Enzymes and metabolism

C1.1 · SL and HL

A catalyst changes the time, not the supply of starch

Explain the reaction before choosing the rate measurement

Amylase can shorten the time until a starch sample no longer gives blue-black iodine colour. It is regenerated overall.

Common content: metabolic networks, catalysis, active sites, induced fit, specificity, rate factors, measurement and energy barriers.

All numerical data and curves are invented teaching models.

AHL extends the model to pathway control

Keep the mechanism and location explicit

AHL content: intracellular/extracellular enzymes, heat, linear/cyclical pathways, reversible inhibition, feedback and mechanism-based inhibition.

Use isoleucine synthesis, statins and penicillin as named biochemical examples.

Data can fit a model without uniquely proving a binding site or reversibility.

Regulation of specific enzymes regulates a network

One enzyme does not catalyse every metabolic reaction

Enzymes speed reactions at temperatures compatible with cells and are regenerated overall.

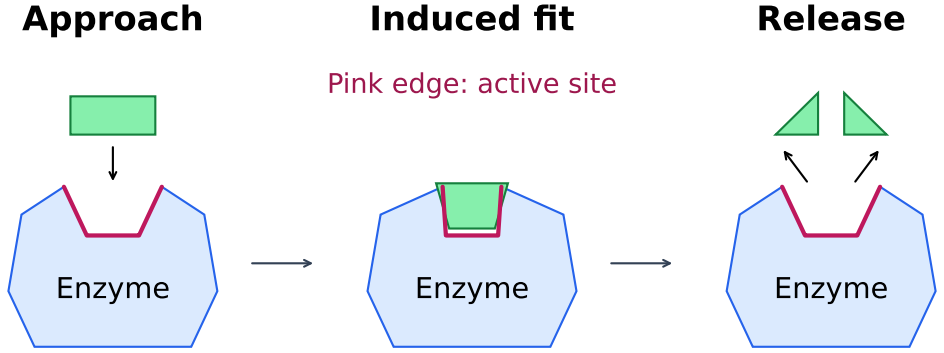
Metabolism is an interacting reaction network: products become substrates, branches share intermediates and reactions depend on matter and energy.

Specificity requires many enzymes; changing their amounts or activities changes pathway flow. A catalyst does not supply unlimited net energy.

Classify the overall process, not one isolated bond

Process	Explanation
Anabolism	Builds larger/complex molecules with an overall energy supply: protein or glycogen synthesis, photosynthesis
Catabolism	Breaks larger molecules down: digestion by hydrolysis, oxidation in respiration
Condensation / hydrolysis	Net removal / use of water when joining / splitting subunits
Energy warning	Bond breaking needs energy; bond formation releases it. Net balance determines the transfer.

Follow binding, adjustment and regeneration



Both enzyme and substrate change shape during binding.

Specificity requires chemical compatibility as well as shape

Folding connects distant parts of a primary sequence

A few amino-acid side chains form the active site. Interactions throughout a globular protein maintain its shape and chemical environment.

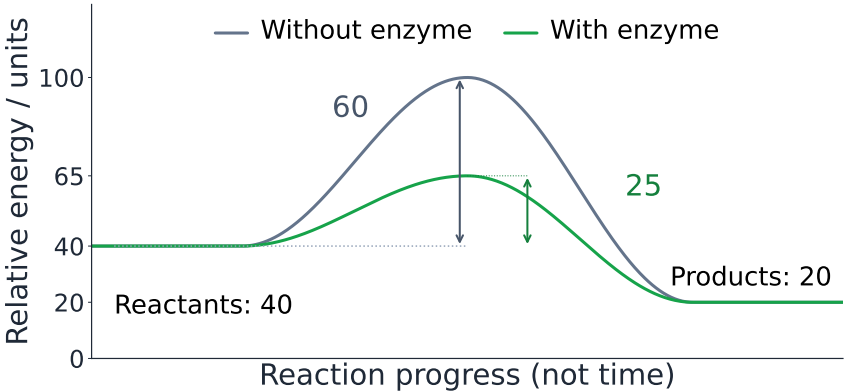
Charges, hydrogen bonding and orientation affect productive binding. A vaguely similar outline does not guarantee catalysis.

Both partners can adjust on binding. Products leave and the enzyme begins another cycle. Protein enzymes are the model here; some catalysts are RNA.

A fixed enzyme can still meet mobile substrate

1. **Molecular motion.** Random motion brings compatible molecules together; suitable orientation and chemistry are needed.
2. **Immobilized example.** An enzyme embedded in a membrane can encounter moving substrate. A mobile enzyme can reach a large fixed substrate.
3. **Conclusion.** Do not infer that both enzyme and substrate must travel freely, or that every collision causes reaction.

Compare the barrier with the starting level



Catalysis lowers the barrier; the net change stays the same.

Subtract the reactant level from each peak

1. **Invented levels.** Reactants 40; products 20; peaks 100 without enzyme and 65 with enzyme.
2. **Barriers.** $100 - 40 = 60$; $65 - 40 = 25$ relative units.
3. **Net transfer.** $20 - 40 = -20$ for both routes. Reaction progress is not time; the catalyst changes neither equilibrium nor the net energy requirement.

An energy-releasing reaction can still need an initial barrier

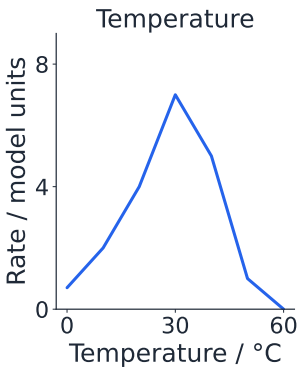
Breaking a bond does not release its stored energy by itself

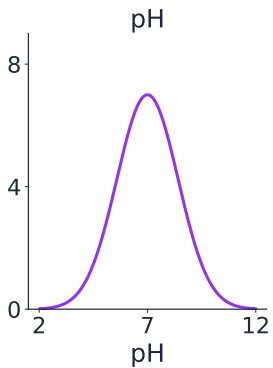
Energy is needed to reach a transition state. A lower-barrier route raises the fraction of encounters that can proceed at the same temperature.

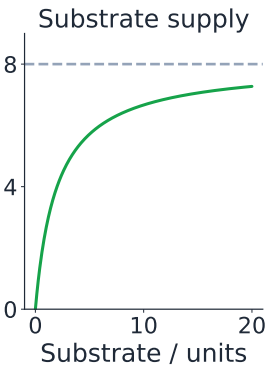
Breaking bonds requires energy; making product bonds releases energy. The net transfer depends on the balance.

A catalyst accelerates both directions without moving equilibrium. Energy-requiring processes still need a suitable energy input or coupling.

Explain three curves using different mechanisms







Peaks and saturation plateaus need different explanations.

Connect the variable to a molecular change

Variable	Reason and condition
Temperature	Increasing motion can raise productive encounters; sufficient heating disrupts folding and the active site
pH	Changed protonation alters charges and catalysis; severe changes can disrupt folding
Substrate concentration	Initially more encounters; at fixed enzyme, occupation and turnover limit the high-substrate rate
Important limits	Optima depend on enzyme and assay; cooling usually slows reversibly; not every pH effect is permanent

The greatest tested value is not necessarily the exact optimum

1. **Invented series.** At 10/20/30/40/50 °C, rates are 2/4/7/5/1 model units.
2. **Observation.** Greatest tested rate = 7 at 30 °C. A peak between tested temperatures is possible.
3. **Test recovery.** Restore cooled/heated samples to common suitable conditions with fresh substrate and an untreated control. Smaller temperature intervals and repeats refine the peak estimate.

Finite-interval means and an initial tangent differ

1. **Invented product volumes.** At 0/20/40/60/80 s: 0/12/21/27/30 mL.
2. **Mean rates.** First interval $12/20 = 0.60$ mL/s; whole period $30/80 = 0.375$ mL/s.
3. **Initial rate.** Estimate a tangent near time zero. Neither finite-interval mean is automatically that exact gradient; volume itself is not rate.

Reciprocal time needs a fixed amount of change

1. **Same endpoint.** 40 s $\rightarrow$ 0.025 per second; 20 s $\rightarrow$ 0.050 per second.
2. **Comparison.** The second proxy is twice the first when endpoint amount/criterion and starting conditions match.
3. **Units.** 1/time is a relative rate proxy, not a measured mL/s. A known product amount is needed to obtain an amount-per-time rate.

Use iodine on withdrawn samples, not in the reacting mixture

A temperature investigation needs controlled pH and timing

Equilibrate separate measured amylase and starch solutions; buffer pH; mix and start consistently. Take equal small samples onto separate iodine spots.

Use the same criterion, concentrations and volumes; fresh mixtures, an appropriate no-active-enzyme control and replicates are needed.

Iodine in the reaction can alter conditions. Follow approved reagent/hot-water procedures and consider treatment order or drift.

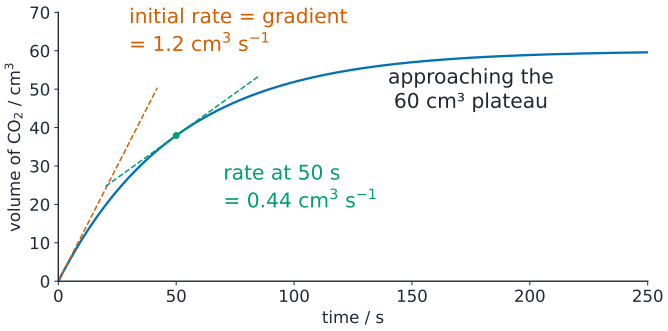
Discrete sampling locates an interval, not an exact endpoint

1. **Observed.** Last blue-black sample: 50 s. First negative sample: 60 s.
2. **Inference.** Disappearance occurred after 50 s and no later than 60 s. Reporting exactly 60 s overstates timing resolution.
3. **Improvement.** Shorter intervals improve resolution but repeated withdrawals can alter volume. Minimize sample sizes or use a suitable separate-tube design.

Match the instrument to the quantity

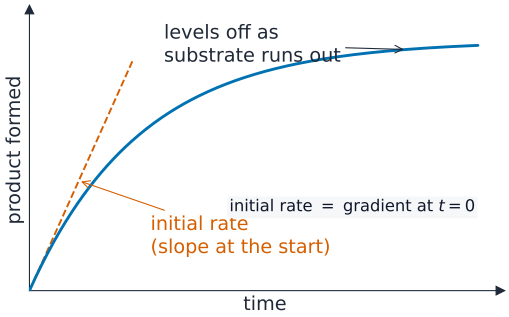
Assay	Controls and interpretation
Gas collection	Measure collected volume, time and leaks; do not assume equal bubble volumes
Colorimetry	Use an appropriate blank and calibration before converting absorbance to concentration
Initial-rate comparison	Match enzyme amount, substrate starting concentration, total volume, pH and temperature as relevant
Repeated readings	Instrument resolution and biological variation are different; retain units and measurement interval

Reading a rate from a curve



The rate falls as substrate is used up

Why use the initial rate?



Conditions are known exactly then

Inside an organism is not necessarily inside a cell

Example	Location and consequence
Glycolysis	Intracellular: cytosol
Krebs cycle in eukaryotes	Intracellular: mainly mitochondrial matrix
Gut-lumen digestion	Extracellular to the secreting cells; food hydrolysis precedes absorption
Metabolic heat	Transfers retain less than all input as specified useful energy; birds and mammals use metabolic heat in temperature maintenance

State the boundary of an efficiency model

1. **Invented accounting.** 200 kJ enters; 70 kJ retained as specified useful output; all remainder becomes heat.
2. **Calculate.** Efficiency $70/200 \times 100 = 35\%$; heat 130 kJ.
3. **Limit.** Other output/stored terms would change this balance. It is not a universal ATP yield; enzymes do not change the net energy balance by accelerating a reaction.

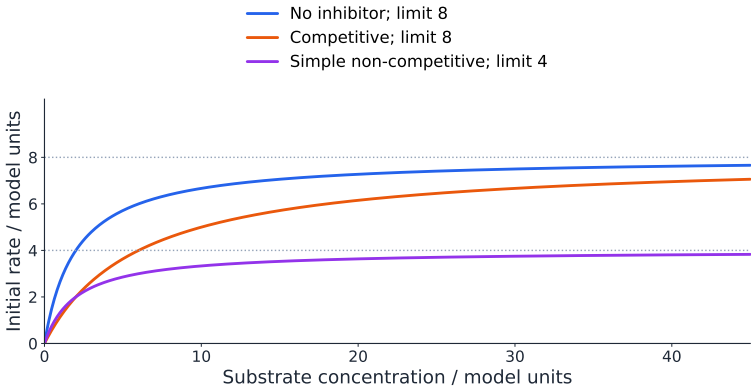
Regeneration of an acceptor is compatible with net output

Pathway	**Organization**
Glycolysis	Linear sequence: glucose $\rightarrow$ intermediates $\rightarrow$ pyruvate; different enzymes catalyse steps
Krebs cycle	Acetyl input and carbon dioxide output while oxaloacetate is regenerated
Calvin cycle	Carbon dioxide incorporation and product withdrawal while ribulose bisphosphate is regenerated
Limits	Cycles require inputs and can produce outputs; entering atoms need not remain forever

A block alters supply as well as accumulation

1. **Linear model.** A $\rightarrow$ B $\rightarrow$ C $\rightarrow$ D; each step has its own enzyme.
2. **Block B $\rightarrow$ C.** Downstream supply can fall and B can accumulate while upstream input continues.
3. **Qualify.** Branches, transport and feedback can modify this simple prediction; the network is more than unrelated reactions.

Compare recovery by high substrate in simple models



Only the competitive model approaches the original maximum.

Binding mechanism predicts a concentration response

Model	Mechanism and named example
Competitive	Reversible active-site binding competes with substrate; high substrate reduces its effect
Statins	Competitive inhibition of HMG-CoA reductase reduces mevalonate production in cholesterol synthesis
Simple non-competitive	Specific allosteric binding changes effective activity; extra substrate cannot restore the original maximum
Scope	Allosteric regulators can also activate; real systems need not match the simplest kinetic model

One concentration cannot identify the model

1. **At substrate 2 units.** No inhibitor / competitive / simple non-competitive rates $= 4 / 2 / 2$.
2. **At substrate 18 units.** Rates $= 7.2 / 6 / 3.6$; the high-substrate pattern helps distinguish the models.
3. **Evidence.** Use a concentration series and binding/recovery evidence. Two rates do not prove the binding site, reversibility or a unique mechanism.

Isoleucine regulates an early step in its own synthesis

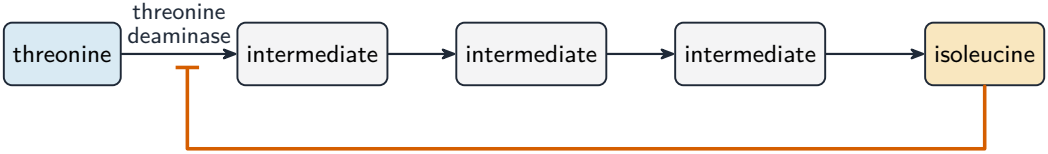
An inhibitory arrow is not a reverse matter pathway

Threonine deaminase catalyses an early step from threonine in bacterial isoleucine synthesis. Isoleucine binds a regulatory site and reduces pathway entry.

As product is used and concentration falls, reversible inhibition is relieved. This limits waste of substrates and energy.

Draw forward intermediate arrows and a separate inhibitory connection back to the early enzyme. Product need not compete at the substrate active site or inhibit every enzyme.

End-product inhibition



> Supply matches demand

Penicillin inactivates a wall-cross-linking target

Covalent modification differs from reversible competition

Reaction forms a stable covalent modification at an active-site serine of bacterial transpeptidases, reducing peptidoglycan cross-linking.

Extra substrate does not quickly remove the modification. The target concerns growing cell-wall construction, not direct digestion of every membrane.

Altered transpeptidases can retain function while binding drug less effectively. Beta-lactamase drug destruction is another route; individual enzymes do not learn resistance.

Attempt the handout questions before checking

Use mechanisms, quantities and evidence together

Explain catalysis, folding, induced fit, motion and metabolic classifications.

Calculate barriers, product-time gradients and reciprocal-time comparisons; evaluate an endpoint investigation.

Explain temperature/pH responses and substrate saturation. Keep units, controls, measurement limits and model conditions.

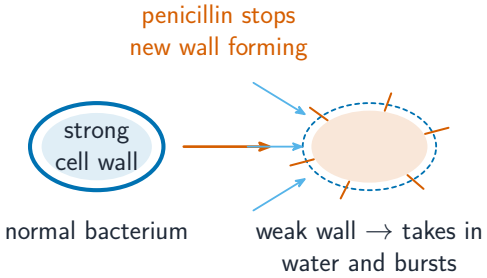
Check the common quantitative answers

1. **Energy levels 30 → 45.** Peaks 95 and 60 give barriers 65 and 30; overall $+15$ remains unchanged.
2. **Product measurements.** First interval $9/15 = 0.60$ mL/s; whole $21/60 = 0.35$ mL/s. A tangent at zero differs.
3. **Endpoints 30 and 75 s.** Reciprocals about 0.0333 and 0.0133 per second; first proxy is 2.5 times the second. Final positive at 40 and first negative at 50 locate (40, 50] s.

AHL answers: efficiency, inhibition and target changes

1. **Accounting.** $90/300 = 30\%$ useful; 210 kJ heat under the specified boundary.
2. **Reversible control.** A fits competitive; B simple non-competitive. Statins target HMG-CoA reductase; isoleucine regulates threonine deaminase.
3. **Mechanism-based inhibition.** Penicillin covalently modifies a transpeptidase active site. Reduced-affinity target variants can retain function; beta-lactamases instead destroy drug.

How penicillin kills bacteria



The bacterium bursts

Check yourself

Name what the evidence measures before explaining it

A substrate plateau is not evidence of denaturation. A tested peak is not an exact universal optimum.

A product volume is not rate; reciprocal time is not volume per time. Repeated endpoint sampling gives a time interval.

Regeneration does not mean rigidity, and a catalyst does not change the overall energy balance or equilibrium.