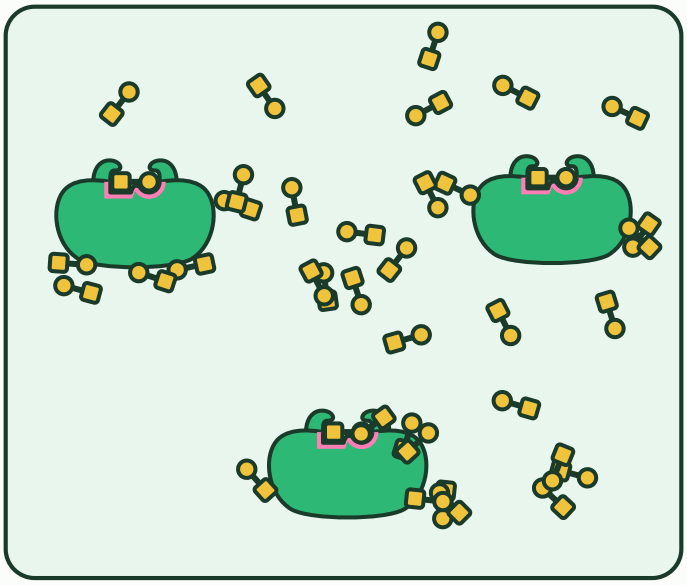
Products matter too. As a reaction runs, substrate is used up and product builds up, so the rate falls: the relative concentrations of substrate and product set how fast it goes. Many reactions are reversible, and the same enzyme speeds whichever direction the concentrations favor.

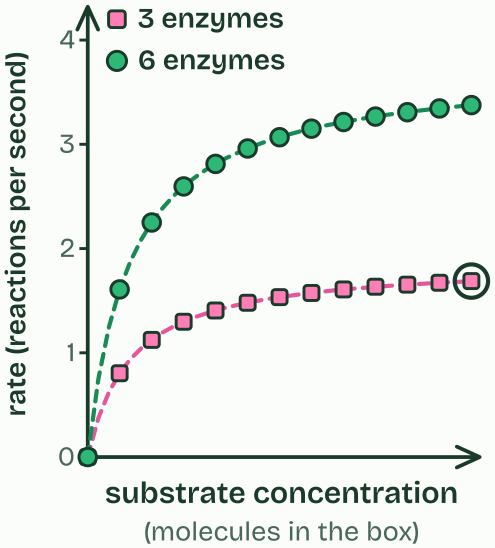
KEY POINTS

- Low substrate: the rate is limited by how often substrate meets a free site.
- High substrate: every site is busy, and the rate is limited by the amount of enzyme.

KEY TERMS

saturated
Working flat out: every active site is occupied all the time, so more substrate cannot raise the rate.



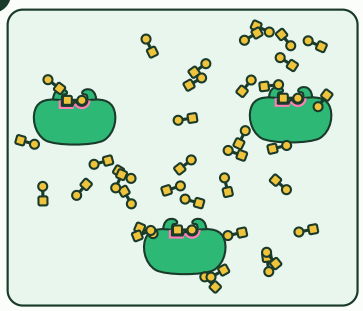


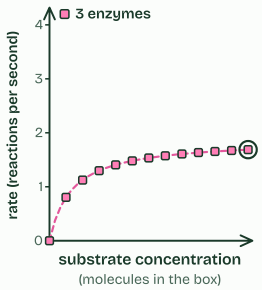
Drag the substrate up a step at a time and watch the active sites fill. Each concentration you try adds a point to the graph.

SWITCH IT

2 SETTINGS

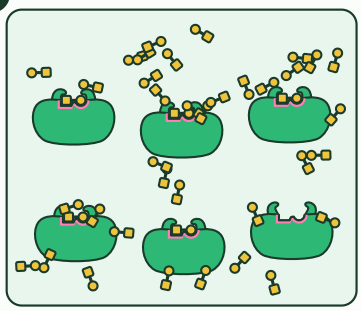
1

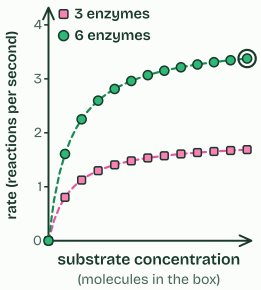




3 enzymes

2





6 enzymes

WATCH OUT

The plateau is not the enzyme being used up or denatured. Every enzyme molecule is still there and still working, flat out. Add more enzyme and the rate climbs again.

EXAMPLE

Carbonic anhydrase in red blood cells turns CO_2 and water into hydrogen carbonate (HCO_3^-) as they pass through your muscles, where CO_2 is high. In your lungs, where CO_2 is low, the same enzyme turns it back into CO_2 to breathe out.

REMEMBER IT

The five things that change an enzyme's rate spell SPITE

S Spicy	P Peppers	I Irritate	T Tired	E Eyes
Substrate how much there is	**pH** charges in the site	**Inhibitors** block or bend the site	**Temperature** collisions, then unfolding	**Enzyme** how much there is

PREDICT

A reaction has reached its plateau: more substrate no longer speeds it up. Which change would raise the rate?

(A) Adding even more substrate to the mixture

(B) Adding more of the enzyme

(C) Taking each product out as soon as it forms

(D) Leaving the reaction to run for longer

Decide before you look. Answer on page 30.

3.2.A 3.2.B TEMPERATURE

04 Heat makes it *faster*, then breaks it

Warm a solution and its molecules move faster. Enzyme and substrate collide more often, and more of those collisions carry enough energy to react, so the rate rises. For many enzymes it roughly doubles with every 10 °C.

But an enzyme's shape is held by weak bonds, mostly hydrogen bonds. Past a point, its atoms vibrate so hard that these bonds break: the chain loosens, the active site loses its shape, the substrate no longer fits, and the rate falls steeply. An enzyme whose structure has been disrupted like this is **denatured**. The temperature where the two effects balance and the rate peaks is the **optimum**.

Denaturing breaks the bonds between R groups, not the peptide bonds, so the amino acid sequence survives. Sometimes, when conditions return to normal, the chain folds back and the enzyme works again: ribonuclease, unfolded by chemicals, refolds fully active once they are washed away. Chains unfolded by heat usually tangle and stick together instead, and the loss is permanent.

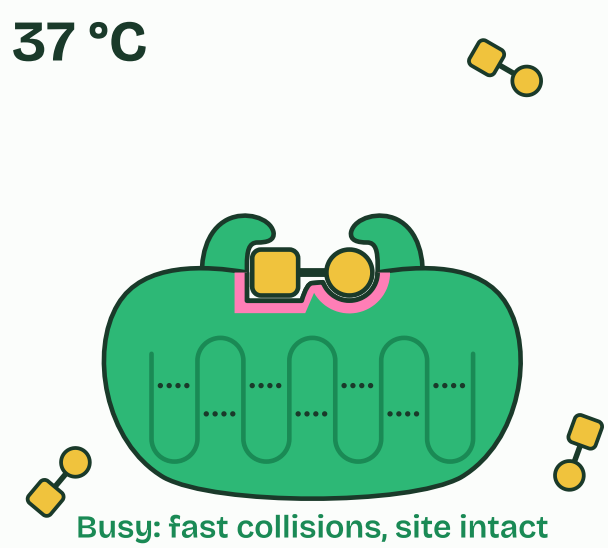
KEY POINTS

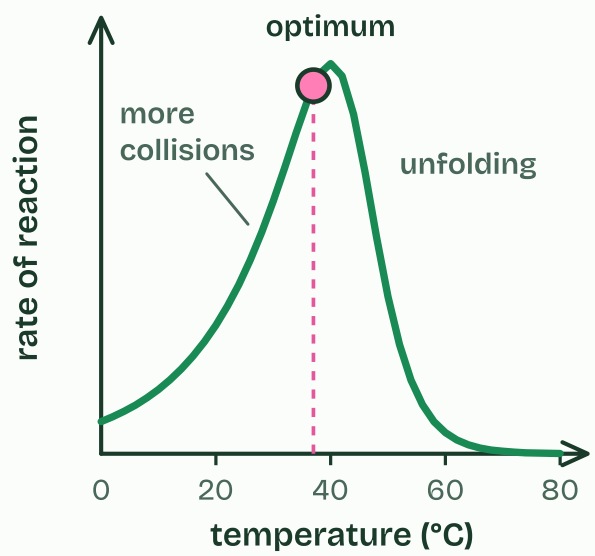
- Rising side: more collisions, and more of them with enough energy.
- Falling side: denaturation, faster the hotter it gets.
- Each enzyme's optimum suits the place it works.

KEY TERMS

denatured
Changed in shape because the weak bonds holding the folded chain have broken, so the active site no longer works. The amino acid sequence is not changed.

optimum
The temperature, or pH, at which an enzyme works fastest.





SLIDE IT

4 SETTINGS

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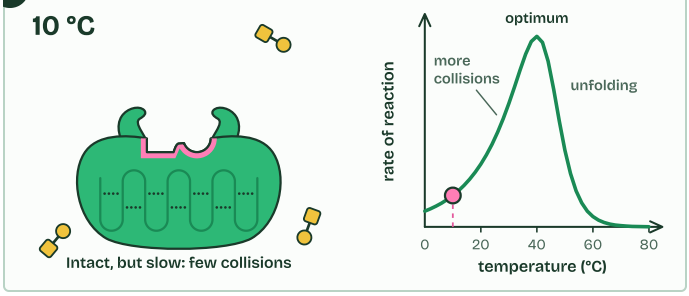

Temperature 10

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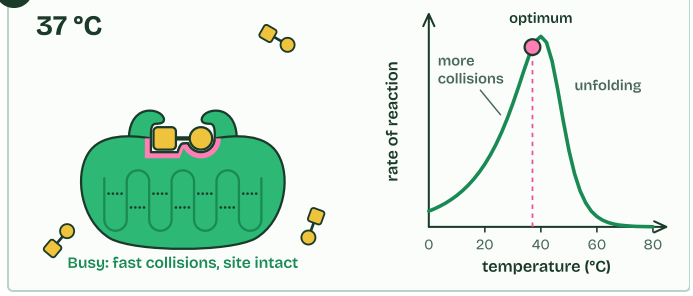

Temperature 37

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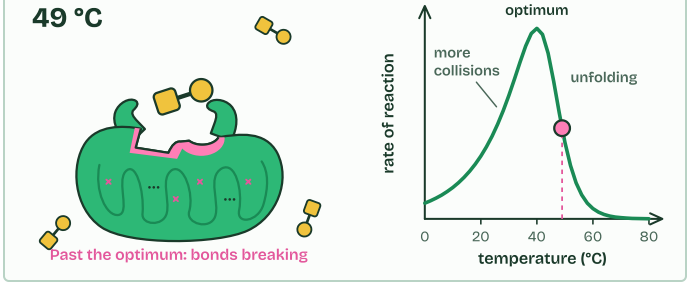
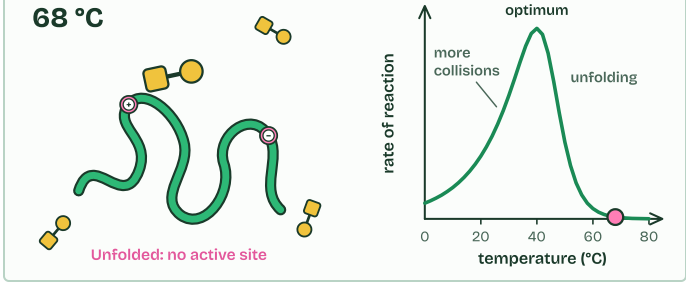


Temperature 49

4

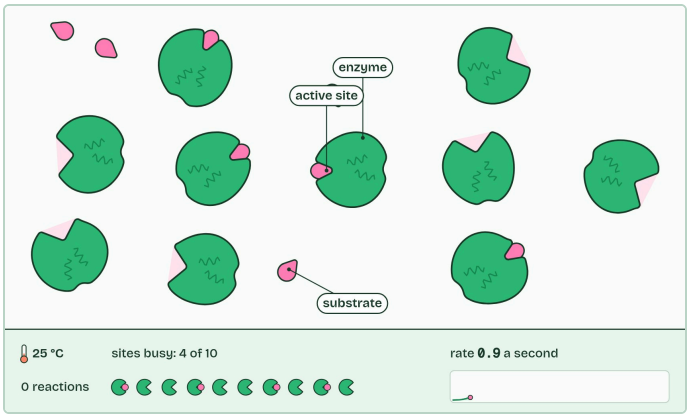


Temperature 68

ANIMATION · WATCH IT HAPPEN: COLLISIONS, HEAT AND INHIBITORS

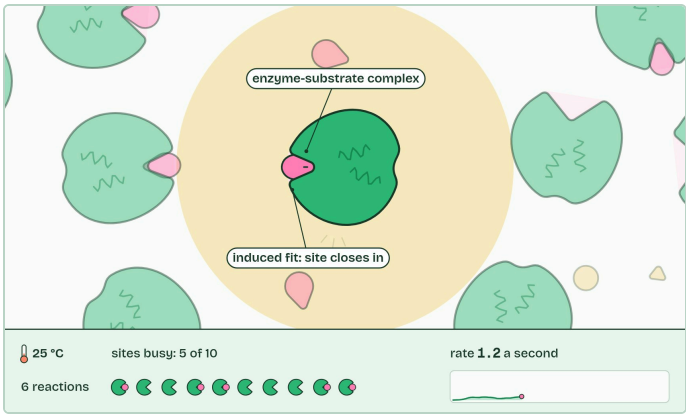
3 MOMENTS

1 0 REACTIONS



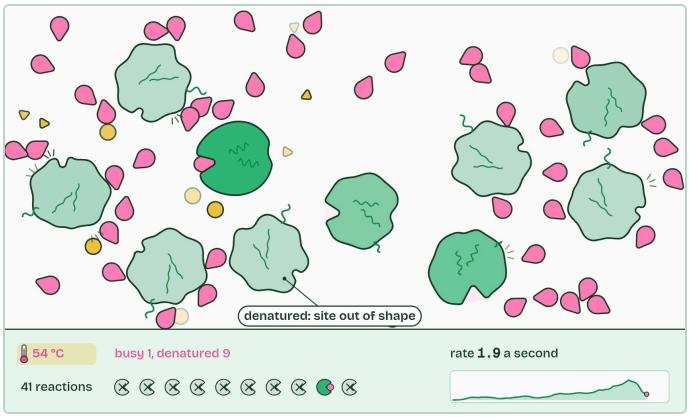
Molecules move at **random**, and most collisions miss the active site.

2 6 REACTIONS



A **successful collision**: it binds and reacts, and the enzyme is unchanged.

3 41 REACTIONS



Too hot: enzymes **denature**. Active sites lose their shape, and the rate falls.

Watch it move, and change it as it runs: biologybybradford.com/guides/ap-biology-unit-3-enzymes#temperature

WATCH OUT

Cold does not denature an enzyme. At 5 °C it is slow because it and its substrate move slowly, but its shape is intact, and it works normally once warmed. Write "denatured" for the hot side only (or an extreme pH).

EXAMPLE

Most human enzymes peak near 37 to 40 °C. The DNA polymerase of *Thermus aquaticus*, a bacterium from hot springs, works best at about 75 °C, which is why PCR uses it. Antarctic icefish have enzymes that work in water at about 0 °C.

PREDICT

Salivary amylase is held at 70 °C for ten minutes, then cooled back to 37 °C. What is its rate now?

(A) Back to normal: 37 °C is near its optimum

(B) Higher than before, from the heat it took in

(C) Far lower than normal: it stays unfolded

(D) Zero at first, then normal again in an hour

Decide before you look. Answer on page 30.

3.2.A 3.2.B PH

05 pH changes the *charges* in the site

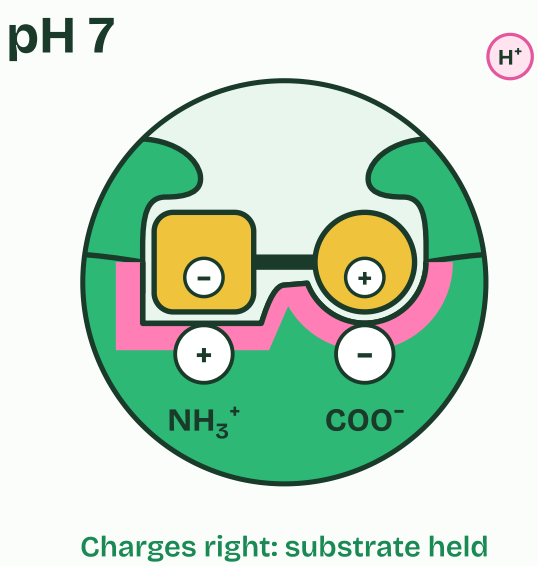
pH measures how many hydrogen ions (H^+) a solution holds: pH = −log[H^+]. The scale is logarithmic, so each step down means ten times more H^+, and pH 4 holds a thousand times the H^+ of pH 7. You need the idea, not the calculation.

Many R groups in an active site can gain or lose H^+. A carboxyl group (−COO^-) picks one up in acid and loses its negative charge; an amino group (−NH_3^+) gives one up in alkali and loses its positive charge. Change those charges and the substrate is no longer held, so the rate drops. Push the pH further and the ionic and hydrogen bonds that hold the fold break too: the enzyme denatures.

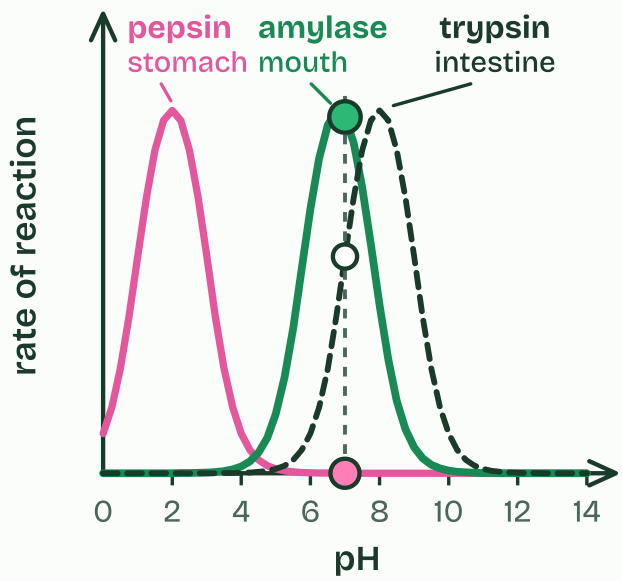
So every enzyme has an **optimum pH**, and it suits the place the enzyme works: pepsin in the acid of the stomach, trypsin in the small intestine, where alkaline juice from the pancreas has neutralized that acid.

KEY POINTS

- A little off the optimum: charges in the site change, and the effect reverses when the pH returns.
- Far off it: the bonds holding the fold break, and the enzyme denatures.



Charges right: substrate held



SLIDE IT

5 SETTINGS

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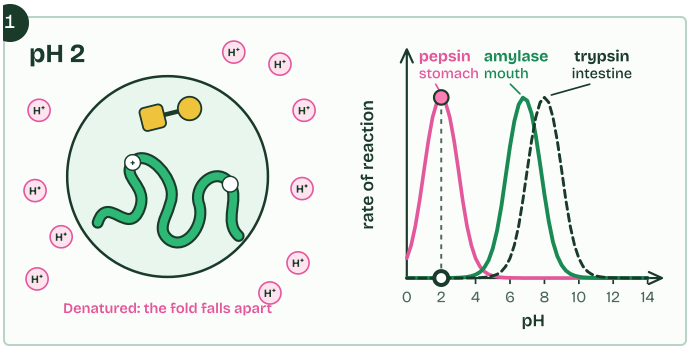
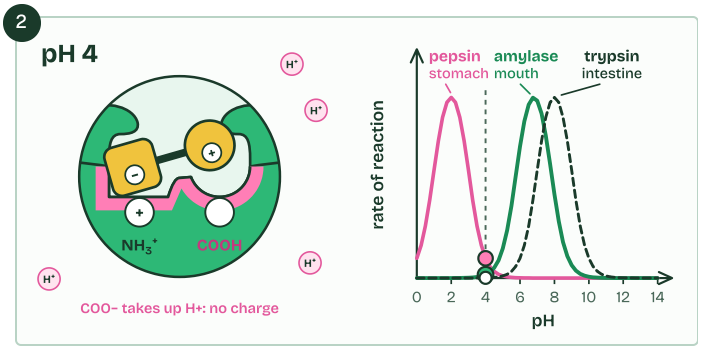


pH 2

2



pH 4

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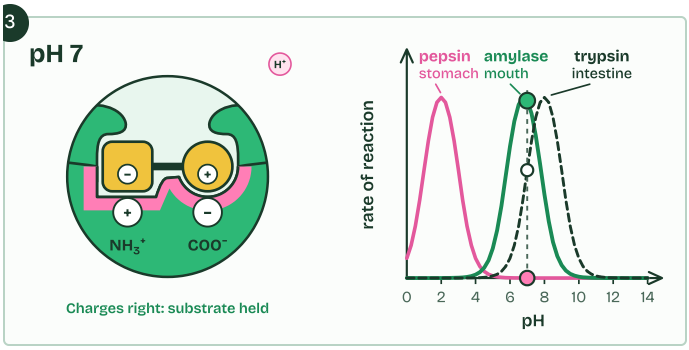
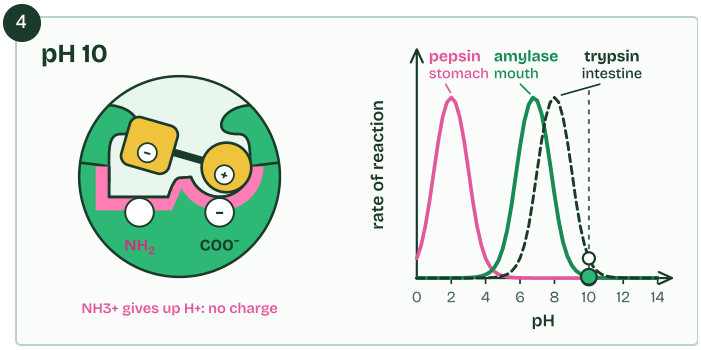


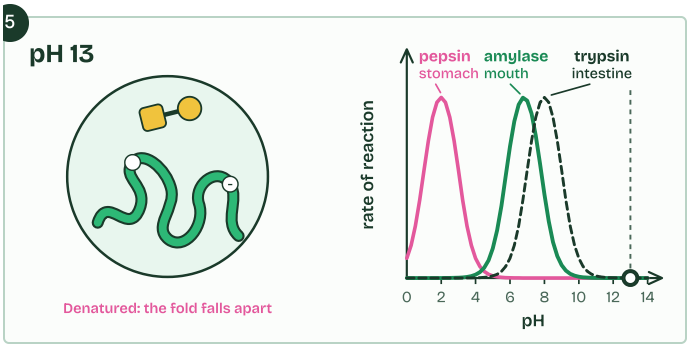
pH 7

4



pH 10

5



pH 13

WATCH OUT

"Enzymes work best at pH 7" is not true of all of them: pepsin barely works at pH 7. And one pH unit is not a small change. It is ten times the H^+.

EXAMPLE

Pepsin starts digesting protein in the stomach at about pH 2. When food moves on into the small intestine, pancreatic juice at about pH 8 neutralizes the acid. Pepsin stops working there, and trypsin, which arrives in that juice and works best near pH 8, takes over.

PREDICT

Amylase is put in a buffer at pH 5 for a minute, then moved back to pH 7. What is its rate now?

(A) About normal: its site's charges come back

(B) Still slow: any change of pH denatures it

(C) Faster than before: it has got used to acid

(D) Zero: an enzyme only works at its optimum

Decide before you look. Answer on page 30.

3.2.B INHIBITORS

06 Inhibitors *block* the site, or *bend* it

An inhibitor is a molecule that binds to an enzyme and slows it down. There are two ways to do it, and a graph of rate against substrate concentration tells them apart.

A **competitive inhibitor** looks enough like the substrate to fit the active site, and while it sits there the substrate cannot bind. Most come and go, so inhibitor and substrate take turns at the sites, and the more substrate there is, the more often the substrate wins. Add enough and the rate climbs back to nearly its normal maximum.

A **noncompetitive inhibitor** binds somewhere else, at an **allosteric site**. It changes the shape of the enzyme, active site included. The substrate is not competing for that spot, so no amount of substrate wins those enzymes back: the maximum rate stays lower.

Most inhibitors bind reversibly and let go again. Some bond to the enzyme for good and take it out of action permanently, whichever site they use.

KEY POINTS

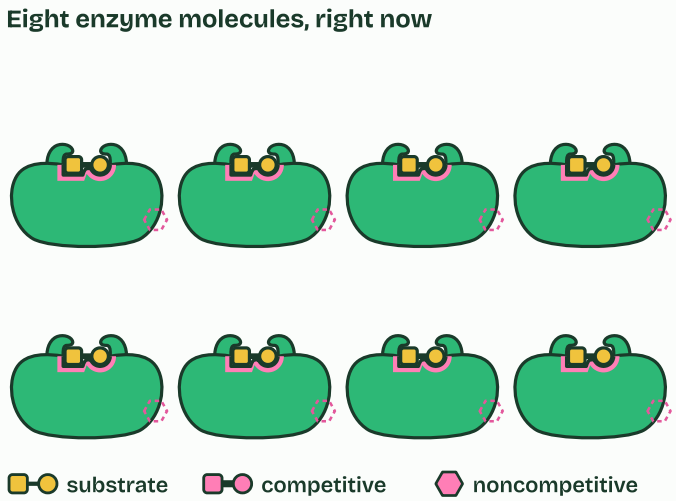
- Competitive: same maximum rate, but more substrate is needed to reach it.
- Noncompetitive: a lower maximum rate, whatever the substrate concentration.

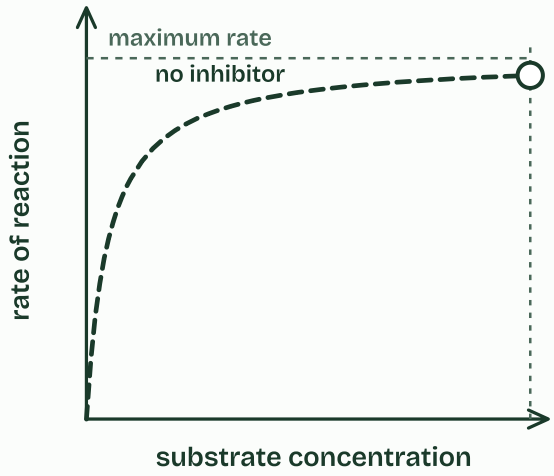
KEY TERMS

competitive inhibitor
An inhibitor that binds in the active site itself, because its shape resembles the substrate's, so the two compete for the site.

noncompetitive inhibitor
An inhibitor that binds somewhere other than the active site and changes the enzyme's shape, so the active site works less well.

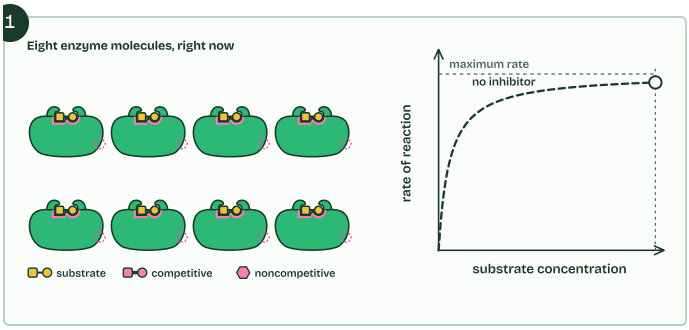
allosteric site
A binding site on an enzyme other than the active site. A molecule binding there changes the enzyme's shape, and so its activity.



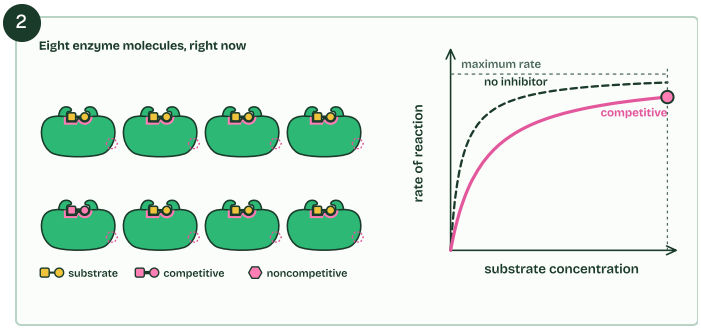


SWITCH IT

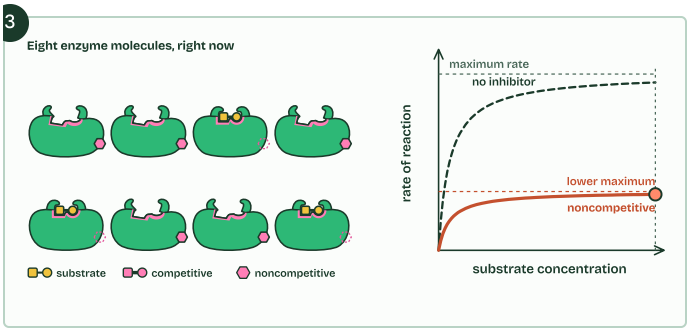
3 SETTINGS



None



Competitive



Noncompetitive

PREDICT

Two reactions are slowed by inhibitors. You add a large excess of substrate to both. One gets back almost its full rate; the other does not. What does the one that recovered contain?

(A) A noncompetitive inhibitor at an allosteric site

(B) A competitive inhibitor, outnumbered by substrate

(C) An inhibitor that the reaction has now used up

(D) Too little enzyme to handle the extra substrate

Decide before you look. Answer on page 30.

EXAMPLES

Zanamivir (Relenza), a flu drug, is shaped like sialic acid. New flu virus particles stay stuck to the cell they leave until the virus's enzyme neuraminidase cuts away the sialic acid they cling to. Zanamivir fills that enzyme's active site, so they stay stuck: a competitive inhibitor. Penicillin binds the active site of the enzyme bacteria use to cross-link their cell walls, and bonds to it for good. Isoleucine shuts down its own production by binding an allosteric site (chapter 8).

WATCH OUT

Cyanide is often given as an allosteric inhibitor. It is not one: it binds the iron and copper center of cytochrome c oxidase, the very place where oxygen should bind at the end of the electron transport chain. And a competitive inhibitor does not lower the maximum rate. It raises the substrate concentration needed to reach it.

REMEMBER IT

Two kinds of inhibitor, and where the second binds

C Copycat	N Nope	A Another
Competitive takes the active site; more substrate wins	**Noncompetitive** bends the site; max rate drops	**Allosteric site** a site that is not the active site

3.3.A CELLULAR ENERGY

07 Staying alive takes a *constant* supply of energy

A cell is a highly ordered place: proteins folded just so, membranes whole, more potassium inside than out. The second law of thermodynamics says that every energy transfer turns some energy into heat, and the disorder of the universe as a whole always increases. Order never keeps itself. A cell holds on to it only by taking in energy all the time, from food or from light, faster than it loses energy. Cut off that flow, or lose the order, and the cell dies.

Life does not break the second law. A cell's order is paid for by the heat and disorder it releases into its surroundings.

Reactions come in two kinds. An **exergonic** reaction releases free energy (ΔG is negative), like the breakdown of glucose. An **endergonic** reaction needs free energy put in (ΔG is positive), like building a protein. A cell runs an endergonic reaction by **coupling** it to an exergonic one, and the usual partner is the hydrolysis of ATP, the cell's energy currency.

KEY POINTS

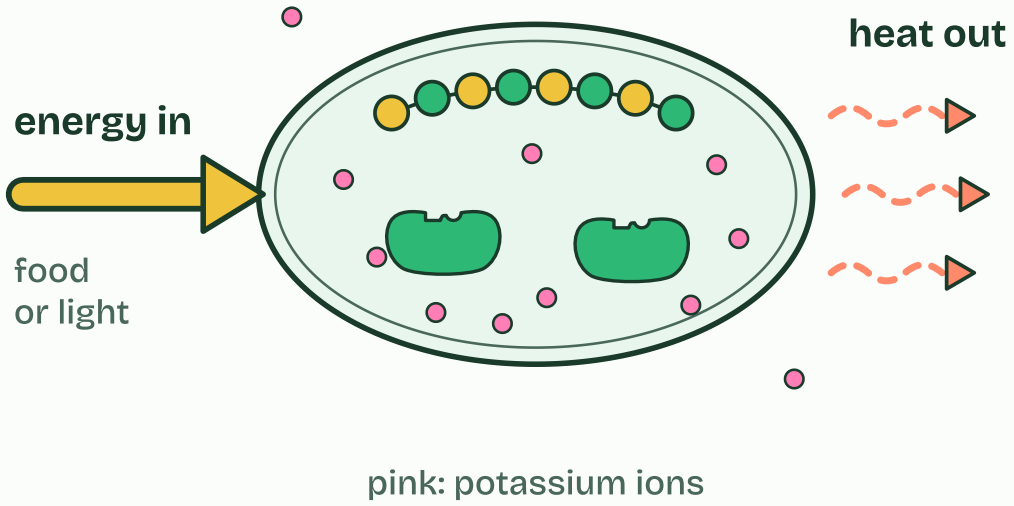
- Energy in must exceed energy lost, to keep order and to do work.
- Coupled to ATP hydrolysis, an uphill step becomes part of a downhill pair.

KEY TERMS

exergonic
Releasing free energy: the products hold less free energy than the reactants, and ΔG is negative.

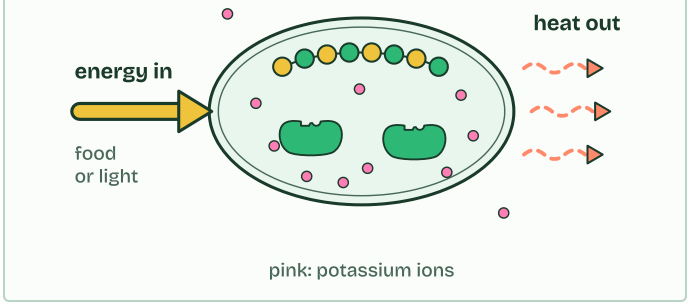
endergonic
Needing free energy put in: the products hold more free energy than the reactants, and ΔG is positive.

coupling
Linking a reaction that releases energy to one that needs it, so that together they release energy and run.



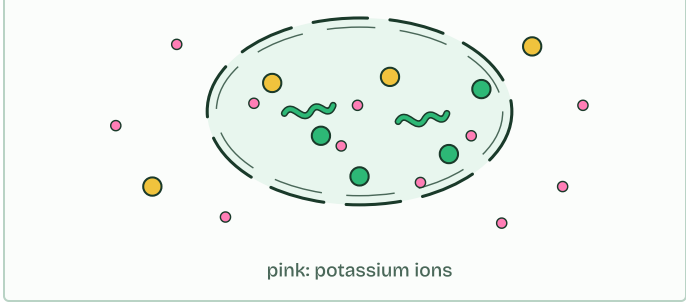
SWITCH IT 2 SETTINGS

1

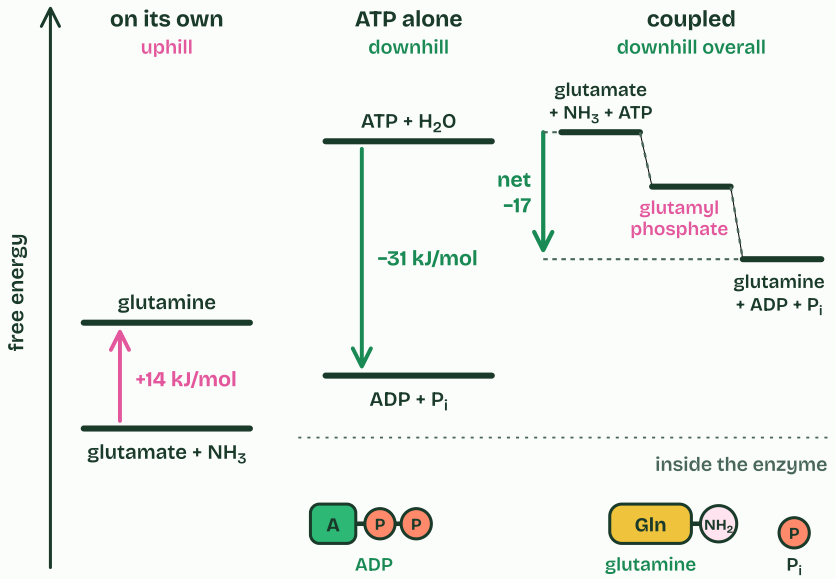


Energy flowing in

2



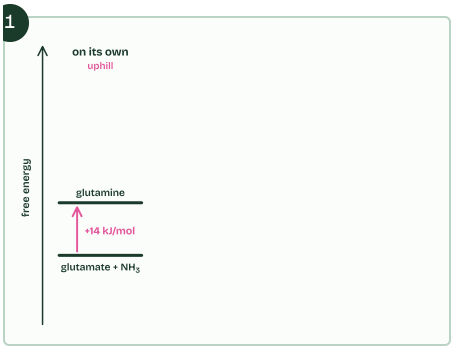
Energy cut off



Standard values. In a living cell, splitting ATP releases even more, about −50 kJ/mol.

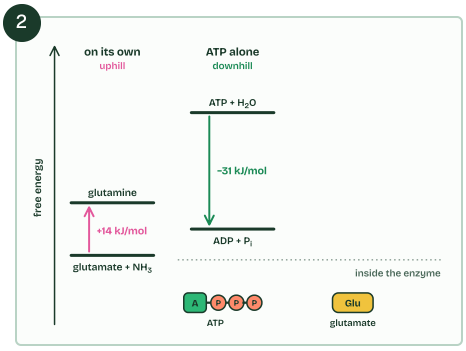
STEP BY STEP 4 STEPS

1



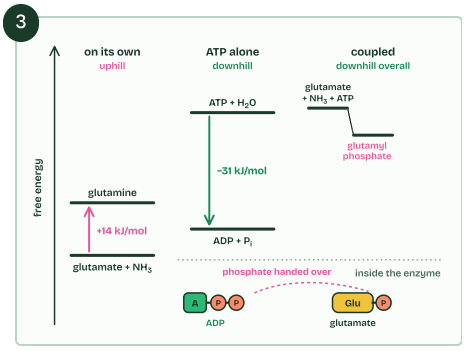
Making glutamine from glutamate and ammonia is **uphill**: ΔG = +14 kJ/mol. On its own it hardly happens.

2



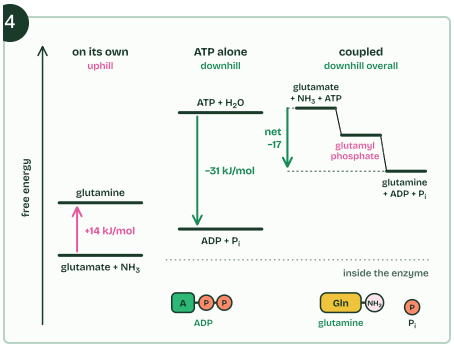
Splitting ATP into ADP and phosphate is **downhill**: ΔG = −31 kJ/mol. The products are more stable than ATP was.

3



The enzyme glutamine synthetase **couples** them: ATP hands its end phosphate to glutamate. Glutamyl phosphate is unstable, ready to react.

4



Ammonia takes the phosphate's place, making glutamine. Overall +14 − 31 = **−17 kJ/mol**: downhill, so it runs. Respiration then rebuilds the ATP.

WATCH OUT

Energy is not stored in ATP's last phosphate bond like a spring, released when the bond snaps. Breaking any bond takes energy in. ATP hydrolysis releases energy because the products, ADP and phosphate, are more stable than ATP and water were: they hold less free energy.

EXAMPLE

Your body holds only about 250 grams of ATP at any moment, yet makes and spends roughly its own weight in ATP every day. Each molecule is split to ADP and rebuilt, over and over, with energy from respiration.

REMEMBER IT

Which way the energy goes: read the first two letters. EXergonic, energy EXits; ENdergonic, energy ENters.

E Exits	**E** Enters
Exergonic	**Endergonic**
energy leaves; ΔG negative	energy goes in; ΔG positive

PREDICT

Reaction X has ΔG = +20 kJ/mol. Which of these would make it run in a cell?

(A) Adding an enzyme that is specific to X

(B) Raising the temperature to speed it up

(C) Coupling X to ATP hydrolysis (−31)

(D) Adding more of X's products to the cell

Decide before you look. Answer on page 30.

3.3.A 3.3.B ENERGY PATHWAYS

08 Pathways go in *small steps*, shared by all life

Cells rarely do anything in one reaction. They use **metabolic pathways**: chains of reactions, each catalyzed by its own enzyme, where the product of one step is the substrate of the next. Set glucose alight and its energy comes out all at once, as heat and light, and none of it is kept. Broken down in a cell, it goes through dozens of steps, each releasing a little energy that the cell can capture as ATP and control.

Control often acts on the first step. When the end product builds up, it binds an allosteric site on the pathway's first enzyme and switches it off: **feedback inhibition**. When the product is used up it lets go, and the pathway runs again. No energy or raw material is wasted making what the cell already has.

The same core pathways turn up everywhere. Bacteria, archaea and eukaryotes all use ATP as their energy currency, and versions of glycolysis run in all three, with several of the same enzymes. Pathways shared and conserved like this are best explained by inheritance from a common ancestor of all living things.

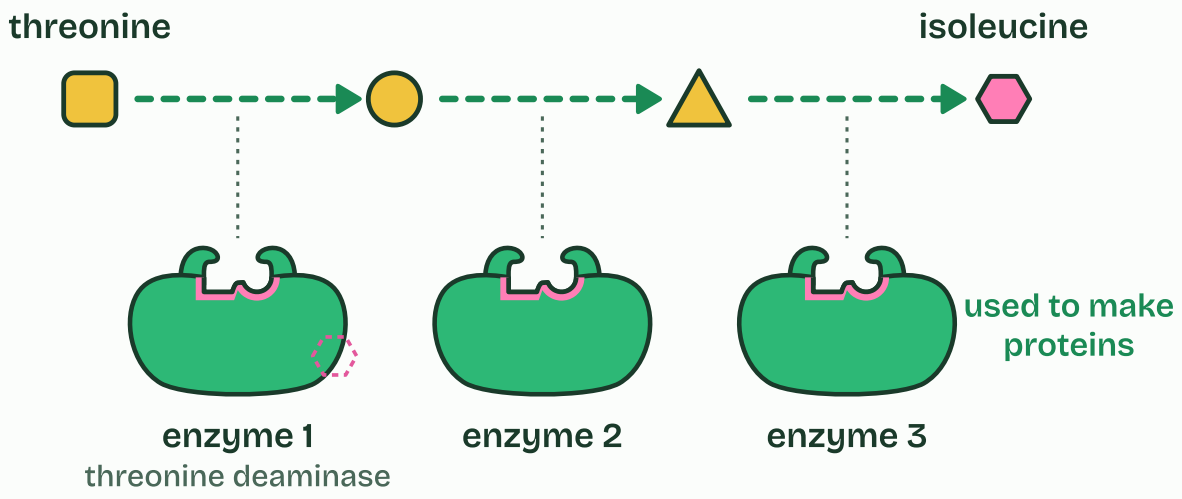
KEY POINTS

- The product of one step is the substrate of the next.
- The end product inhibits the first enzyme: negative feedback.
- Conserved pathways in every domain are evidence of common ancestry.

KEY TERMS

metabolic pathways
A series of reactions in which the product of each step is the substrate of the next, each step catalyzed by its own enzyme.

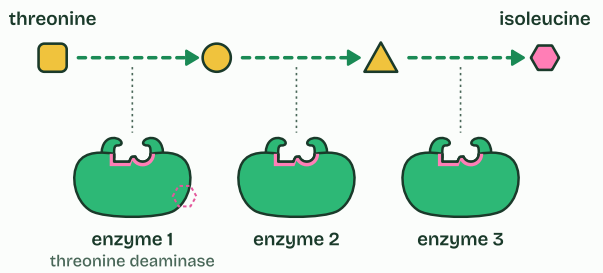
feedback inhibition
Control in which the end product of a pathway inhibits an enzyme early in the pathway, so the product limits its own production.



STEP BY STEP

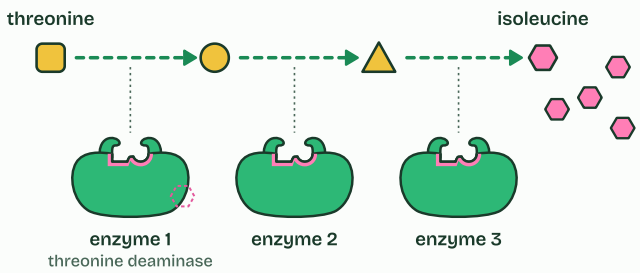
4 STEPS

1



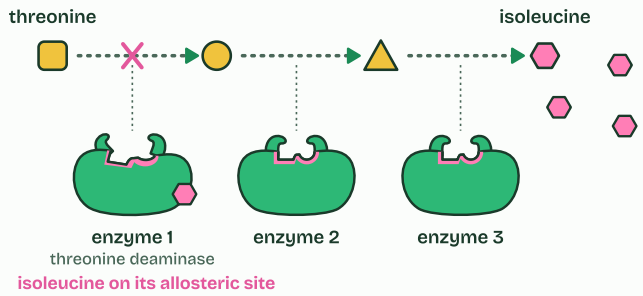
A pathway: **each product is the next enzyme's substrate**, so threonine is turned into isoleucine step by step (three steps here; the real pathway has five).

2



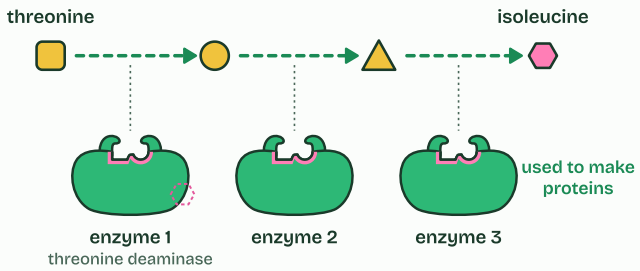
The cell makes more isoleucine than it is using, and it **builds up**.

3

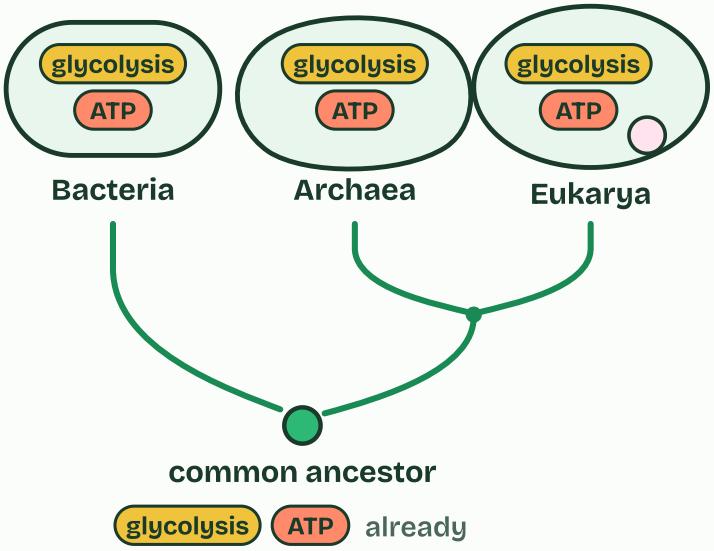


Isoleucine binds an **allosteric site** on enzyme 1 and bends its active site. The first step stops, so the whole pathway stops.

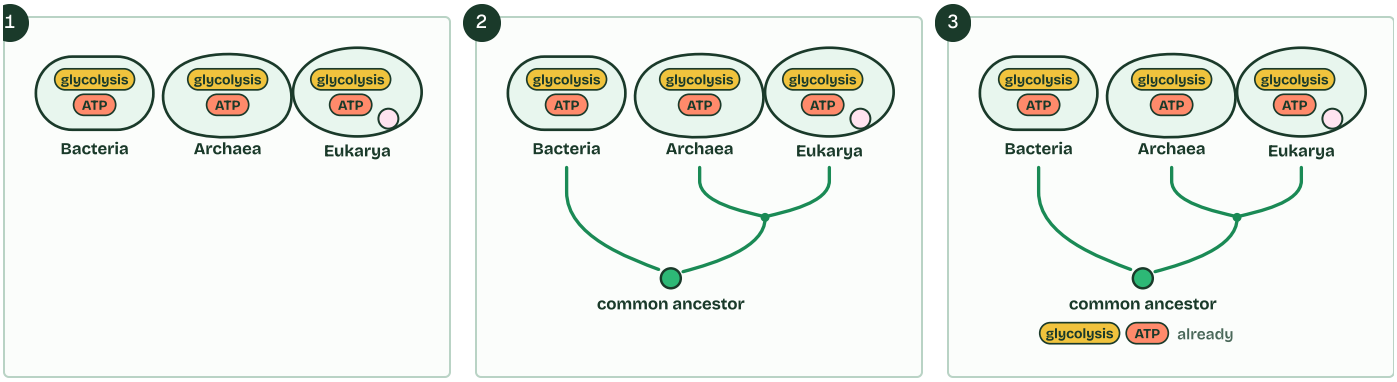
4



As the cell uses isoleucine to build proteins, the level falls, isoleucine **lets go**, and the pathway runs again: negative feedback.



STEP BY STEP 3 STEPS



Bacteria, archaea and eukaryotes all **run on ATP**, and all break glucose down by versions of **glycolysis**, several of whose enzymes have closely similar sequences in all three.

All three groups trace back to **one common ancestor**. Archaea and eukaryotes share a more recent one as well.

The simplest explanation: the ancestor **already had** ATP and glycolysis, and every line of descent kept them. Shared, conserved pathways are evidence of common ancestry.

WATCH OUT

Feedback inhibition does not destroy the first enzyme. The end product binds reversibly: as its level falls it lets go, and the enzyme works again. That is what makes it a switch.

EXAMPLE

Your liver breaks alcohol down in steps: ethanol to acetaldehyde (alcohol dehydrogenase), acetaldehyde to acetate (aldehyde dehydrogenase), then into the Krebs cycle. Some people inherit a slow version of the second enzyme. Toxic acetaldehyde builds up, and their face flushes after a drink.

PREDICT

The end product of a pathway builds up in a cell. What happens to the first enzyme of that pathway?

(A) It is denatured by the build-up of product

(B) It speeds up to clear the extra product

(C) It is unaffected; only the last enzyme stops

(D) It is slowed: the product binds it elsewhere

Decide before you look. Answer on page 31.

Key terms

Every term this guide defines, A to Z, with the chapter it is in. Cover the right-hand side and test yourself.

activation energy 02
The energy reactants must take in before a reaction can start: enough to strain and begin to break their bonds.

active site 01
The pocket on an enzyme where the substrate binds and the reaction happens. Its shape and its charges match the substrate.

allosteric site 06
A binding site on an enzyme other than the active site. A molecule binding there changes the enzyme's shape, and so its activity.

competitive inhibitor 06
An inhibitor that binds in the active site itself, because its shape resembles the substrate's, so the two compete for the site.

coupling 07
Linking a reaction that releases energy to one that needs it, so that together they release energy and run.

denatured 04
Changed in shape because the weak bonds holding the folded chain have broken, so the active site no longer works. The amino acid sequence is not changed.

endergonic 07
Needing free energy put in: the products hold more free energy than the reactants, and ΔG is positive.

enzyme 01
A protein that speeds up one chemical reaction in a cell and comes out of it unchanged, ready to work again.

exergonic 07
Releasing free energy: the products hold less free energy than the reactants, and ΔG is negative.

feedback inhibition 08
Control in which the end product of a pathway inhibits an enzyme early in the pathway, so the product limits its own production.

induced fit 01
The change in shape of an active site as the substrate binds. The site closes around the substrate and strains its bonds.

metabolic pathways 08
A series of reactions in which the product of each step is the substrate of the next, each step catalyzed by its own enzyme.

noncompetitive inhibitor 06
An inhibitor that binds somewhere other than the active site and changes the enzyme's shape, so the active site works less well.

optimum 04
The temperature, or pH, at which an enzyme works fastest.

saturated 03
Working flat out: every active site is occupied all the time, so more substrate cannot raise the rate.

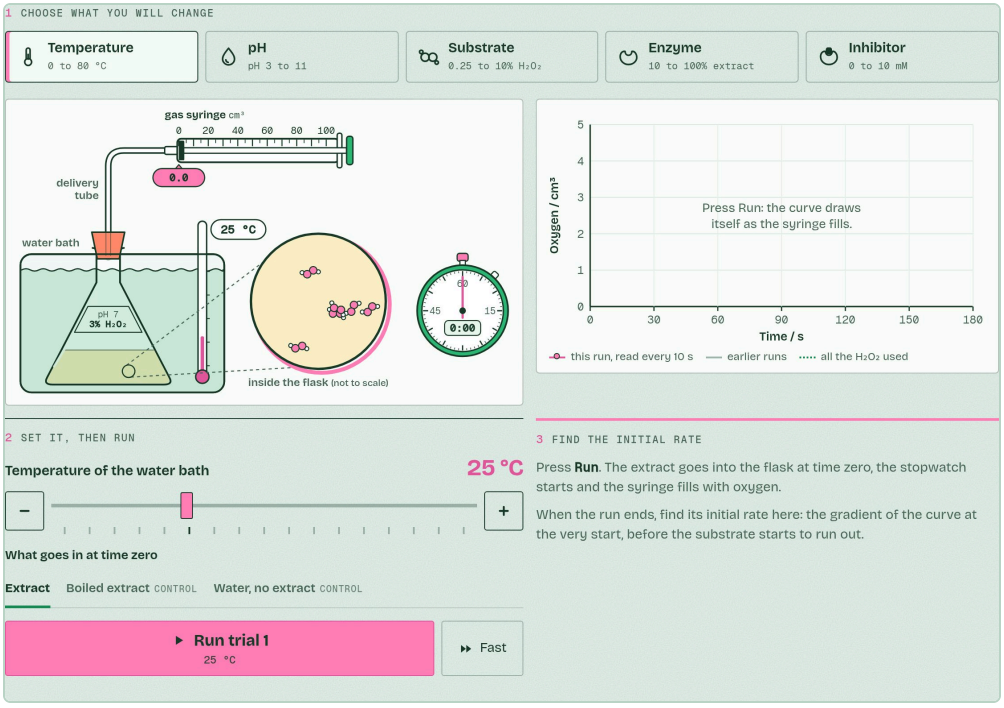
specific 01
Acting on one substrate, or a few very similar ones, because only they fit the active site.

substrate 01
The molecule an enzyme acts on. It binds in the active site and is turned into products.

ΔG 02
The change in free energy in a reaction. Negative when free energy is released (exergonic), positive when it must be put in (endergonic).

Dry lab: *Catalase and hydrogen peroxide*

Catalase, from a cell extract, breaks hydrogen peroxide down to water and oxygen. Collect the oxygen in a gas syringe, find the initial rate, and see what changes it.



Run it online: change the variables, run the trials, and the table and graph fill in as you go.
`biologybybradford.com/guides/ap-biology-unit-3-enzymes#lab`

Your question

How does one factor change the rate at which catalase breaks down hydrogen peroxide? Start with temperature: where is the optimum, and what happens beyond it?

What you can change

One independent variable at a time: temperature, pH, hydrogen peroxide concentration, extract concentration, or an inhibitor, competitive or noncompetitive. Everything else stays fixed unless you change it on purpose.

What to record

The volume of oxygen every 10 seconds, then the initial rate in cm³/min: the gradient at the very start. Three trials at each value, a mean, and a graph of the means.

TASK 1

Find the optimum temperature

Choose **Temperature**. Run 0, 10, 20 and so on up to 80 °C, three trials at each, and press Add to table after every run. Plot the means, then go back and fill in every 5 °C around the peak.

Record: the optimum to the nearest 5 °C, and how a run at 65 °C or above behaves after its first 30 seconds.

TASK 2

Saturate the enzyme

Choose **Substrate** and run hydrogen peroxide from 0.25% to 10%. Find where doubling the concentration stops doubling the rate. Then set **Extract concentration** to 50% under Kept the same and run the series again.

Record: where each curve levels off, and how halving the enzyme changes the plateau.

TASK 3

Outcompete an inhibitor

Choose **Substrate** and run a series with no inhibitor. Under Kept the same, set **Inhibitor** to Competitive at 1 mM and run the series again; then Noncompetitive at 1 mM. Compare all three at 0.5% and at 10% peroxide.

Record: each series' rate at 0.5% and at 10%, as a percentage of the no-inhibitor rate.

TASK 4

Run the controls

At 25 °C and pH 7, run **Boiled extract** and **Water, no extract** beside a normal run.

Record: the oxygen each gives in 3 minutes, and what each control rules out.

QUESTIONS ON THE LAB

QUESTION 1

At 65 °C the oxygen curve rises for the first minute, then flattens, far below the volume the peroxide could give. Why?

(A) The peroxide runs out sooner when it is hot

(B) The enzyme unfolds as the run goes on

(C) Hot oxygen escapes through the syringe

(D) The molecules collide less often over time

Answer on page 31.

QUESTION 2

In Task 3, the competitive inhibitor costs a lot of rate at 0.5% peroxide but little at 10%. The noncompetitive one halves the rate at both. Why the difference?

(A) More peroxide breaks the competitive inhibitor down

(B) The noncompetitive inhibitor denatures the catalase

(C) Peroxide wins the shared site, not a site elsewhere

(D) At 10% peroxide the catalase makes more enzyme

Answer on page 31.

QUESTION 3

Why does the lab ask for the initial rate, not the total oxygen after 3 minutes?

(A) The syringe can only be read accurately for small volumes

(B) The enzyme has all been used up by the end of the first minute

(C) Oxygen made later in the run dissolves back into the solution

(D) The total is set mostly by how much peroxide went in

Answer on page 31.

Check yourself

The check for this guide is online: a fresh paper of past-paper style questions from the exam bank on 3.1 to 3.3 each time, marked as you go, and saved to your Exam practice progress when you are signed in. Scan the code, or type the link.

biologybybradford.com/exam-maker/sit?program=ap&units=U3&ced=3.1,3.2,3.3&paper=quiz&marks=15&src=ep

Answers

PREDICTIONS IN THE CHAPTERS

01 A molecule has exactly the substrate's shape, but its two charges are the other way round. What happens at the active site?

C It is pushed away, because like charges repel

Shape is not enough. Each of its charges now faces a like charge in the site, and like charges repel, so it never settles in. Offer the enzyme the **Look-alike** in the figure to see it.

---

02 An enzyme is added to a reaction with ΔG = −20 kJ/mol. What is ΔG now?

B −20 kJ/mol: the enzyme does not change it

The enzyme lowers the peak, not the start or the finish. Play the figure to step 4: both routes begin at the same level and end at the same level, so ΔG is −20 kJ/mol either way. Only the activation energy drops.

---

03 A reaction has reached its plateau: more substrate no longer speeds it up. Which change would raise the rate?

B Adding more of the enzyme

At the plateau every active site is busy, so the number of sites is the limit. More enzyme means more sites. Try it: push the substrate high, then switch from 3 enzymes to 6, and watch the graph.

---

04 Salivary amylase is held at 70 °C for ten minutes, then cooled back to 37 °C. What is its rate now?

C Far lower than normal: it stays unfolded

At 70 °C the chains unfold, and as they cool most of them tangle with each other instead of refolding, so their active sites are gone for good. Compare the cold side: drag the slider down to 5 °C and the enzyme is slow but whole.

---

05 Amylase is put in a buffer at pH 5 for a minute, then moved back to pH 7. What is its rate now?

A About normal: its site's charges come back

At pH 5 some carboxyl groups in the site pick up H^+ and lose their charge, so the substrate is held less well. Nothing is broken: back at pH 7 they give the H^+ up again, the charges return and so does the rate. Only a pH far from the optimum breaks the bonds that hold the fold. Slide the figure from 7 to 4 and on down to 2 to see the difference.

---

06 Two reactions are slowed by inhibitors. You add a large excess of substrate to both. One gets back almost its full rate; the other does not. What does the one that recovered contain?

B A competitive inhibitor, outnumbered by substrate

A competitive inhibitor takes turns with the substrate for the same site, so flooding the mixture with substrate wins nearly every site back. A noncompetitive one holds its enzymes out of shape whatever the substrate does. Choose each type in the figure and drag the substrate up.

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07 Reaction X has ΔG = +20 kJ/mol. Which of these would make it run in a cell?

C Coupling X to ATP hydrolysis (−31)

An enzyme only lowers the activation energy and heat only speeds up collisions: neither changes ΔG, so X stays uphill. Coupled to ATP hydrolysis, the pair has ΔG = +20 − 31 = −11 kJ/mol, downhill, so it runs. More product would push X backwards.

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08 The end product of a pathway builds up in a cell. What happens to the first enzyme of that pathway?

D **It is slowed: the product binds it elsewhere**

Feedback inhibition: the end product binds an allosteric site on the first enzyme and bends its active site, so the whole pathway slows. Play the top figure to step 3, then step 4, when the product has been used and lets go.

QUESTIONS ON THE DRY LAB

Q1 At 65 °C the oxygen curve rises for the first minute, then flattens, far below the volume the peroxide could give. Why?

B **The enzyme unfolds as the run goes on**

At 65 °C the catalase is unfolding all through the run, and the unfolded molecules never refold. Fewer and fewer active sites are left, so the curve flattens long before the peroxide is used up. The same peroxide at 25 °C gets close to the dashed line within the 3 minutes.

Q2 In Task 3, the competitive inhibitor costs a lot of rate at 0.5% peroxide but little at 10%. The noncompetitive one halves the rate at both. Why the difference?

C **Peroxide wins the shared site, not a site elsewhere**

The competitive inhibitor takes turns with peroxide at the active site, so a flood of peroxide wins nearly every site back. The noncompetitive one binds an allosteric site and changes the enzyme's shape. Peroxide is not competing for that site, so no amount of it helps: the enzyme molecules holding the inhibitor stay out of action.

Q3 Why does the lab ask for the initial rate, not the total oxygen after 3 minutes?

D **The total is set mostly by how much peroxide went in**

Given time, every working run levels off near the volume its peroxide can give: 2 cm^3 of 3% gives 21.2 cm^3, however fast the enzyme. The initial rate, taken before the substrate starts to run low, is the measure of how fast the enzyme works under each set of conditions.

How this was made. The course structure, the activities and the teaching choices come from my own classroom, my presentations and my years of teaching IB and AP Biology. I use Claude, an AI, to help write and build the interactive guides, lesson plans, slides and practice questions, and to draw the diagrams and code the interactives. I direct the work and review it against the IB and AP specifications before it goes live.

Written and drawn for Biology by Bradford, biologybybradford.com. Free to print and share with a class.