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Enzyme Kinetics

Enzyme Catalysis

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Outline: Enzyme catalysis

- enzymes and non-bonding interactions (review)
- catalysis (review - see section 9.2 of A&D)
  - general principles of catalysis
  - differential binding
  - types of catalysis
    - approximation
    - electrostatic
    - covalent
    - acid-base catalysis
    - strain and distortion
- enzyme catalysis and energy diagrams

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Enzymes

- proteins that play functional biological roles
- responsible for the catalysis of nearly all chemical reactions that take place in living organisms
  - acceleration of reactions by factors of 10^6 to 10^{17}
- biological catalysts that bind and catalyse the transformation of substrates
- the three-dimensional structures of many enzymes have been solved (through X-ray crystallography)

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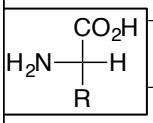
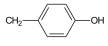
Reminder: Amino acid structures

- as proteins, enzymes are polymers of amino acids whose side chains interact with bound ligands (substrates)



R	Name	3 Letter Code	1 Letter Code
H	Glycine	Gly	G
CH_3	Alanine	Ala	A
$CH(CH_3)_2$	Valine	Val	V
$CH_2CH(CH_3)_2$	Leucine	Leu	L
$CHCH_2CH_3$ CH_3	Isoleucine	Ile	I
	Phenylalanine	Phe	F
	Proline	Pro	P
CH_2OH	Serine	Ser	S
$CHOH$ CH_3	Threonine	Thr	T
	Tyrosine	Tyr	Y

R	Name	3 Letter Code	1 Letter Code
CH_2SH	Cysteine	Cys	C
$CH_2CH_2SCH_3$	Methionine	Met	M
$(CH_2)_4NH_2$	Lysine	Lys	K
$(CH_2)_3NHCNH_2$ NH	Arginine	Arg	R
	Histidine	His	H
	Tryptophan	Trp	W
CH_2COH O	Aspartic acid	Asp	D
CH_2CNH_2 O	Asparagine	Asn	N
CH_2CH_2COH O	Glutamic acid	Glu	E
$CH_2CH_2CNH_2$ O	Glutamine	Gln	Q

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Coenzymes and cofactors

- indispensable for the activity of some enzymes
- can regulate enzymatic activity
- the active enzyme-cofactor complex is called a *haloenzyme*
- an enzyme without its cofactor is called an *apoenzyme*

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Cofactors

- metal ions (Mg^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, etc.)
- three possible modes of action:
  1. primary catalytic centre
  2. facilitate substrate binding (through coordination bonding)
  3. stabilise the three-dimensional conformation of an enzyme

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Coenzymes

- organic molecules, very often vitamins
  - e.g.: nicotinic acid gives NAD; pantothenic acid gives CoA
- intermediates in the transport of functional groups
  - e.g. H (NAD), acyl (CoA), CO_2 (biotin), etc
- also known as *prosthetic groups*

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Protein-ligand interactions

- covalent bonds
- ionic bonds
- ion-dipole and dipole-dipole interactions
- hydrogen bonds
- charge transfer complexes
- hydrophobic interactions
- van der Waals interactions

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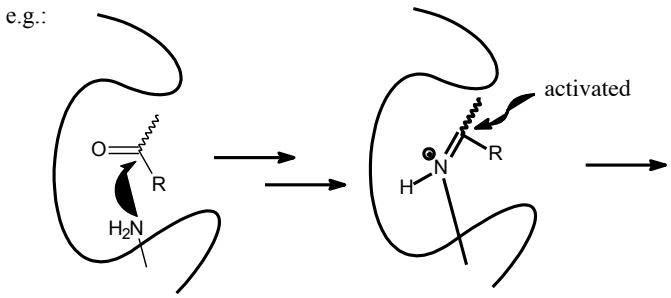
Covalent bond

- the formation of a covalent bond can represent a stabilisation of 40 to 110 kcal/mol



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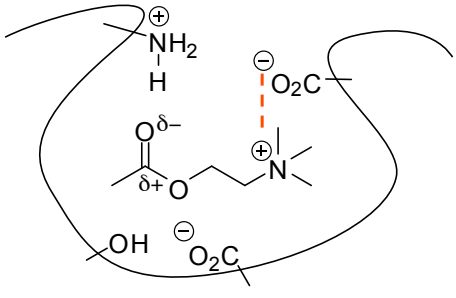
Ionic bonds

- Coulombic attraction between full positive and negative charges
  - ~5 kcal/mol of stabilisation



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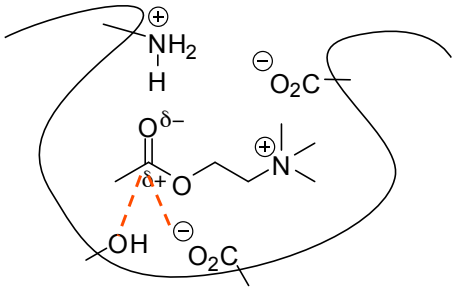
Ion-dipole and dipole-dipole interactions

- electrostatic interactions that involve partial charges
  - ~1 kcal/mol of stabilisation



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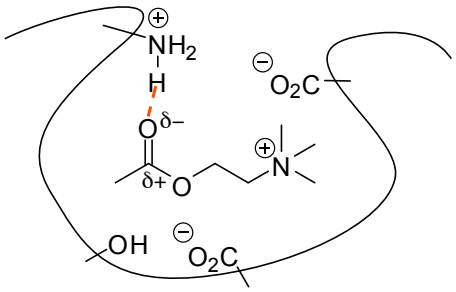
Hydrogen bonds

- special type of dipole-dipole interaction
  - donors / acceptors : N, O, F
  - stabilisation of around 3-10 kcal/mol



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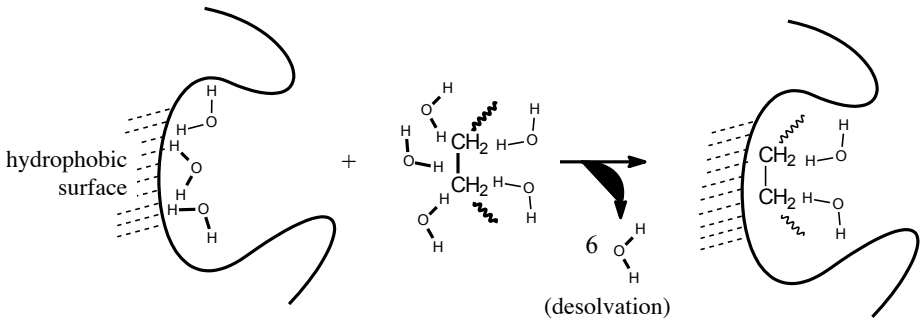
Charge transfer complex

- special type of dipole-dipole interaction
- involves π electrons, often in aromatic rings (Phe, Tyr, Trp, His)
  - stabilisation : < 3 kcal/mol

Hydrophobic interactions

- stabilisation largely due to desolvatation (entropy increase)
  - stabilisation : ~0.5 kcal/mol



van der Waals interactions

- special type of dipole-dipole interaction
  - movement of electrons in electron cloud of alkyl chains induces the formation of temporary dipoles
  - very important over short distances
  - stabilisation : ~0.5 kcal/mol (per interaction)

Outline: General principles of catalysis

- see section 9.1 of A&D
  - principles of catalysis
  - differential bonding

General principles

- a **catalyst** accelerates a reaction without being consumed
- the rate of catalysis is given by the **turnover number**
- a reaction may alternatively be "**promoted**" (accelerated, rather than catalysed) by an **additive** that is consumed
- a **heterogeneous** catalyst is not dissolved in solution; catalysis typically takes place on its surface
- a **homogeneous** catalyst is dissolved in solution, where catalysis takes place
- all catalysis is due to a **decrease in the activation barrier, $\Delta G^{\ddagger}$**

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Catalysts

- efficient at low concentrations
  - e.g. $[Enz]_{cell} << 10^{-5}$ M; $[Substrates]_{cell} < 10^{-4} - 10^{-5}$ M
- not consumed during the reaction
  - e.g. each enzyme molecule can catalyse the transformation of 20 - 36 x 10^6 molecules of substrate per minute
- do not affect the equilibrium of reversible chemical reactions
  - only accelerate the rate of approach to equilibrium end point
- most chemical catalysts operate in extreme reaction conditions while enzymes generally operate under mild conditions (10° - 50 °C, neutral pH)
- enzymes are specific to a reaction and to substrates; chemical catalysts are far less selective

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Catalysis and free energy

- catalysis accelerates a reaction by stabilising a TS relative to the ground state
  - free energy of activation, $\Delta G^{\ddagger}$, *decreases*
  - rate constant, *k*, *increases*
- catalysis does not affect the end point of an equilibrium, but only accelerates how quickly equilibrium is attained
  - free energy of the reaction, ΔG°, *remains unchanged*
  - equilibrium constant, K_{eq}, *remains unchanged*

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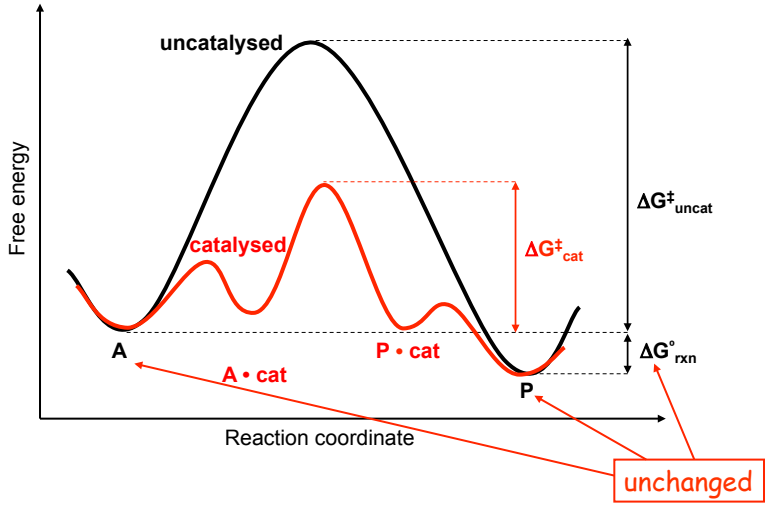
Energy profile of catalysis



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Transition state binding

- interaction between a catalyst and reactant or activated complex can stabilise one or the other
- if the activated complex is bound more strongly than the substrate, the activation barrier will be decreased
- HOWEVER, the activated complex is not a molecule – so the catalysts must first of all interact with the substrate, and then release the product at the end of the reaction :

$$A + cat \rightleftharpoons A{\bullet}cat \rightleftharpoons P{\bullet}cat \rightleftharpoons P + cat$$

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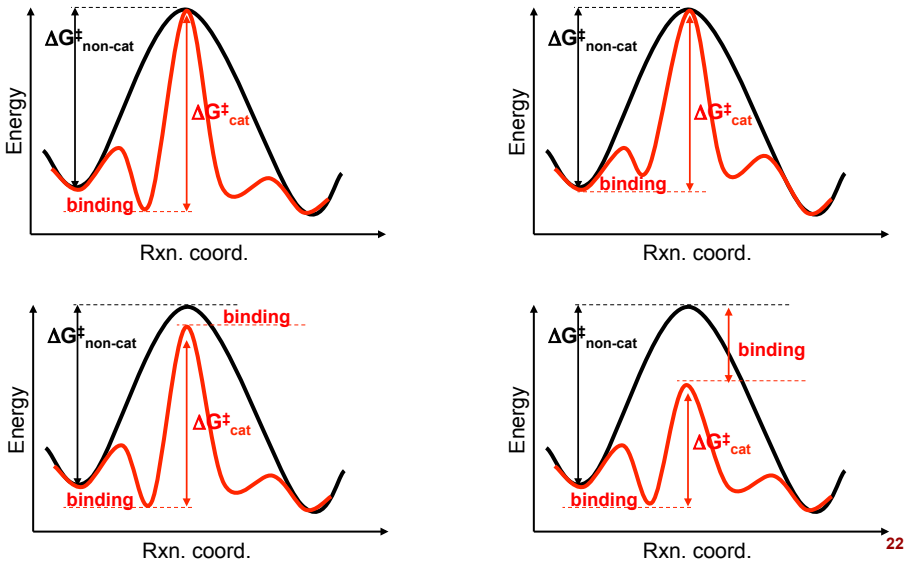
Differential binding

- consider 4 scenarios :



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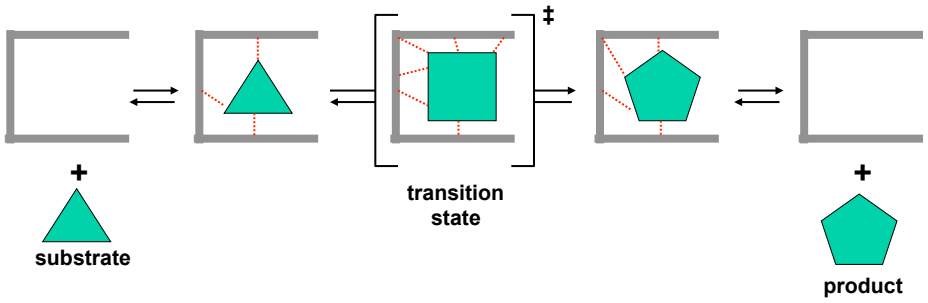
Differential binding

- to accelerate a reaction, a catalyst must stabilise the TS more than it stabilises the substrate
  - even if this stabilisation takes place over less time than that of a bond vibration, by definition



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Outline: Types of catalysis

- see section 9.2 of A&D
  - approximation
  - electrostatic
  - covalent
  - strain and distortion

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Catalysis by approximation

- the catalyst *brings together* the reactants, increasing their *effective concentrations*, and *orients* them with respect to the reactive groups

Jencks :

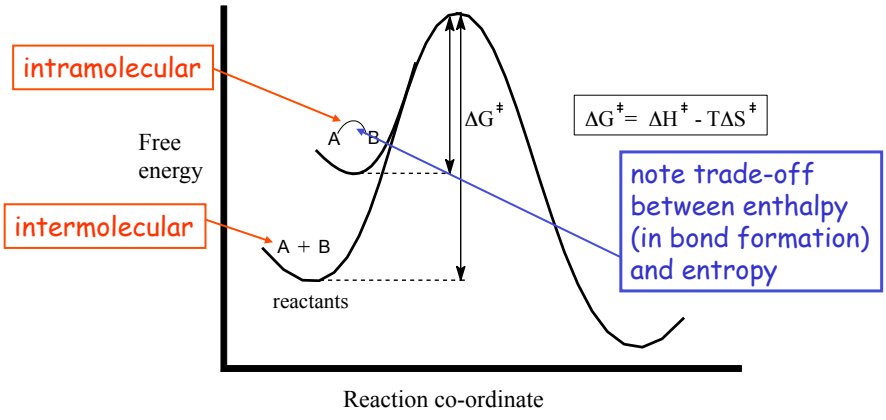
- the loss of entropy associated with the restriction of rotation and translation of substrate must be compensated by the *intrinsic energy of binding* (favourable non-bonding interactions)

Bruice / Kirby :

- the magnitude of this effect is given by the *effective concentration*, determined by comparison if the rate constants of the bimolecular and intramolecular reactions

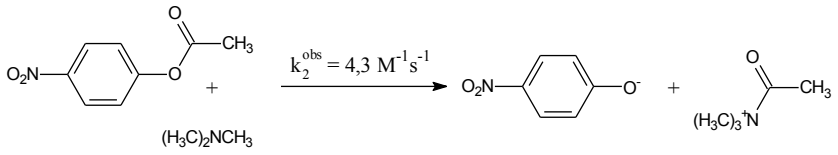
Intramolecular approximation

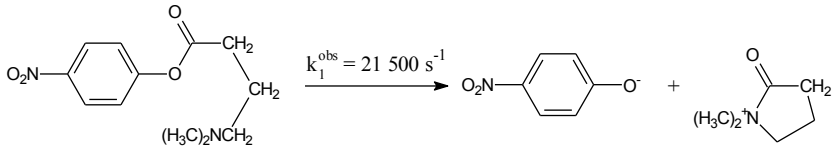
- an intramolecular reaction implies a smaller decrease in entropy (and therefore a decrease in the free energy of activation)



Example of catalysis by approximation

- the catalyst *brings together* the reactants, increasing their *effective concentrations*, and *orients* them with respect to the reactive groups

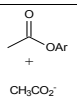
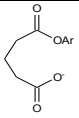
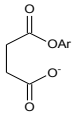
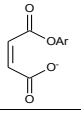
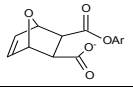




$$\frac{k_1^{obs}}{k_2^{obs}} = 5000\ \text{M} =$$ *effective concentration*, or *effective molarity (EM)*

Example: Ester hydrolysis

parent reaction

Reaction	k_{rel}	Effective concentration (M)
	1 $M^{-1}s^{-1}$	-
	220 s^{-1}	220 M
	5.1×10^4 s^{-1}	5.1×10^4 M
	2.3×10^6 s^{-1}	2.3×10^6 M
	1.2×10^7 s^{-1}	1.2×10^7 M

decreasing entropy of rotation and translation

More notions of catalysis by approximation

- many notions have been advanced by many different researchers, to describe the subtleties of catalysis by approximation:
  - *orbital steering*: the alignment of orbitals is proposed to accelerate the reaction
  - *stereopopulation control*: one reactive conformer among several is favoured
  - *near attack conformations*: conformations are favoured whose spatial orientation lead to the desired reaction

CAUTION: one must not forget the Curtin-Hammett principle!!

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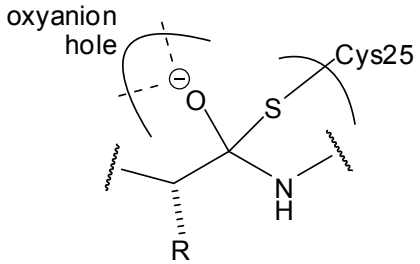
Electrostatic catalysis

- stabilisation of charge developed at TS
- for example, serine and cysteine proteases favour the formation of a tetrahedral intermediate by stabilising the negative charge developed on oxygen, in an *oxyanion hole*
  - e.g.: consider papain, a Cys protease



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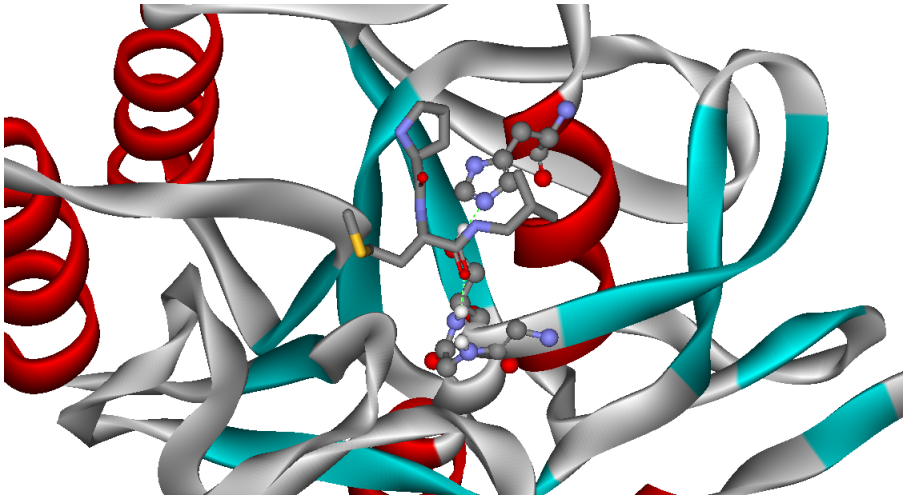
Electrostatic catalysis

- e.g.: oxyanion hole of subtilisin:



J. Mol. Biol. **1991**, *221*, 309-325.

Electrostatic catalysis

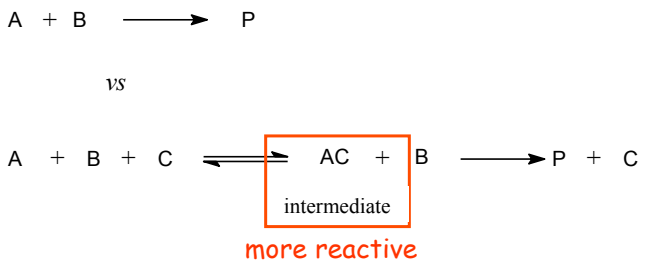
- can be very important :
  - consider the triple mutant of subtilisin where each residue of its catalytic triad is replaced (S221A-H64A-D32A)
    - catalyses proteolysis 10^6-fold less than the native enzyme
    - BUT the reaction with the mutant is still 10^3-fold faster than the uncatalysed reaction!!
    - an important part of catalysis is due to the electrostatic environment

Metal catalysis

- electrostatic charges developed at the TS can also be stabilised by metal ions
- coordination of a ligand by a metal (as a Lewis acid) can also lead to polarisation of a ligand
  - e.g. pK_a of metal-bound H_2O is 7.2, making it easier to deprotonate, therby generating ^-OH as a nucleophile
    - for example, zinc-bound water in carbonic anhydrase, a highly efficient metalloenzyme as well as certain enzyme models

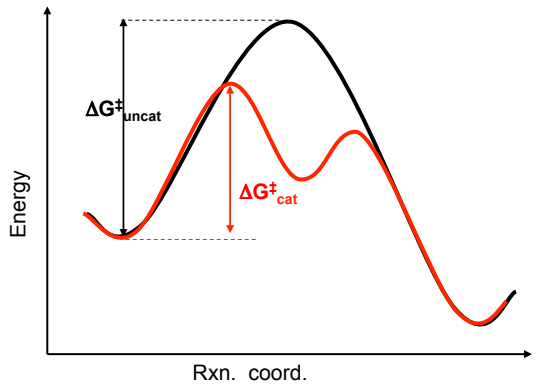
Covalent catalysis

- catalyst forms a covalent intermediate that reacts faster than uncatalysed reaction :



Covalent catalysis

- in order for catalysis to be efficient, the activation energy for formation of the intermediate and for its subsequent reaction must both be lower than that of the uncatalysed reaction :



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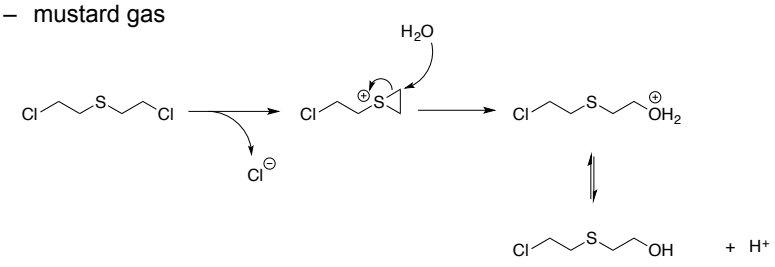
Covalent catalysis

- an example of non-enzymatic covalent catalysis and *anchimeric assistance* :
  - mustard gas



- enzymes use nucleophilic groups (e.g. Asp, Glu, Ser, Cys, Lys, His, Arg) and cofactors to form covalent bonds (*nucleophilic catalysis*)

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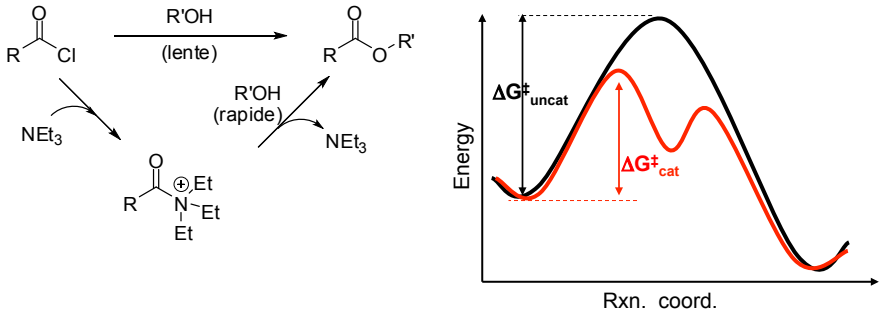
Nucleophilic catalysis

- catalyst attacks substrate to form intermediate that is even more susceptible to nucleophilic attack, by a second reactant
  - e.g. reaction of acid chlorides with alcohols, catalysed by addition of a tertiary amine:



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Acid-base catalysis

- the catalyst (namely an acid or a base) accelerates the reaction through *protonation* or *deprotonation*

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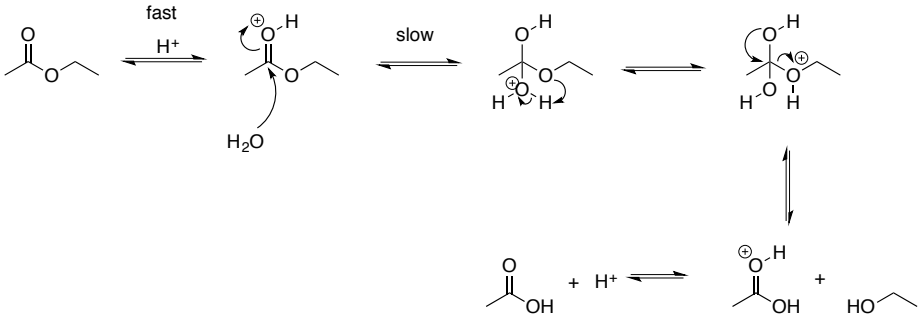
Specific acid-base catalysis

- catalysis by H^+ or ^-OH, controlled only by pH, where a *fast equilibrium* precedes the rls :
  - e.g.:



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Rate laws of specific acid-base catalysis

- when a substrate must be protonated *before* its reaction in the rls, this appears as a pH dependence in the rate law:
  - e.g.: $v = k[R]\times[H^+]/K_{a,RH}$

- when a substrate must be deprotonated *before* its reaction in the rls, this appears as a pH dependence in the rate law:
  - e.g.: $v = k[RH]\times K_{a,RH}/[H^+]$

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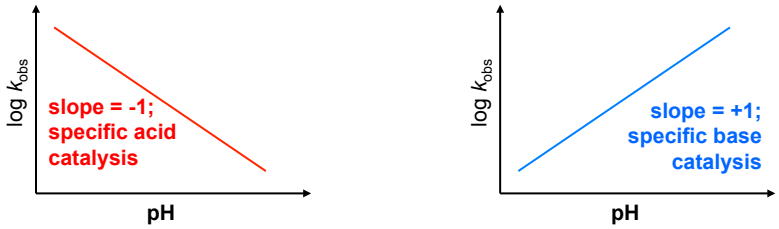
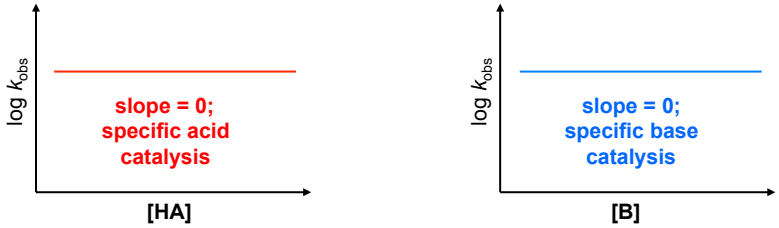
Specific acid-base catalysis plots

- pH dependence :



- at constant pH, independence of [HA] or [B] :



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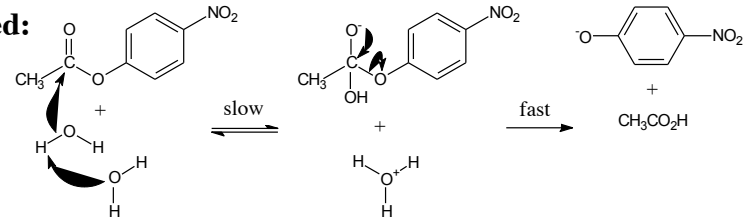
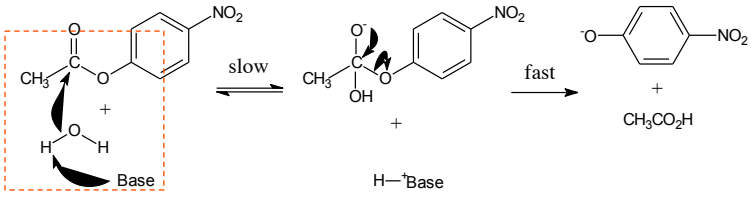
General acid catalysis

- catalysis by an acid or a base (not H^+ nor ^-OH) where a proton is transferred *during the rls*
  - rate is proportional to the concentration of acid or base, at constant pH

uncatalysed:



catalysed:



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Rate laws

- if a substrate is protonated *during* the rls, this appears as a dependence on [HA] in the rate law :
  - e.g.: $v = k[R]\times[HA]$ → $= k_{obs}[R]$ where $k_{obs} = k[HA]$
- if a substrate is deprotonated *during* the rls, this appears as a dependence on [B] in the rate law:
  - e.g.: $v = k[R]\times[B]$ → $= k_{obs}[R]$ where $k_{obs} = k[B]$

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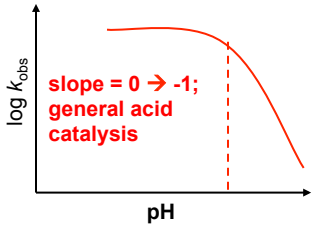
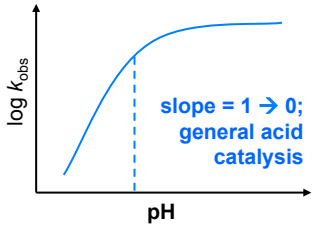
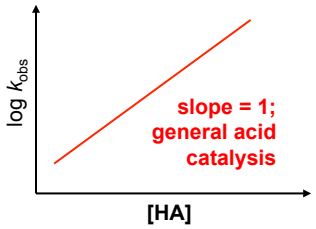
General acid-base catalysis plots

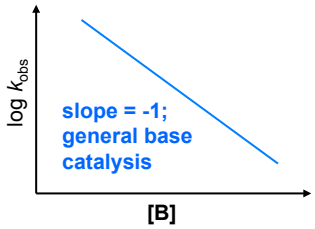
- pH dependence :





- at constant pH, dependence on [HA] or [B]:





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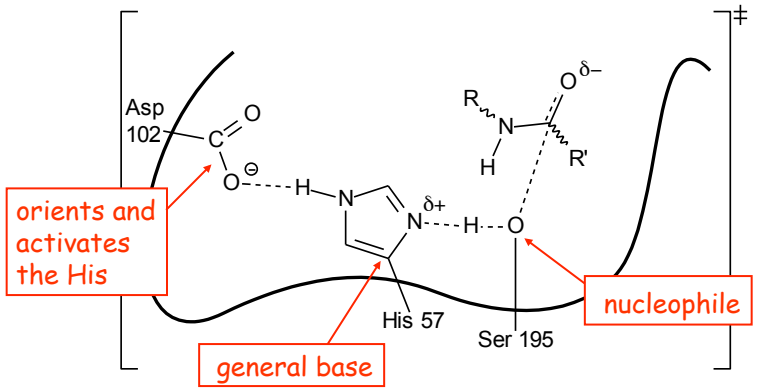
General acid-base catalysis

- very common in enzymes
  - e.g. chymotrypsin uses a catalytic triad whose His plays the role of a general base :



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Strain and distortion

- *destabilisation of the ground state* induced in the substrate ***or*** in the catalyst (such as an enzyme)

Koshland :
- *induced complementarity* hypothesis: the approach of substrate serves to provoke a conformational change in the enzyme, to adopt a form that better binds the substrate, but in a higher energy (strained) form and/or to better orient reactive groups (*"orbital steering"*)
- the substrate can also be deformed to adopt a strained form

Jencks :
- strain and distortion in the substrate are *essential* for the catalysis
- TSs are stabilised, rather than E•S and E•P complexes (so as not to form overly stable intermediates)
- *binding energy must therefore be used to destabilise* the E•S and E•P complexes

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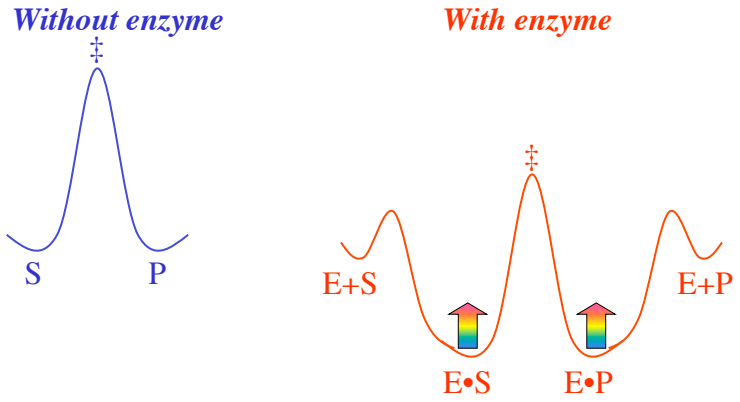
Strain and distortion

- *binding energy is used to destabilise* the E•S and E•P complexes



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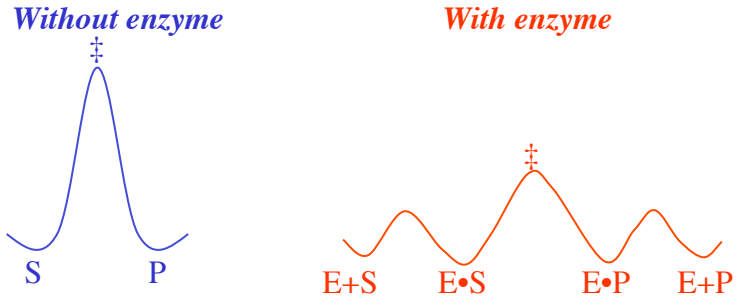
Strain and distortion

- *binding energy is used to destabilise* the E•S and E•P complexes



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Productive strain

- in order for a reaction to be facilitated by strain, two conditions must be met:

1. the strain must be along the reaction pathway
   - strain "pushes" the reactants towards the TS

2. the strain must be at least partly alleviated at the TS
   - if the strain were still present at the TS, it would not contribute to catalysis

Enzymes as catalysts

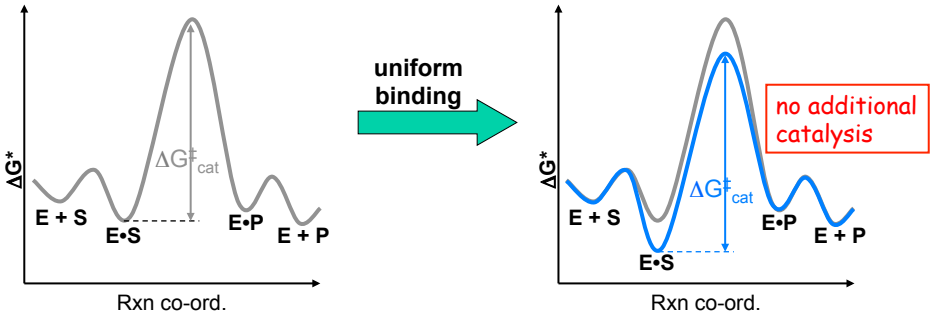
Jencks :
- enzymes use *binding* energy to effect catalysis

Wolfenden :
- reaction acceleration is proportional to the affinity of an enzyme for the *transition state* of the catalysed reaction
- the reaction rate is proportional to the concentration of *substrate in the activated complex* at the TS
- substrate affinity is therefore also important and enzymes use *protein conformational changes* during the reaction to better *stabilise* the TS

Knowles :
- often the various steps of an enzymatic reaction are stabilised so as to *level* the energies of the various ground states and TSs

Energy diagrams

- consider energy profiles for enzymatic reactions, at the native concentration of substrates:
- for K_M << [S] (binding of substrate heavily favoured)
  - the *uniform* binding of substrate and activated complex would not lead to catalysis :



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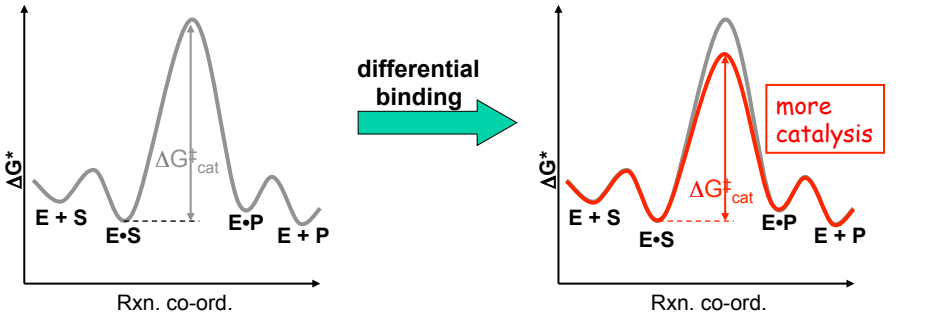
Energy diagrams

- consider energy profiles for enzymatic reactions, at the native concentration of substrates:
- for K_M << [S] (binding of substrate heavily favoured)
  - the *differential* binding of substrate and activated complex can lead to catalysis :



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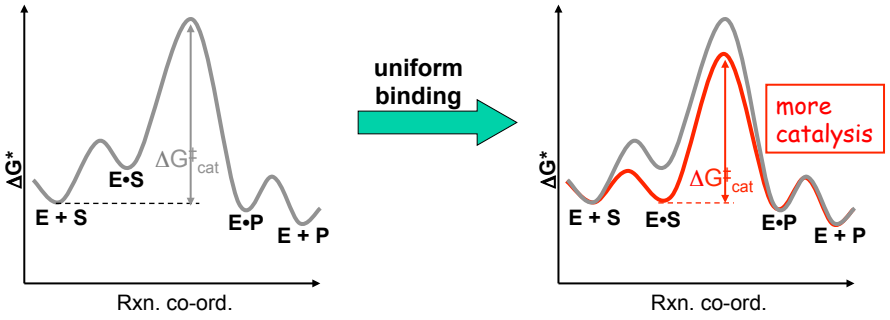
Energy diagrams

- consider energy profiles for enzymatic reactions, at the native concentration of substrates:
- for K_M > [S] (binding of substrate *not* favoured)
  - the *uniform* binding of substrate and activated complex can lead to catalysis :



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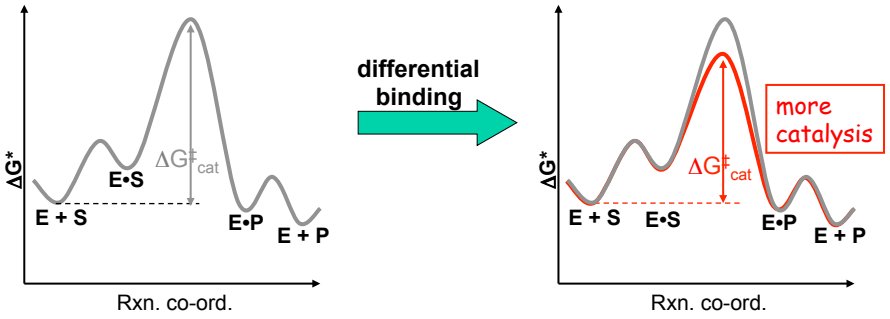
Energy diagrams

- consider energy profiles for enzymatic reactions, at the native concentration of substrates:
- for K_M > [S]
  - the *differential* binding of substrate and activated complex can lead to catalysis :



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