

## Binding energy

The substrate binds to the active site of an enzyme by multiple weak non-covalent interactions. The free energy released in the formation of a large no. of weak interactions between the enzyme & the substrate is termed binding energy. This binding energy is used to lower the activation energy.

## Different approaches

### → Entropy reduction

Benefit of binding substrate to enzyme is a large restriction in the relative motions of 2 substrates that are to react. Substrates can be precisely aligned on the enzyme, with many weak interactions bet<sup>n</sup> each substrate & strategically located groups on the enzyme clamping the substrate molecules into the proper position.

### → Desolvation

Formation of weak bonds bet<sup>n</sup> substrate & enzyme results in desolvation of substrate. The solvation shell of hydrogen bonded water that surrounds & helps to stabilize most biomolecules in aqueous sol<sup>n</sup> is removed by desolvation. Enzyme substrate interactions replace most or all of the hydrogen bonds bet<sup>n</sup> the substrate & water.

→

Distortion of substrate

Binding energy, involving weak interaction formed only in the reaction transition state helps to compensate thermodynamically for any distortion, primarily electron redistribution, that the substrate must undergo to react.

→

Microenvironment

Some enzymes create an environment inside the active site that's favorable to the reaction

→

Direct participation

Some enzymes lower activation energies by taking part in the chemical reaction themselves. That is, active site residues may form temporary covalent bonds with substrate molecules as part of the reaction process.



## Factors affecting enzyme activity

### → Temperature

- Increasing temperature increases the kinetic energy that molecules possess, in a fluid, this means that there are more random collisions bet<sup>n</sup> molecules per unit time.
- Since enzymes catalyze reactions by randomly colliding with substrate molecules, increasing temperature increases the rate of reaction forming more product.
- However, increasing temperature also increases the vibrational energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.
- As temperature increases, more bonds, specially the weaker hydrogen & ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the active site to change shape.
- This change in shape means that the active site is less complementary to the shape of the substrate, so that it is <sup>less</sup> likely to ~~to~~ catalyze the reaction. Eventually the enzyme will become denatured & will no longer function.

- As temperature increases, active site shapes of more enzymes will be less complementary to the shape of their substrate & more enzymes will be denatured. This will decrease the rate of react<sup>n</sup>.

- In summary, as temp. increases, initially the rate of reaction will increase because of increased kinetic energy. However the effect of bond breaking will become greater & the rate of react<sup>n</sup> will begin to decrease.

- The temperature at which the maximum rate of react<sup>n</sup> occurs is called the enzyme's optimum temperature. This is different for different enzymes. Most enzymes in the human body have an optimum temp of around 37°C.

→ pH

- pH measures the acidity & basicity of a solution. It is a measure of the hydrogen ion (H<sup>+</sup>) conc<sup>n</sup>, & therefore a good indicator of the hydroxide ion (OH<sup>-</sup>) concentration. It ranges from pH 1 to pH 14. Lower pH values mean higher H<sup>+</sup> concentrations & lower OH<sup>-</sup> concentrations.

- H<sup>+</sup> & OH<sup>-</sup> ions are charged & therefore interfere with hydrogen & ionic bonds that hold together an enzyme since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme & importantly, its active site.



- Different enzymes have different optimum  $p^H$  values. This is the  $p^H$  value at which the bonds within them are influenced by  $H^+$  &  $OH^-$  ions such that the shape of their active site is most complementary to the shape of their substrate. At the optimum  $p^H$ , the rate of  $cat^{\Delta}$  is at an optimum.
- Any change in  $p^H$  above or below the optimum will quickly cause a decrease in the rate of  $cat^{\Delta}$  since more of the enzyme molecules will have active sites whose shapes are not (or at least are less) complementary to the shape of their substrate.
- Small changes in  $p^H$  above or below the optimum do not cause a permanent change to the enzymes since the bonds can be reformed. However extreme changes in  $p^H$  can cause enzymes to denature & permanently lose their function.
- Enzymes in different locations have different optimum  $p^H$  values since their environmental cond<sup>ns</sup> may be different. For example the enzyme pepsin functions best at around  $p^H$  2 & is found in the stomach, which contains hydrochloric acid ( $p^H$  2).

→ Concentration

- Changing the enzyme & substrate conc<sup>n</sup> affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity & so its metabolism.
- Changing the conc<sup>n</sup> of a substance only affects the rate of rxn<sup>n</sup> if it is the limiting factor. i.e. it is the factor that is stopping a rxn<sup>n</sup> from proceeding at a higher rate.
- If it is the limiting factor, increasing conc<sup>n</sup> will increase the rate of rxn<sup>n</sup> upto a point, after which any increase will not affect the rate of rxn<sup>n</sup>. This is because it will no longer be the limiting factor & another factor will be limiting the max<sup>m</sup> rate of rxn<sup>n</sup>.
- As a rxn<sup>n</sup> proceeds, the rate of rxn<sup>n</sup> will decrease, since the substrate will get used up.

→ Substrate concentration

- Increasing substrate conc<sup>n</sup> increases the rate of rxn<sup>n</sup>. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain conc<sup>n</sup> any increase will have no effect on the rate of rxn<sup>n</sup>, since substrate conc<sup>n</sup> will no longer be the limiting factor.



\_ / \_ / \_

The enzymes will effectively become saturated & will be working at their max<sup>m</sup> possible rate.

### → Enzyme concentration

- Increasing enzyme conc<sup>n</sup> will increase the rate of rxn<sup>n</sup> since more enzyme will be colliding with substrate molecules.
- However this too will only have an effect up to a certain conc<sup>n</sup>, where the enzyme conc<sup>n</sup> is no longer the limiting factor.