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Physical Organic Chemistry

Catalysis

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Outline: General principles of catalysis

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

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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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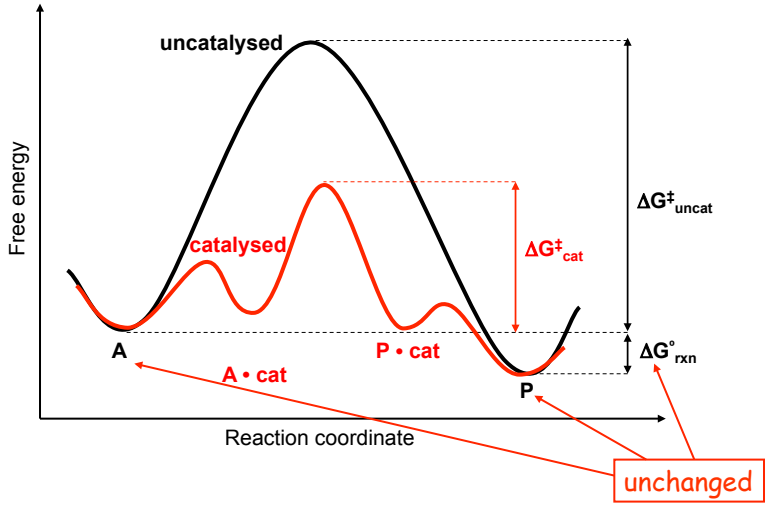
Energy profile of catalysis



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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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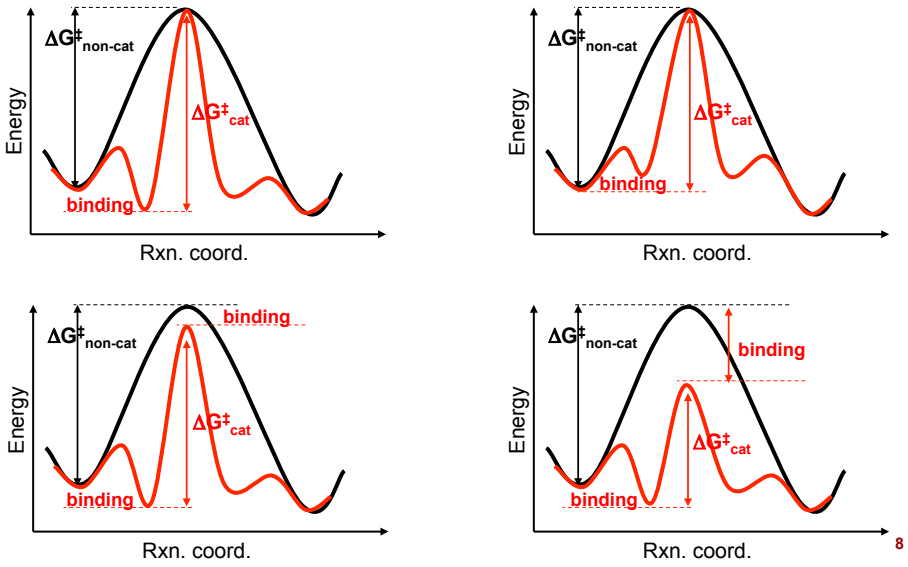
Differential binding

- consider 4 scenarios :



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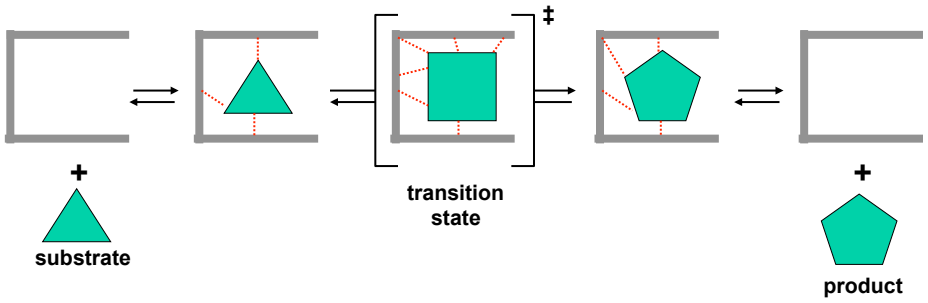
Differential binding

- 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 :

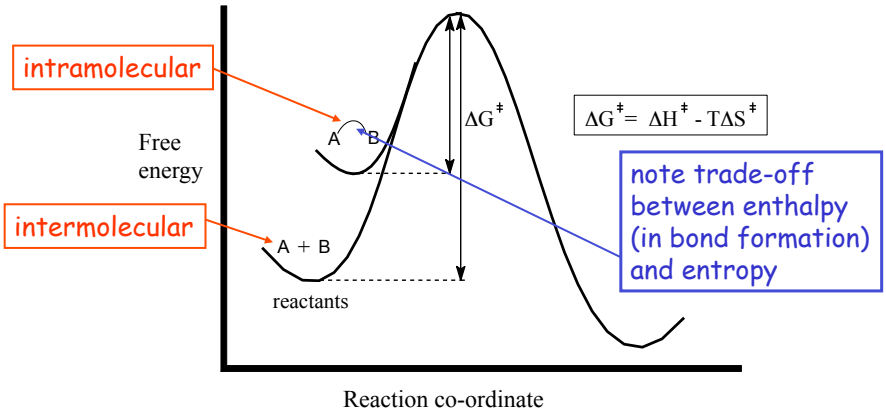
- the loss of entropy associated with the restriction of rotation and translation of substrate must be compensated by the *intrinsic energy of binding* (favourable non-bonding interactions)

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

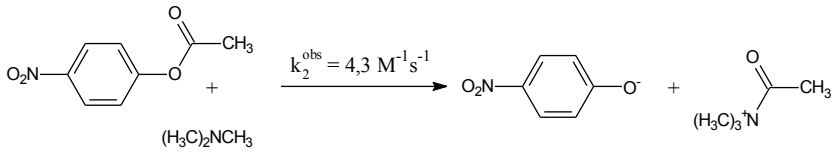
Intramolecular approximation

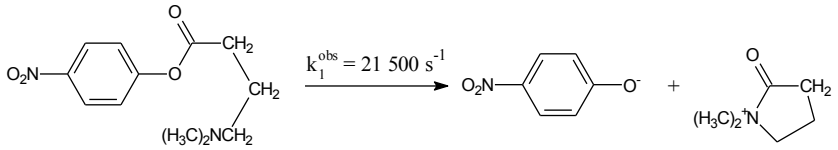
- an intramolecular reaction implies a smaller decrease in entropy (and therefore a decrease in the free energy of activation)



Example of catalysis by approximation

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

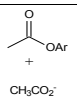
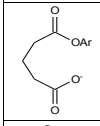
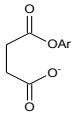
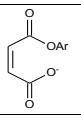
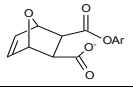




$$\frac{k_1^{obs}}{k_2^{obs}} = 5000\ \text{M} =$$ *effective concentration*, or *effective molarity (EM)*

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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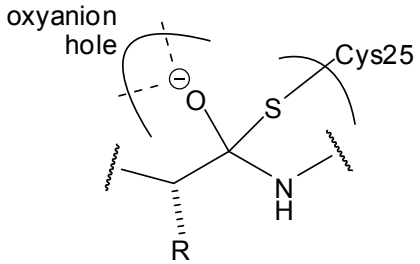
Electrostatic catalysis

- 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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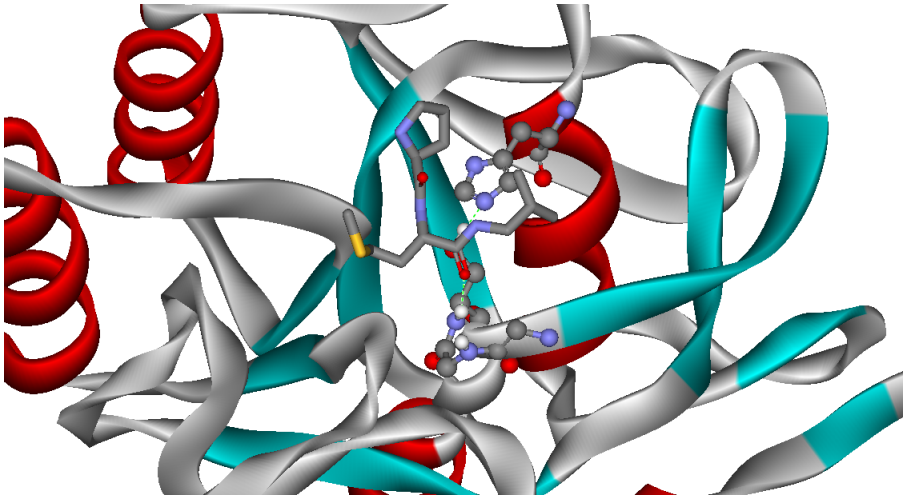
Electrostatic catalysis

- e.g.: oxyanion hole of subtilisin:



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

Electrostatic catalysis

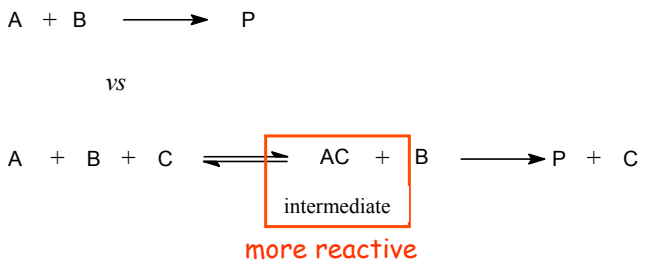
- can be very important :
  - consider the triple mutant of subtilisin where each residue of its catalytic triad is replaced (S221A-H64A-D32A)
    - catalyses proteolysis 10^6-fold less than the native enzyme
    - BUT the reaction with the mutant is still 10^3-fold faster than the uncatalysed reaction!!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

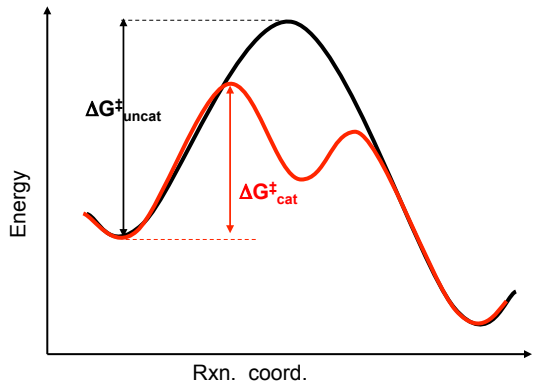
Covalent catalysis

- 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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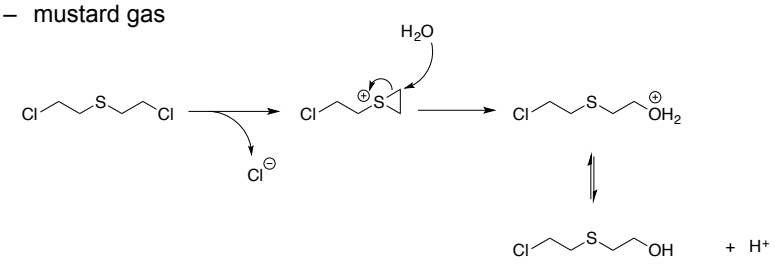
Covalent catalysis

- 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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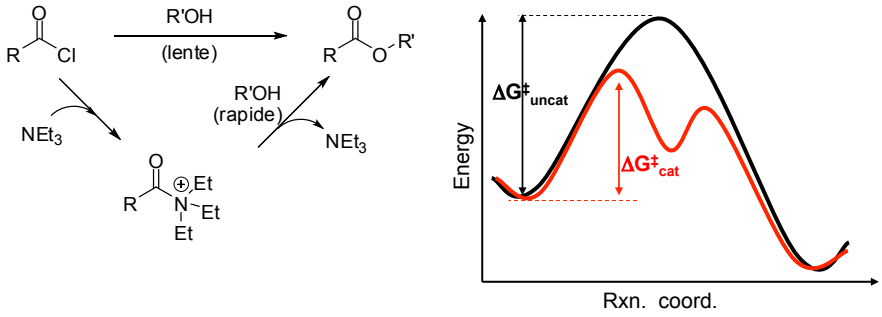
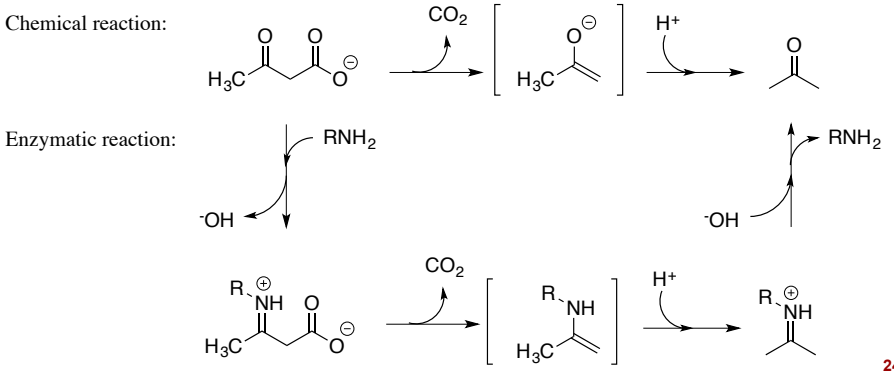
Nucleophilic catalysis

- 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:



Covalent catalysis

- another important example is activation of a carbonyl by formation of an imine
  - very common in *organocatalysis*
  - used by many *enzymes*
    - e.g.: acetoacetate decarboxylase :



Strain and distortion

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

Koshland :

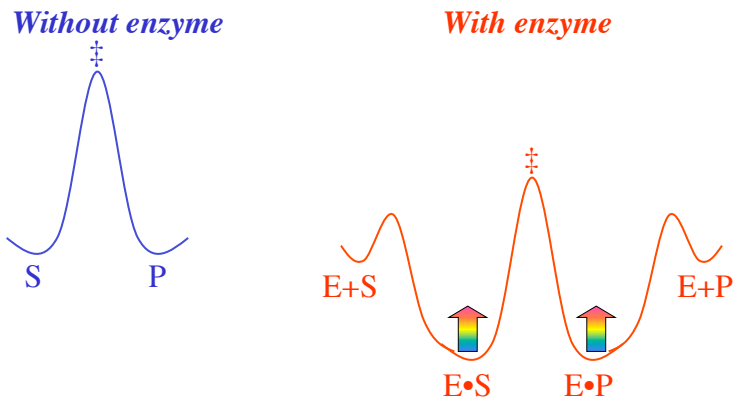
- *induced complementarity* hypothesis: the approach of substrate serves to provoke a conformational change in the enzyme, to adopt a form that better binds the substrate, but in a higher energy (strained) form and/or to better orient reactive groups (*"orbital steering"*)
- the substrate can also be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

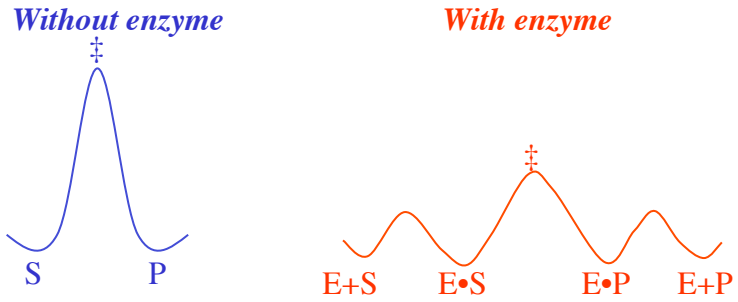
Strain and distortion

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



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

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Outline: Acid-base catalysis

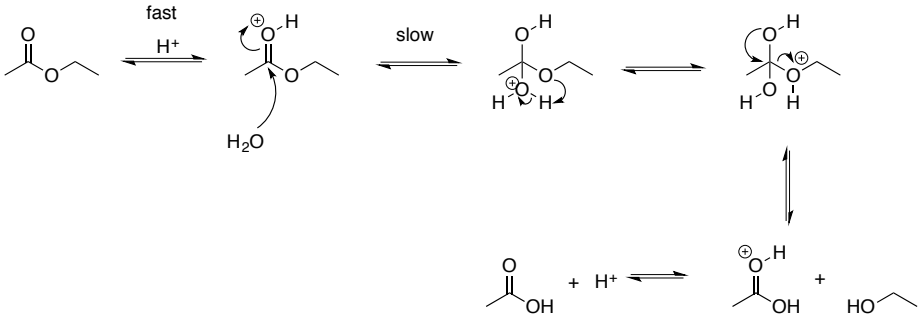
- see section 9.3 of A&D
  - specific acid/base catalysis
  - general acid/base catalysis
  - kinetic equivalence
  - Brønsted catalysis law
  - prediction of acid-base catalysis
  - energy surfaces of acid-base catalysis

Acid-base catalysis

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

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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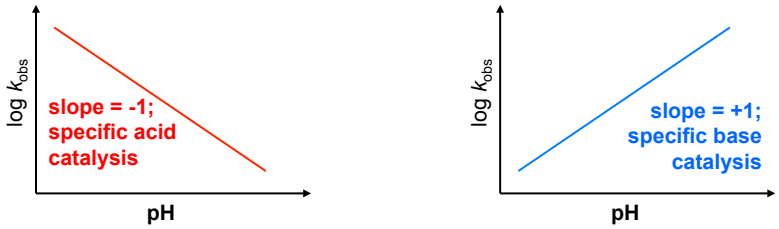
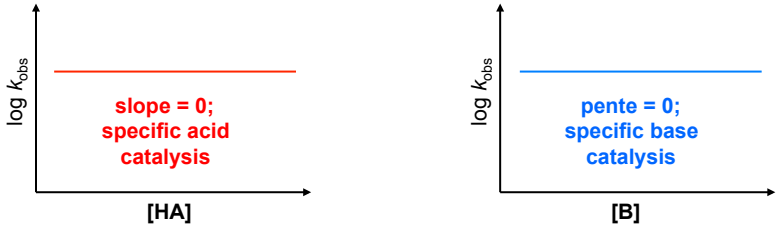
Specific acid-base catalysis plots

- pH dependence :



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



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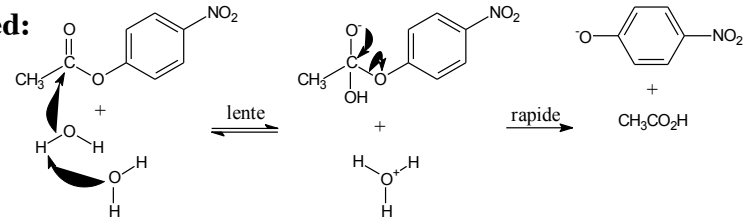
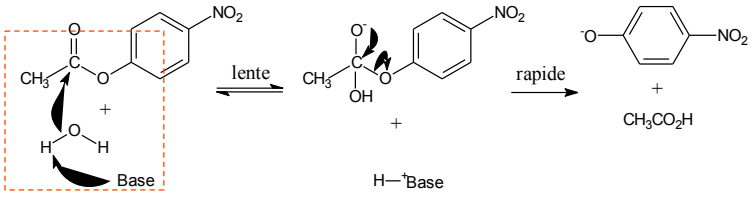
General acid catalysis

- 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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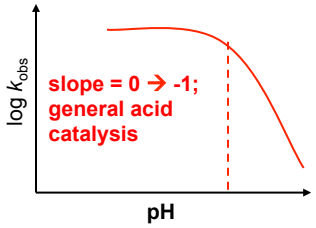
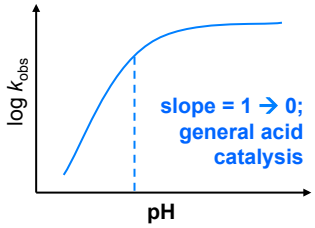
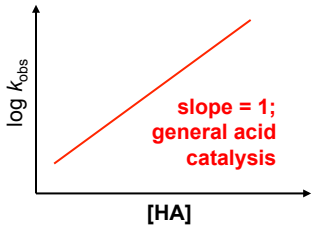
General acid-base catalysis plots

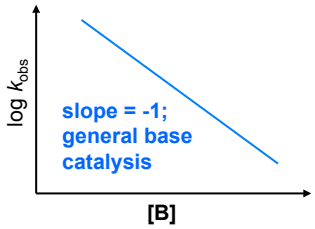
- pH dependence :





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





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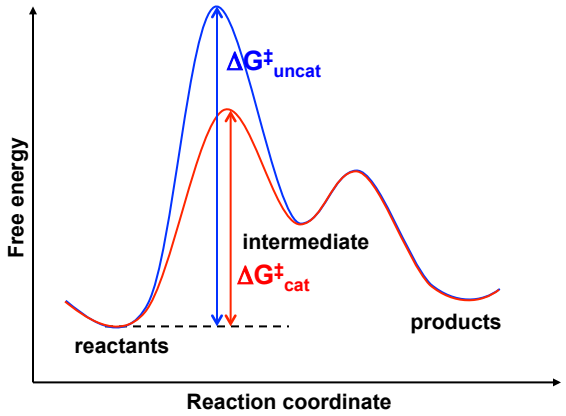
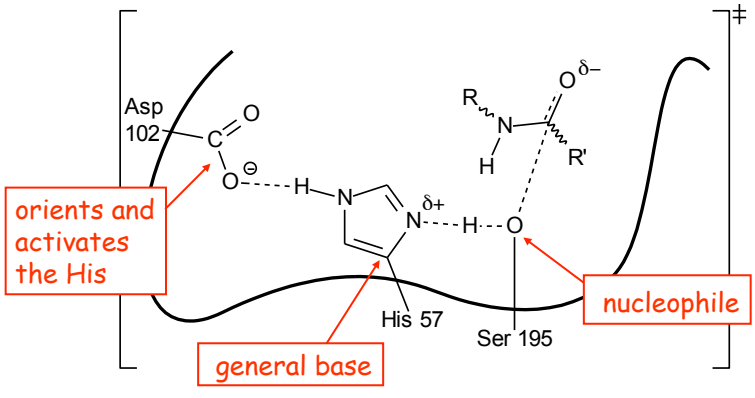
General acid-base catalysis

- can increase the nucleophilicity or the electrophilicity of a functional group



General acid-base catalysis

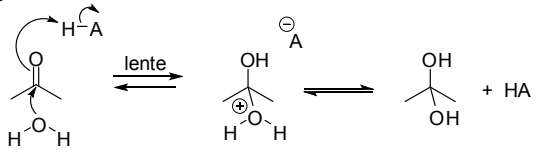
- very common in enzymes
  - e.g. chymotrypsin uses a catalytic triad whose His plays the roles of a general base :



Kinetic equivalence

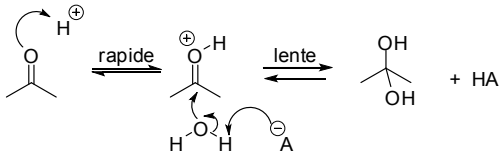
- one cannot distinguish, kinetically, between :
  1. general acid catalysis;

     $v = k_{obs}[R][HA]$:



  2. specific acid catalysis followed by general base catalysis;

     $v = k_{obs}[R][H^+][A^-]$:



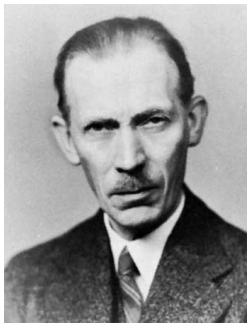
Kinetic equivalence

- by analogy, one cannot distinguish between :
  1. general base catalysis and
  2. specific base catalysis followed by general acid catalysis

$$v = k_{obs}[R][B] = k_{obs}[R][OH^-][HB^+]$$

Brønsted

- **Johannes Brønsted** (1879-1947)
  - Danish physical chemist (Copenhagen)
  - studied protonic theory of acid-base reactions (as did Lowry)
  - acid-base catalysis



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Brønsted catalysis law

- Brønsted noted that the rate constants for reactions catalysed by a general acid (having a proton *in flight* in the rate limiting transition state) are proportional to the acidity constants of the general acids :

$$k_{obs} \propto K_a^{\alpha} \qquad \boxed{\log k_{obs} = \alpha \cdot \log K_a = -\alpha \cdot pK_a}$$

and for general bases : $k_{obs} \propto K_b^{\beta}$ $\boxed{\log k_{obs} = \beta \cdot \log K_b = -\beta \cdot pK_b}$

- Brønsted plots (log *k* vs p*K*) have slopes between 0 and 1:
  - slope of 0 : no proton transfer in rds
  - slope of 1 : proton already transferred before rds
  - intermediate slope: *proportional with charge developed at TS of rds*

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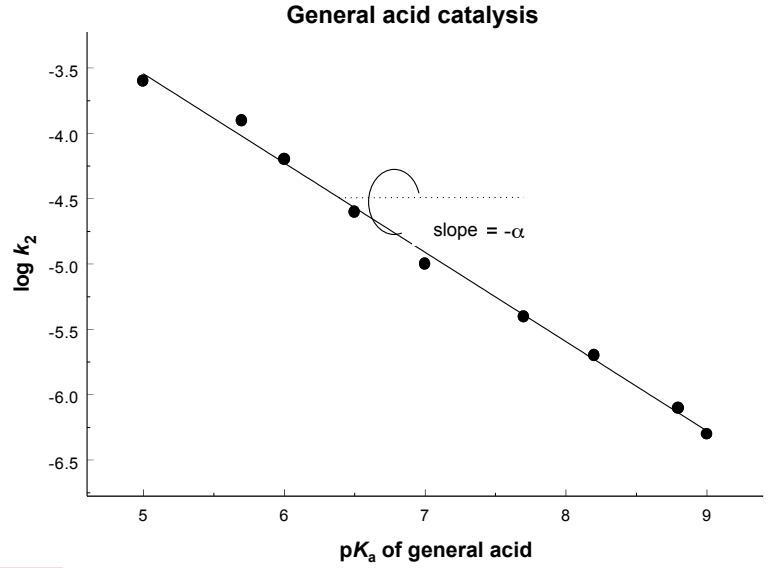
Brønsted plot (acids)



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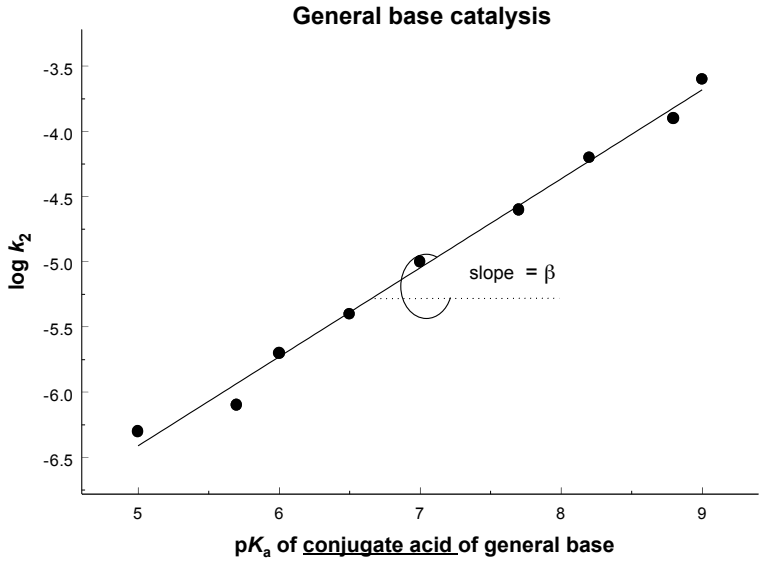
Brønsted plot (bases)



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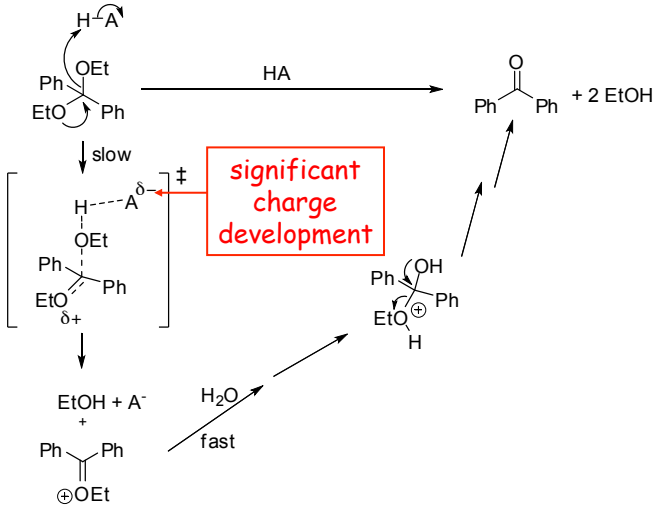
Example: gac ketal hydrolysis

- Brønsted coefficient α = 0.78
  - significant (partial) proton transfer at TS of rds



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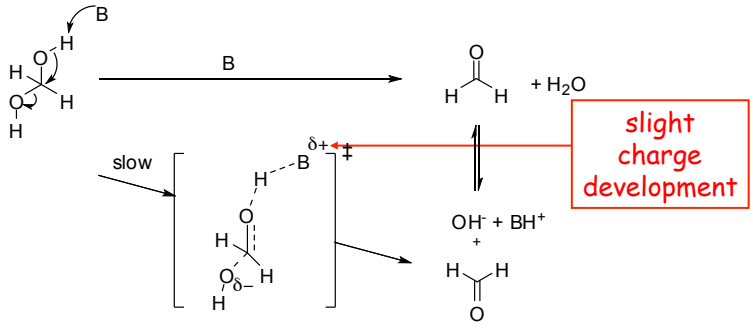
Example: gbc *gem*-diol dehydration

- Brønsted coefficient β = 0.4
  - slight (partial) proton transfer at TS of rds



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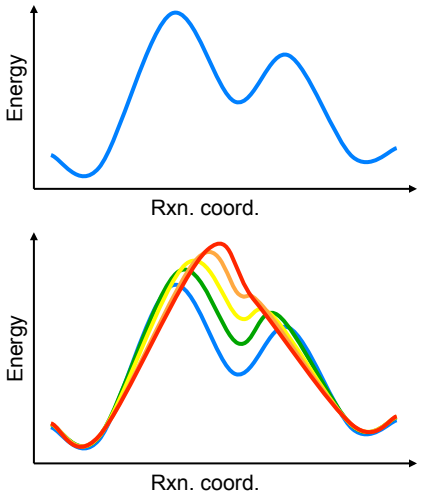
Step-wise *vs* concerted mechanism

- in general, a reaction will take place by a *step-wise* mechanism unless it is *forced* to take place by a concerted mechanism
  - if the intermediates of a reaction pathway are all fairly stable, this pathway will have the lowest energy….
  - but when an intermediate becomes too unstable to exist, it becomes a TS:



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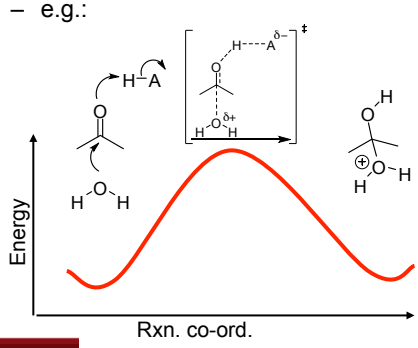
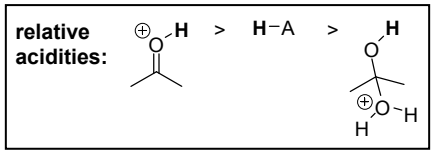
Prediction of general acid-base catalysis

- catalysis by a general acid or base is a *concerted* mechanistic step
  - proton transfer AND heavy atom bond formation/cleavage, in one step
  - *cf* specific acid/base catalysis, which is *step-wise*
- concerted catalysis becomes *necessary* when proton transfer to or from the reactant *is only possible at the TS*, owing to changes in heavy atom bonding
  - predictable, according to relative acidities
  - e.g.:





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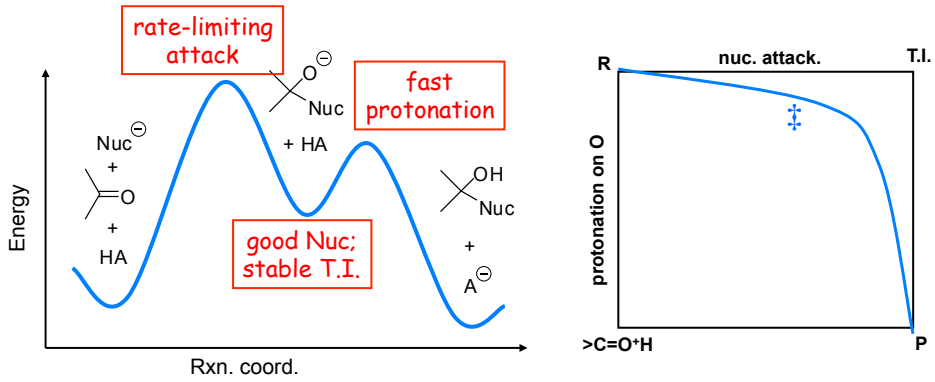
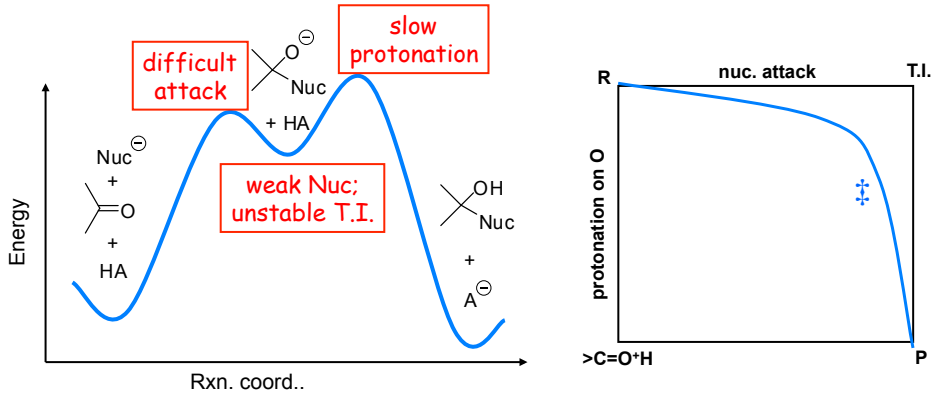
Energy surfaces and catalysis

- the order of the reaction steps of a mechanism can vary according to the stability of the corresponding intermediates
  - e.g.: addition/elimination on carbonyl
  - Example #1: strong nucleophile, no acid catalysis:



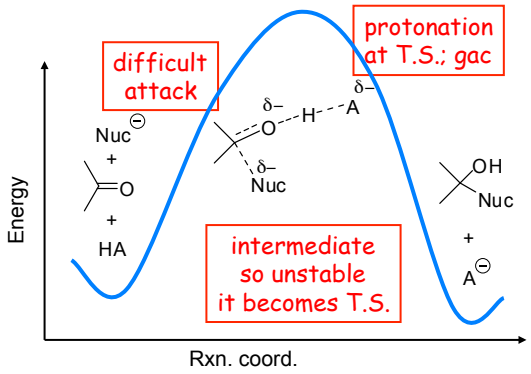
Energy surfaces and catalysis

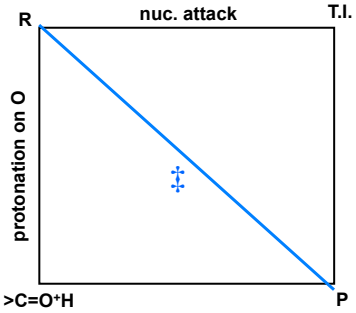
- the order of the reaction steps of a mechanism can vary according to the stability of the corresponding intermediates
  - e.g.: addition/elimination on carbonyl
  - Example #2: weak nucleophile, "required" catalysis:



Energy surfaces and catalysis

- the order of the reaction steps of a mechanism can vary according to the stability of the corresponding intermediates
  - e.g.: addition/elimination on carbonyl
  - Example #3: both nuc. attack AND protonation are difficult, and concerted





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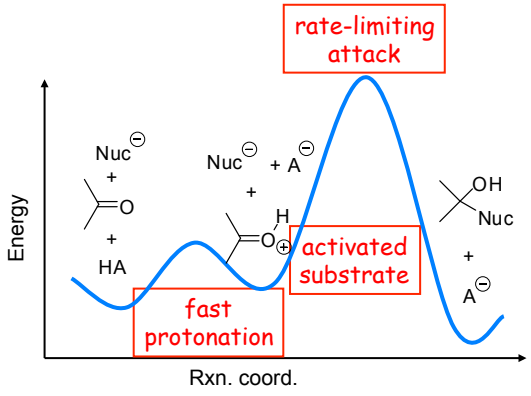
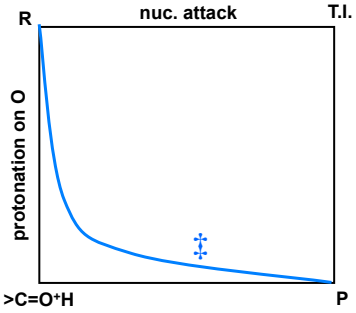
Energy surfaces and catalysis

- the order of the reaction steps of a mechanism can vary according to the stability of the corresponding intermediates
  - e.g.: addition/elimination on carbonyl
  - Example #4: weak electrophile, protonation required, spec. acid catalysis:





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Outline: Enzyme catalysis

- see section 9.4 of A&D
  - enzymes and non-bonding interactions (review)
  - Michaelis-Menten kinetics
  - significance of kinetic parameters
  - energy diagrams
  - enzyme catalysis and "supramolecular"
    - example: chymotrypsin and an enzyme model

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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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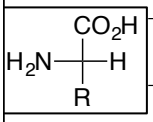
Rappel: Structures des acides aminés

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



R	Name	3 Letter Code	1 Letter Code	R	Name	3 Letter Code	1 Letter Code
H	Glycine	Gly	G	CH_2SH	Cysteine	Cys	C
CH_3	Alanine	Ala	A	$CH_2CH_2SCH_3$	Methionine	Met	M
$CH(CH_3)_2$	Valine	Val	V	$(CH_2)_4NH_2$	Lysine	Lys	K
$CH_2CH(CH_3)_2$	Leucine	Leu	L	$(CH_2)_3NHCNH_2$ (NH)	Arginine	Arg	R
$CHCH_2CH_3$ (CH_3)	Isoleucine	Ile	I	H_2C, H, N, N	Histidine	His	H
CH_2	Phenylalanine	Phe	F	H_2C, N, H	Tryptophan	Trp	W
CO_2H, HN, H, CH_2	Proline	Pro	P	O, CH_2COH	Aspartic acid	Asp	D
CH_2OH	Serine	Ser	S	O, CH_2CNH_2	Asparagine	Asn	N
$CHOH$ (CH_3)	Threonine	Thr	T	O, CH_2CH_2COH	Glutamic acid	Glu	E
CH_2, OH	Tyrosine	Tyr	Y	O, $CH_2CH_2CNH_2$	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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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

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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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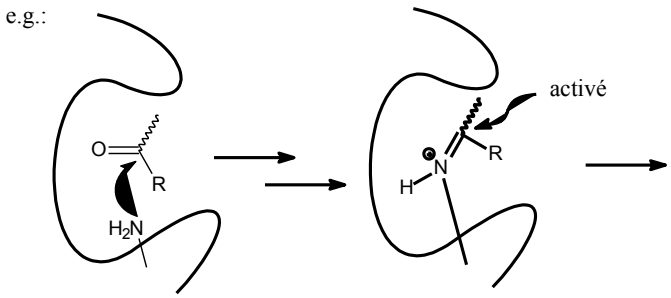
Covalent bond

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



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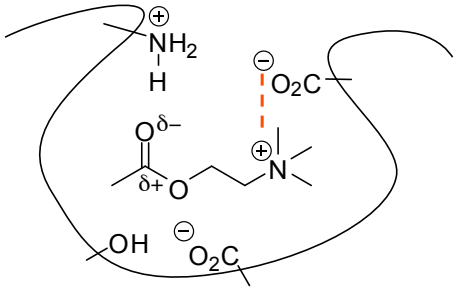
Ionic bonds

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



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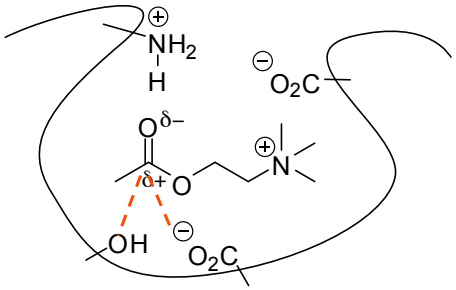
Ion-dipole and dipole-dipole interactions

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



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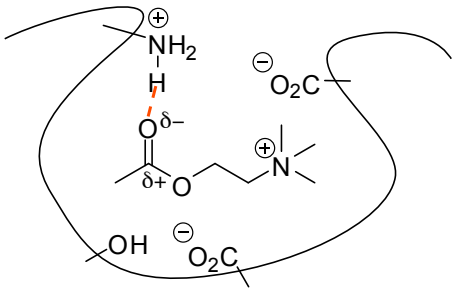
Hydrogen bonds

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



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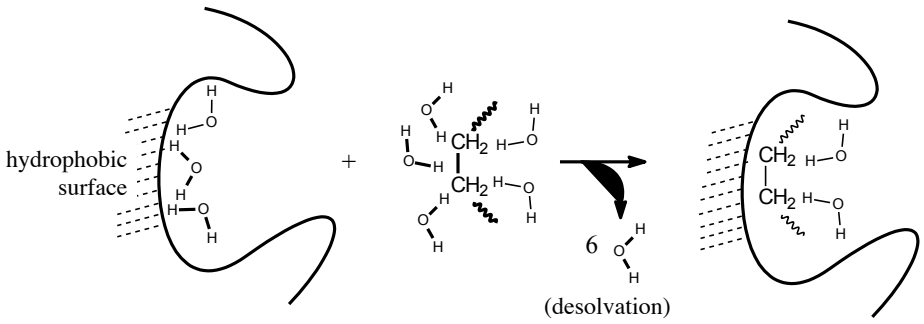
Charge transfer complex

- 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



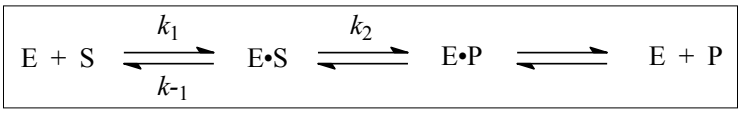
van der Waals interactions

- 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)

Enzyme kinetics

- same rules, laws and methods as analysis of non-enzymatic ("chemical") kinetics
- treated separately simply to emphasise the kinetic equations of enzyme activity and inhibition that are so pertinent in bioorganic and medicinal chemistry

Steady state



- at the beginning of an enzymatic reaction, there is an induction period (see the treatment of consecutive reactions) where the concentrations of intermediates build to a certain level
- when the rate of formation of these intermediates equals their rate of disappearance, they are said to be in a *steady state*
- *enzymatic reaction rates are typically measured during this time period*

Initial rates

- normally, $[E]_0 << [S]$
- at the beginning of the reaction (<10%), $[S] \approx [S]_0$
- under these conditions, [E•S] doesn't change appreciably and the rate is therefore considered to be constant

Saturation kinetics

- the rate of an enzymatic reaction is linearly proportional to the concentration of enzyme
- however, these rates show *saturation kinetic* (hyperbolic) behaviour with respect to the concentration of substrate
  - at low concentrations of S, the rate increases linearly with [S]
  - at higher concentrations of S, the rate increases less and less with increasing [S]
  - at saturating concentrations of S, the rate approaches a limiting value called the *maximum rate*, $\mathbf{V_{max}}$

Michaelis

- **Leonor Michaelis** (1875 – 1949)
  - German biochemist and physician (Berlin, Johns Hopkins, Rockefeller)
  - develop enzyme kinetic equations with Menten
  - studied urinary tract infections
  - developed chemical denaturation of keratin ('perm'!) and depilation

Menten

- **Maude Menten** (1879-1960)
  - Canadian medical scientist
  - MD/PhD with Michaelis
  - developed enzyme kinetics equations
  - later became pathologist (Pittsburgh)
  - developed enzyme assays and electrophoretic separation of proteins

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Michaelis-Menten equation

- in 1913, Michaelis and Menten proposed the following simplified kinetic scheme:
  - NB: rapid equilibrium to form the *Michaelis complex*, followed by its reaction in the slow step

$$\mathrm{E} + \mathrm{S} \underset{}{\overset{K_S}{\rightleftharpoons}} \mathrm{E{\bullet}S} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$$

$$v = k_{cat}[\mathrm{E{\bullet}S}] \quad \text{and} \quad K_S = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{E{\bullet}S}]} \quad \text{and} \quad [\mathrm{E}]_0 = [\mathrm{E}] + [\mathrm{E{\bullet}S}]$$

$$[\mathrm{E}] = \frac{K_S[\mathrm{E{\bullet}S}]}{[\mathrm{S}]} = [\mathrm{E}]_0 - [\mathrm{E{\bullet}S}] \qquad K_S[\mathrm{E{\bullet}S}] = [\mathrm{E}]_0[\mathrm{S}] - [\mathrm{E{\bullet}S}][\mathrm{S}] \qquad [\mathrm{E{\bullet}S}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_S + [\mathrm{S}]}$$

hyperbolic equation → $$v = \frac{[\mathrm{E}]_0[\mathrm{S}]k_{cat}}{K_S + [\mathrm{S}]}$$

$$V_{max} = k_{cat}[\mathrm{E}]_0$$

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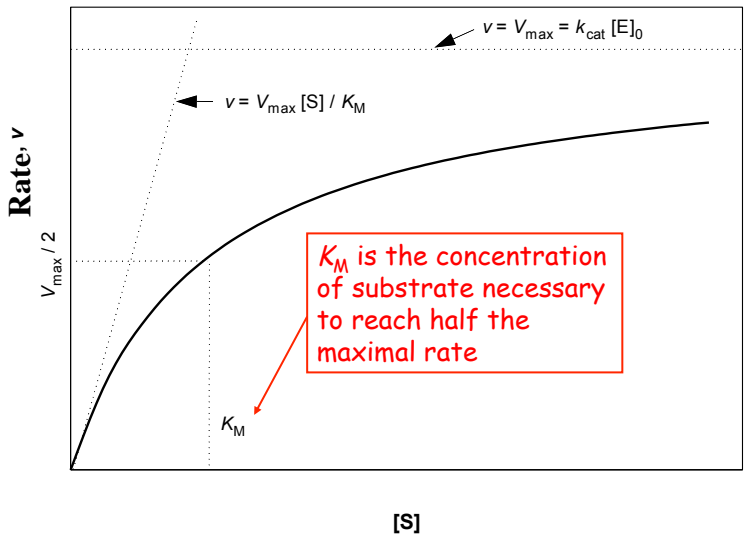
Michaelis-Menten plot



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M-M equation *cf* M-M mechanism

- the Michaelis-Menten equation (hyperbolic) accurately describes the kinetics observed for most enzymatic reactions
- however, the mechanism suggested by the simplistic Michaelis-Menten scheme (fast equilibrium binding, followed by *one* slow step) is rarely appropriate
- a more rigorous kinetic treatment of the same scheme invokes the *steady state approximation*

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Steady state equation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_2} E + P$$

- at the steady state, the rate of formation of E•S equals that of its disappearance:

$$\frac{d[E{\bullet}S]}{dt} = k_1[E][S] - (k_2 + k_{-1})[E{\bullet}S] = 0 \qquad \frac{k_2 + k_{-1}}{k_1} = \frac{[E][S]}{[E{\bullet}S]}$$

- this is the only difference compared to the previous treatment; K_S is simply replaced by K_M, the *Michaelis constant* :

$$\frac{k_2 + k_{-1}}{k_1} = K_M \qquad v = \frac{[E]_0[S]k_{cat}}{K_M + [S]} \qquad v = \frac{V_{max}[S]}{K_M + [S]} \qquad V_{max} = k_{cat}[E]_0$$

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Mechanistic implication

- saturation kinetics refers to the the hyperbolic relation between the reaction rate and the concentration of reactant (substrate)
- in general, this is consistent with (and often due to) the *rapid pre-formation of a complex* before its reaction to give product

$$A + C \rightleftharpoons A{\bullet}C \longrightarrow P + C$$

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Constant catalytic : k_{cat}

- in general, k_{cat} represents the rate constant of the rds, the slowest step of the enzymatic reaction
  - more strictly speaking, it is affected by first order rate constants of all steps in the mechanism
- also called the *turnover number* because it represents the number of substrate molecules converted into product, per enzyme active site, per unit of time

Apparent equilibrium constant : K_M

- related to K_S, the dissociation constant
- can be considered as an *apparent* dissociation constant
  - more strictly, it is the dissociation constant for the sum of *all* enzyme species bound by substrate
  - related to the *affinity* of the enzyme for the substrate
    - lower K_M corresponds to higher affinity
      - less substrate necessary to saturate enzyme
- always represents the concentration of substrate necessary to give half of the maximal rate
  - (derives mathematically from the hyperbolic equation)

Specificity constant : k_{cat} / K_M

- second order rate constant for the reaction of enzyme and substrate
- since the value of (k_{cat} / K_M) varies for each substrate and its affinity for the enzyme, this ratio is also called the *specificity constant*
- can be considered as an indicator of the efficiency of the reaction of free enzyme with a given substrate

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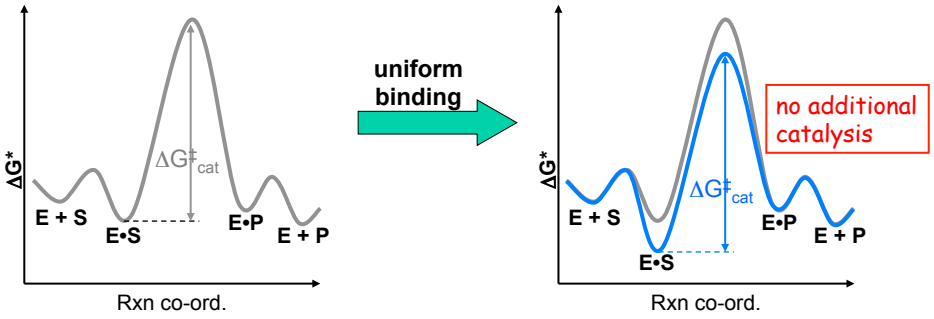
Energy diagrams

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



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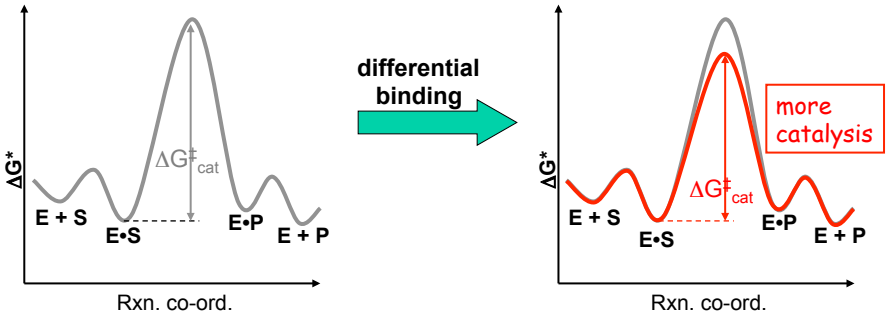
Energy diagrams

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



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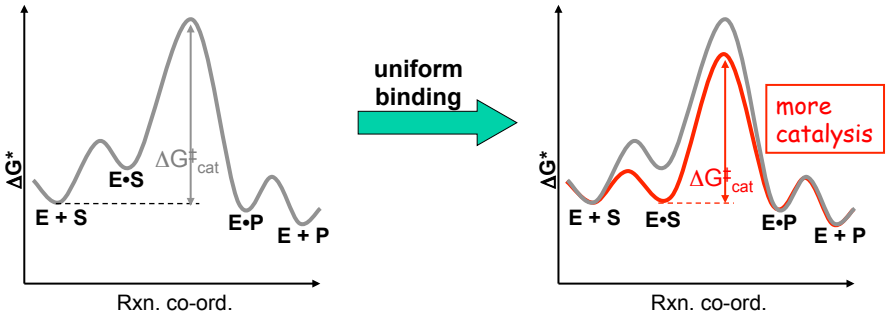
Energy diagrams

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



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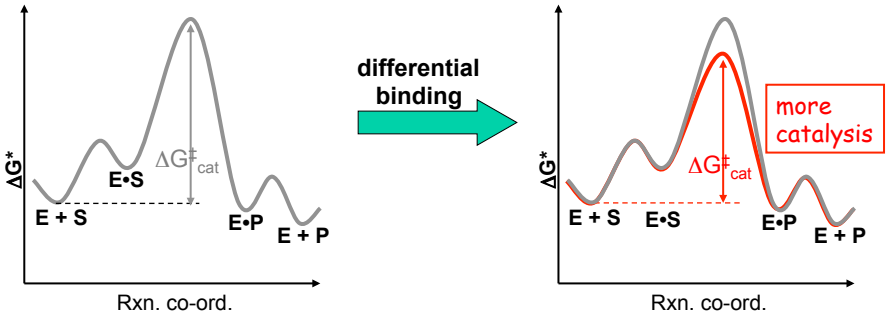
Energy diagrams

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



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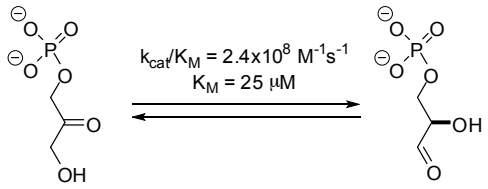
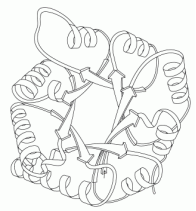
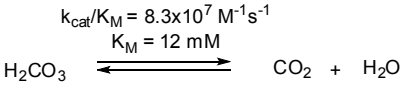
"Perfect" enzymes

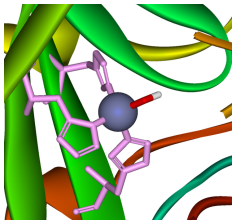
- some enzymes are so efficient, their k_{cat}/K_M values approach the diffusion-controlled limit, at [S]<K_M
  - e.g.: triosephosphate isomerase





  - e.g. carbonic anhydrase





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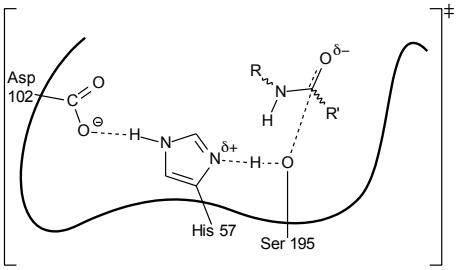
Serine proteases

- catalyses the hydrolysis of proteins and peptides
  - esters, too
- studied for over 40 years, their mechanism is very well known
  - especially true for trypsin, chymotrypsin, elastase and subtilisin
- contain a *catalytic triad*, composed of Ser, His and Asp



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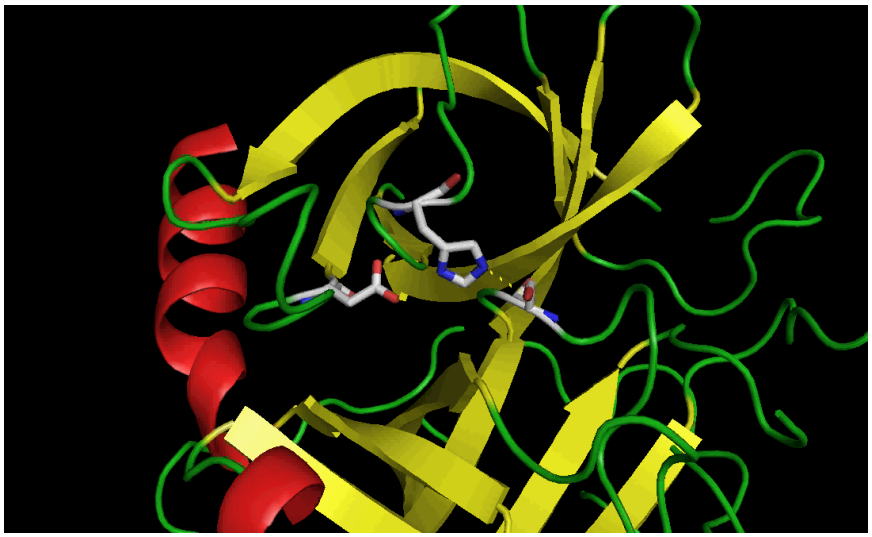
Chymotrypsin



J. Mol. Biol. **1985**, *184*, 703-711.

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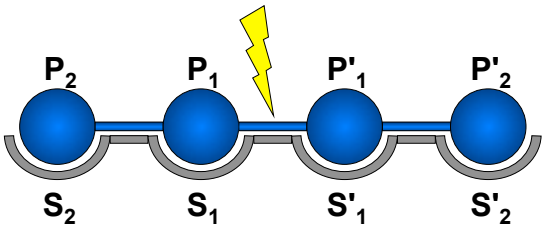
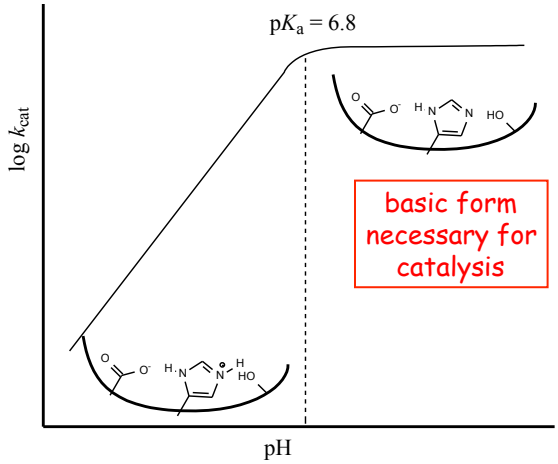
Chymotrypsin

- secreted by the pancreas, it aids in the digestion proteins in the intestine
- catalyses the hydrolysis of the peptide bond on the C-terminal side of an amino acid having a side chain containing an aromatic group :
  - P_1 = Phe, Trp, Tyr

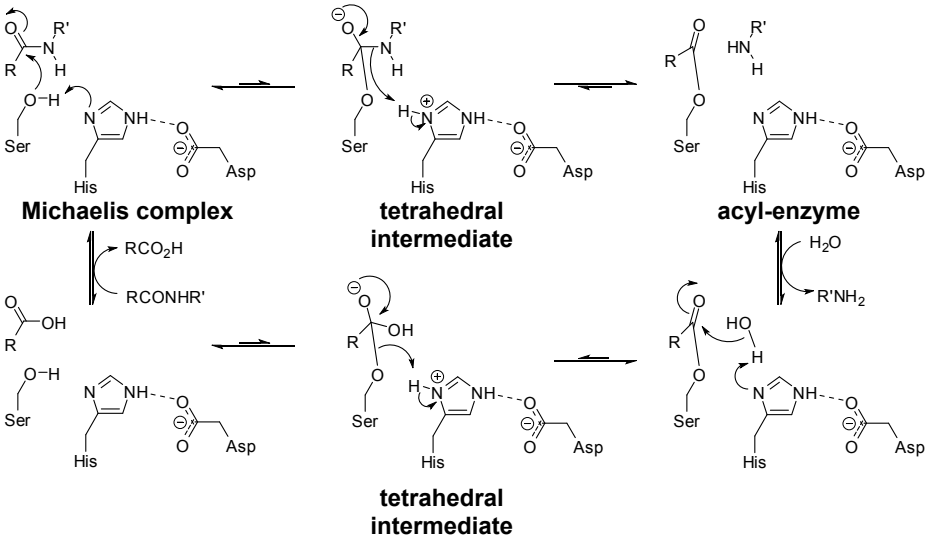


pH-rate profile of chymotrypsin

- plateau in pH-rate profile implies dependence on ionisation state of *one* residue (His57)



Chymotrypsin mechanism



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Bender's model (*Acc. Chem. Res.* 1987, *20*, 146)

- Bender coupled a binding site model (a cyclodextrin) with a model of the catalytic triad of the serine proteases
- one of the first enzyme models to incorporate all the components of an enzymatic system in the same molecule
- particpates in an *intermolecular* reaction with a model substrate, *p-t*-butylphenyl acetate
  - caution! a phenyl ester is not an amide!

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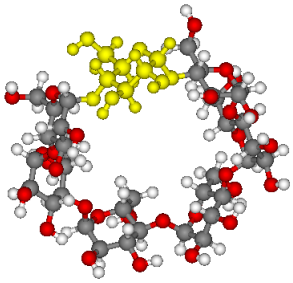
Cyclodextrin

- cyclic oligomer of 1,4-α-D-glucose units
  - 6 glucoses = α-CD, 7 glucoses = β-CD, 8 glucoses = γ-CD
- hydrophobic cavity, with OH groups around the entry, often used as sites for attachment of other functional groups



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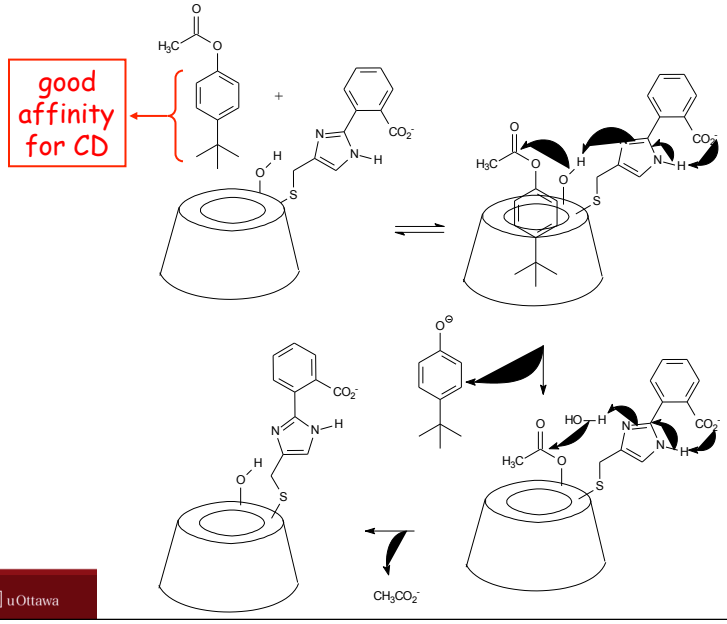
Bender's model (*Acc. Chem. Res.* 1987, *20*, 146)



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Bender's model (*Acc. Chem. Res.* 1987, *20*, 146)

- saturation kinetics observed
  - consistent with formation of a bound complex
  - allows the measurement of k_{cat} and K_M values
- 1 mmol of catalyst effected the hydrolysis of > 10 mmol of substrate
  - regeneration of catalyst; true catalysis

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Bender's model (*Acc. Chem. Res.* 1987, *20*, 146)

- reaction is three-fold slower in D_2O than in water
  - proton in flight at TS
  - consistent with general base catalysis
  - not consistent with nucleophilic catalysis (which is also possible with imidazoles)
- pH-k_{cat} profile plateaus above pH 10
  - basic form is active
  - supports a role for a general base

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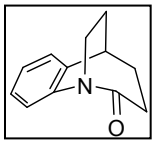
Bender's model (*Acc. Chem. Res.* 1987, *20*, 146)

- comparisons (made by the author) between chymotrypsin and his "artificial chymotrypsin":
  - model is more stable with respect to pH and temperature
  - model is more efficient than the enzyme, with respect to their molecular weights
  - starting point for the synthesis of "artificial enzymes"
  - "*the ultimate proof of the mechanism of chymotrypsin catalysis*"
- HOWEVER, chymotrypsin catalyses the hydrolysis of *amides* at pH 7...
- note that other authors have used more realistic substrate models, namely amides
  - see Brown *et al.*, *JACS* **1989**, *111*, 1445 :



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