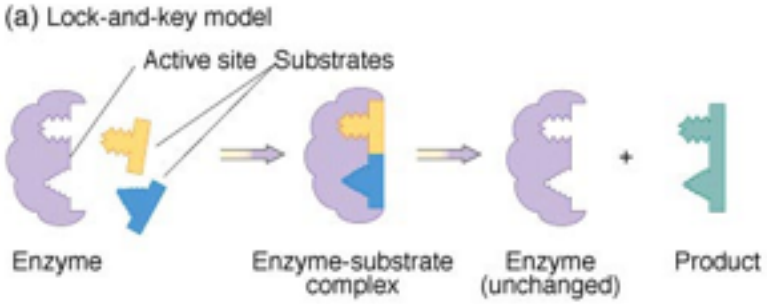
- the region where the substrate binds
- steric relationship with substrate (complementary shape and complementary charges)
- site of catalytic activity

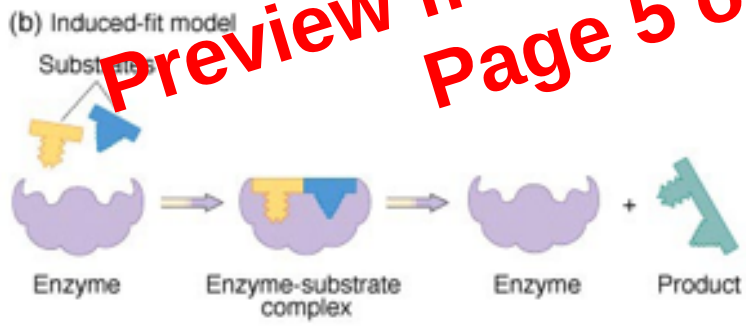
- The substrate molecules attach to the active site of the enzyme to form an **enzyme-substrate complex**. This complex only exists for only a fraction of a second.

'Lock and Key' hypothesis



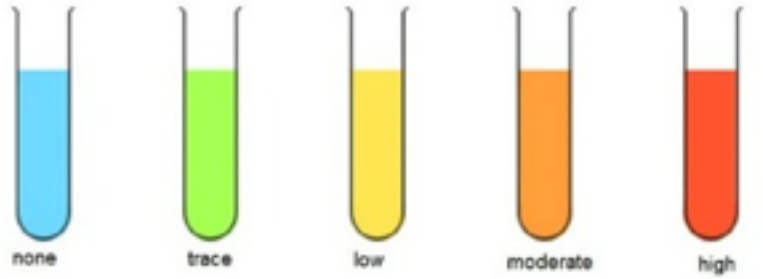
- Enzyme = lock; Substrates = key
- This theory supposes that the shape of the substrate is directly complementary to the shape of the active site. i.e. only substrates with the exact shape will fit into the enzyme and undergo catalysis

'Induced Fit' theory



- Observations show that not all enzymes have permanent active sites; they only develop when they come into close proximity the substrates.
- Before substrate binding, the active site is **relaxed**. When the substrate binds, a small change occurs in the structure of the enzyme and the active site is pulled into the correct shape by the molecular interactions between the two molecules and an enzyme-substrate complex is formed.
- This can happen because the bonds between the amino acids of the active site are relatively flexible.
- After the products are released, the enzyme molecule becomes relaxed again.

- The Benedict's test is semi-quantitative as different concentrations of reducing sugars will produce different colours which can be used to estimate the amount of reducing sugar in a sample.



Non-reducing sugars

- To detect a non-reducing sugar, it must be broken into its monosaccharide components by hydrolysis.
- Test:
  1. Add 2cm³ of sample (liquid form or grind it up in water) into a test tube
  2. Add 2cm³ of Benedict's reagent to the sample
  3. Heat the mixture gently in a boiling water bath for 5 minutes
  4. If the Benedict's reagent doesn't change colour, then a reducing sugar is not present
  5. Add another 2cm³ of the food sample and 2cm³ of dilute hydrochloric acid into a test tube and place it into a gently boiling water bath for 5 minutes. The HCL will hydrolyse any disaccharides into monosaccharides.
  6. Add sodium hydroxide to solution to neutralise the hydrochloric acid
  7. Heat solution with 2cm³ of Benedict's reagent in a gently boiling water bath for 5 minutes.
  8. If a non-reducing sugar was present in the original sample, the Benedict's reagent will now turn from blue to a brick red precipitate.

This is due to the reducing sugars produced by the hydrolysis of the non-reducing sugar.