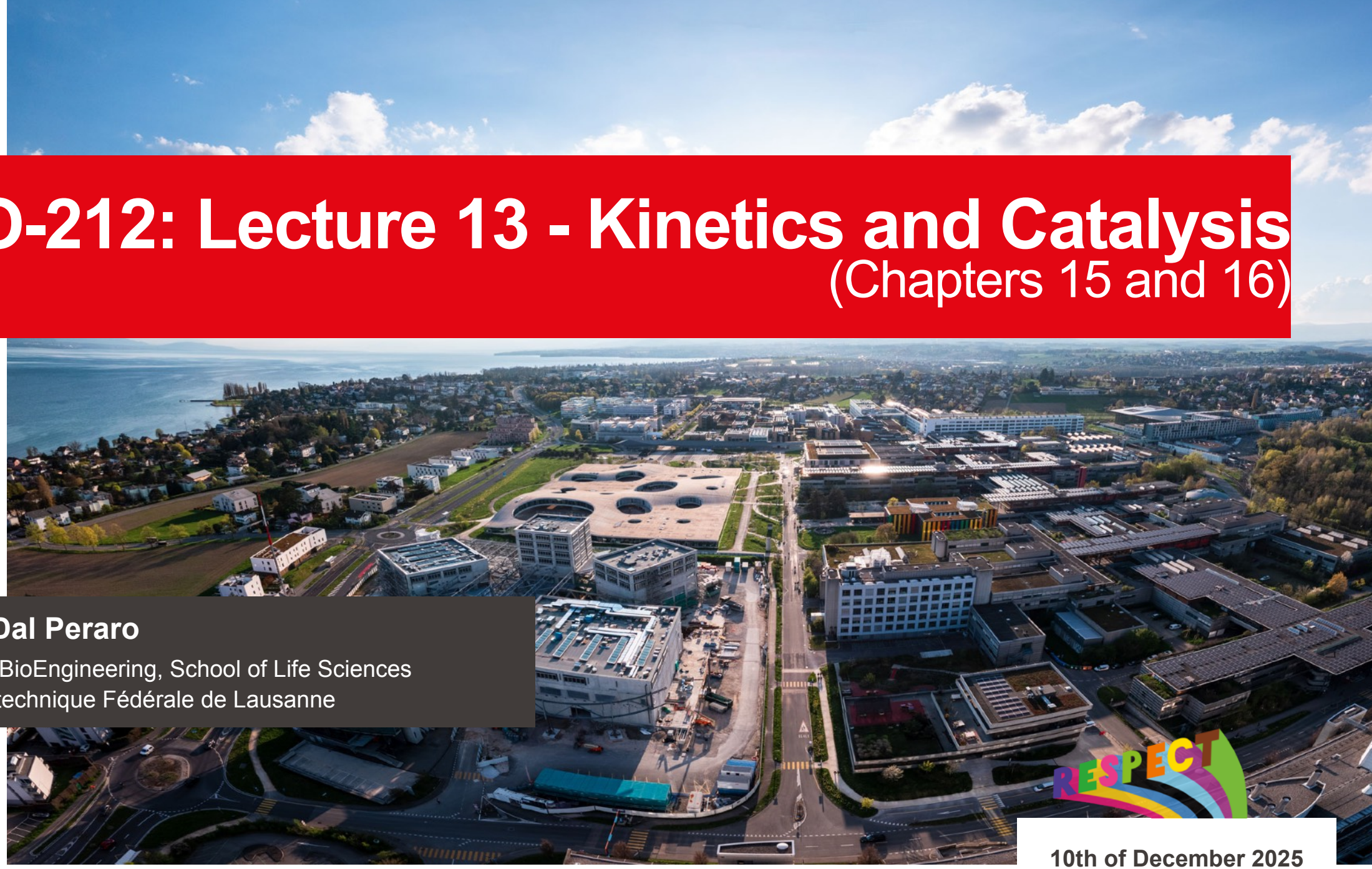
BIO-212: Lecture 13 - Kinetics and Catalysis
(Chapters 15 and 16)



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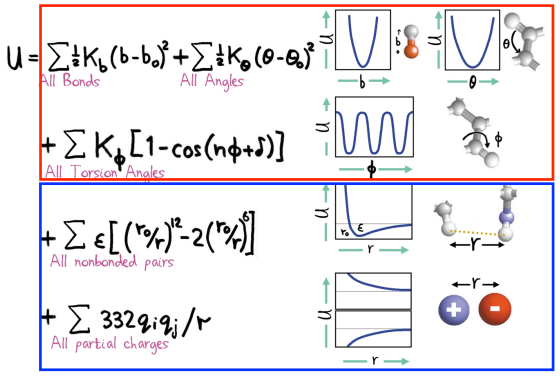
10th of December 2025

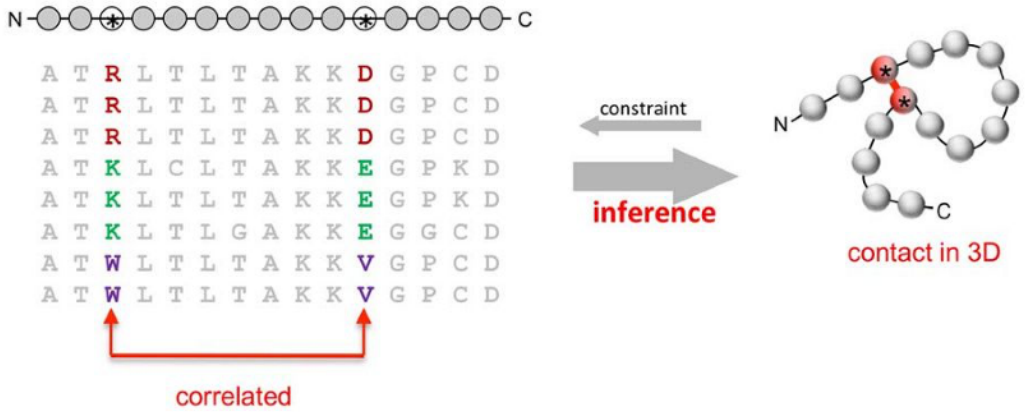
Lecture 12 - Summary

Molecular simulations



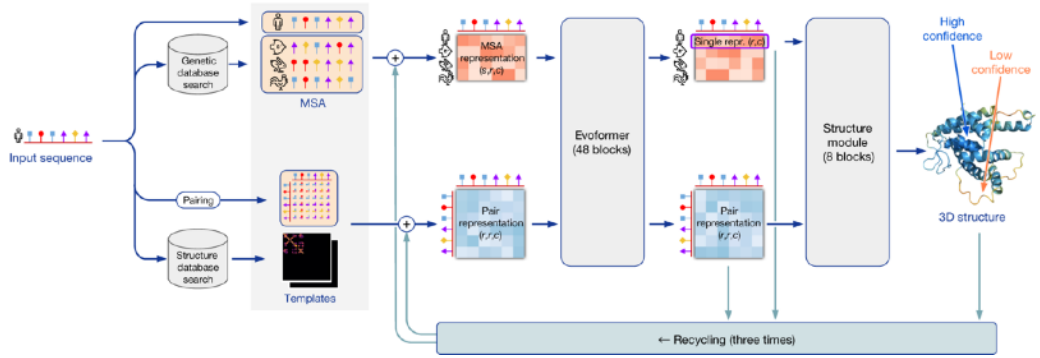
- Physics-based models allow to generate molecular movies of biological systems

Evolutionary couplings



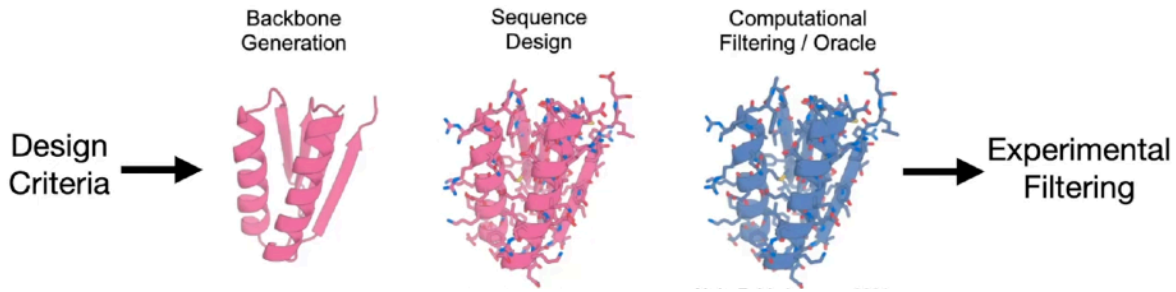
- EVs extracts structural information from sequence data

AlphaFold 2



AF2 solved the problem of protein structure prediction and produced models for >2 200M sequences

Protein design



A variety of AI-driven methods allow to accurately design new to nature molecular systems

The many timescales of biological processes

- biological nanoscale spans **different timescales**
- **small** things function at **higher** rate

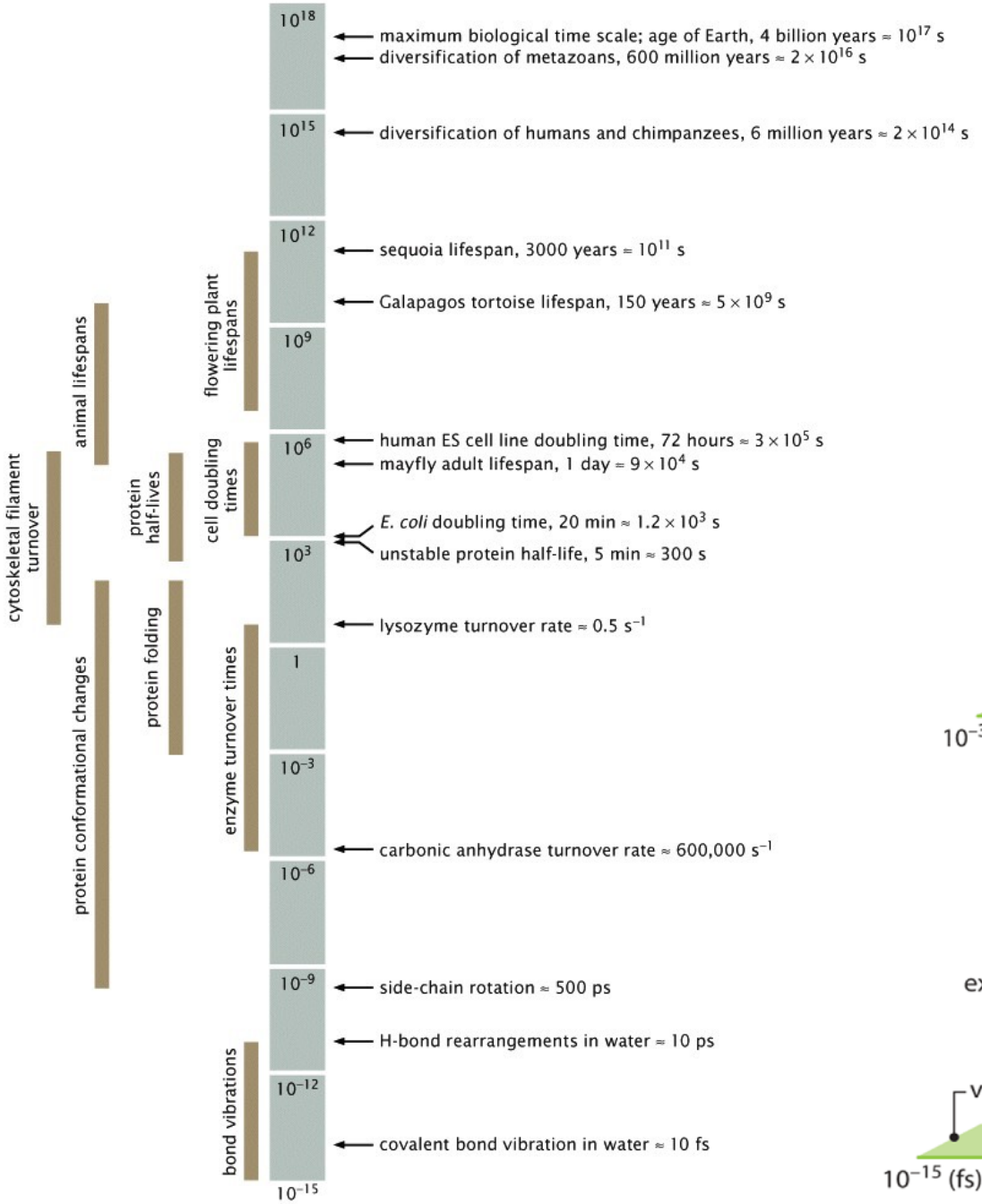
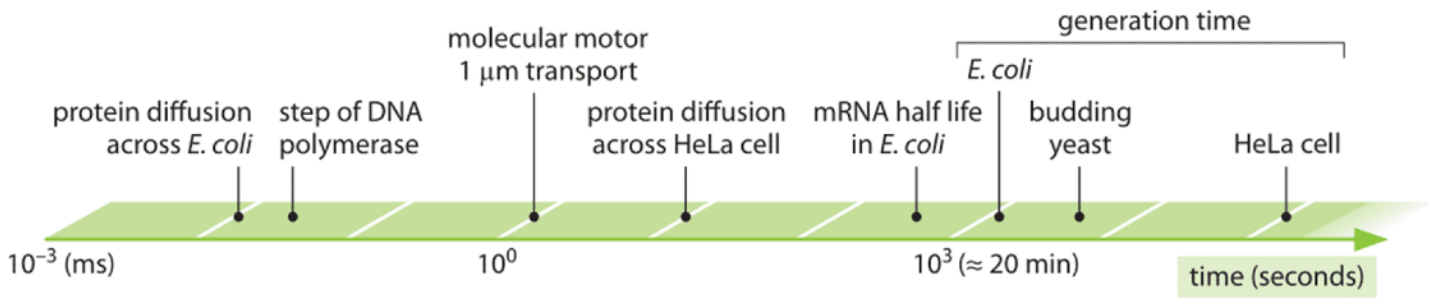
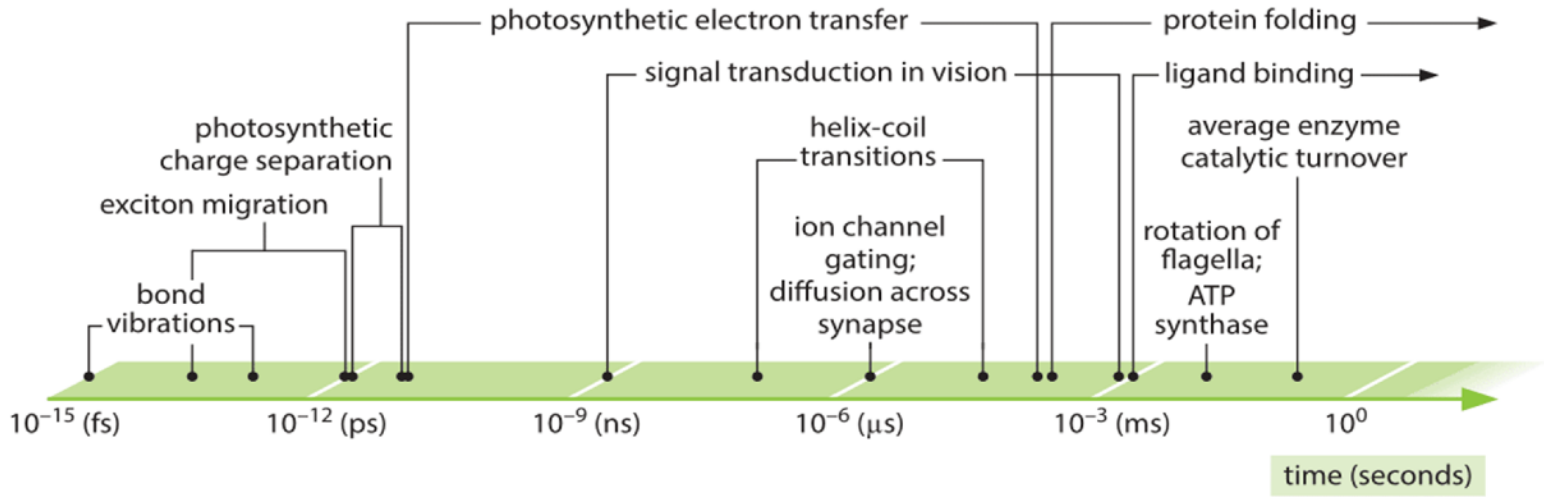


Figure 3.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)





The many timescales of biological processes

from days to seconds ...

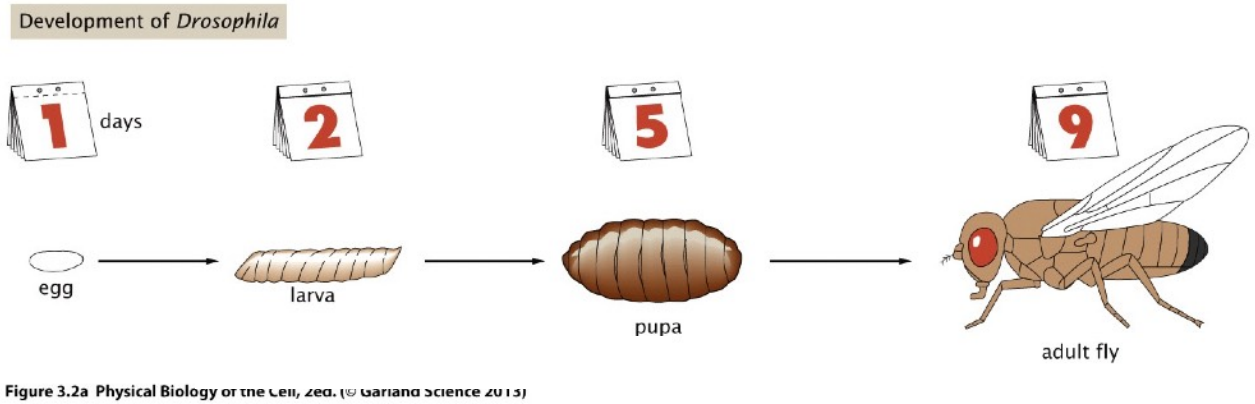
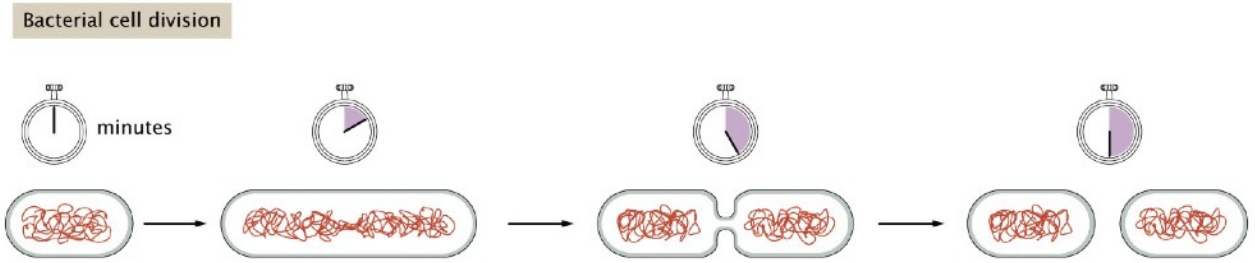
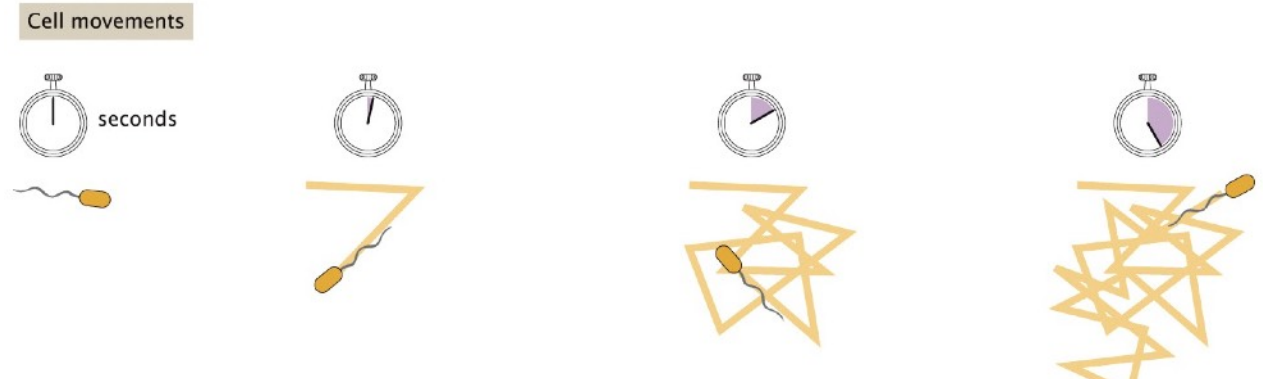


Figure 3.2a Physical Biology of the Cell, 2ed. (© Garland Science 2013)





The many timescales of biological processes

... and from seconds to microseconds

Transcription

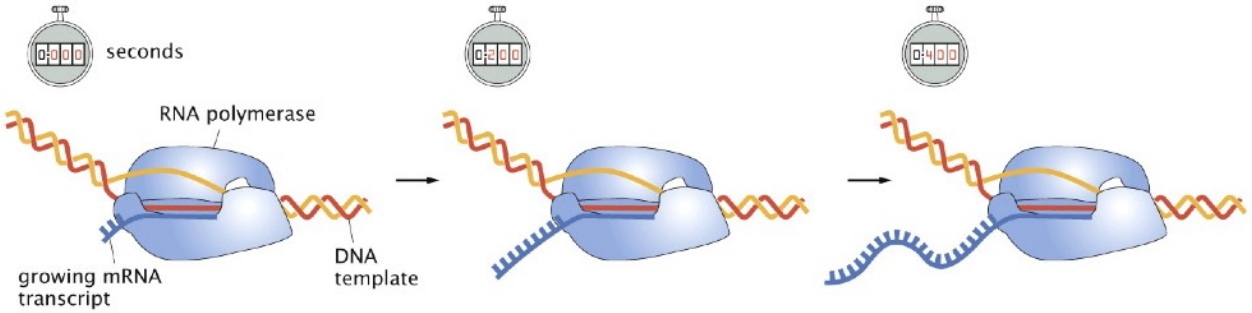
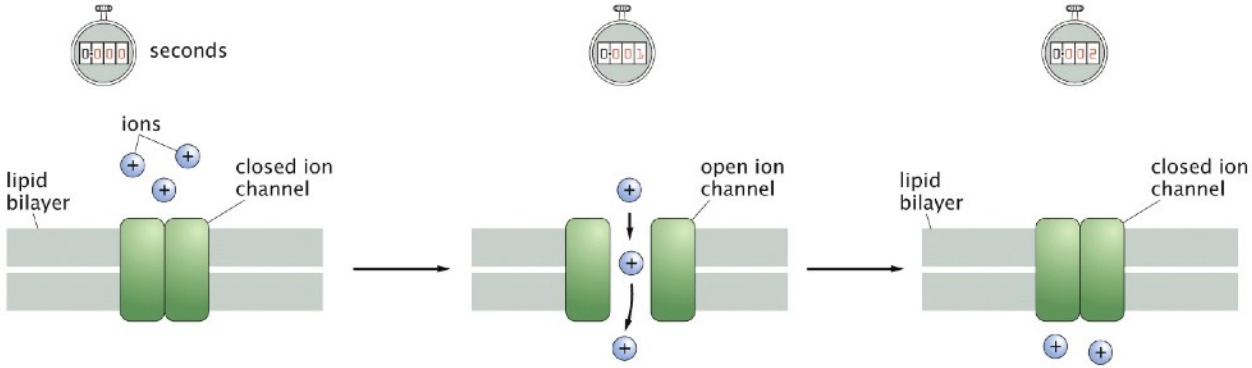
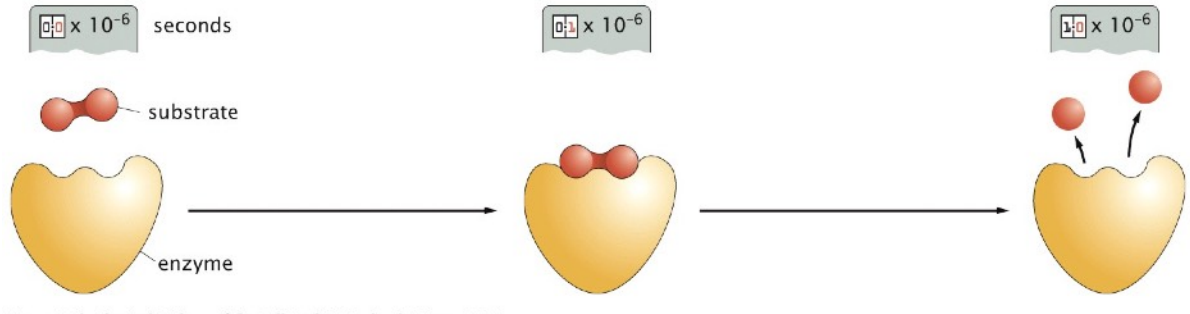


Figure 3.2f Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Gating of ion channels



Enzyme catalysis



Two different ways of assessing binding

Thermodynamic approach

What is the difference in free energy between the unbound and bound state?

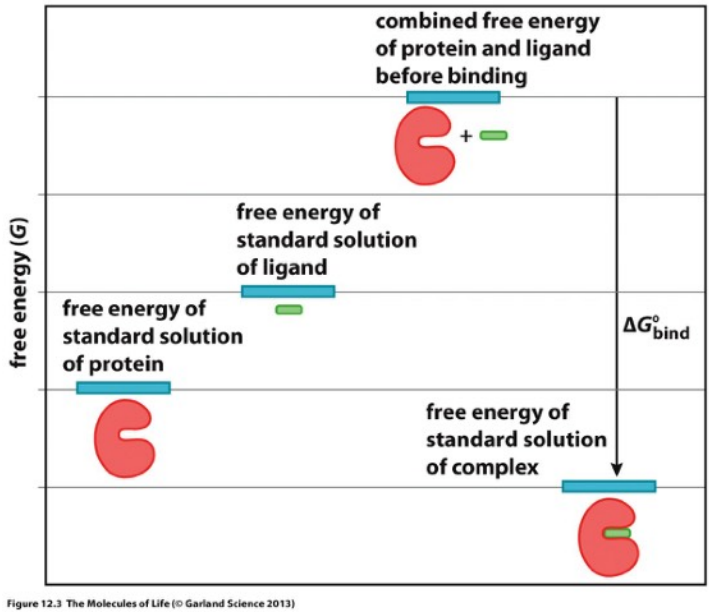


Figure 12.3 The Molecules of Life (© Garland Science 2013)

Measuring the energy change at steady state to calculate K_D

$$\Delta G^{\circ} = RT \ln K_D$$

Kinetic approach

How fast does the ligand bind the protein?
How fast does the complex dissociate?

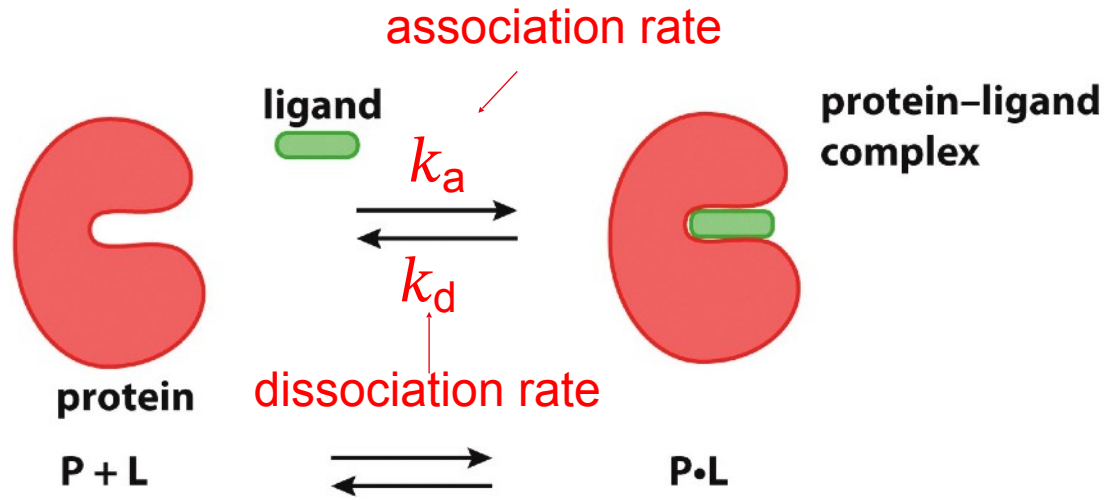


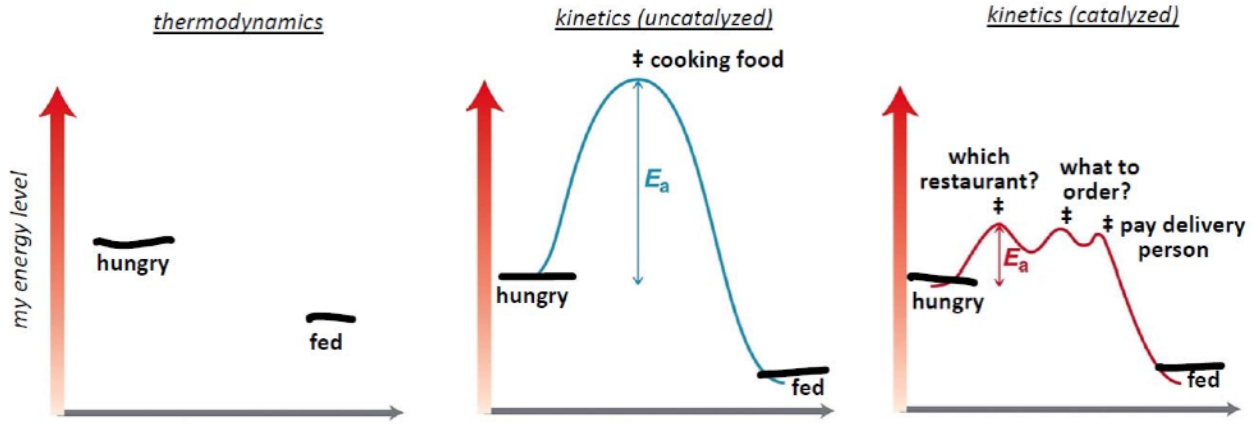
Figure 12.2a The Molecules of Life (© Garland Science 2013)

Measuring the rates at which system achieves equilibrium (k_a and k_d) to calculate K_D

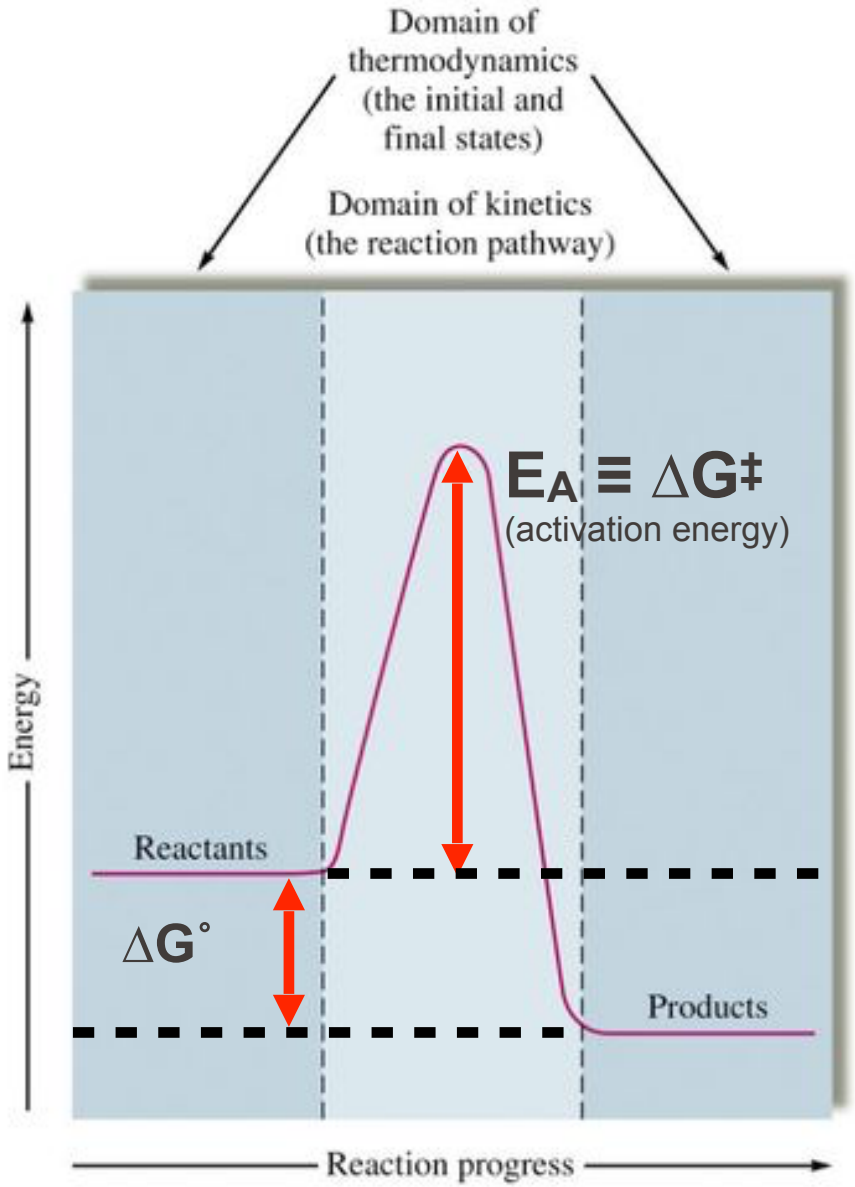
$$\frac{k_d}{k_a} = \frac{k_{off}}{k_{on}} = \frac{[P][L]}{[P \bullet L]} = K_D = \frac{1}{K_A}$$

Thermodynamics *vs.* Kinetics

- **Thermodynamics** has no concept of time
- Thermodynamics does not consider the pathway only considers initial state and final state and tell us whether a reaction is spontaneous based on that
- **Kinetics** considers the rate of changes and the reaction pathway from reactants to products

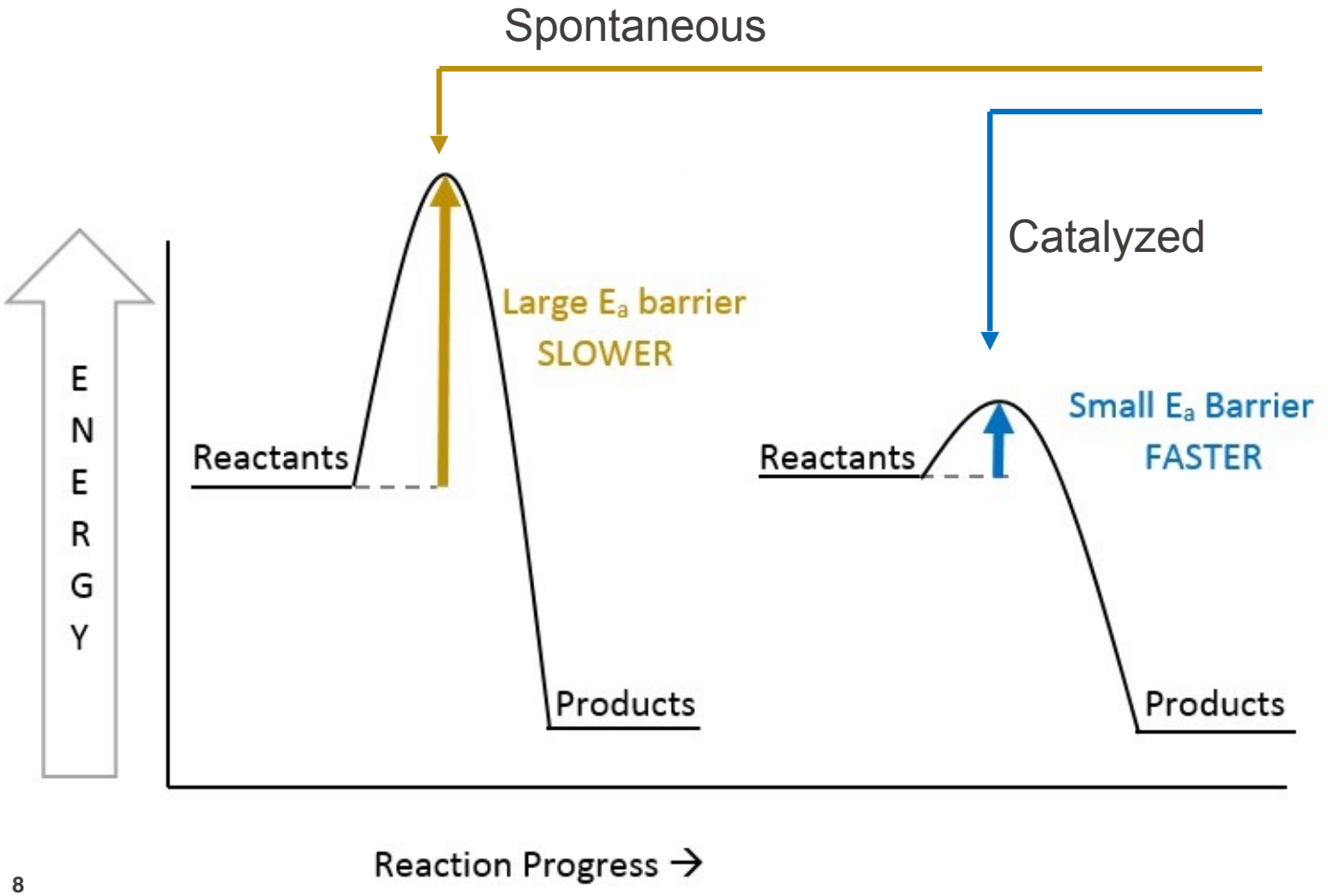


@ChemKritzer on Twitter

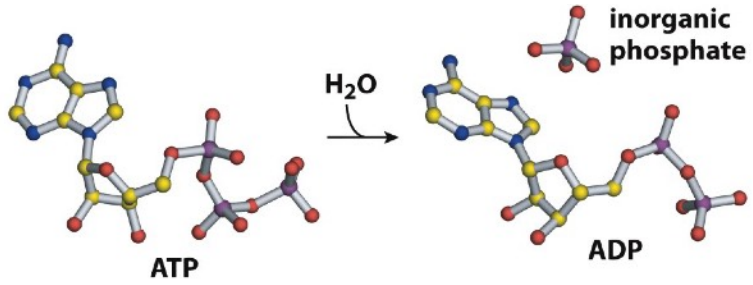


Activation energy and reaction rate

- Reaction rates are **exponentially and inversely correlated** to the activation energy
- The reactions below have the **same ΔG° and K_{eq}** but **different rates**



e.g. ATP hydrolysis



ATPase enzymes accelerate the reaction rates by factors of 10^7 from ~0.3 s^{-1} (eg actin) to > 240 s^{-1} (kinesin).

- in water: E_a ~ 170 kJ/mol
- catalysed: E_a ~ as low as ~12 kJ/mol

Rate equations

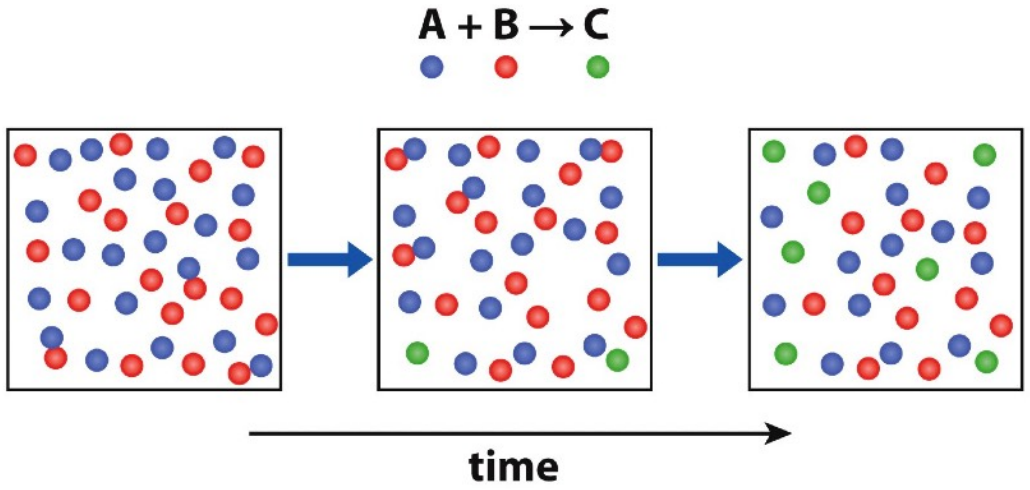
- for quantification of reactions we will use chemical **concentration** (# molecules/moles per unit of volume)
- in general concentration for a species *i* depends upon space and time, thus $c_i(\mathbf{r}, t)$
- in cells, local concentration is more appropriated than a global value (not homogenous)
- if concentration is very low, stochastic behavior needs to be taken into account
- if $c_i(t)$, and $\{c_j\} = (c_1, c_2, ..., c_n)$, then the time evolution of the species *i* is:

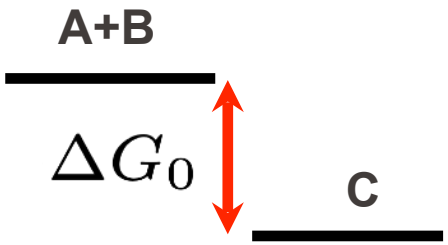
$$\frac{dc_i(t)}{dt} = f(\{c_j\}; \{k_i\})$$

k_i are called **rate constants**

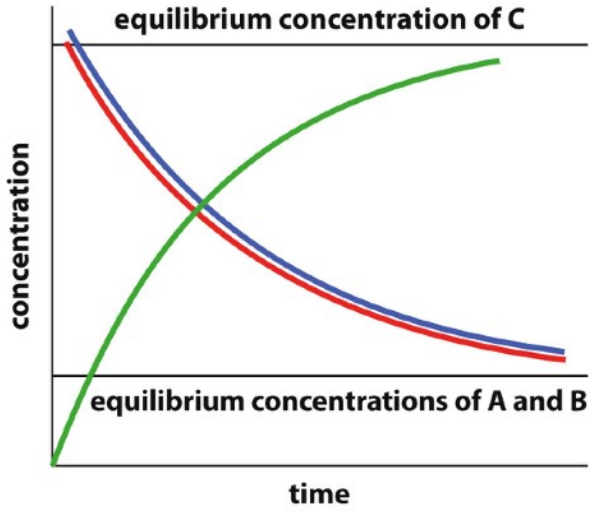
Kinetics of simple reactions

- Let's look at a simple chemical reaction where A and B react to produce C (can also be thought of as binding)
- The system is initially not in equilibrium but with time A and B convert to C





- Thermodynamically this reaction is favorable
- However, that doesn't automatically mean that it will be fast



- For quantification **molecular concentration** (# molecules/moles per unit of volume) are used

- The **rate of reaction** defines how efficiently the reactants change to products with time

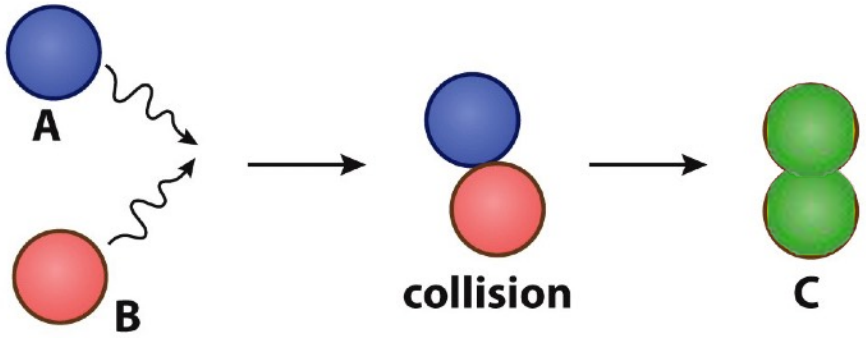
$$rate = -\frac{d[A]}{dt} = \frac{d[C]}{dt} = k[A][B]$$

- The rates of reactants are always negative and the rates of products are always positive

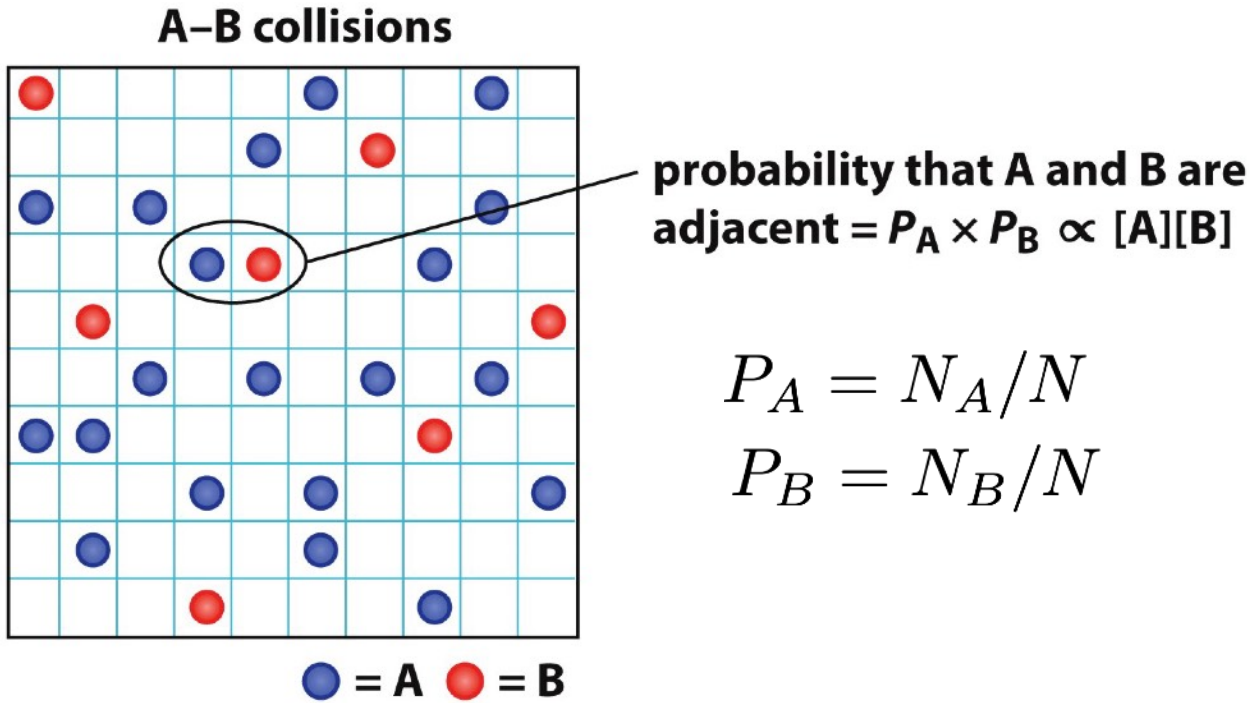
- ***k*** is called **reaction rate constant** (equivalent to k_a and k_d discussed in Lecture 10)

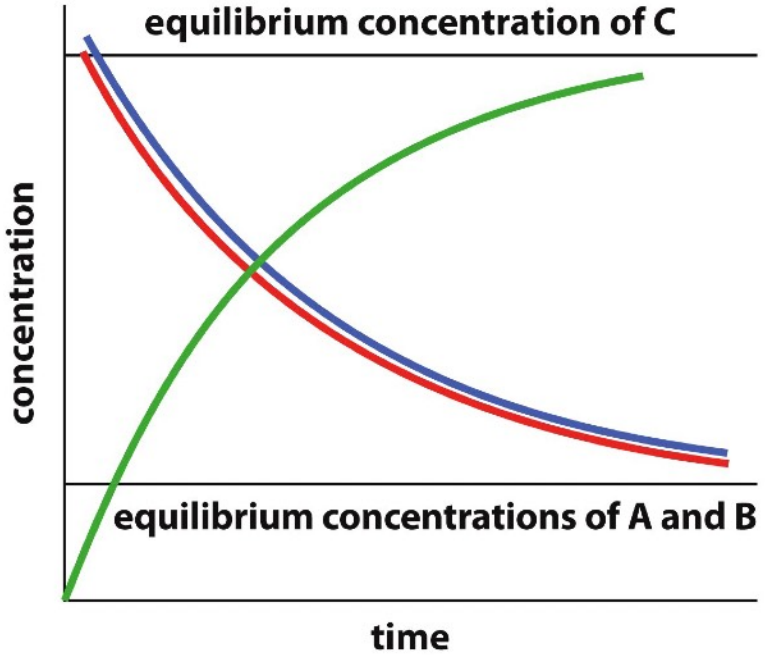
Reaction rates depend on molecular collisions

- The collision rate is proportional to the product of concentrations of reactants
- Higher temperature increases the diffusion rates of molecules and the likelihood of collisions

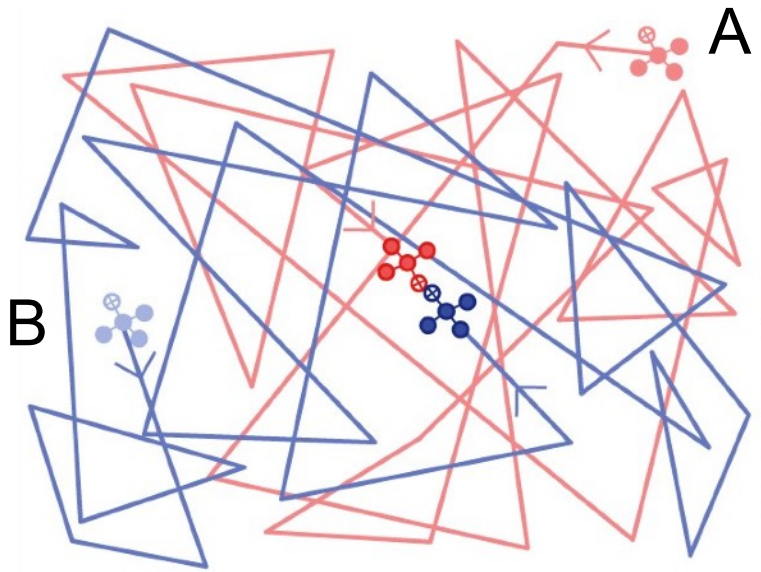


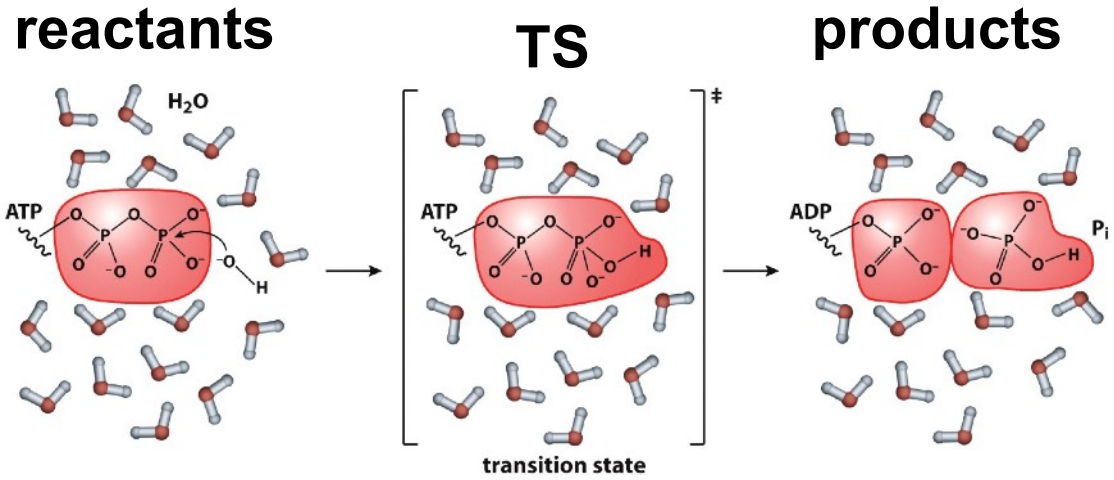
$$reaction\ rate = -\frac{d[A]}{dt} = \frac{d[C]}{dt} = k[A][B]$$





Reactions and diffusion





- ***rate of collision N*** depends on the frequency of collisions and if they lead to products (effective collisions)

$$N_{coll} = k_{coll}[A][B]$$

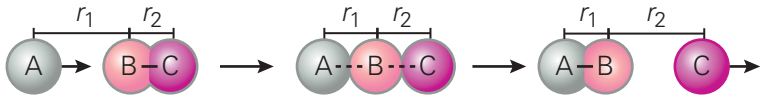
k_{coll} ~10^{10} M^{-1} s^{-1}

- **diffusion-limited** reactions are the fastest
- usual fast reactions have k_{obs}~10^{6} M^{-1} s^{-1}
- need for optimal orientation for formation of the **transition state (TS)**
- effective # of collisions are weighted with f_p (fraction of collisions that can actually lead to product)

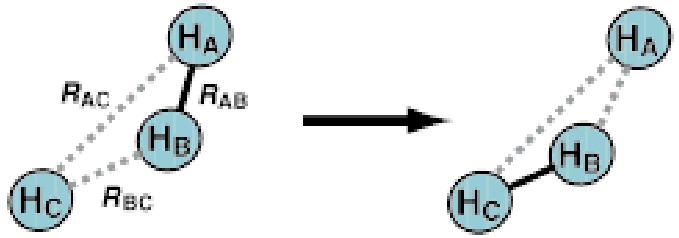
$$A = k_{coll} f_p$$

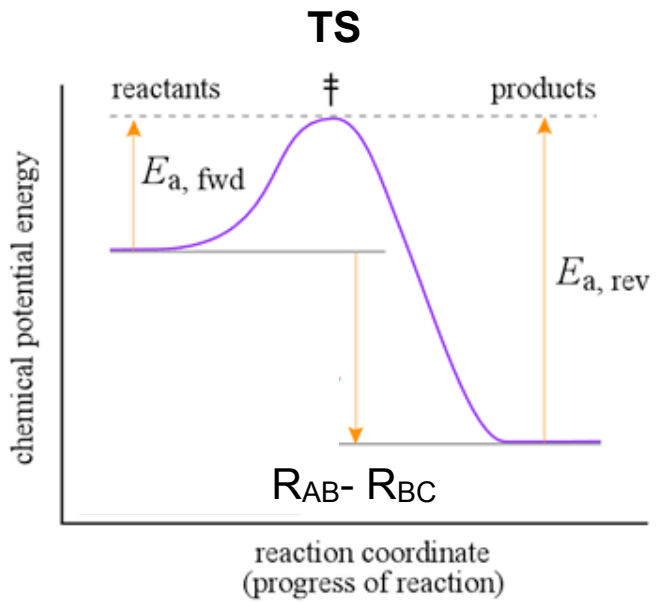
***A* = pre-exponential factor**

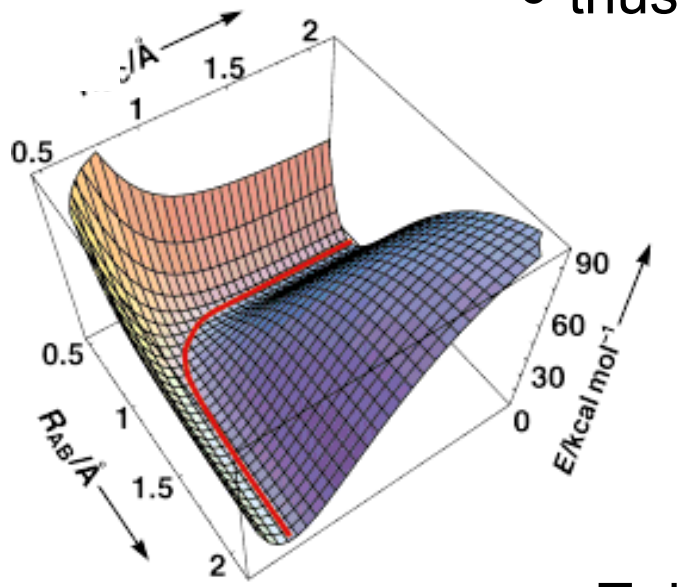
Arrhenius equation



$$H + H_2 \rightleftarrows H_2 + H$$







- a given energy is required to reach the **TS**
- **activation energy E_A**
- fraction of molecules having such energy is

$$e^{-E_A/RT}$$

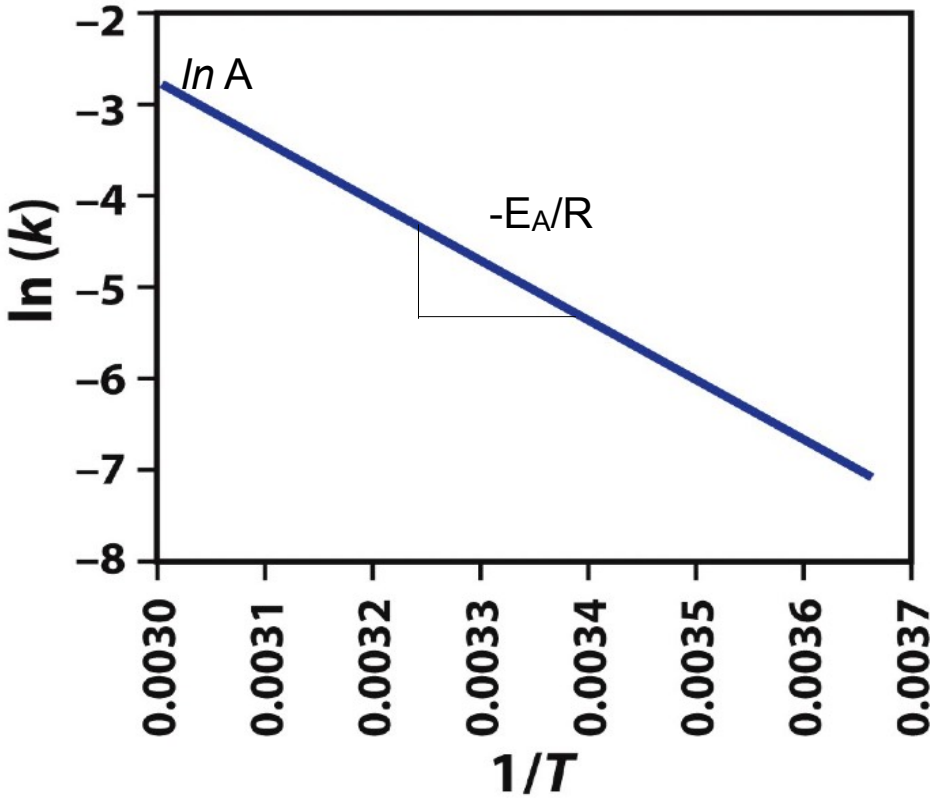
- thus the rate constant:

$$k = Ae^{-E_A/RT}$$

$$\frac{d[C]}{dt} = Ae^{-E_A/RT}[A][B]$$

- T dependency of rate constant *k*

Arrhenius equation - linear version



- if $k = Ae^{-E_A/RT}$

$$ln\ k = \left(\frac{-E_A}{R}\right)\frac{1}{T} + lnA$$

- linear version of the **Arrhenius equation** used to calculate the activation energy of a reaction

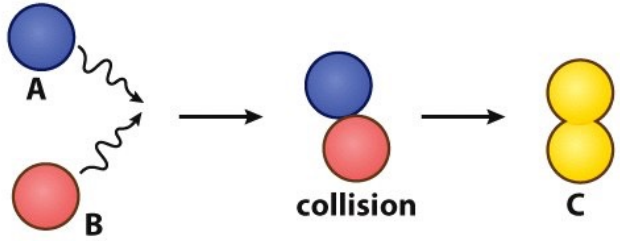
Concentration and order of the reaction

Fundamental reaction types:

(A) first-order reaction

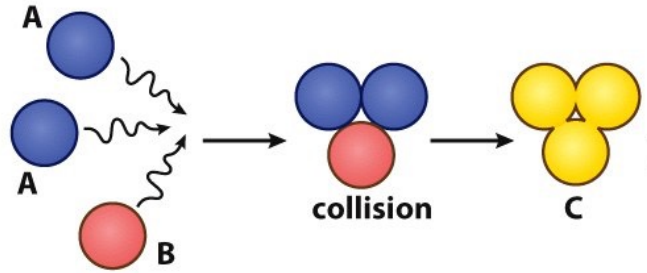
$$\frac{d[A]}{dt} = -k[A]$$

(B) second-order reaction



$$\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k[A][B]$$

(C) third-order reaction



$$\frac{1}{2}\frac{d[A]}{dt} = \frac{d[B]}{dt} = -k[A]^2[B]$$

k unit
sec^{-1}
$M^{-1}\ sec^{-1}$
$M^{-2}\ sec^{-1}$

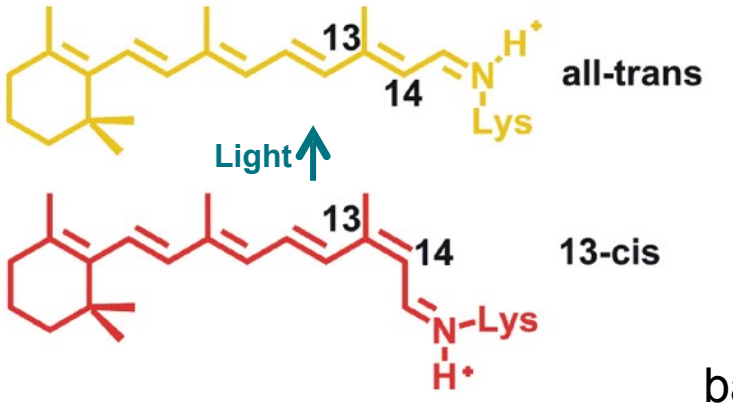
- *k* is related to the frequency of collision depending of the environment
- It also depends on collisions that actually form products (not always the case)
- **Order of the reaction** is the number of molecules
- Unit of *k* depends on the reaction order (s^{-1}, $M^{-1}s^{-1}$, etc.)

the slower the reaction the higher is the order

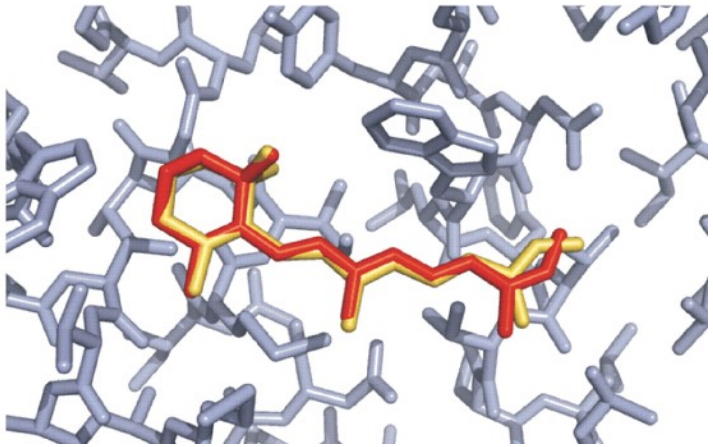
Examples of 1st order reactions

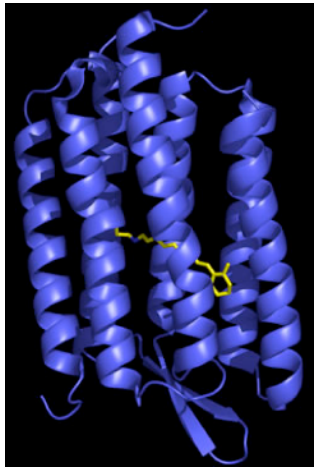
- Reaction that proceeds at a rate that depends linearly on only one reactant concentration
- Examples include decay, degradation, isomerization…

Retinal isomerization by photons



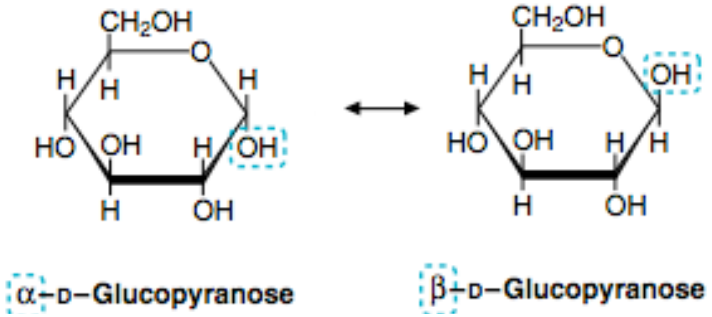
bacteriorhodopsin



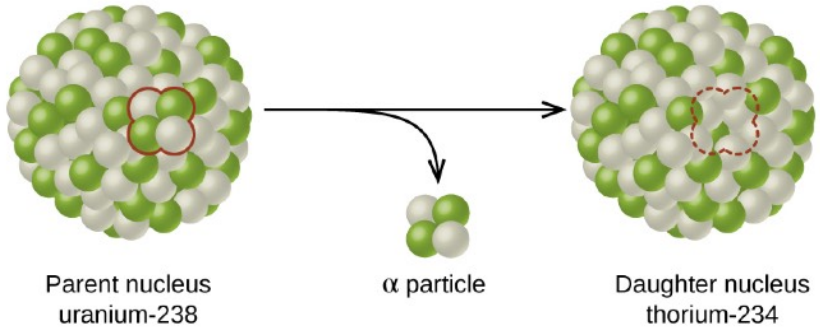


Important for photosynthesis and vision

Carbohydrate mutarotation



Spontaneous decay of radioactive nuclei



1st Order Reactions: Determining kinetic parameters

- Molecular decay or retinol isomerization can be modeled as

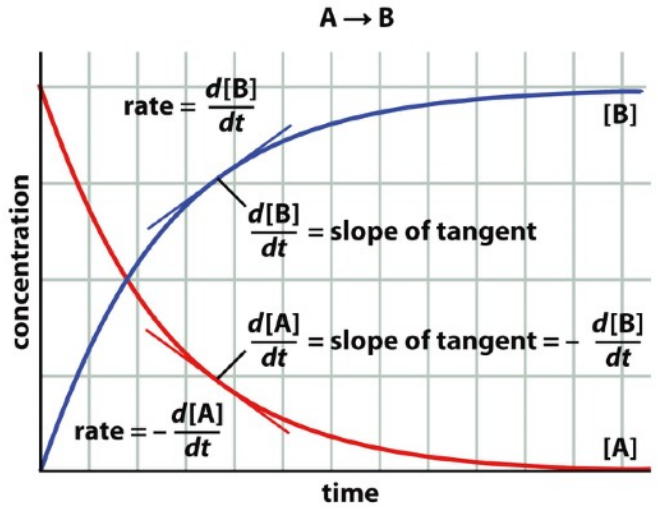
$$A \rightarrow B \qquad \frac{d[A]}{dt} = -\frac{d[B]}{dt} = -k[A]$$

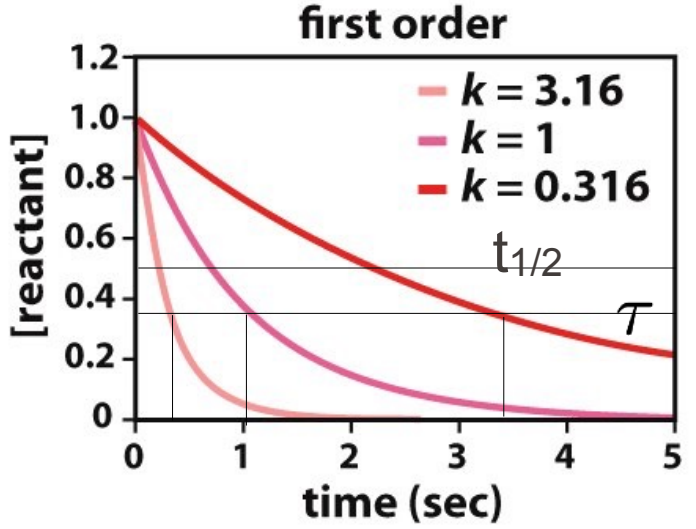
- Rate of decay of reactant is proportional to its concentration via *k* [s^{-1}] constant; solving the 1st-order linear differential equation:

$$[A](t) = [A]_0 e^{-kt} = [A]_0 e^{-t/\tau} \qquad \tau = 1/k$$

- With $[A]_0 = c_{(t=0)}$ is the initial concentration, τ is the characteristic time of decay (**lifetime or time constant**)
- Rate equations describe the time evolution of average A and B amounts (concentrations)
- **Half-life $t_{1/2}$** (= ln2/k =0.693/k) is the time needed for the concentration of [A] to drop to half of its initial value

$$\frac{d[B]}{dt} = k[A] = k\{[A]_0 - [B]\} \Rightarrow [B](t) = [A]_0(1 - e^{-kt})$$





Protein folding can be considered a series of 1st order reactions

- Protein folding is a reaction with many intermediate steps.
- For simpler proteins (monomeric, no post-translational modifications), the folding process can be approximated as a series of 1st order reactions

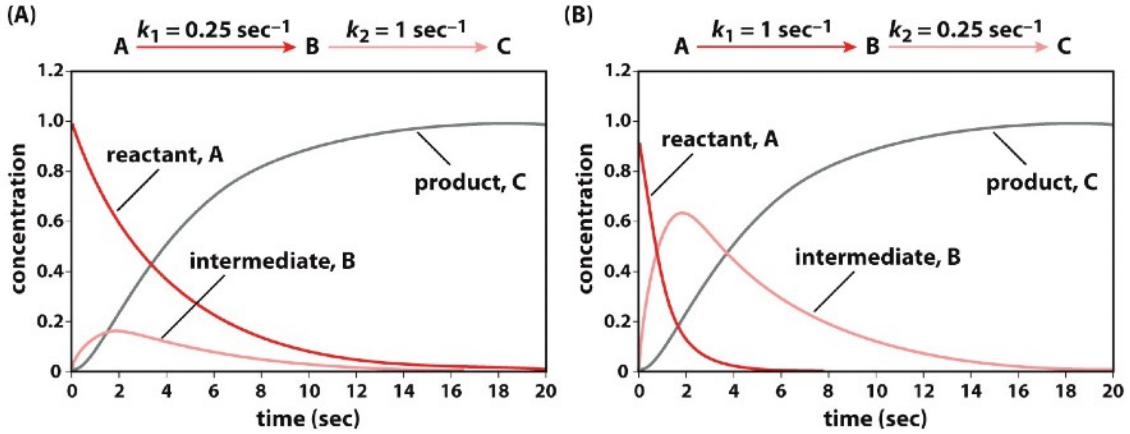
$$\frac{d[A]}{dt} = -k_1[A] \qquad \frac{d[B]}{dt} = k_1[A] - k_2[B] \qquad \frac{d[C]}{dt} = k_2[B]$$

- solutions

$$[A] = [A]_0 e^{-k_1 t}$$

$$[B] = \frac{k_1[A]_0}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t})$$

$$[C] = [A]_0 \left[1 - \frac{1}{k_2 - k_1}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t})\right]$$

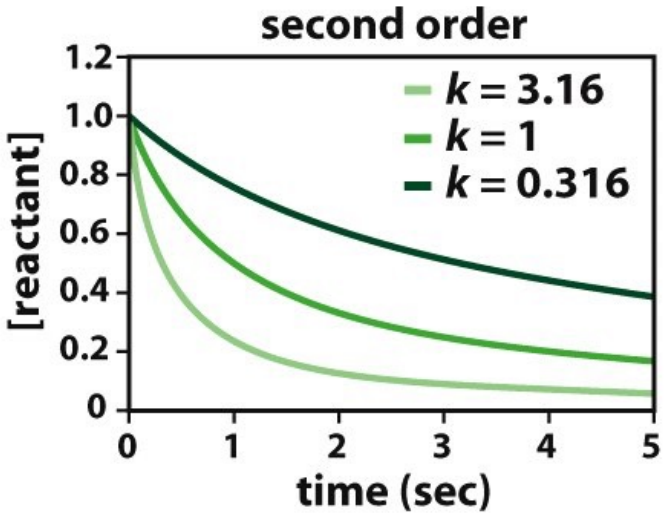


- To determine the concentration of each reaction component at each time point these equations need to be integrated
- the slowest step is called **rate-determining step**

2nd order reactions

- More general case including **2 reactants** whose concentrations impact reaction kinetics
- If A and B reactants come together to form a product C

$$A + B \xrightarrow{k} C$$



- The rate equation can be written as:

$$\frac{d[C]}{dt} = k[A][B] \qquad \frac{d[A]}{dt} = -k[A][B]$$

- The rate of reaction (i.e. forming C) is proportional to the probability that A and B are at the same place (i.e., undergoing collision). Higher temperature promotes the reaction.

- Unlike the 1st order kinetics this case the dynamical equation of different species are **coupled** (i.e., you need to know the concentration of both A and B to determine reaction rate)

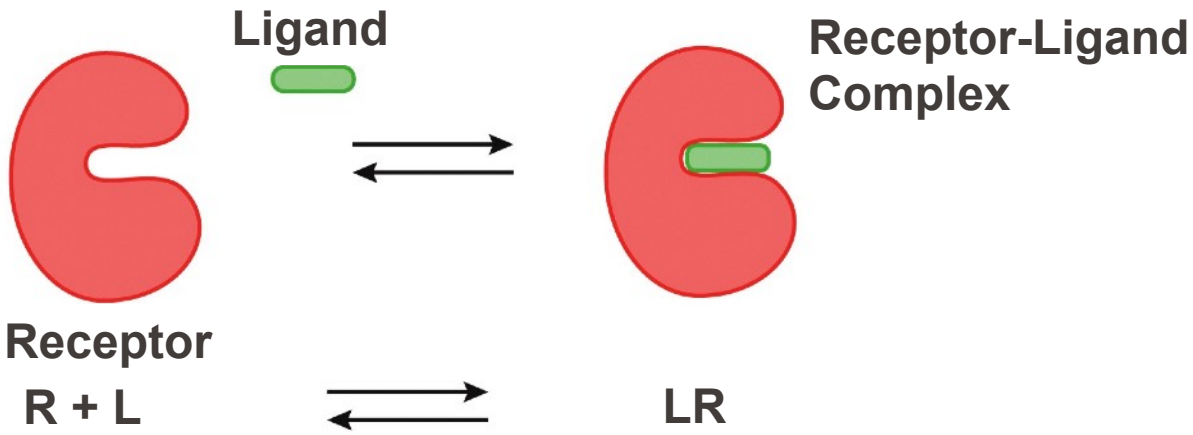
Reactions can be reversible

- Molecule binding can be approximated using 2nd order reversible reaction
- The complex is equivalent to a ”reaction product” while the two binding partners are “reactants”
- For example, consider ligand-receptor case or drug binding:

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} LR$$

$$\frac{d[LR]}{dt} = -k_{off}[LR] + k_{on}[L][R]$$

- *L* = concentration of ligands, R = concentration of receptors. But when L >> R:



$$\frac{d[LR]}{dt} = -k_{off}[LR] + k_{on}^{'}[R]$$

- with

$$k_{off} = [s^{-1}]$$

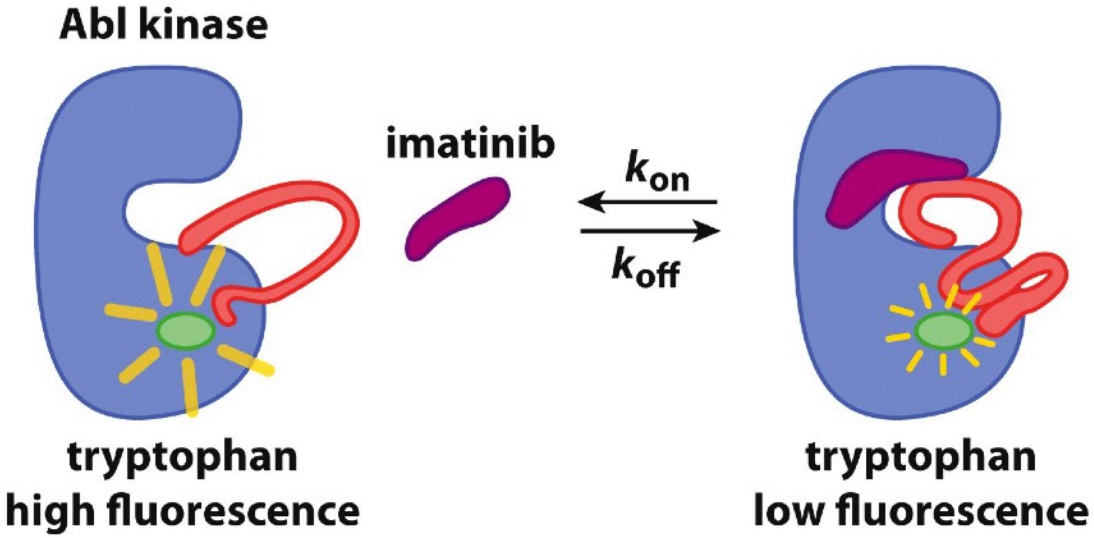
$$k_{on} = [M^{-1}s^{-1}]$$

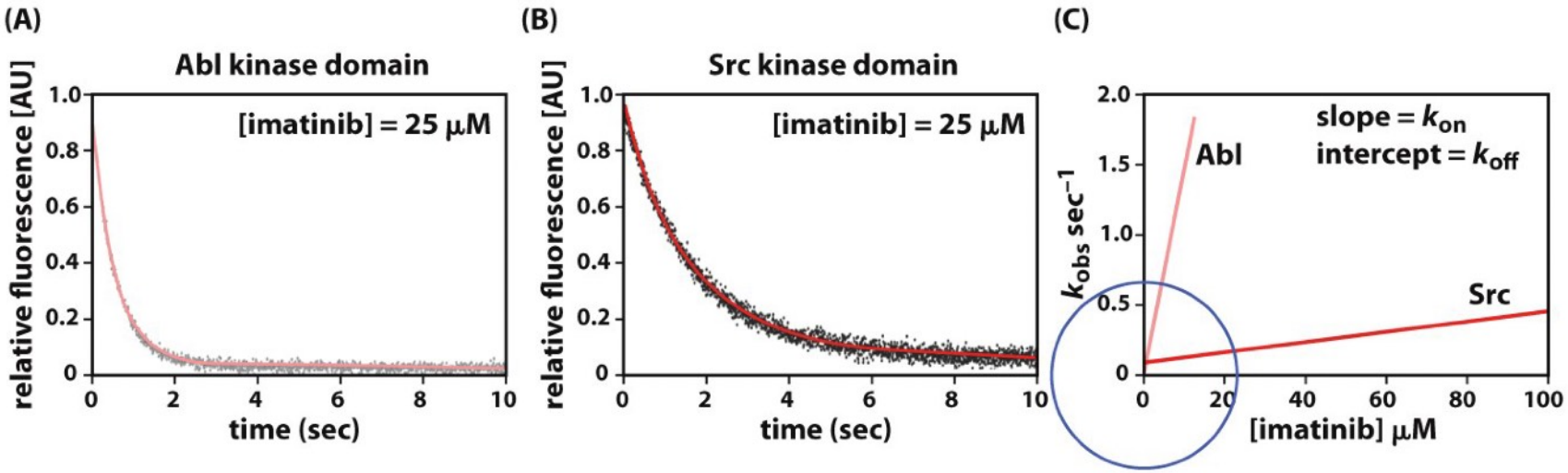
pseudo 1st-order rate constant

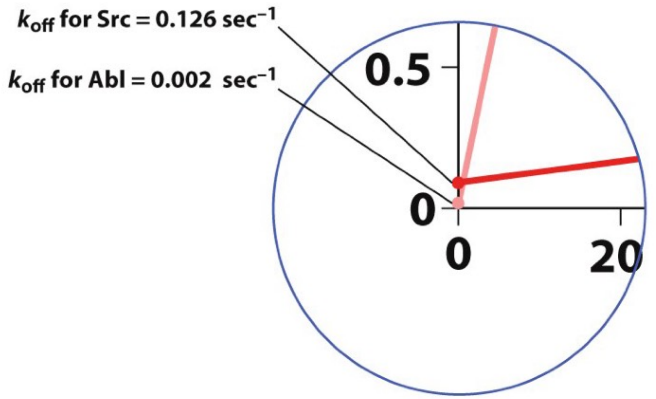
Ligand binding

$$[R] = ([R]_0 - [R]_{eq})e^{-(k'_{on}+k_{off})t} + [R]_{eq}$$

$$k_{obs} = k'_{on} + k_{off} = k_{on}[L] + k_{off}$$







Equilibrium and Steady-State conditions

- At the **equilibrium** the reaction is at its **steady state**, thus the rate of LR product release is zero:

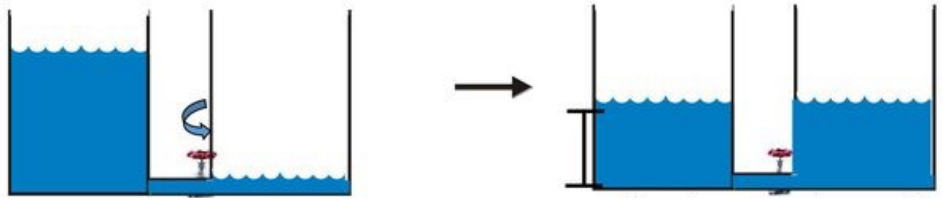
$$-k_{off}[LR]_{eq} + k_{on}[L]_{eq}[R]_{eq} = 0$$

- solving for : $\frac{k_{off}}{k_{on}} = \frac{[L]_{eq}[R]_{eq}}{[LR]_{eq}} = K_D$

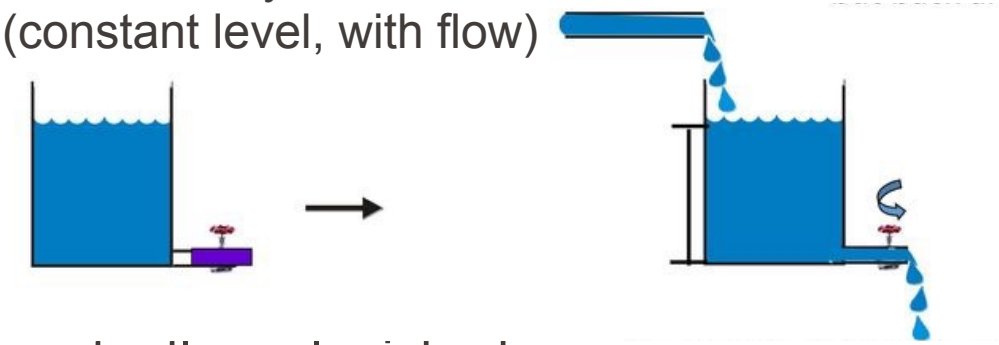
$$\boxed{K_D = \frac{k_{off}}{k_{on}}}$$

- **Steady state** conditions can be reached also out-of-equilibrium, when concentrations do not change with time. This is always the case in cellular metabolic pathways (BC2)

Equilibrium
(constant level, no net flow)



Steady state
(constant level, with flow)



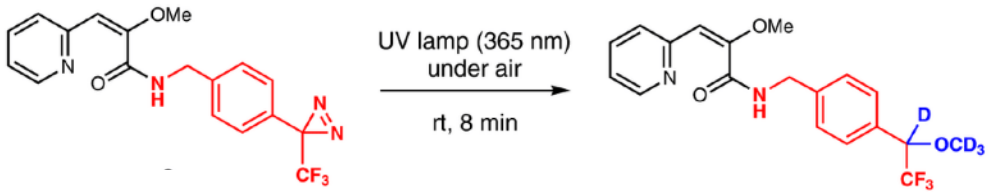
- In cells, basic metabolites and building blocks are constantly replenished

Catalysis and Enzymes

Catalysis of chemical reactions

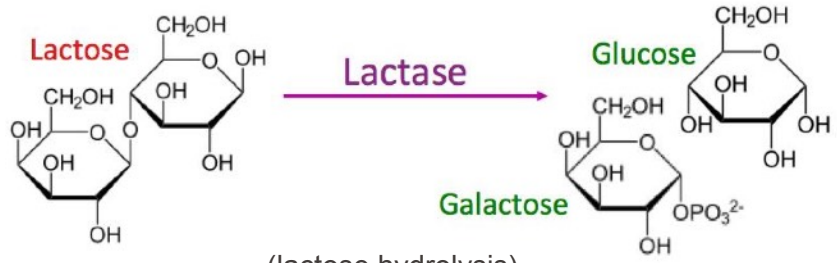
- The modification of the rate of a chemical reaction, usually an acceleration, by addition of a substance (catalyst) not consumed during the reaction
- Catalysis can be achieved by light, chemical methods, proteins (i.e., enzymes) and even RNA (i.e., ribozymes)

Photocatalysis



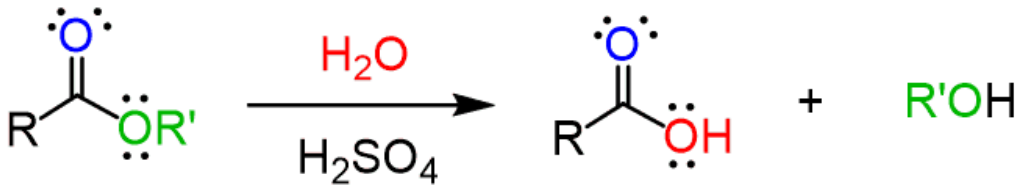
(diazirine group cross-linking)

Enzymatic catalysis



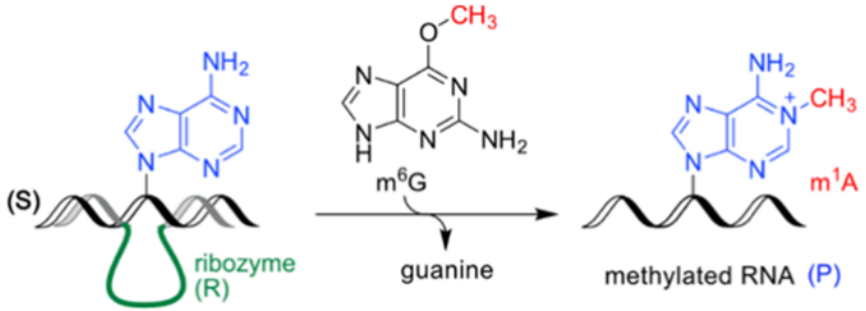
(lactose hydrolysis)

Chemical catalysis



(ester hydrolysis in the presence of acids)

RNA catalysis



(RNA-catalyzed methylation of RNA)

Enzymes

- Enzymes catalyze hundreds of stepwise reactions of metabolism, conserve and transform chemical energy, and make biological macromolecules from simple precursors.
- While all proteins interact with other biomolecules, only ~10% of all proteins possess enzymatic activity.
- Enzymes are named by adding the suffix "**-ase**" to the name of their substrate or of their activity.
- Substrate binding site on the enzyme is known as the **active site.**
- They are named and **classified based on the type of reaction catalyzed**

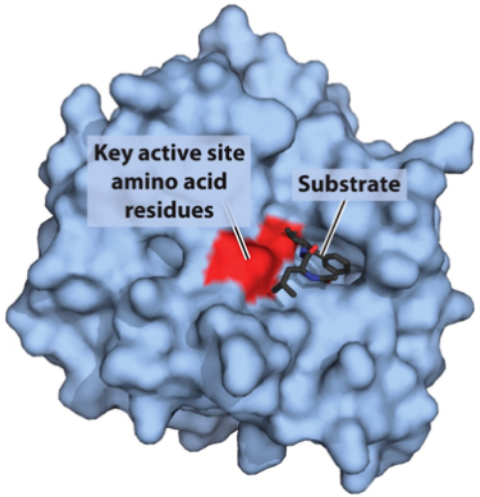
TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

- The name enzyme comes from Greek *én* (within) and *zymē (yeast)*

EC Number (Enzyme Commission number)

Example : Alcohol Dehydrogenase (EC 1.1.1.1)
- 1. Oxidoreductase (catalyzes redox reactions).
- 1.1. Acting on the CH-OH group of donors (alcohols).
- 1.1.1. With NAD+ or NADP+ as acceptor.
- 1.1.1.1 Alcohol dehydrogenase.



Enzyme cofactors

- Some enzymes require an additional chemical component called a **cofactor**.
- Cofactors can be inorganic ions, or complex organic or metalloorganic molecules called **coenzymes**.
- **Vitamins** are organic molecules essential for normal cellular processes, serving as **precursors of coenzymes**

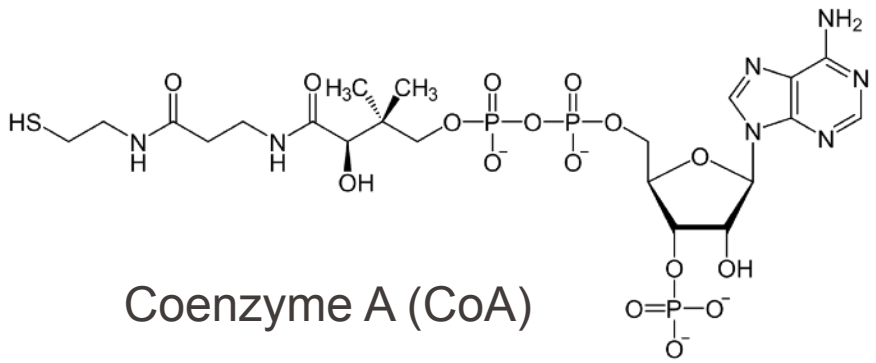
TABLE 6–1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

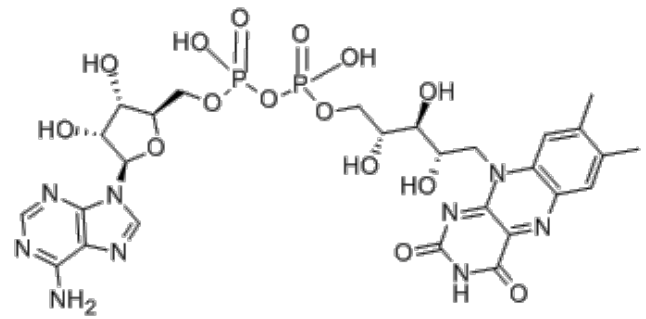
metallo-enzymes

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)



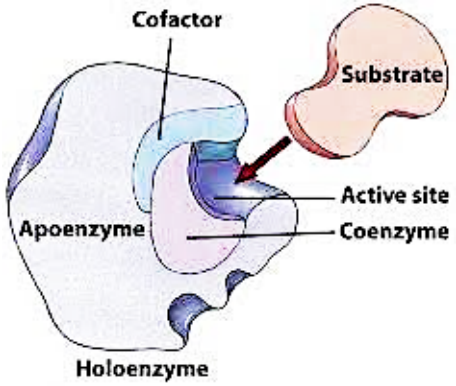
Coenzyme A (CoA)



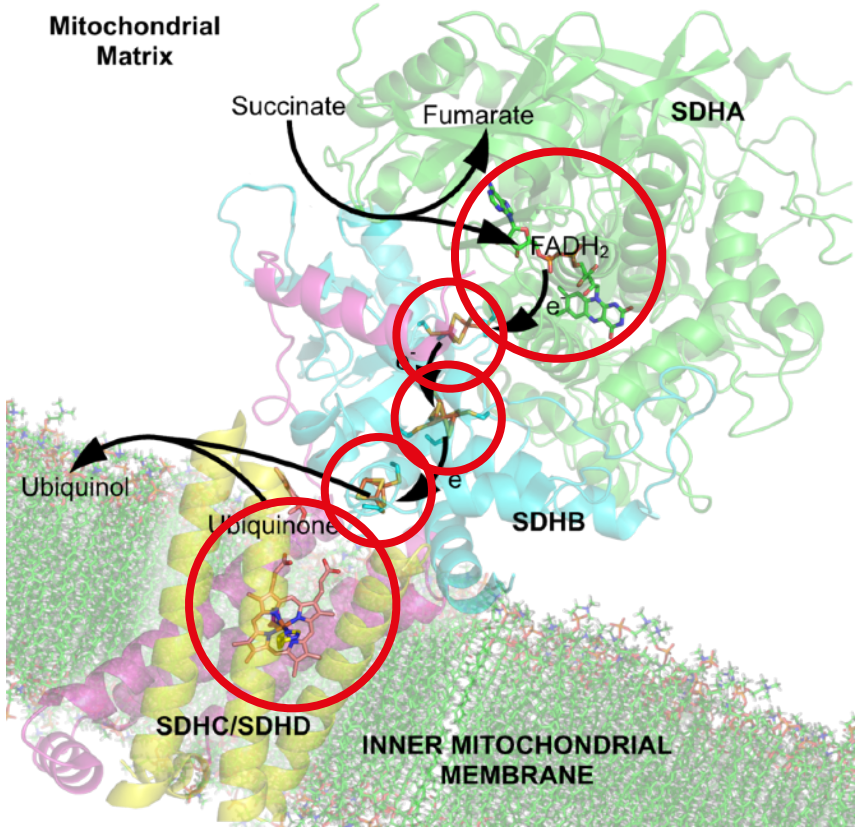
Flavin adenine dinucleotide (FAD)

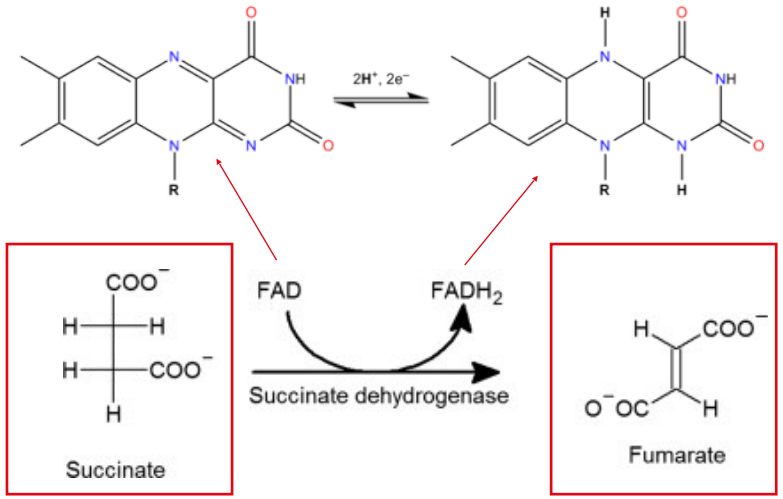
Enzyme cofactors

- Cofactors are often found in enzyme active sites where they actively contribute to catalysis of reactions
- In the absence of cofactor, enzyme is inactive (**apoenzyme**) and often unstable in solution
- Cofactor binding provides functionality, and this active complex is referred to as **holoenzyme**



Succinate dehydrogenase (SDH) has several cofactors





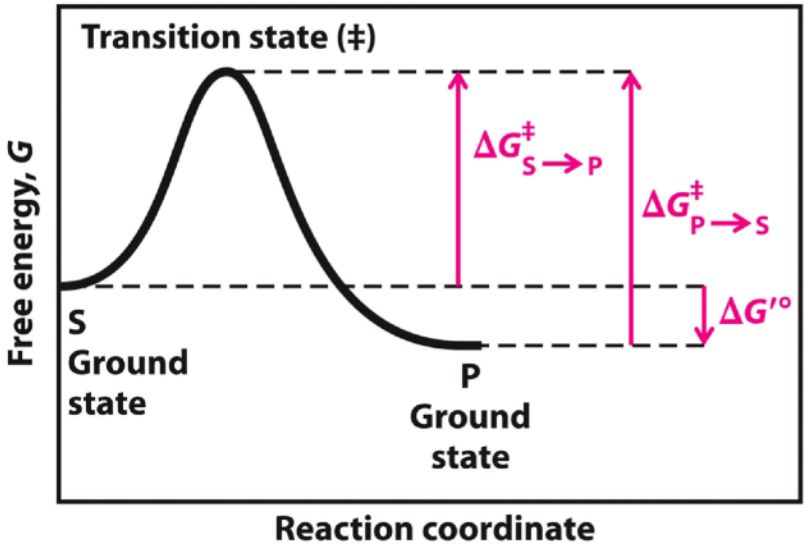
Background info:
- SDH is a crucial enzyme in cellular respiration, acting in both the Krebs cycle (oxidizing succinate to fumarate) and the electron transport chain (transferring electrons to ubiquinone).
- Both processes are essential for energy metabolism inside the cell

Enzyme properties

- Enzymes have extraordinary catalytic power and a **high degree of specificity for their substrates** and they accelerate chemical reactions tremendously (**10^5 to 10^{17}-fold**).
- They function in aqueous solutions under very **mild conditions of temperature and pH**, unlike many catalysts used in organic chemistry.
- Like other catalysts, enzymes **enhance reaction rates** by lowering activation energies. They have no effect on the **position of reaction equilibria**.

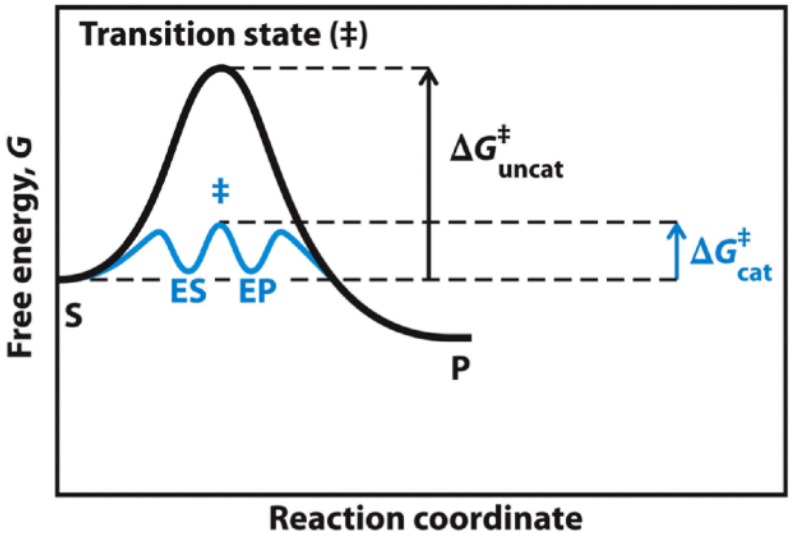
uncatalyzed reaction

$$S \rightleftarrows P$$



catalyzed reaction

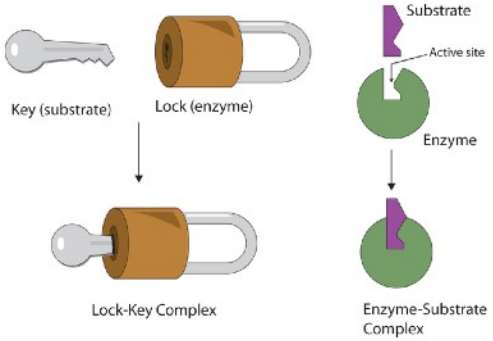
$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$

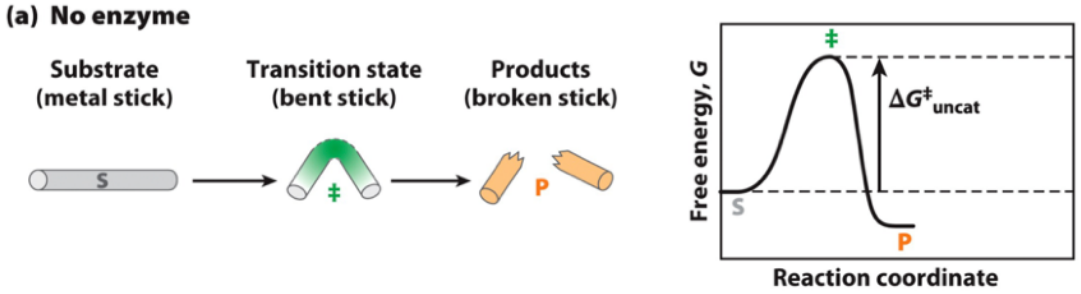


$E_a \equiv \Delta G^{\ddagger}$
(activation energy)

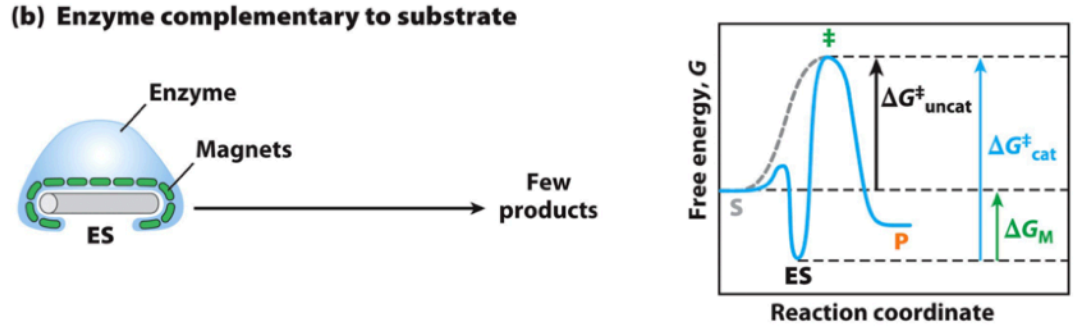
Transition state complementarity and rate enhancement

- Early theory from Fischer et al (1890s) proposed a “lock and key” model for enzyme-substrate interaction which explains the specificity, but does not explain why reaction rate increases
- Let’s imagine a scenario where a substrate is converted into a product:

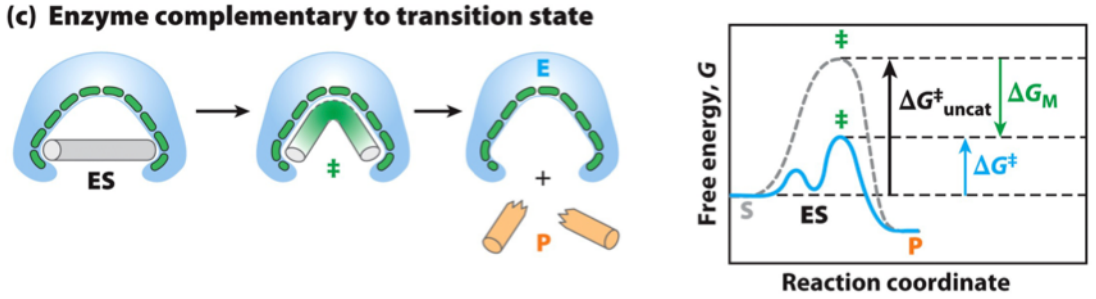




- Uncatalyzed
- High activation energy



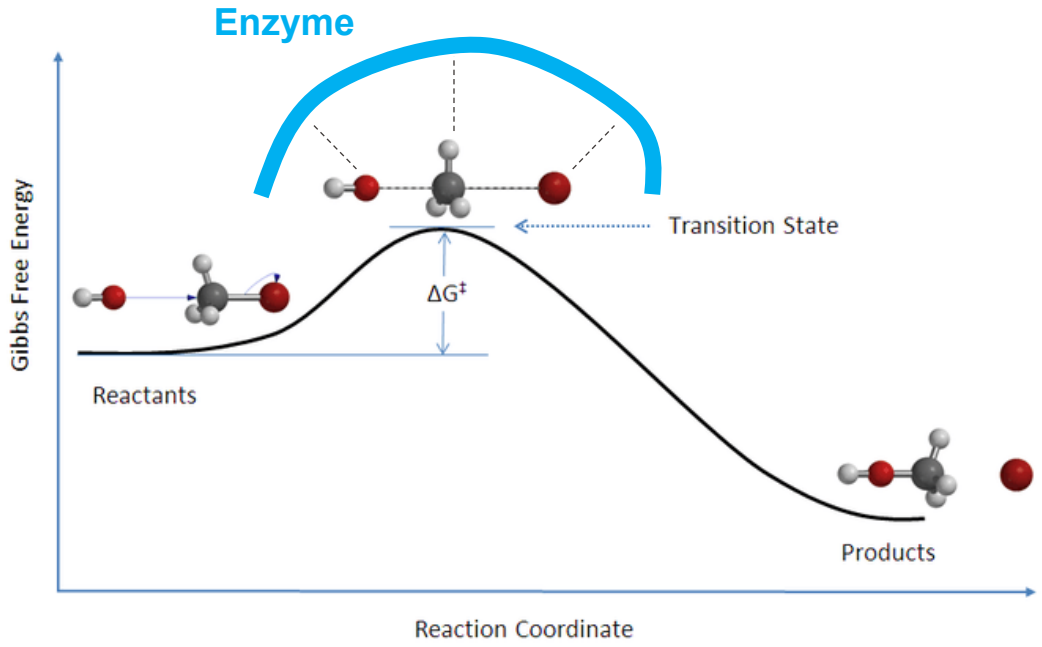
- Enzyme binds substrate well
- The activation energy increases



- Enzyme binds substrate less-well compared to (b)
- The activation energy decreases

Transition state complementarity and rate enhancement

- Enzyme-substrate interactions need to be sufficiently strong to lead to complex formation, but the full complement of such interactions is created with **substrate in a transition state conformation**
- The free energy (binding energy) released by the formation of these interactions partially offsets the energy required to reach the top of the energy hill.
- **The transition state is not a stable species**, but is a brief point in time that the substrate spends atop an energy hill
- for this reason is not easy to capture the structure of such a fleeting state (e.g. by X-ray crystallography)

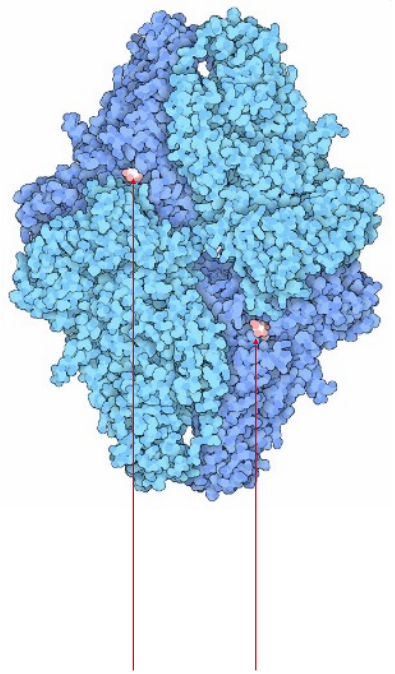


The enzyme brings these two reactants into close proximity and coordinates them in such a way that they form an unstable transition intermediate ultimately leading to the departure of the red group on the right side and formation of products.

Examples of enzymatic reactions

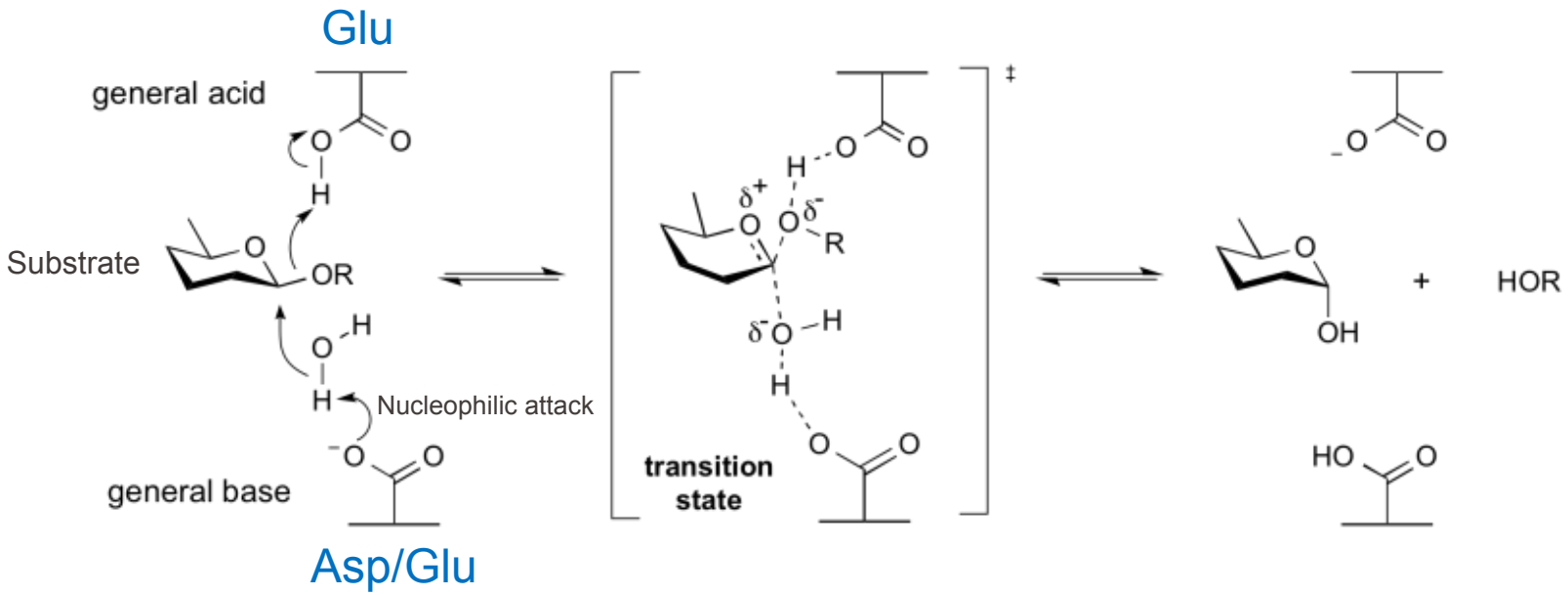
• Many enzymatic mechanisms involve transfer of protons (general acid-base catalysis) or the formation of transient covalent bonds in the active site via amino-acids or cofactors

β-galactosidase



Active sites

β-galactosidase cleaves galactose from carbohydrates (e.g., lactose)

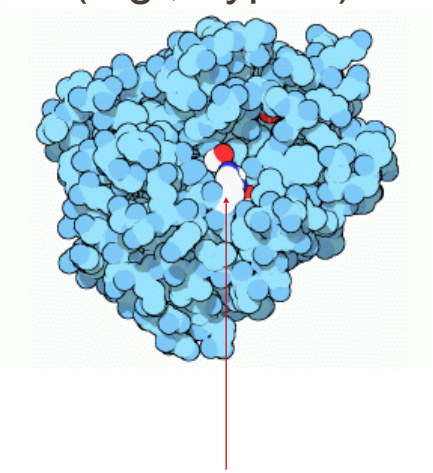


Nucleophile is a chemical group that forms bonds by donating electron pair
Electrophile forms bonds with nucleophiles by accepting electrons

Examples of enzymatic reactions

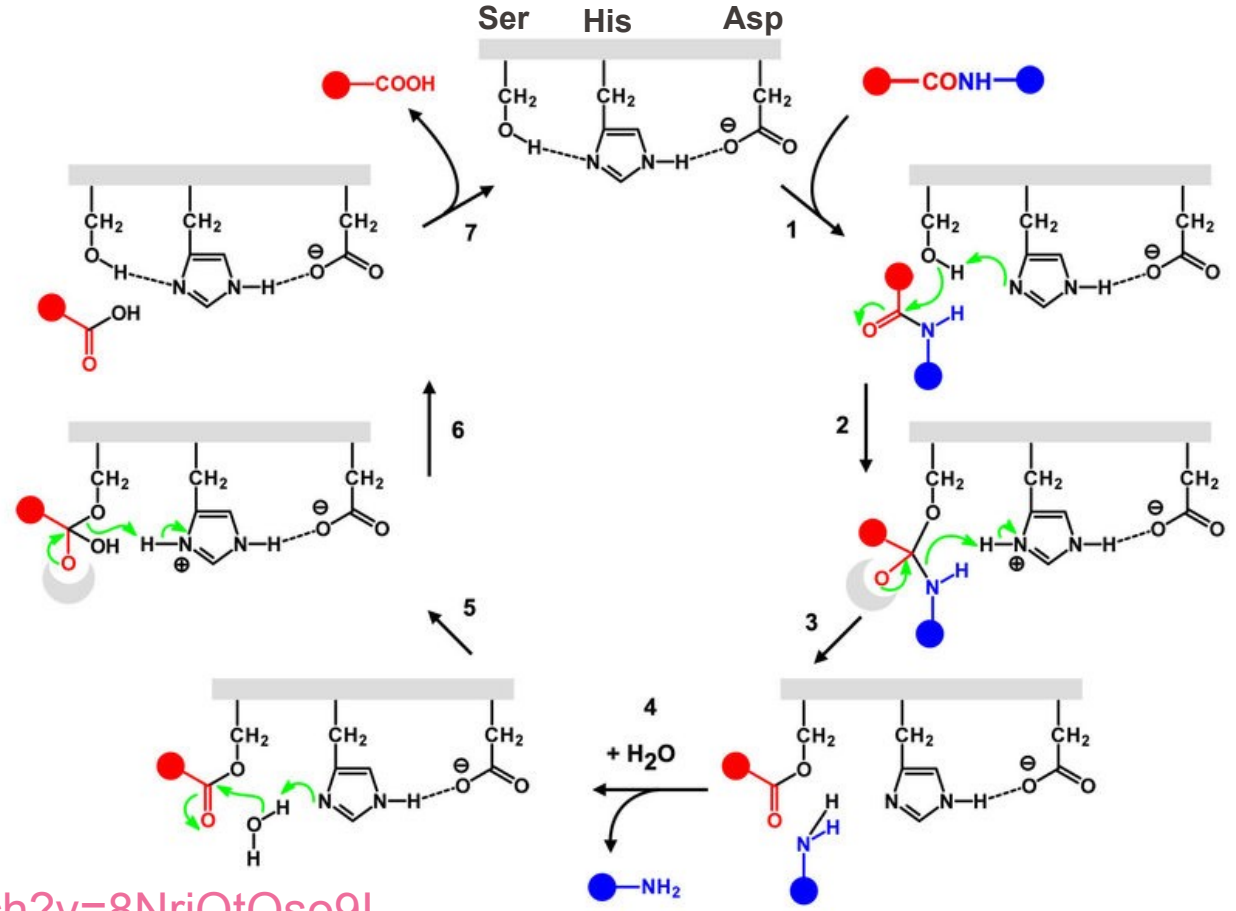
• Many enzymatic mechanisms involve transfer of protons (general acid-base catalysis) or the formation of transient covalent bonds in the active site via amino-acids or cofactors

Serine protease
(e.g., trypsin)



Active site

Serine proteases (comprising digestive enzymes) cleave peptide bonds in proteins



Green arrows indicate the nucleophilic attacks (electron transfer)

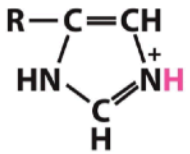
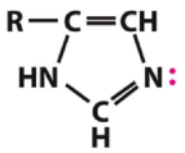
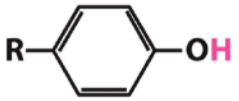
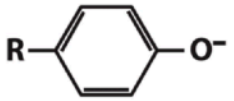
Ser + His + Asp
=
Catalytic triad

https://www.youtube.com/watch?v=8NriQtOso9I

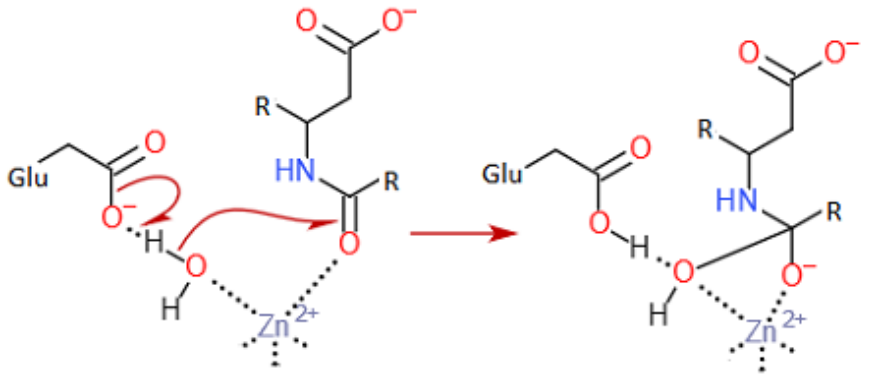
Groups commonly involved in catalysis

- Amino-acids with exchangeable hydrogens in their side chains (i.e., bound to O or N) are particularly important for reaction catalysis
- Active site residues are often not proximal in sequence, but they are brought together in 3D structure
- Metal ions are also very important for certain enzymes and contribute either by (1) coordinating the substrate in the active site through ionic interactions or (2) by catalyzing oxidation/reduction reactions

Amino-acids with general acid:base properties

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—$\overset{+}{N}$H$_3$	R—NH$_2$
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

Example of metal ion (Zn^{2+}) assisted catalysis



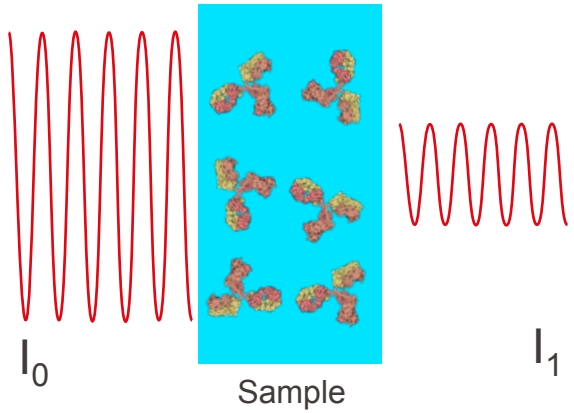
Enzyme substrate complex Tetrahedral intermediate

Enzyme kinetics

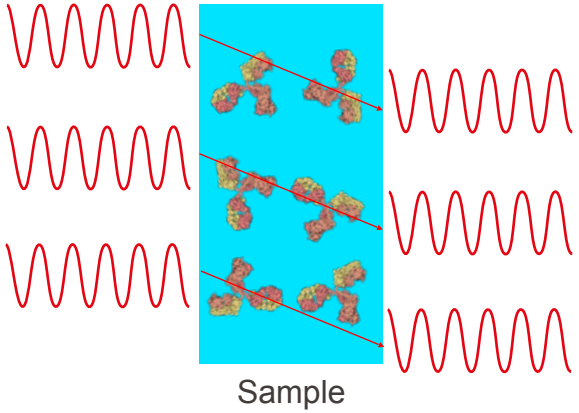
Biophysics is applied to measure reaction kinetics

- Different spectroscopic methods are exploited for the purpose of measuring reaction progress
- Reactants are combined and reaction progress monitored over time by a suitable detection method

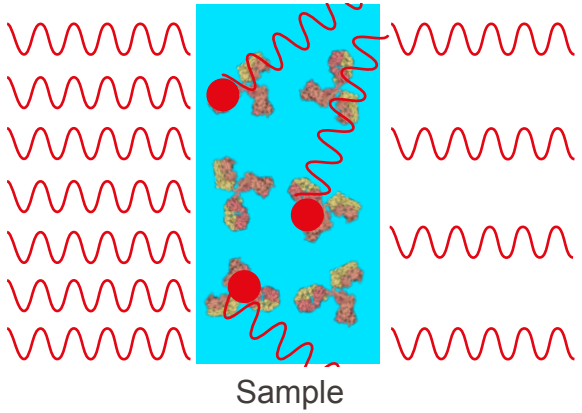
Absorption:



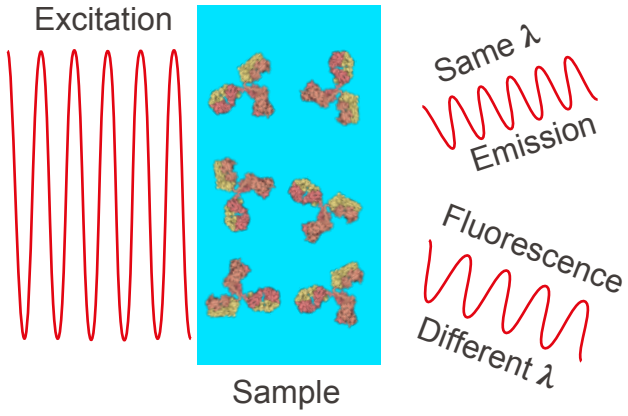
Refraction:



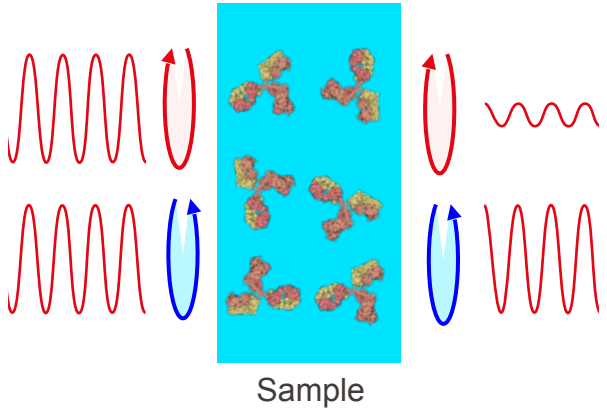
Scattering:



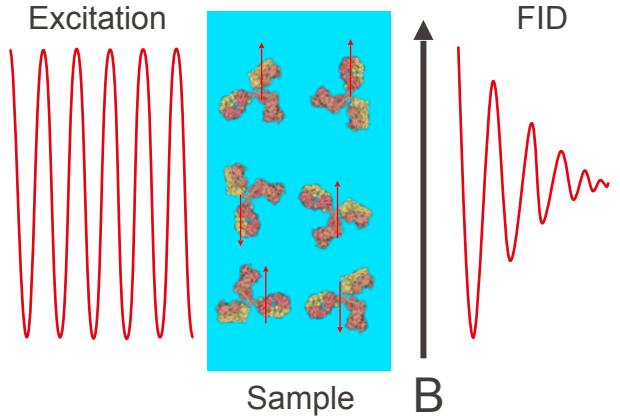
Emmission/Fluorescence:



Polarization:

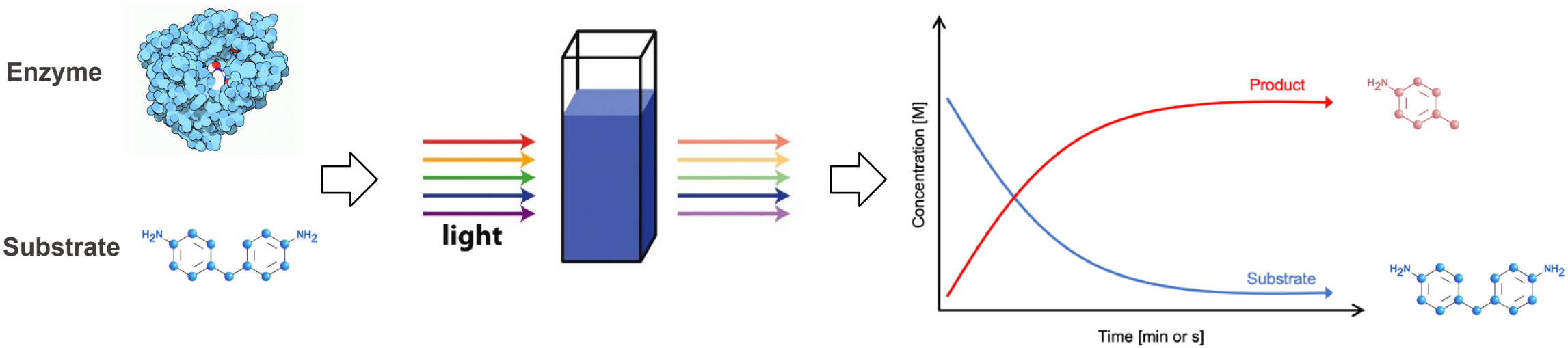


Resonance:



Quantitative analyses of reaction rates

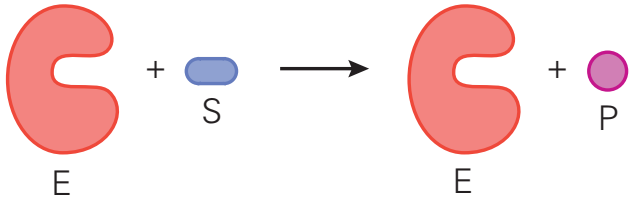
- If you consider a simple reaction where a substrate get converted into a product through the action of an enzyme:



- The amount of product will gradually increase while the amount of substrate decreases proportionally
- The rate at which the reaction takes place will differ depending on the experimental conditions (e.g., enzyme concentration, substrate concentration temperature), but it will also be dependent on the enzyme's capacity to catalyze reactions (i.e., reduce the activation energy).
- Measuring the catalytic properties of different enzymes is a discipline known as **enzyme kinetics**.

Measuring kinetic parameters of enzymes

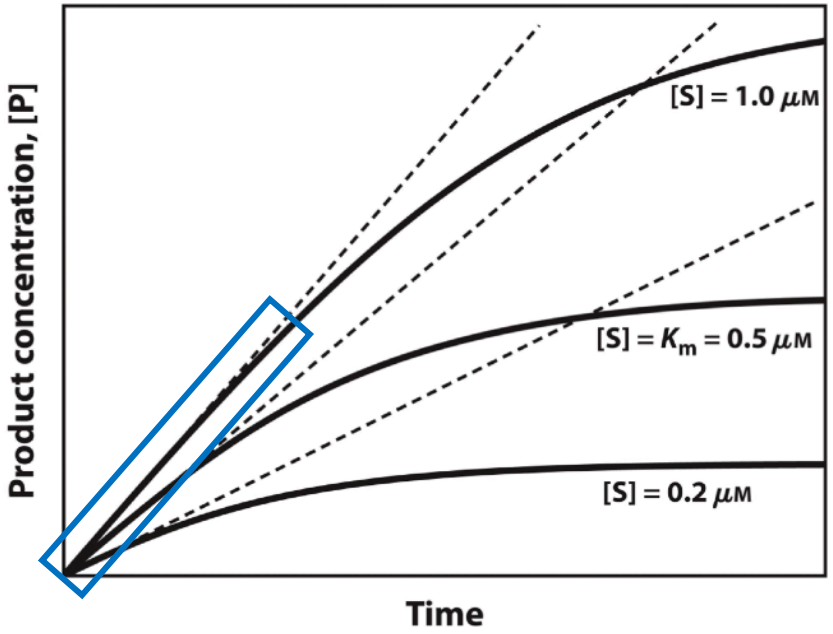
- Studying the effects of substrate [S] or product [P] concentration is complicated as it changes with time
- the rate of P production is called **velocity of the reaction**, it changes proportionally with substrate concentration, during the reaction [E] is usually constant
- the velocity of the reaction is linear with time during the initial phase



$$S + E \underset{k_r}{\overset{k_f}{\rightleftharpoons}} P + E$$

$$v = \frac{d[P]}{dt} = k_f[S][E]$$

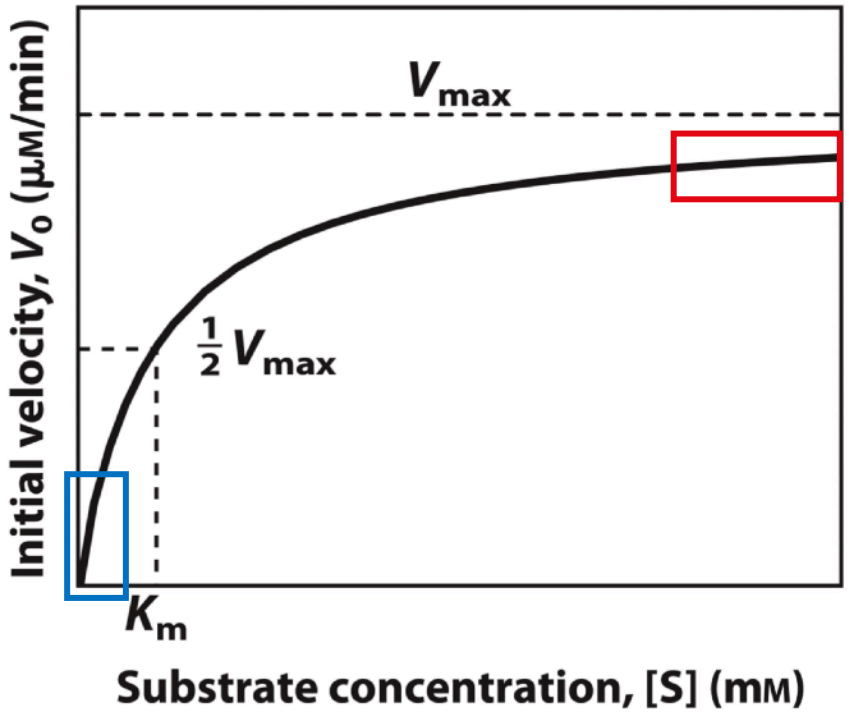
v - velocity of the reaction depends on time t
k - rate constant (Arrhenius equation)



- To treat the problem, we only measure the **initial velocity of the reaction** (designated V_0), when the velocity regime is linear

Measuring kinetic parameters of enzymes

• The initial velocity (V_0) expressed as a function of starting substrate concentration [S] provides the typical enzyme kinetics plot from which catalytic parameters can be extracted
• Note that all initial velocity measurements need to be performed under constant experimental conditions including temperature, enzyme concentration, buffer composition etc. The only variable is [S].



(reminiscent of hyperbolic binding isotherm)

• At relatively low concentrations of substrate relative to the enzyme, V_0 increases almost linearly with an increase in [S]

If [E] > [S] then V_0 ~ k[S]

• At higher substrate concentrations, V_0 increases by smaller and smaller amounts in response to increases in [S].

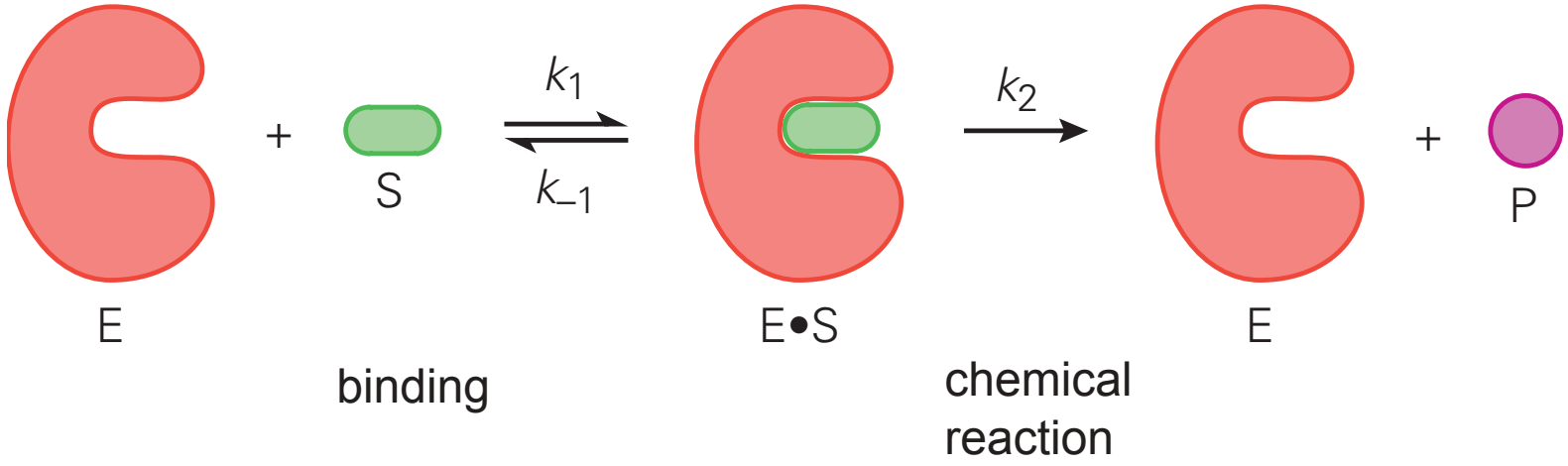
If [S] >> [E] then all E is **saturated** with S

• The maximum value of reaction velocity that can be achieved under given experimental conditions is called V_{max}

• the **Michaelis constant (K_M)** specifies the concentration of substrate required to reach half of V_{max}

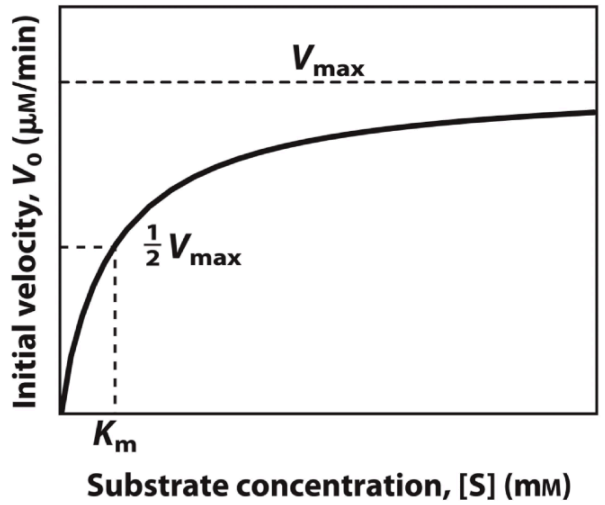
Michaelis-Menten kinetics (1913)

There is a close connection between the thermodynamics of binding and the kinetics of enzyme-catalyzed reactions, because the first step in catalysis is the binding of substrate to the enzyme. If the on- and off -rates for the substrate binding to the enzyme are fast compared to the catalytic step, then the binding can be considered to be a reversible event



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

Michaelis-Menten kinetics (1913)



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

$$v_0 = \frac{d[\mathrm{P}]}{dt} = k_2[\mathrm{E}\bullet\mathrm{S}]$$ initial velocity of the reaction

After mixing enzyme with substrate, the rates of formation and dissociation of the enzyme complex will become equal, and so the concentrations of the free enzyme and the enzyme–substrate complex will reach constant values - **steady state conditions**

$$\frac{d[\mathrm{E}\bullet\mathrm{S}]_{ss}}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{E}\bullet\mathrm{S}]_{ss} - k_2[\mathrm{E}\bullet\mathrm{S}]_{ss} = 0 \implies [\mathrm{E}\bullet\mathrm{S}]_{ss} = \frac{k_1[\mathrm{E}][\mathrm{S}]}{k_{-1} + k_2}$$

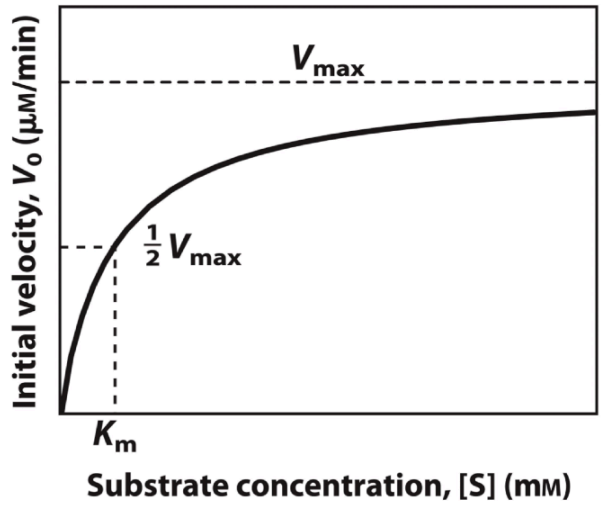
[E] (free enzyme) is difficult to measure, thus we can express in terms of total E concentration, $[E]_0$

$[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{E}\bullet\mathrm{S}]_{ss}$, substituting and solving for $[ES]_{ss}$ we obtain

$$[\mathrm{E}\bullet\mathrm{S}]_{ss} = \frac{[\mathrm{E}]_0}{\left(1 + \frac{k_{-1} + k_2}{k_1[\mathrm{S}]}\right)}$$, if we define $K_M = \frac{k_{-1} + k_2}{k_1}$ we obtain $[\mathrm{E}\bullet\mathrm{S}]_{ss} = \frac{[\mathrm{E}]_0}{\left(1 + \frac{K_M}{[\mathrm{S}]}\right)}$

Michaelis-Menten equation (1913)

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



$$[E\bullet S]_{ss} = \frac{[E]_0}{\left(1 + \frac{k_{-1} + k_2}{k_1[S]}\right)}$$, if we define $K_M = \frac{k_{-1} + k_2}{k_1}$ we obtain $[E\bullet S]_{ss} = \frac{[E]_0}{\left(1 + \frac{K_M}{[S]}\right)}$

which multiplied by k_2 will give the initial velocity of the reaction $v_0 = k_2[E\bullet S]_{ss} = \frac{k_2[E]_0}{\left(1 + \frac{K_M}{[S]}\right)}$

Now, the maximum velocity, ***V_{max}***, of the enzyme-catalyzed reaction occurs when all of the enzyme is bound to substrate—that is, when [E•S] is equal to $[E]_0$. And so the value of $V_{max} = k_2[E]_0$. Finally:

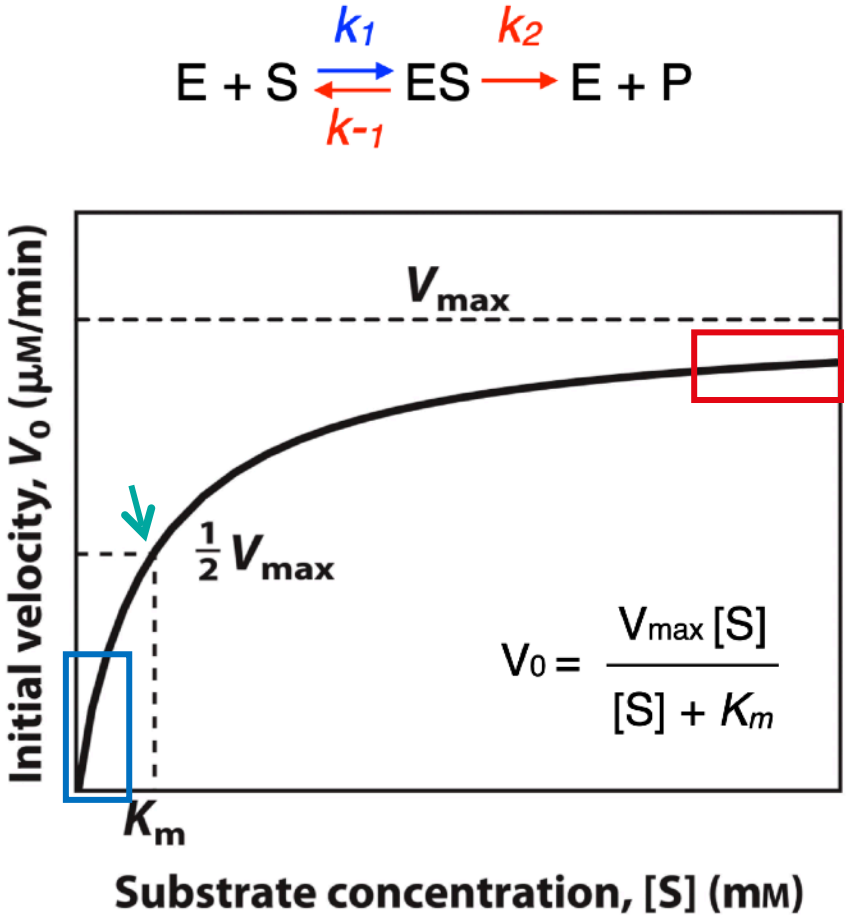
$$v_0 = \frac{V_{max}}{\left(1 + \frac{K_M}{[S]}\right)}$$

Michaelis–Menten equation

Michaelis-Menten equation

$$v_0 = \frac{V_{max}}{\left(1 + \frac{K_M}{[S]}\right)}$$

- used to fit experimental data and calculate K_m, V_{max} or k_2 for any enzyme
- Different sections of the curve can be rationalized through this equation



- When [S] is low (i.e., [S] << K_M)

$$v_0 = \frac{V_{max}}{1 + \frac{K_M}{[S]}} \approx \frac{V_{max}}{\frac{K_M}{[S]}} = \left(\frac{k_2}{K_M}\right)[E]_0[S]$$

Linear increase of V_0 with [S]

- When [S] is high (i.e., [S] >> K_m)

$$v_0 = \frac{V_{max}}{1 + \frac{K_M}{[S]}} \approx \frac{V_{max}}{1} = k_2[E]_0$$

Plateau at high [S]

- When [S] = K_M $\quad v_0 = \frac{V_{max}}{2}$

[S] at ½ V_{max} equals to K_M

What is the meaning of different constants

- The K_m is sometimes used as an indicator of the **affinity of an enzyme for its substrate**.
- Under the conditions that k_2 represents the rate limiting step the reaction ($k_2 << k_{-1}$) then the K_m expression can be simplified to k_{-1}/k_1 which is essentially a dissociation constant for the ES complex reaction
- It has concentration of K_D units but the values vary greatly even for different substrates of the same enzyme

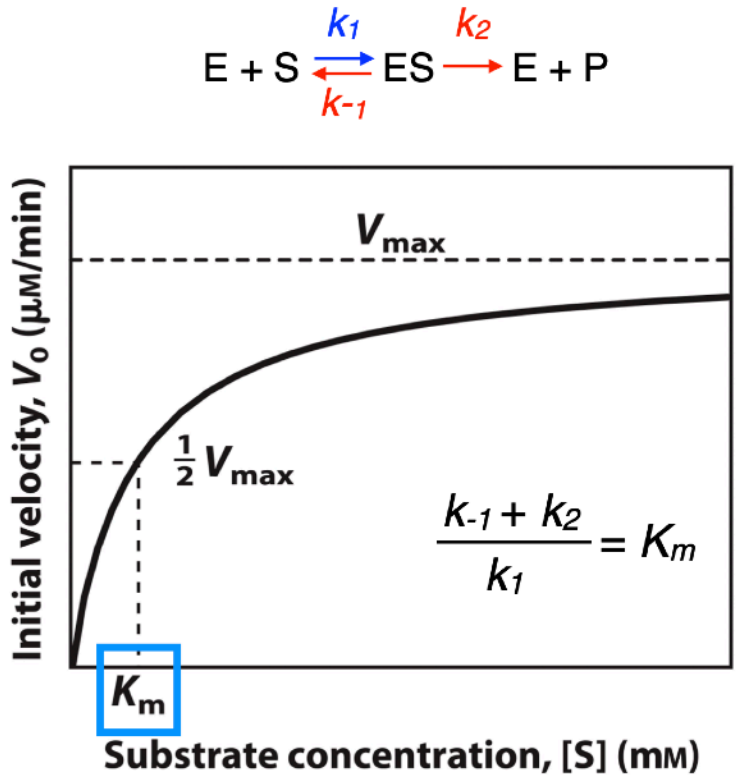


TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

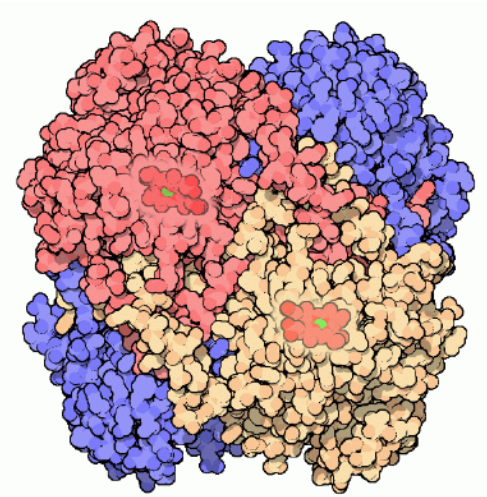
V_{max} and k_{cat}

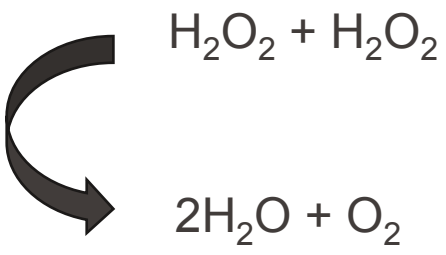
- V_{max} and k_2 are connected through the equation: $V_{max} = k_2[E]_0$
- For simple 2-step M-M mechanism, $k_2 \equiv k_{cat}$, called **catalytic rate constant** or **turnover number** for the enzyme
- k_{cat} can also be used more generally to depict the overall rate constant of a multi-step reaction.
- k_{cat} / K_M is called the **catalytic efficiency** of the enzyme (optimal, diffusion-limited enzymes reach ~10^{10} $M^{-1} \cdot sec^{-1}$)

TABLE 6–7 Turnover Number, k_{cat}, of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

Catalase



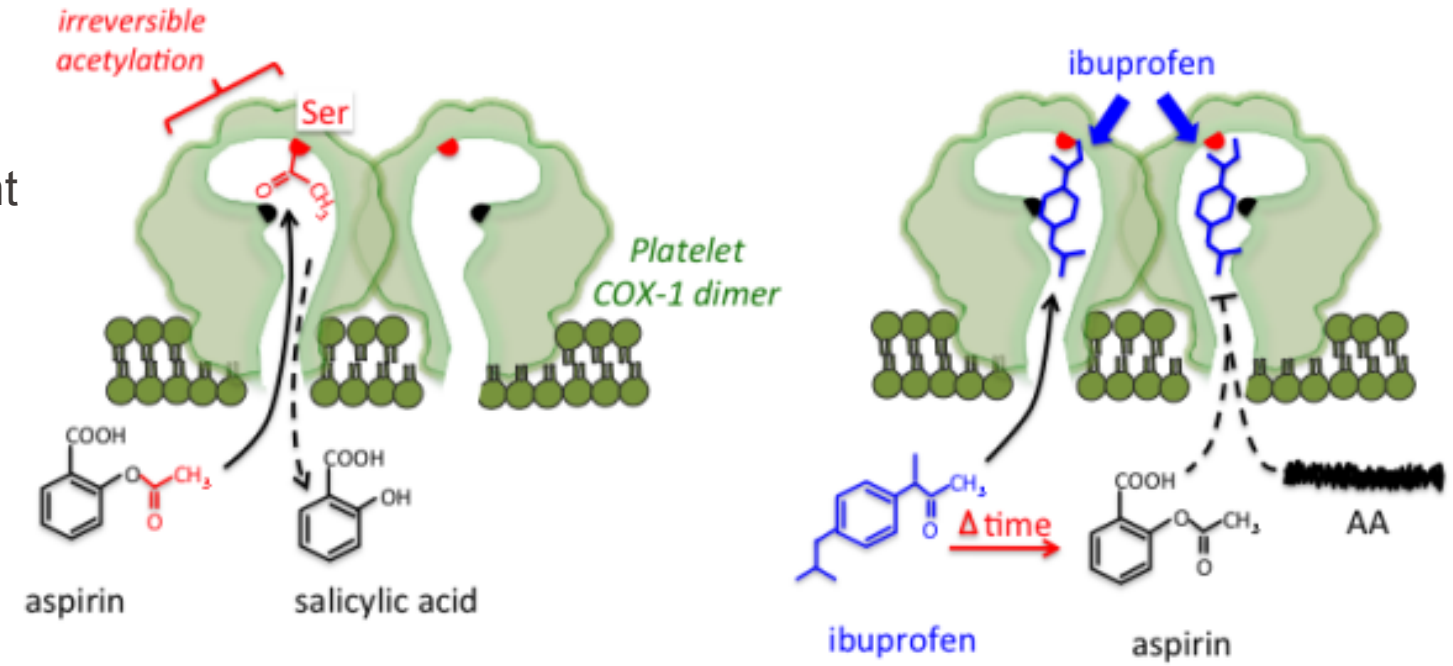


- k_{cat} is a 1st order rate constant and has units of s^{-1}.
- It is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule.

Enzyme inhibition

- Enzyme inhibitors are molecules that **interfere with catalysis, slowing or halting enzymatic reactions**.
- Enzyme inhibitors are among the most important pharmaceutical agents known (~50% of drugs on the market)
- Ibuprofen and Aspirin (acetylsalicylate) inhibits the enzyme called COX-1 that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that cause pain.

Covalent attachment
=
Irreversible



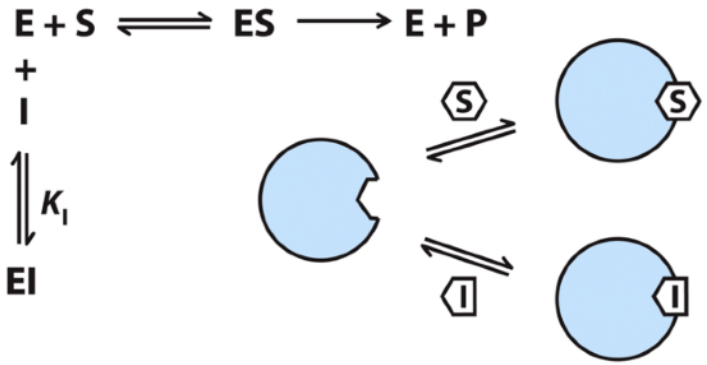
Transient binding
=
Reversible

- Inhibitors can be broadly divided into **reversible** and **irreversible** based on whether they achieve the desired outcome by transiently affecting to the target (reversible) or permanently (irreversible)

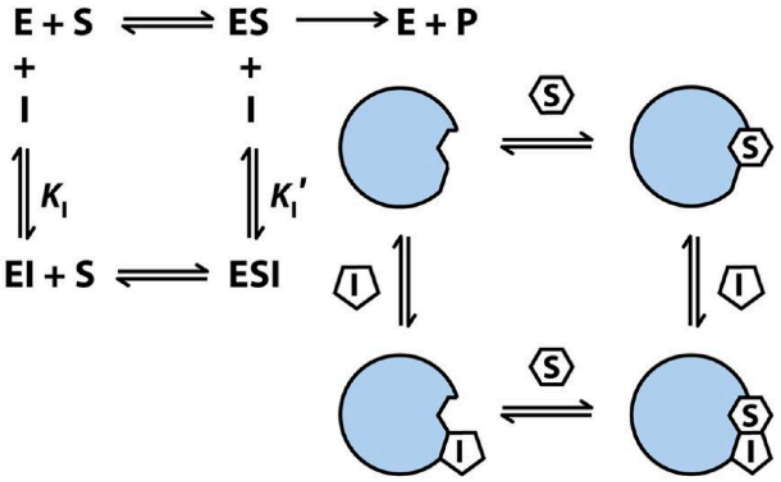
Reversible enzyme inhibition

- inhibitors can act by **competitive, non-competitive** and **substrate-dependent non-competitive** mechanisms
- An inhibition constant (K_I) quantifies the strength of inhibitor in blocking the activity of an enzyme

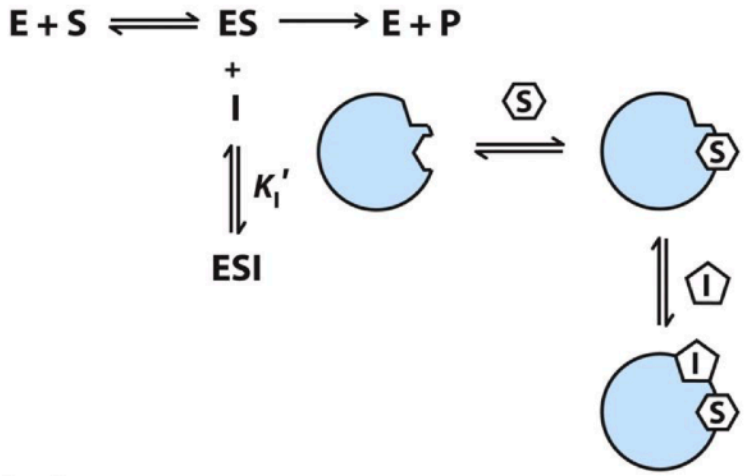
Competitive



Non-competitive



Substrate-dependent non-competitive



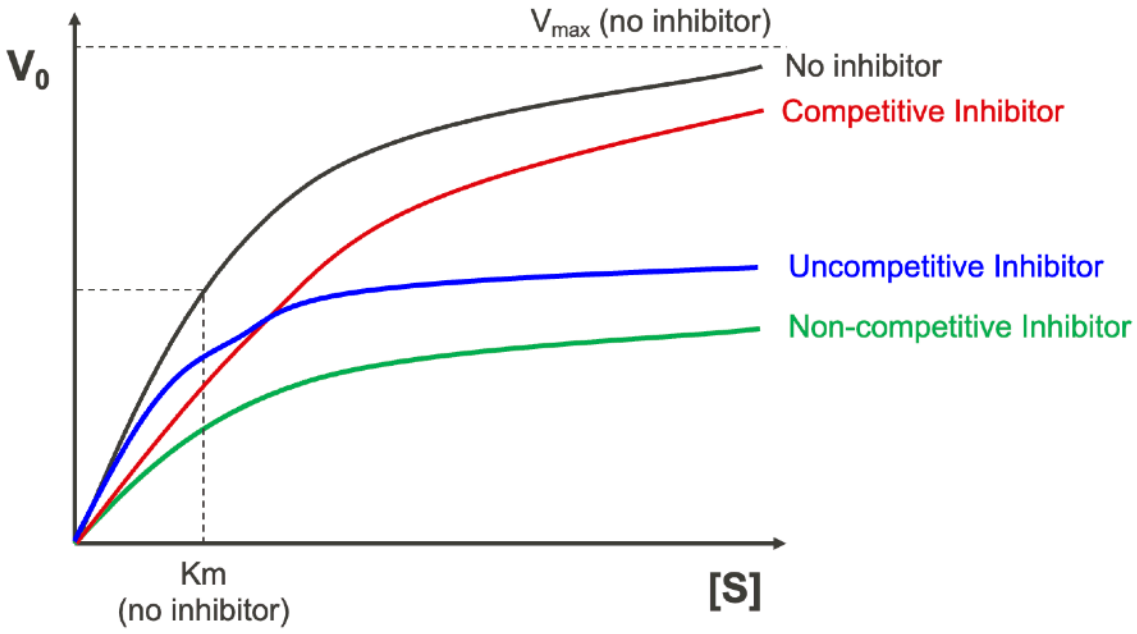
A competitive inhibitor (I) directly competes with the substrate for binding to the active site of an enzyme. They cannot bind at the same time.

A non-competitive inhibitor (I) binds the enzyme in a different site and independently from S. It does not directly interfere with the binding of S to E but reduces the [ ] of active ES through an **allosteric** mechanism

An uncompetitive inhibitor (I) binds the enzyme in a different site but **it does bind only** the ES complex. It does not directly interfere with the binding of S to E but reduces the [ ] of active ES

Reversible enzyme inhibition

- Depending on the inhibition mechanism the kinetic parameters are impacted differently
- Inhibition is also dose dependent (i.e., the higher the inhibitor concentration the greater the inhibitory effect)



Competitve: K_m is higher V_{max} stays the same

Uncompetitive: K_m is lower V_{max} is lower

Non-competitive: K_m stays the same V_{max} is lower

* These are apparent Km and V_{max} constants since kinetics is measured in the presence of an inhibitor. It does not mean that the enzyme itself changed but that rather the measured readout is altered.

Example for competitive inhibition:

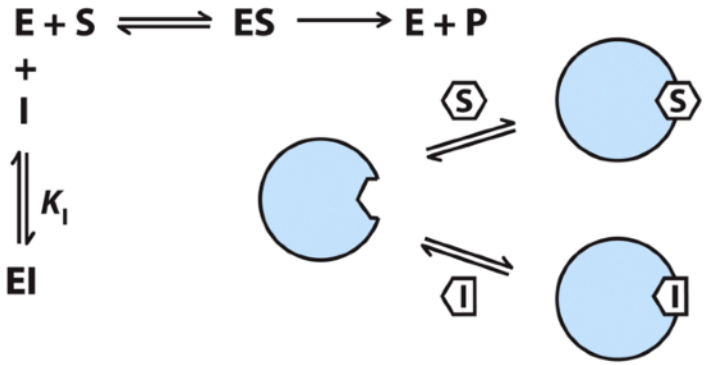
$$\alpha = 1 + \frac{[I]}{k_I} \qquad V_0 = \frac{V_{max}[S]}{[S] + \alpha K_m}$$

- Impact on kinetic parameters is quantified through parameter α which is dependent on K_I and the concentration of inhibitor [I]

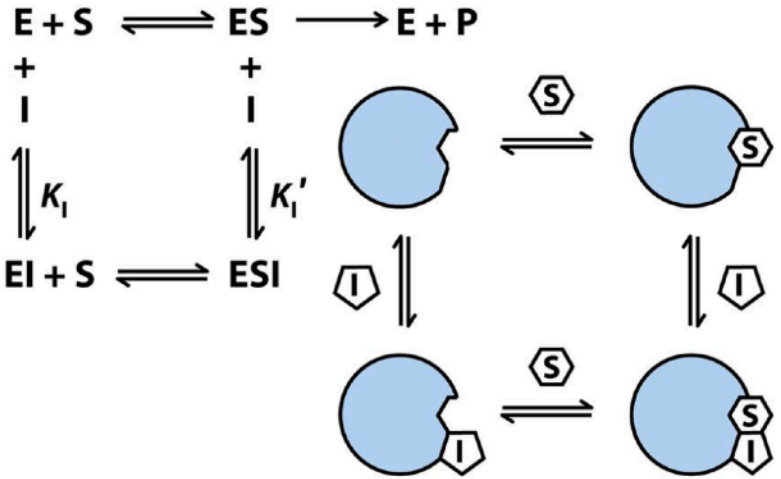
Reversible enzyme inhibition

- Equations used to describe enzyme kinetics in the presence of different types of inhibitors

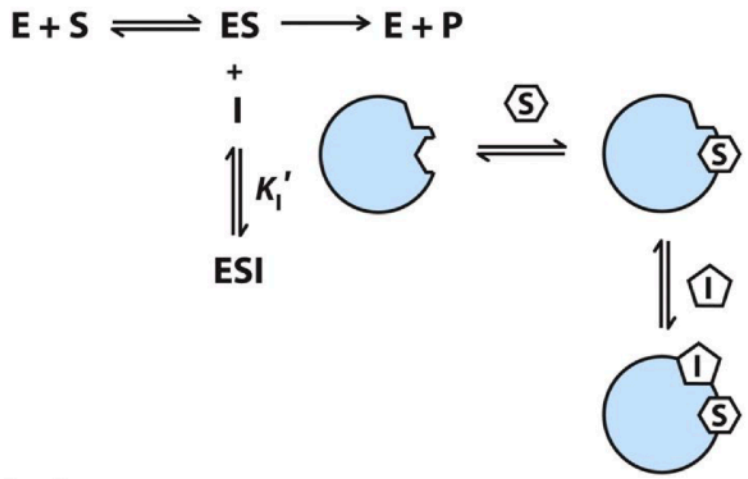
Competitive



Non-competitive



Substrate-dependent non-competitive



$$V_0 = \frac{V_{max}[S]}{[S] + \alpha K_m}$$

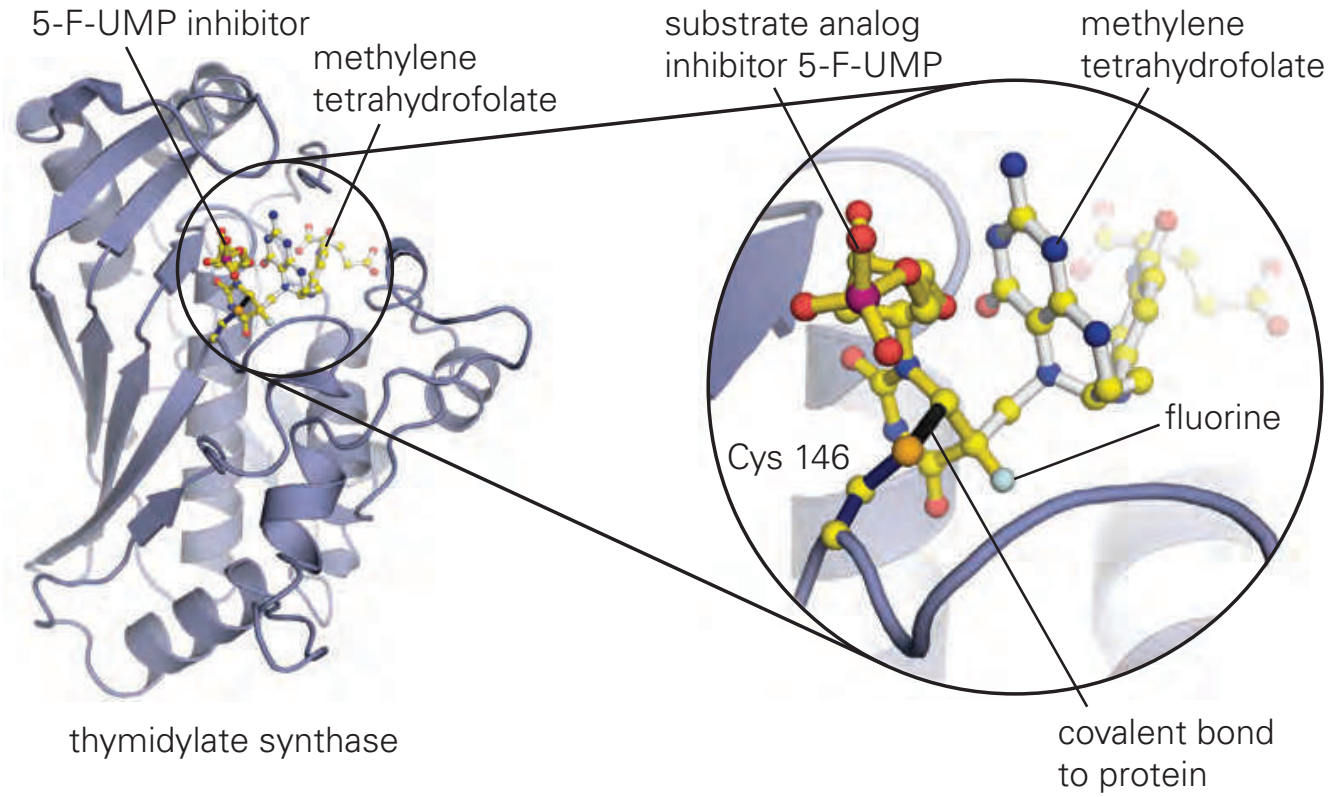
$$V_0 = \frac{V_{max}[S]}{\alpha'[S] + \alpha K_m}$$

$$V_0 = \frac{V_{max}[S]}{\alpha'[S] + K_m}$$

- α is derived for K_I (binding to enzyme), while α' corresponds to K'_I (binding to enzyme-substrate complex)

Irreversible enzyme inhibition

- Irreversible inhibitors bind covalently to an enzyme that is essential for the enzyme's activity.



- A special class of irreversible inhibitors are the **suicide** inactivators.

- A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted into a very reactive compound that combines irreversibly with the enzyme.

Summary

- Reaction rates are inversely correlated to the activation energy (Arrenhius equation)
- The half-life of a reaction provides a measure of the relative speed at which the product is generated
- Multistep reactions have intermediates that build up as the reaction is initiated, but disappear as the reaction goes to completion
- A steady state condition in a reaction means that a concentration does not change with time although the reaction is occurring
- Enzymes reduce the activation energy required for chemical reactions
- Enzymes are proteins often complexed with cofactors/ coenzymes
- The ratio of forward and reverse reactions must be considered in calculating the approach to equilibrium
- Enzymes kinetics describes how reactions change in response to changes in experimental parameters
- Concepts of V_0, V_{max} K_m, k_{cat} and catalytic efficiency (k_{cat} / K_m)
- The principle of reversible and irreversible enzyme inhibition