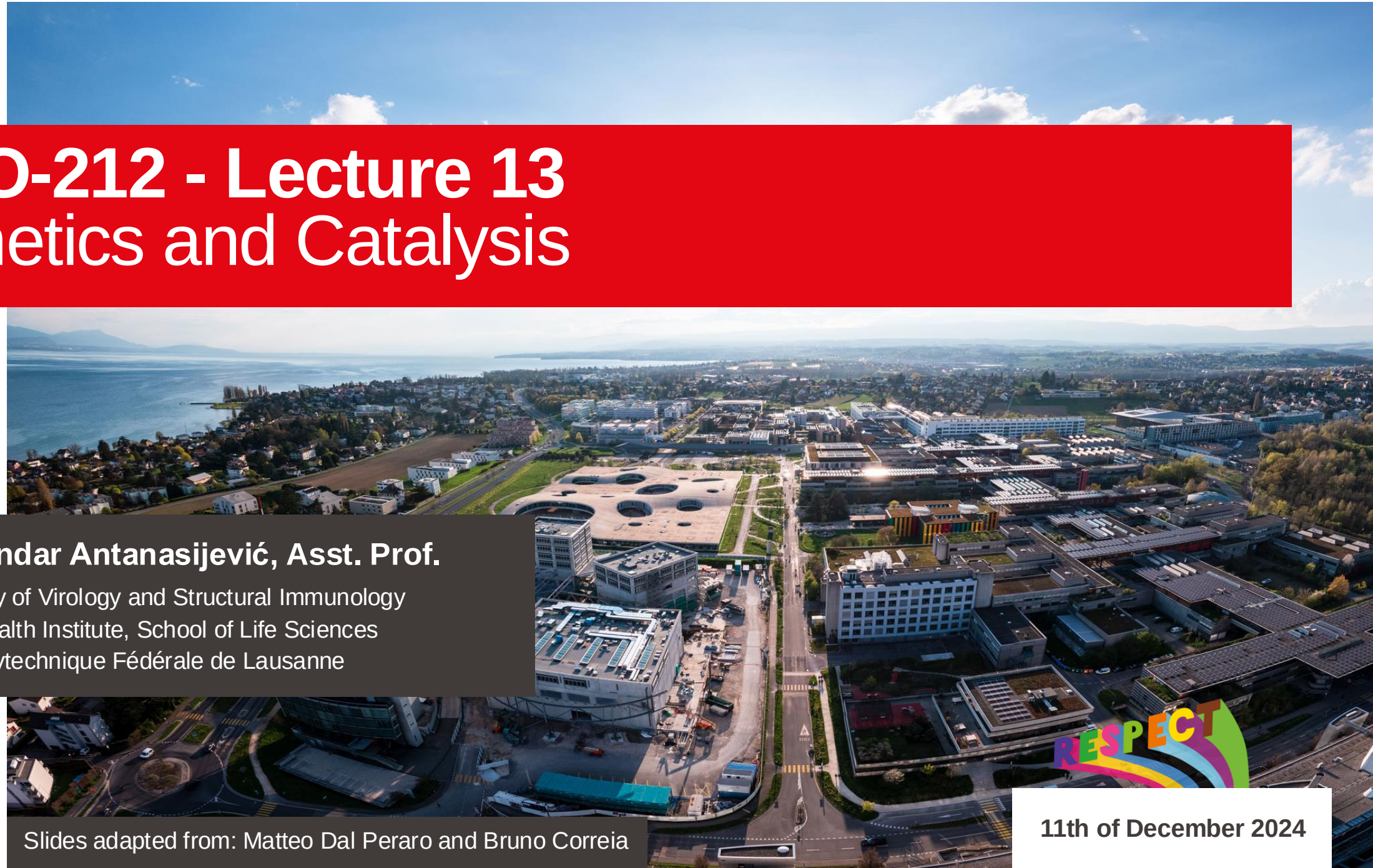
BIO-212 - Lecture 13
Kinetics and Catalysis



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Slides adapted from: Matteo Dal Peraro and Bruno Correia

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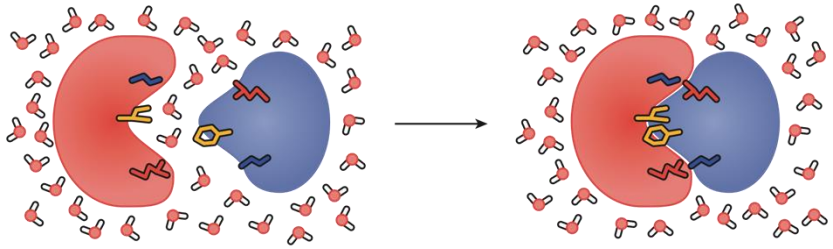
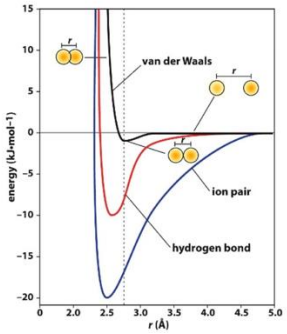
Lecture 12 - Summary

• Biomolecular interactions and binding





- Small energy contributions from hydrogen bonds, vdW, ionic and hydrophobic interactions
- Water can have a positive and negative impact on binding

• Dissociation constants and affinity

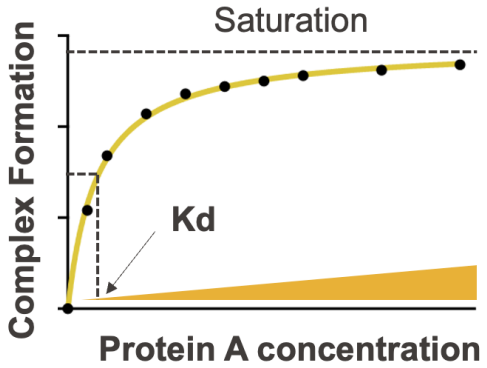
$$\Delta G^\circ = RT \ln K_D = \Delta H - T\Delta S$$

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

- Thermodynamic and kinetic analysis of binding

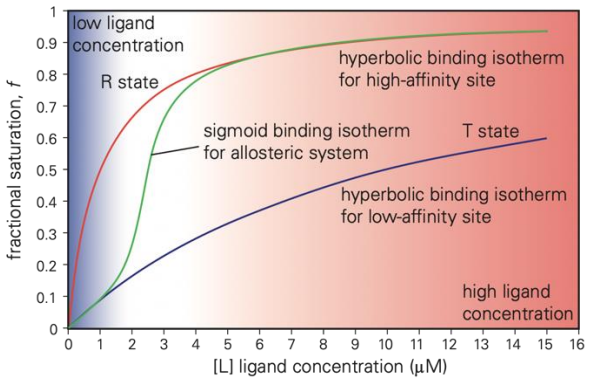
• Experimental methods for Kd determination

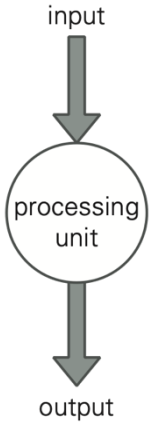
- Titrate one binding partner and measure complex formation



- Calorimetric (ITC) or spectroscopic (FP, SPR, NMR) measurements

• Cooperativity and Allostery





Two different ways of assessing binding

Thermodynamic approach

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

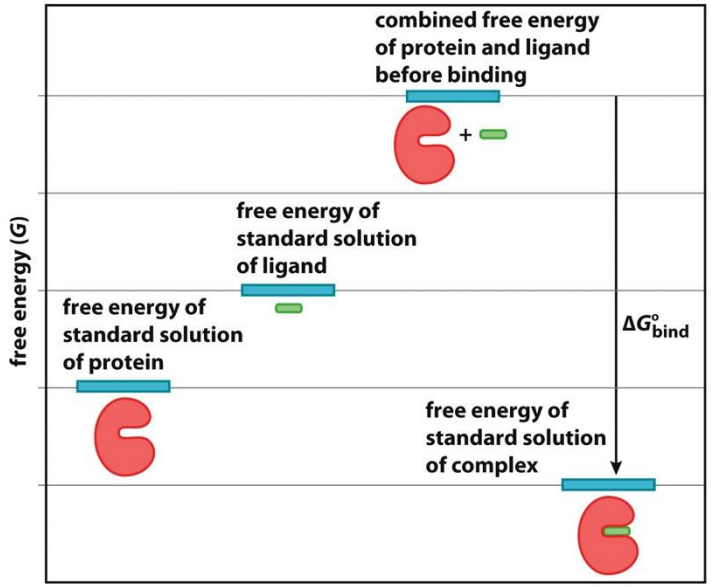


Figure 12.3 The Molecules of Life (© Garland Science 2013)

Measuring the energy change at steady state to calculate K_d

$$\Delta G^{o} = RT \ln K_d$$

Kinetic approach

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

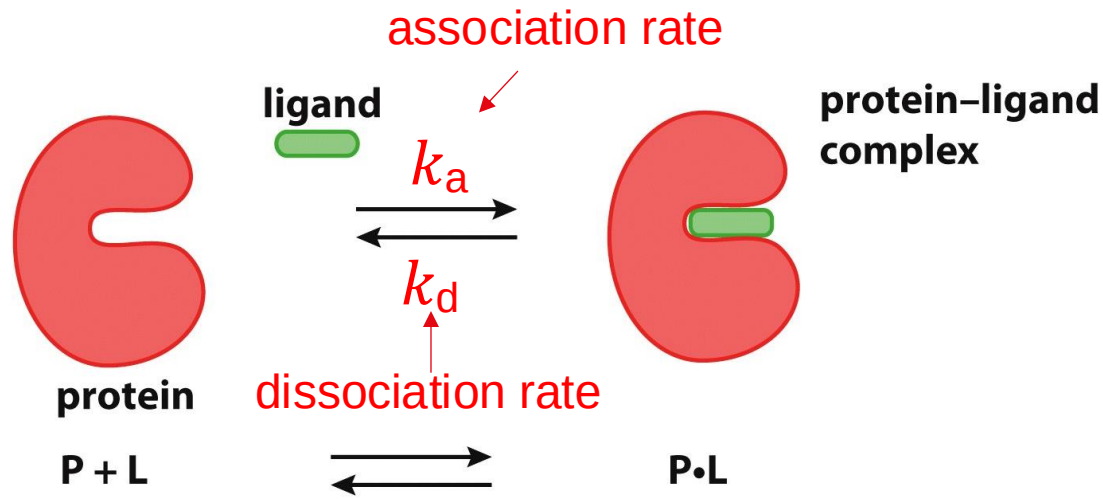


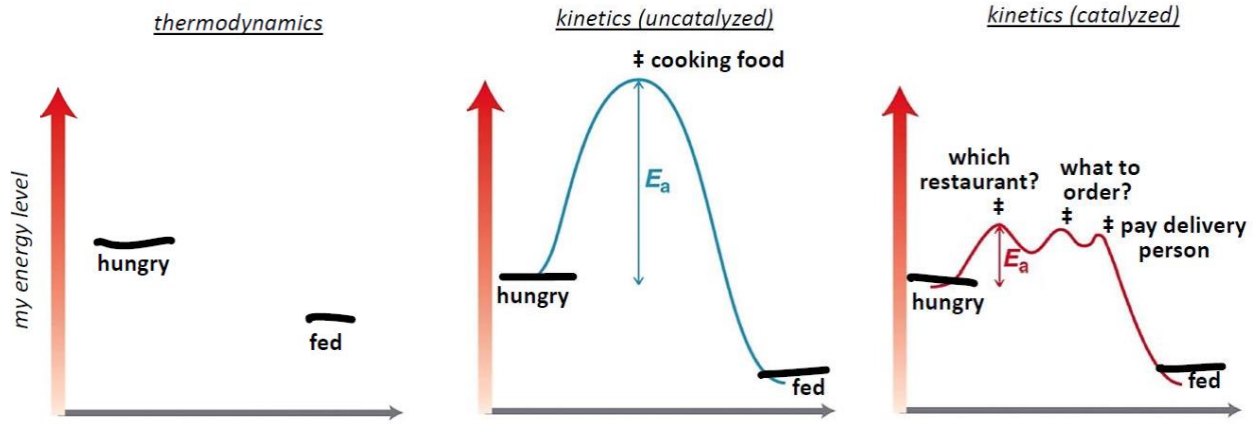
Figure 12.2a The Molecules of Life (© Garland Science 2013)

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

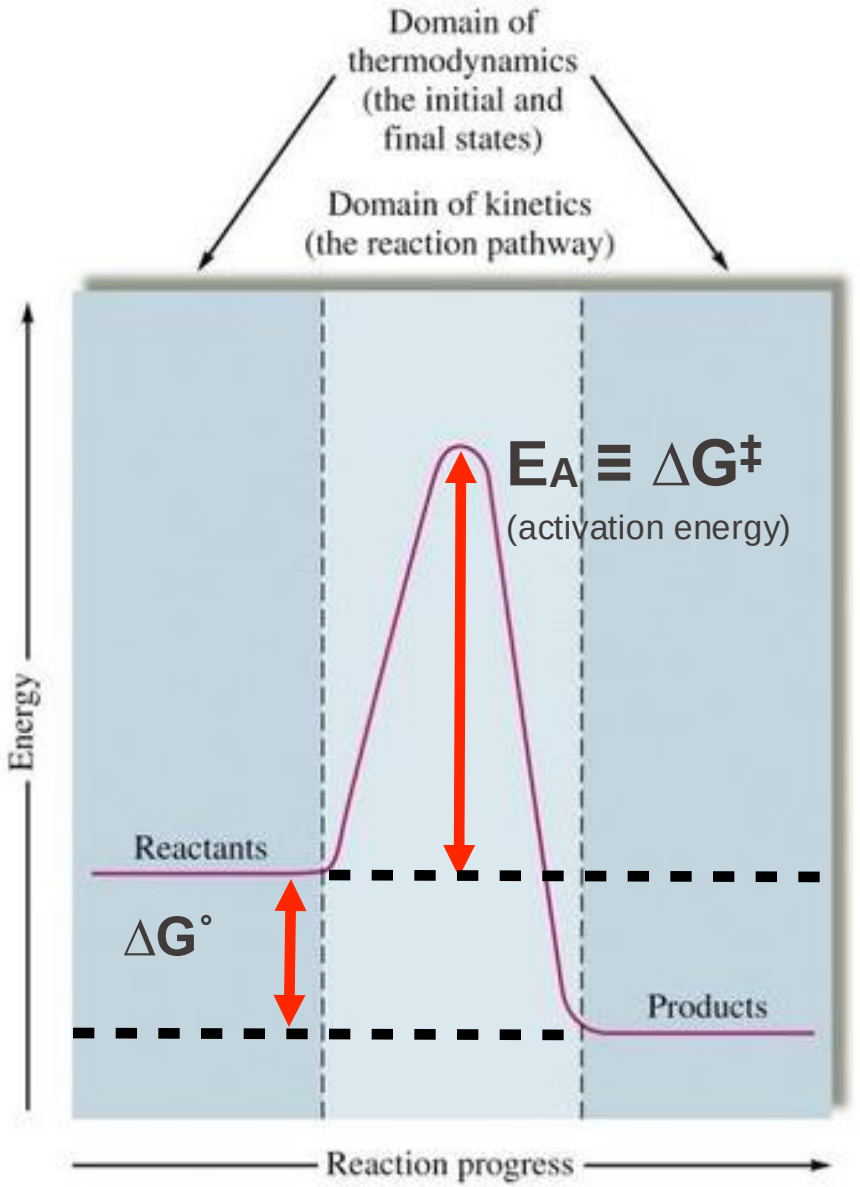
$$\frac{k_d}{k_a} = \frac{k_{off}}{k_{on}} = \frac{[P][L]}{[P \bullet L]} = K_D = \frac{1}{K_A}$$

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

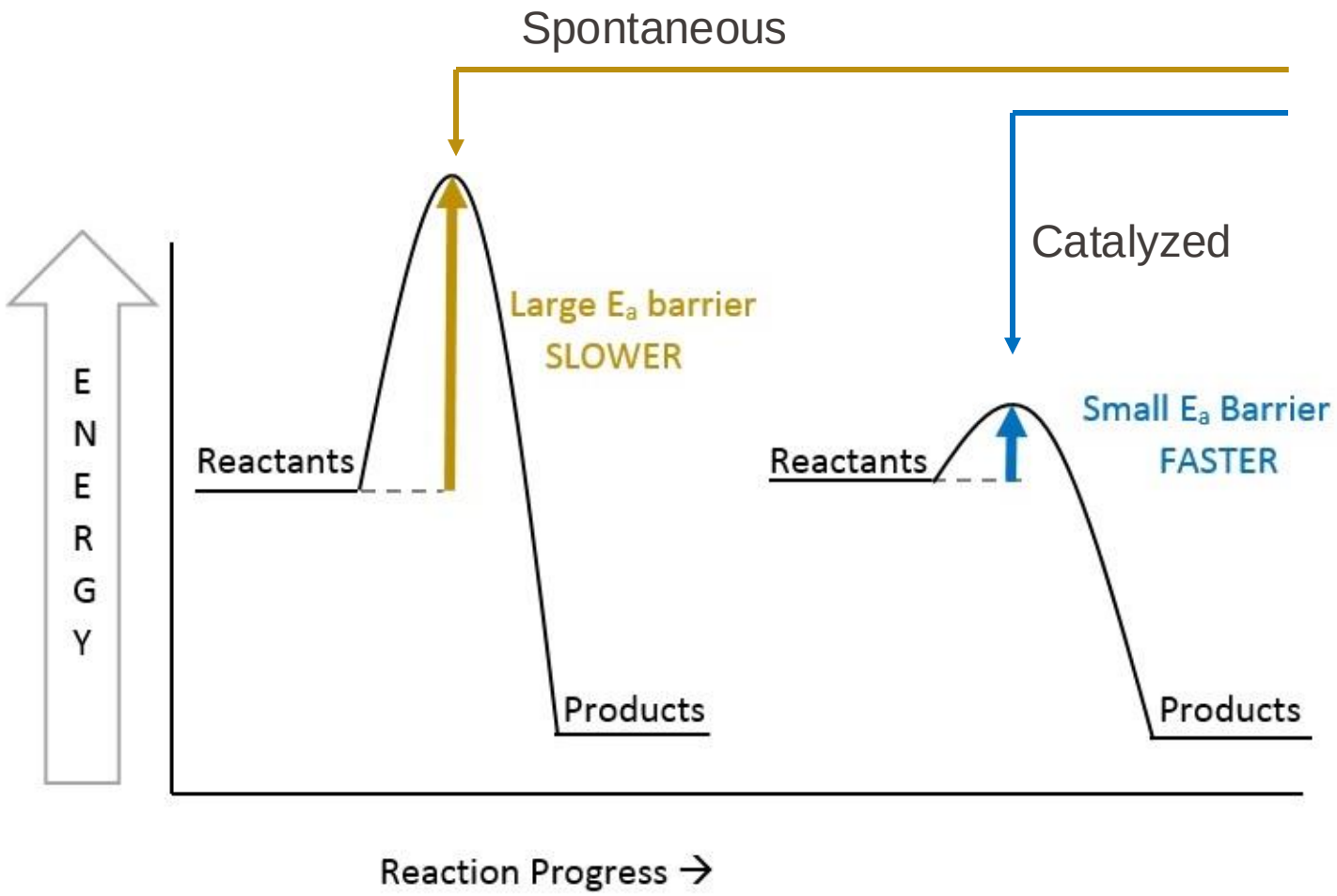


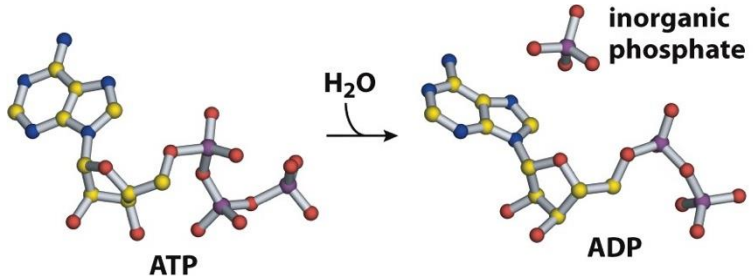
@ChemKritzer on Twitter



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**





ATP hydrolysis

Arrhenius equation

$$k = Ae^{\frac{-Ea}{RT}}$$

$Ea \downarrow \quad k \uparrow$

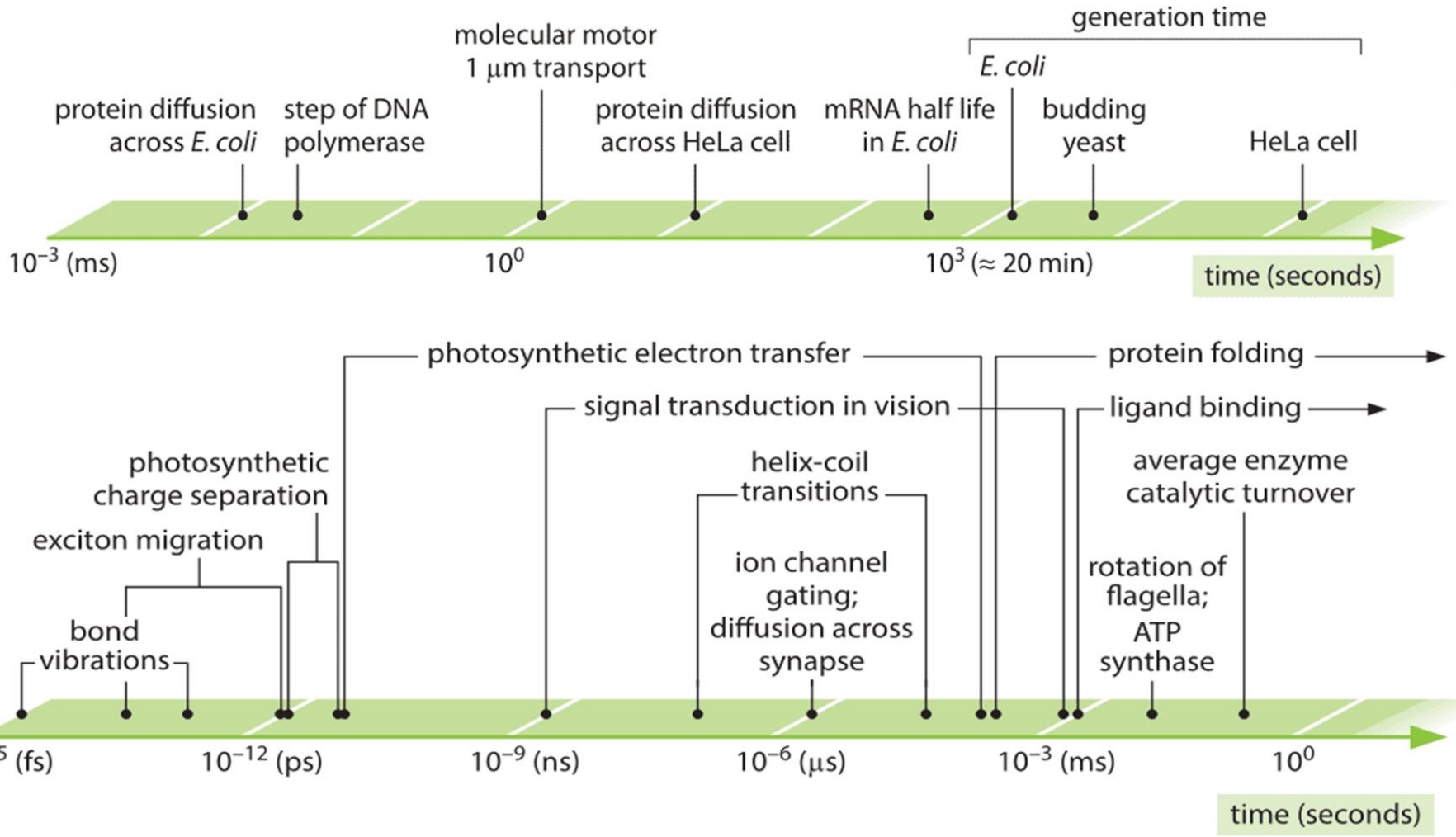
$T \uparrow \quad k \uparrow$

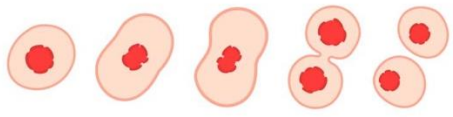
k - Chemical Reaction Rate
A - Pre-exponential factor
E_a - Activation energy
R - Universal gas constant
T - Temperature in K

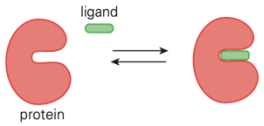
General aspects of reaction kinetics

Kinetics of biological processes

- Biological processes span over a wide range of timescales
- Typically, smaller systems will exhibit faster turnovers (e.g., chemical bond vibrations vs cell division)

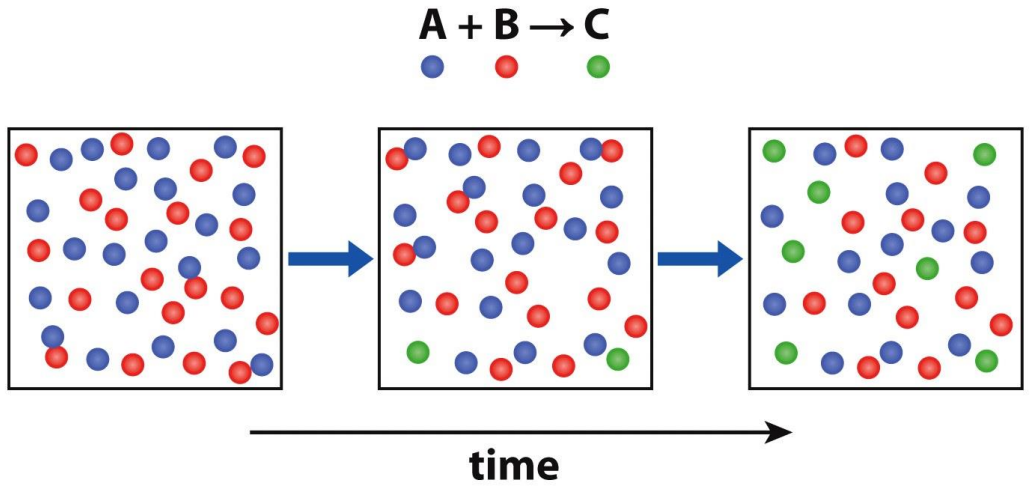


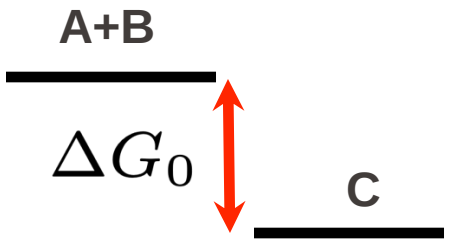




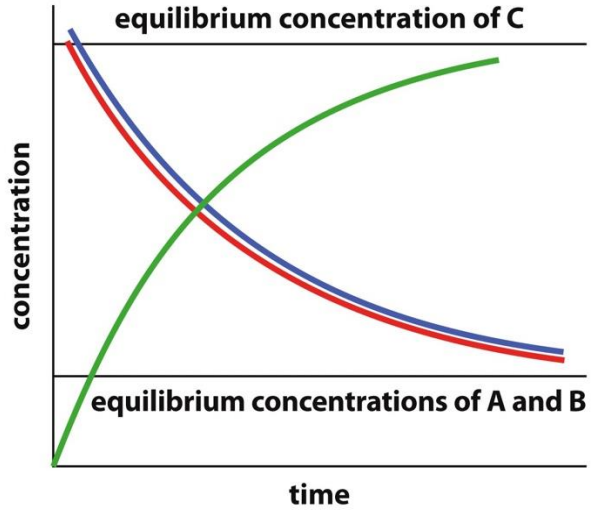
Kinetics of simple reactions

- 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

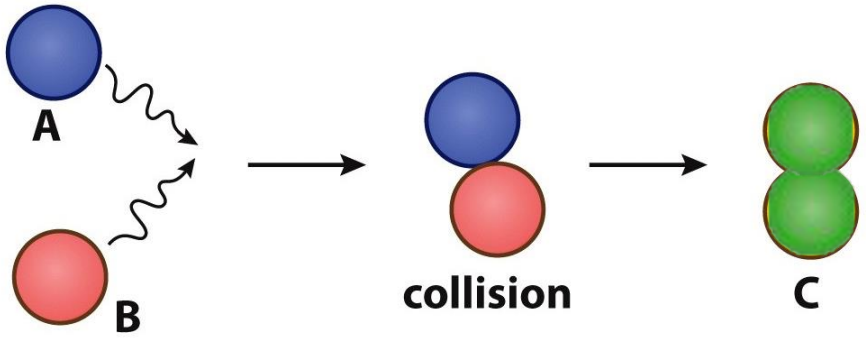
$$rate = -\frac{d[A]}{dt} = \frac{d[C]}{dt} = k[A][B]$$

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

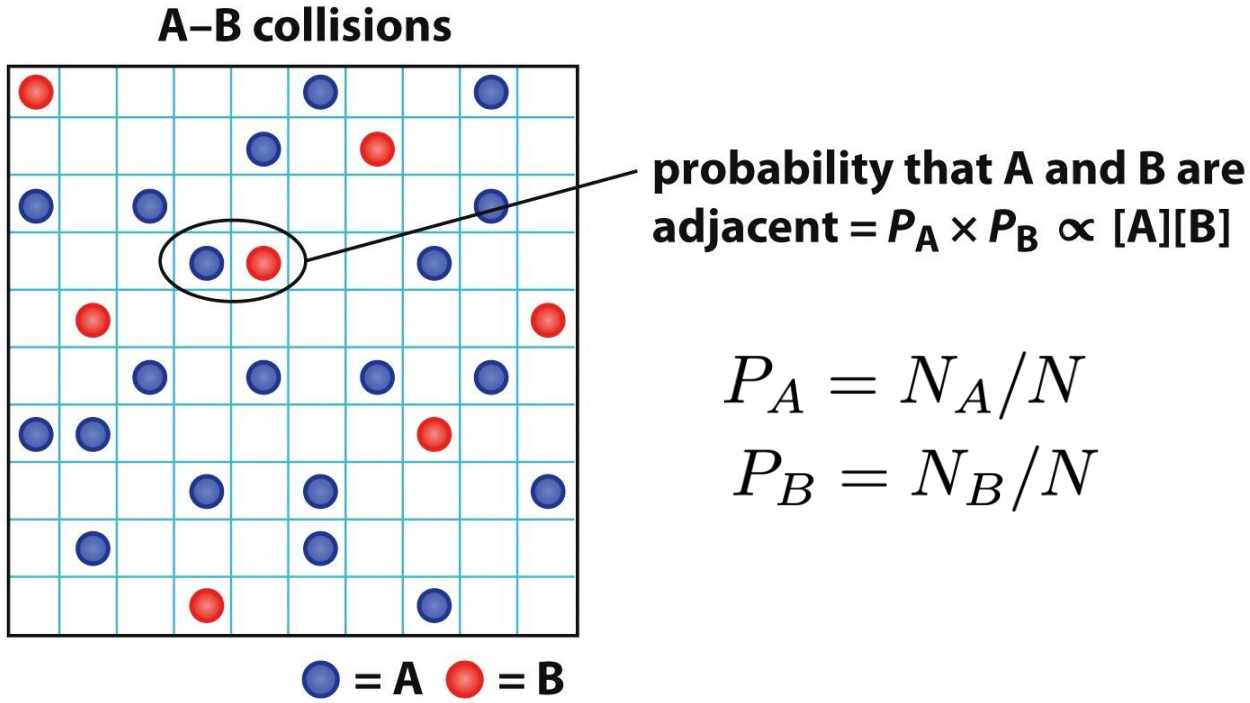
- **k is called a reaction rate constant** (equivalent to k_a and k_d we discussed in Lecture 12)

Reaction rates depend on molecular collisions

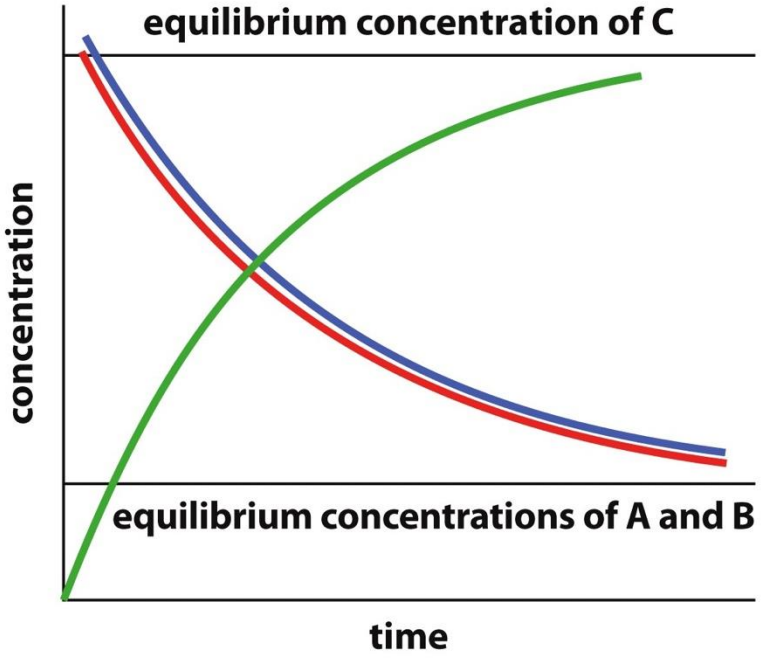
- The collision rate is proportional to the product of concentrations of reactants
- Higher temperature increases the diffusion rates of molecules and the likelihood of collisions



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

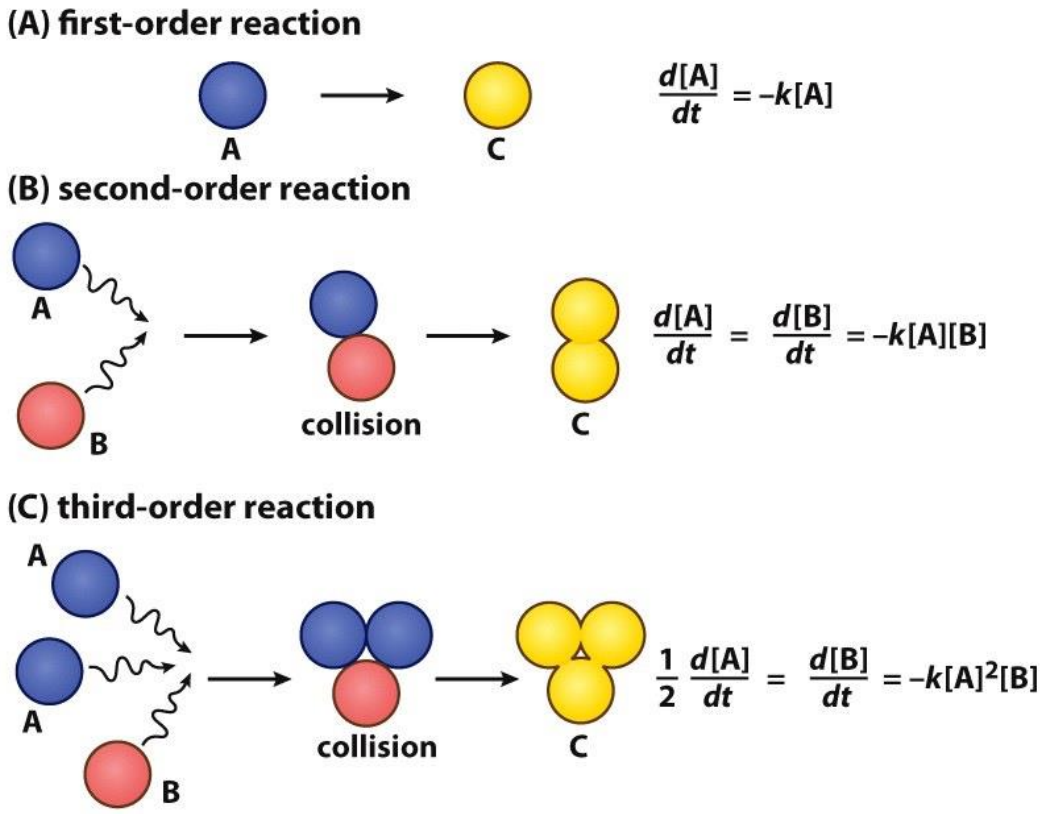


$$P_A = N_A/N$$
$$P_B = N_B/N$$



Concentration and order of the reaction

Fundamental reaction types:



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

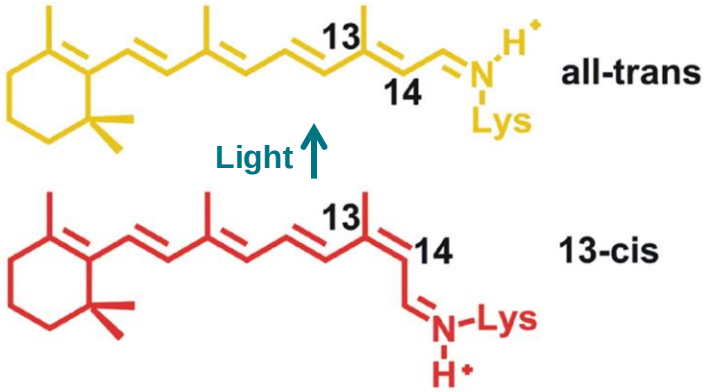
- *k* is related to the frequency of collision depending of the environment
- It also depends on collisions that actually form products (not always the case)
- **Order of the reaction** is the number of molecules
- Unit of *k* depends on the reaction order (s^{-1}, $M^{-1}s^{-1}$, etc.)

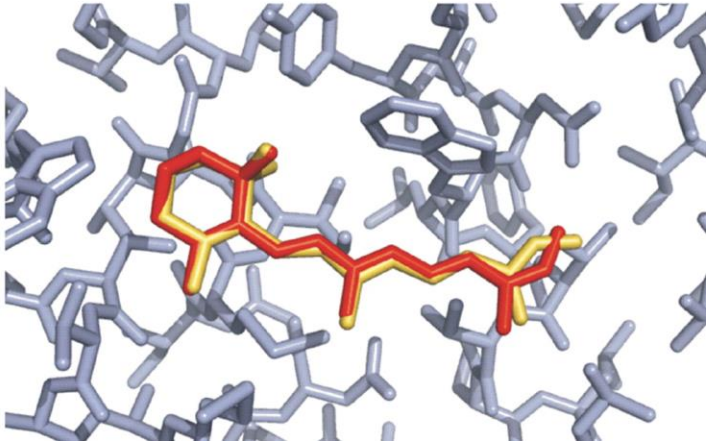
the slower the reaction the higher is the order

Examples of 1st order reactions

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

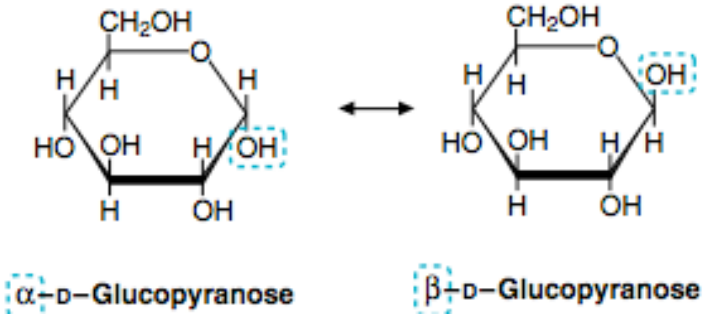
Retinal isomerization by photons



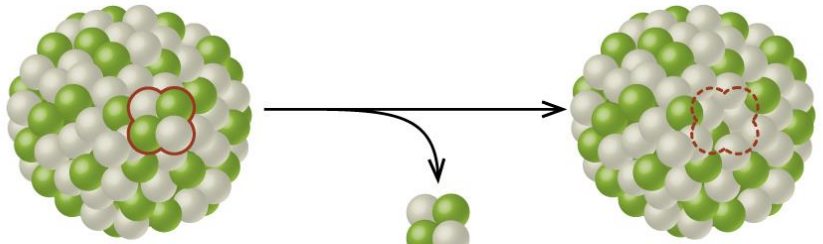


Important for photosynthesis and vision

Carbohydrate mutarotation



Spontaneous decay of radioactive nuclei



1st Order Reactions: Determining kinetic parameters

- Molecular decay or retinol isomerization can be modeled as

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

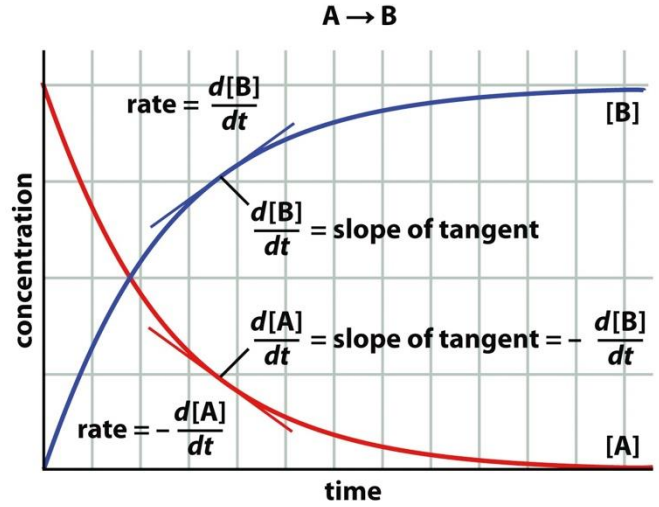
- Rate of decay of reactant is proportional to its concentration via k [s^{-1}] constant; solving the 1st-order linear differential equation:

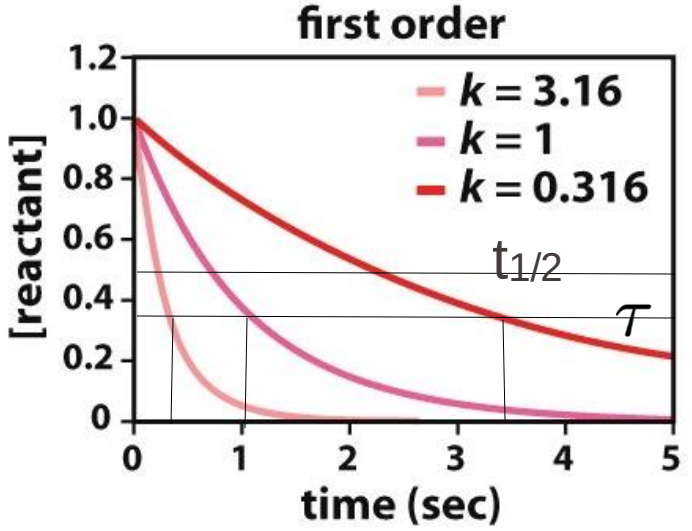
$$[A](t) = [A]_0 e^{-kt} = [A]_0 e^{-t/\tau} \qquad \tau = 1/k$$

- 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)

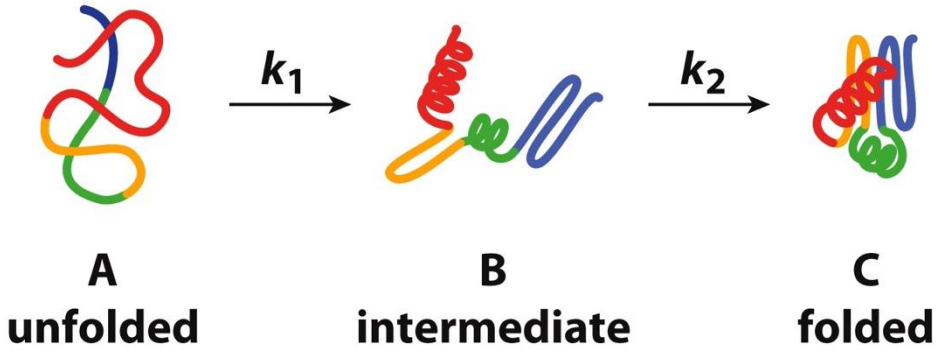
- **Half-life $t_{1/2}$** (= ln2/k =0.693/k) is the time needed for the concentration of [A] to drop to half of its initial value

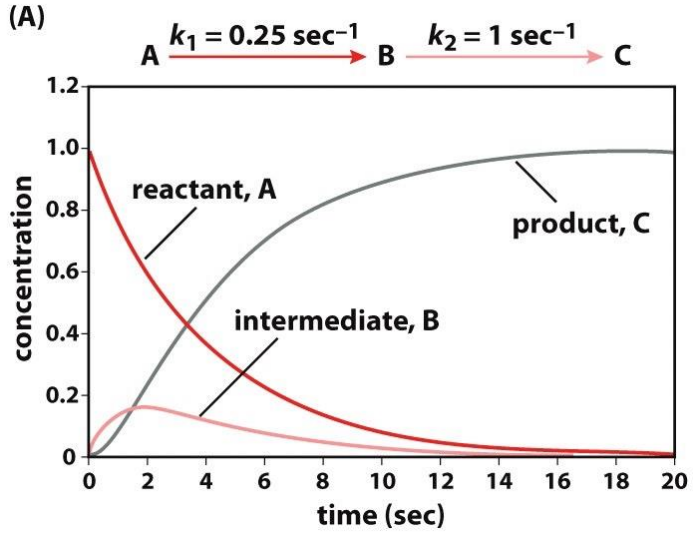




Protein folding can be considered a series of 1st order reactions

- 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





$$\frac{d[A]}{dt} = -k_1[A]$$

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

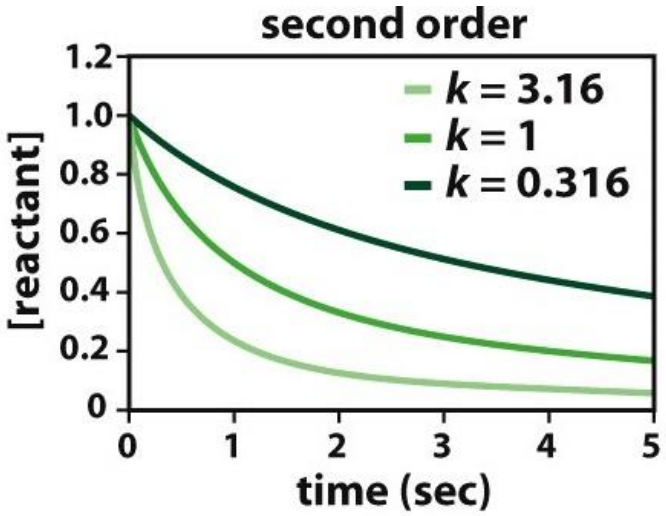
$$\frac{d[C]}{dt} = k_2[C]$$

- To determine the concentration of each reaction component at each time point these equations need to be integrated

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:

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

- *L* = concentration of ligands, R = concentration of receptors

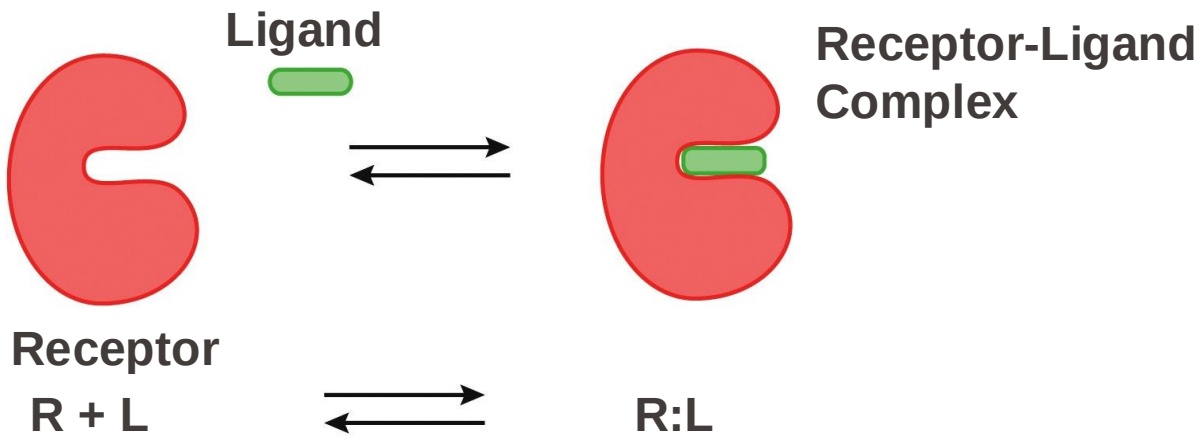


Figure 12.2a The Molecules of Life (© Garland Science 2013)

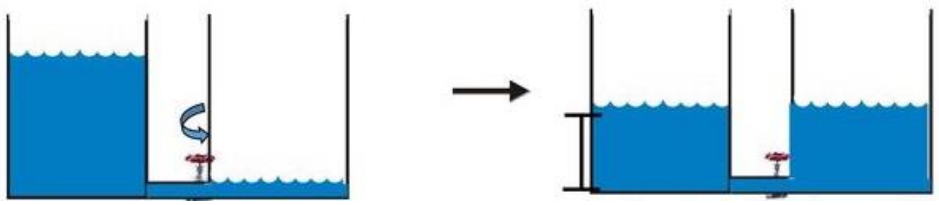
Equilibrium and Steady-State conditions

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

$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} C \qquad K_{eq} = \frac{k_2}{k_1} = \frac{[A]_{eq}\,[B]_{eq}}{[C]_{eq}}$$

- **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)

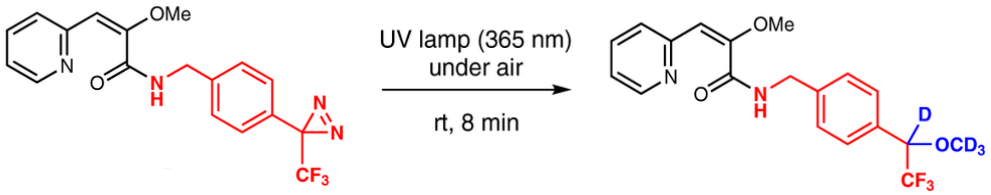
- In cells, basic metabolites and building blocks are constantly replenished

Catalysis and Enzymes

Catalysis of chemical reactions

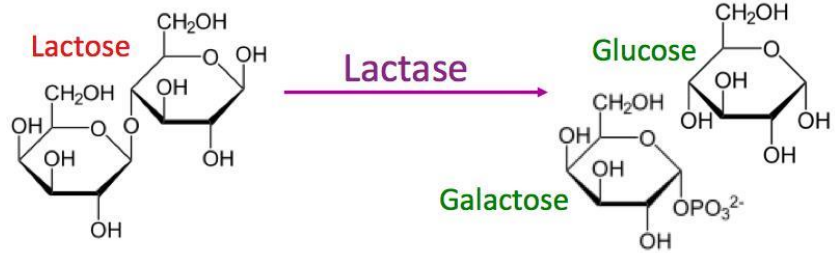
- The modification of the rate of a chemical reaction, usually an acceleration, by addition of a substance (catalyst) not consumed during the reaction
- Catalysis can be achieved by light, chemical methods, proteins (i.e., enzymes) and even RNA (i.e., ribozymes)

Photocatalysis



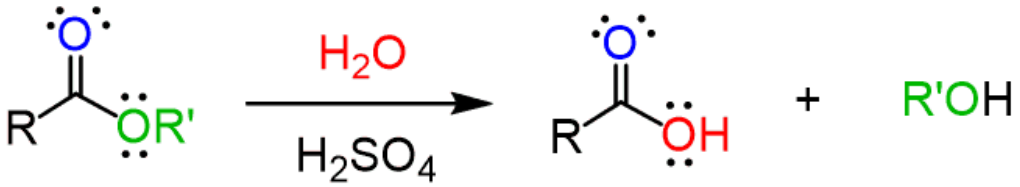
(diazirine group cross-linking)

Enzymatic catalysis



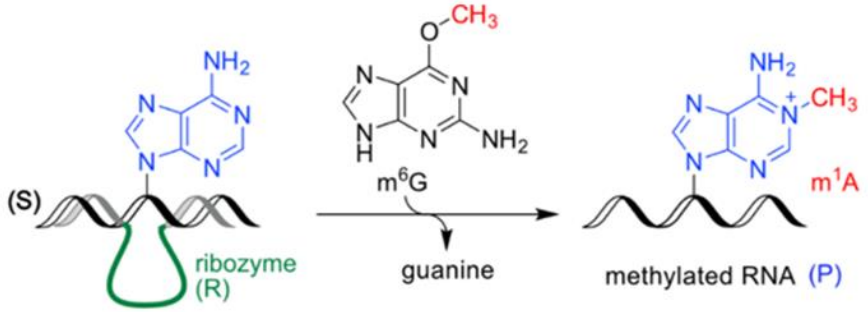
(lactose hydrolysis)

Chemical catalysis



(ester hydrolysis in the presence of acids)

RNA catalysis



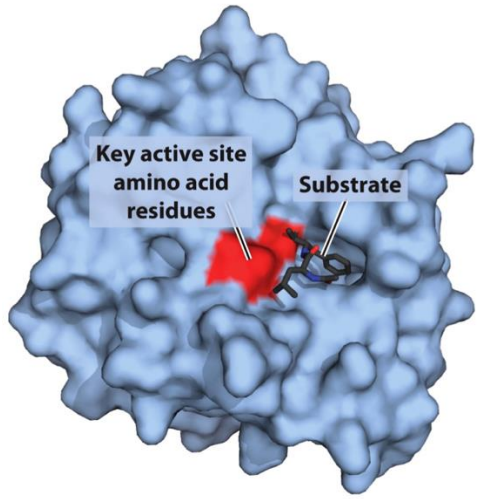
(RNA-catalyzed methylation of RNA)

Enzymes

- Enzymes catalyze the 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 "*énzymon*" which means "leavened with yeast"

Enzyme cofactors

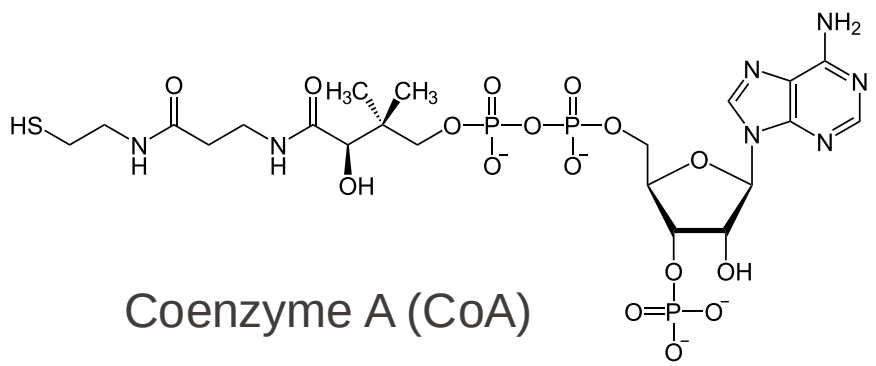
- Some enzymes require an additional chemical component called a **cofactor**.
- Cofactors can be inorganic ions, or complex organic or metalloorganic molecules called **coenzymes**.
- **Vitamins** are organic molecules essential for normal cellular processes, serving as **precursors of coenzymes**

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

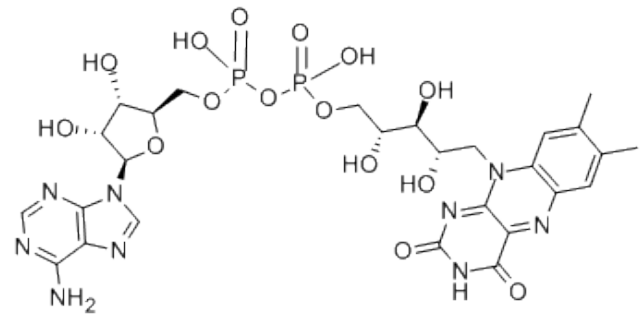
Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase,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

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)



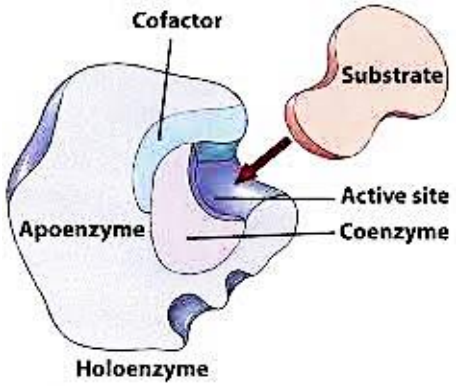
Coenzyme A (CoA)



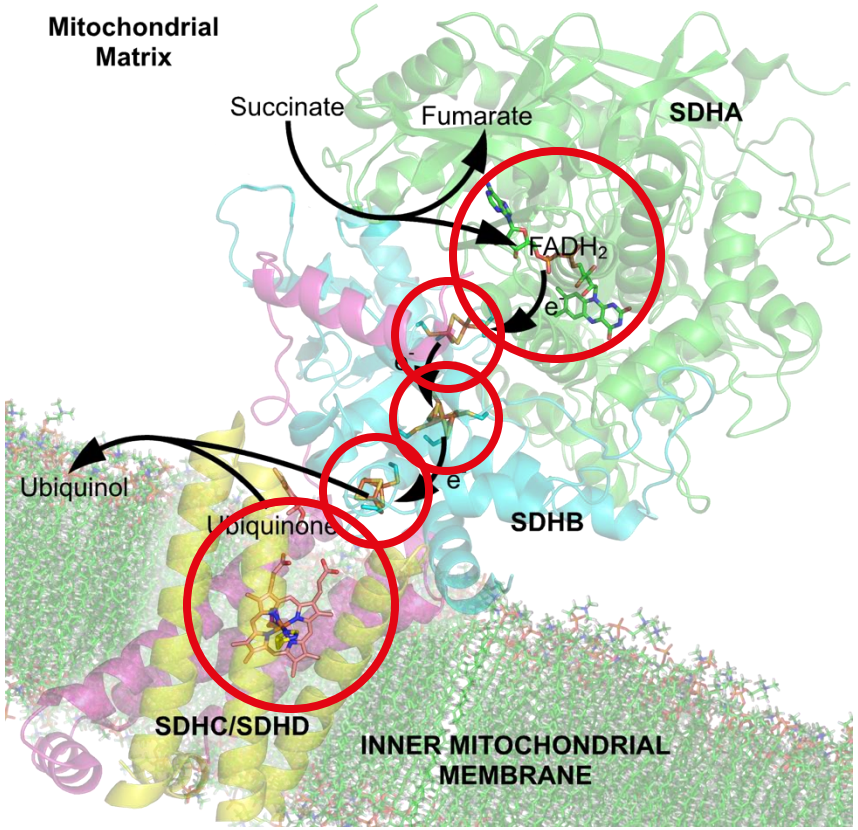
Flavin adenine dinucleotide (FAD)

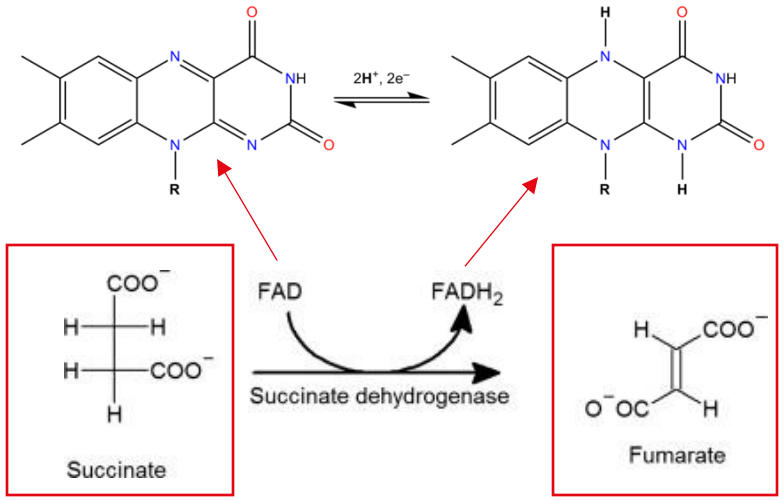
Enzyme cofactors

- 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



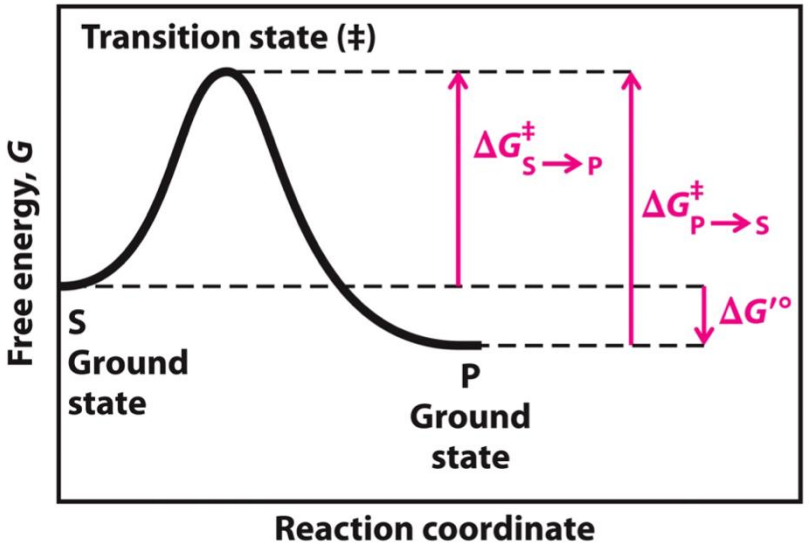


Background info:
- SDH is an enzyme component of oxidative phosphorilation and citric acid cycle
- Both processes are essential for energy metabolism inside the cell

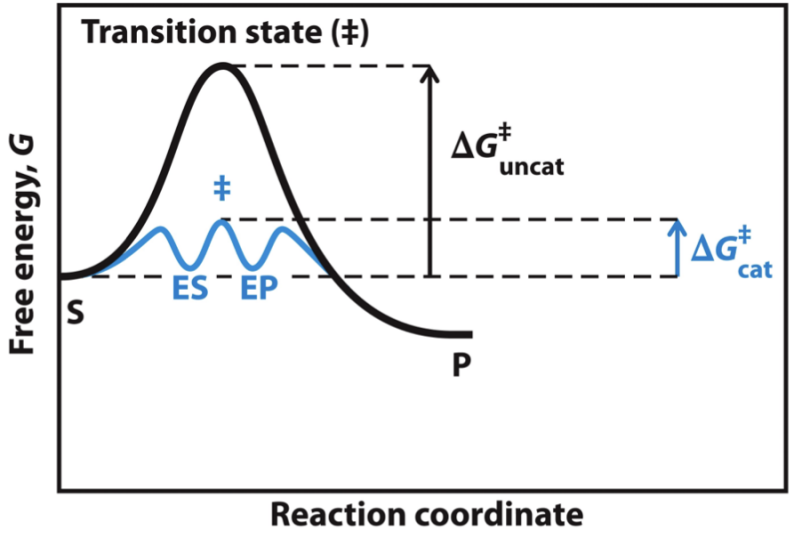
Enzyme properties

- 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**.

$$S \rightleftarrows P$$



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

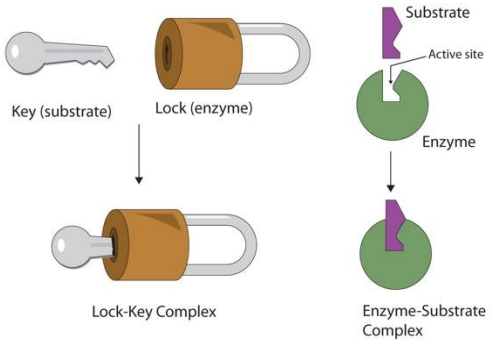


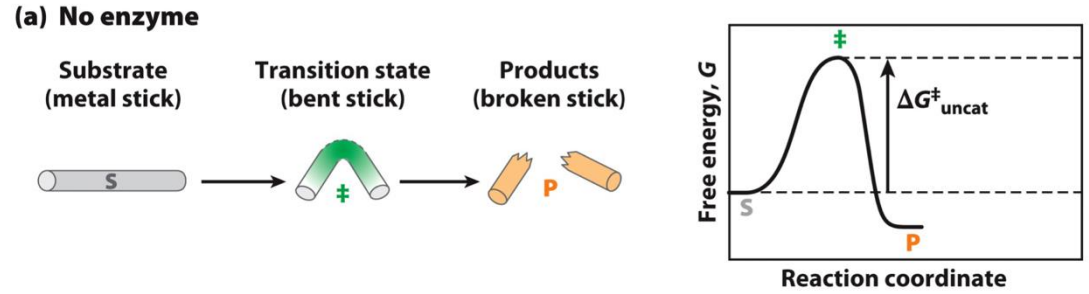
$$E_A \equiv \Delta G^{\ddagger}$$

(activation energy)

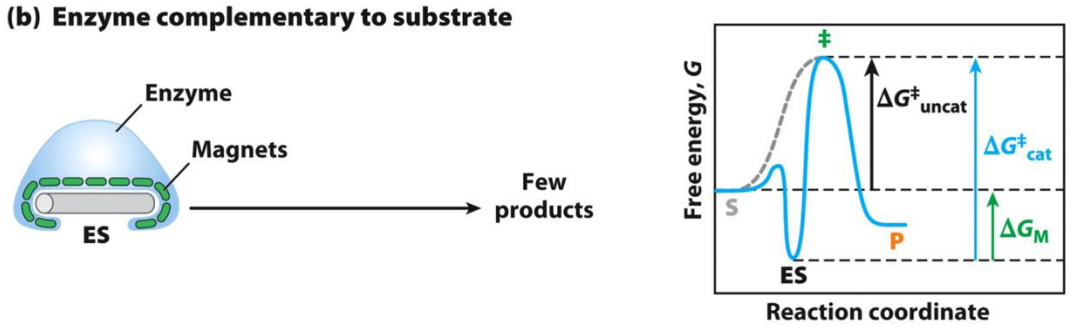
Transition state complementarity and rate enhancement

- 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:

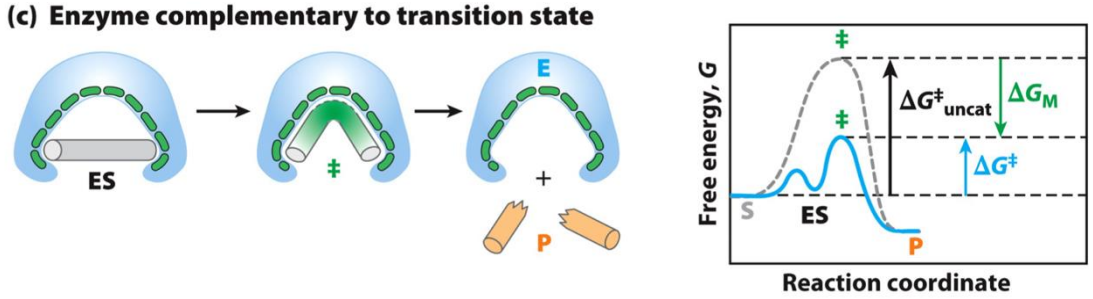




- Uncatalyzed
- High activation energy



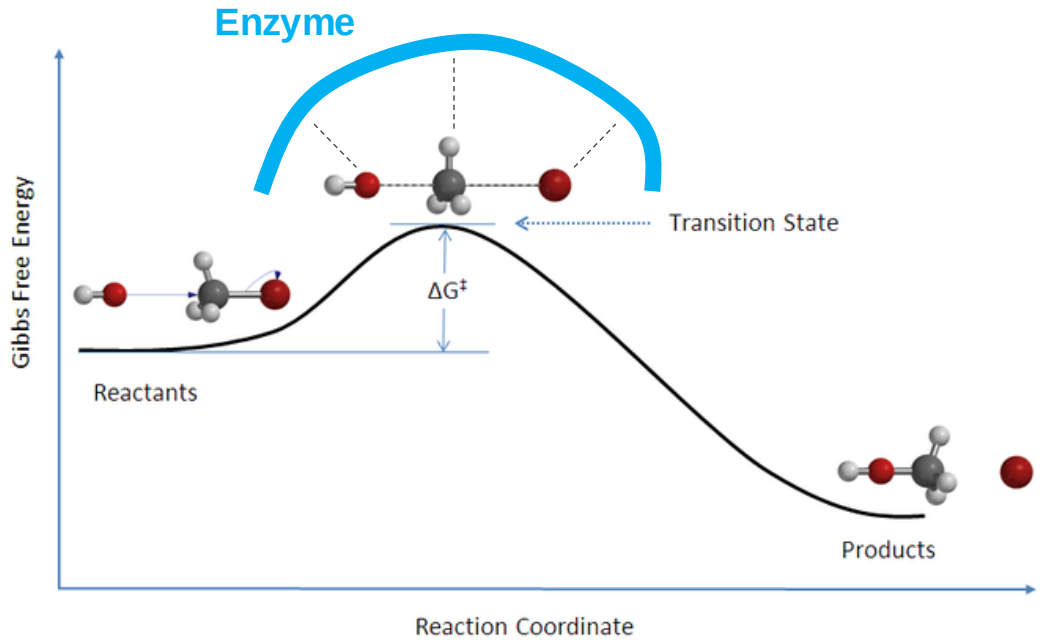
- Enzyme binds substrate well
- The activation energy increases



- 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

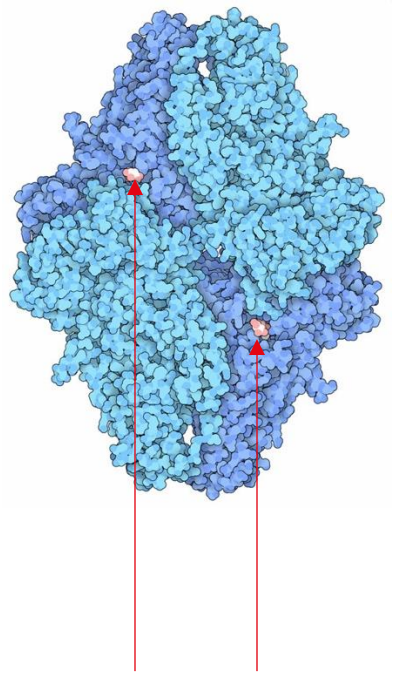


The enzyme brings these two reactants into close proximity and coordinates them in such a way that they form an unstable transition intermediate ultimately leading to the departure of the red group on the right side and formation of products.

Examples of enzymatic reactions

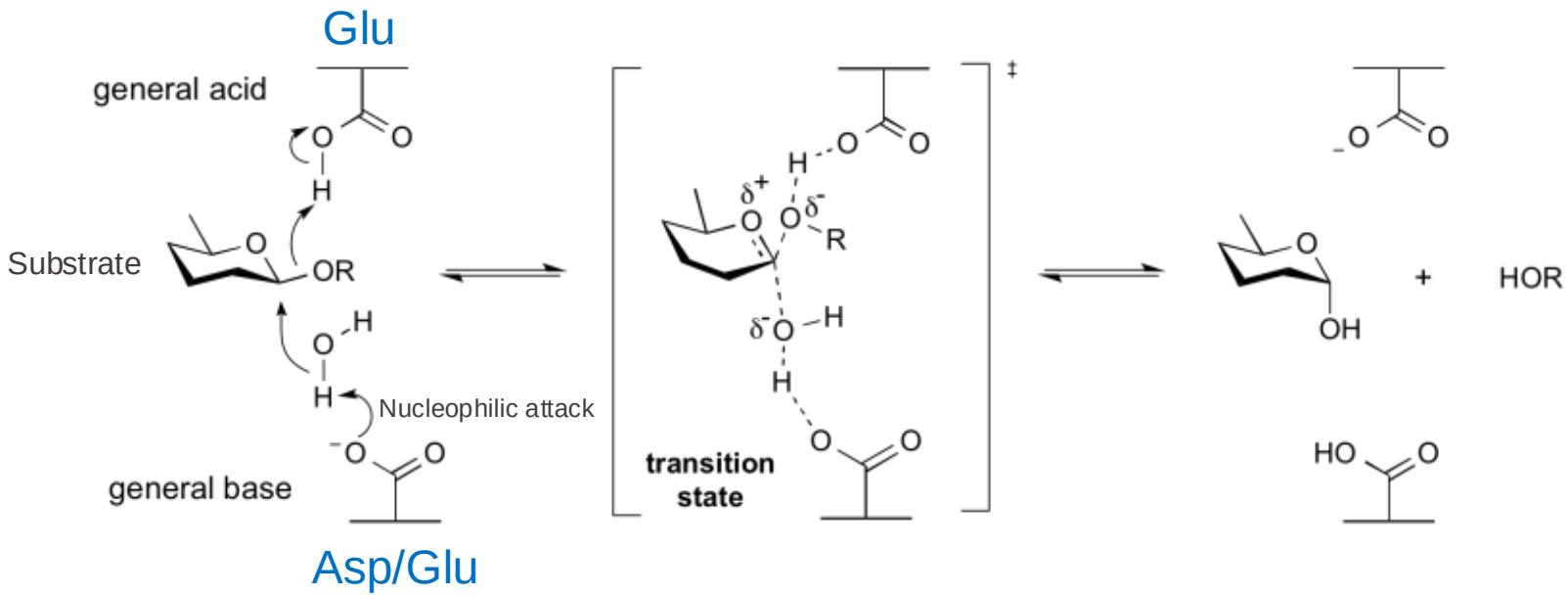
• Many enzymatic mechanisms involve transfer of protons (general acid-base catalysis) or the formation of transient covalent bonds in the active site via amino-acids or cofactors

β-galactosidase



Active sites

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

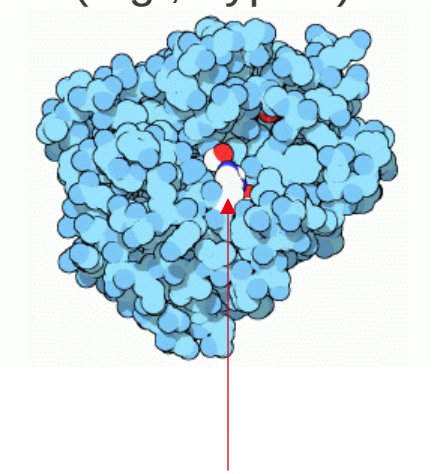


Nucleophile is a chemical group that forms bonds by donating electron pair
Electrophile forms bonds with nucleophiles by accepting electrons

Examples of enzymatic reactions

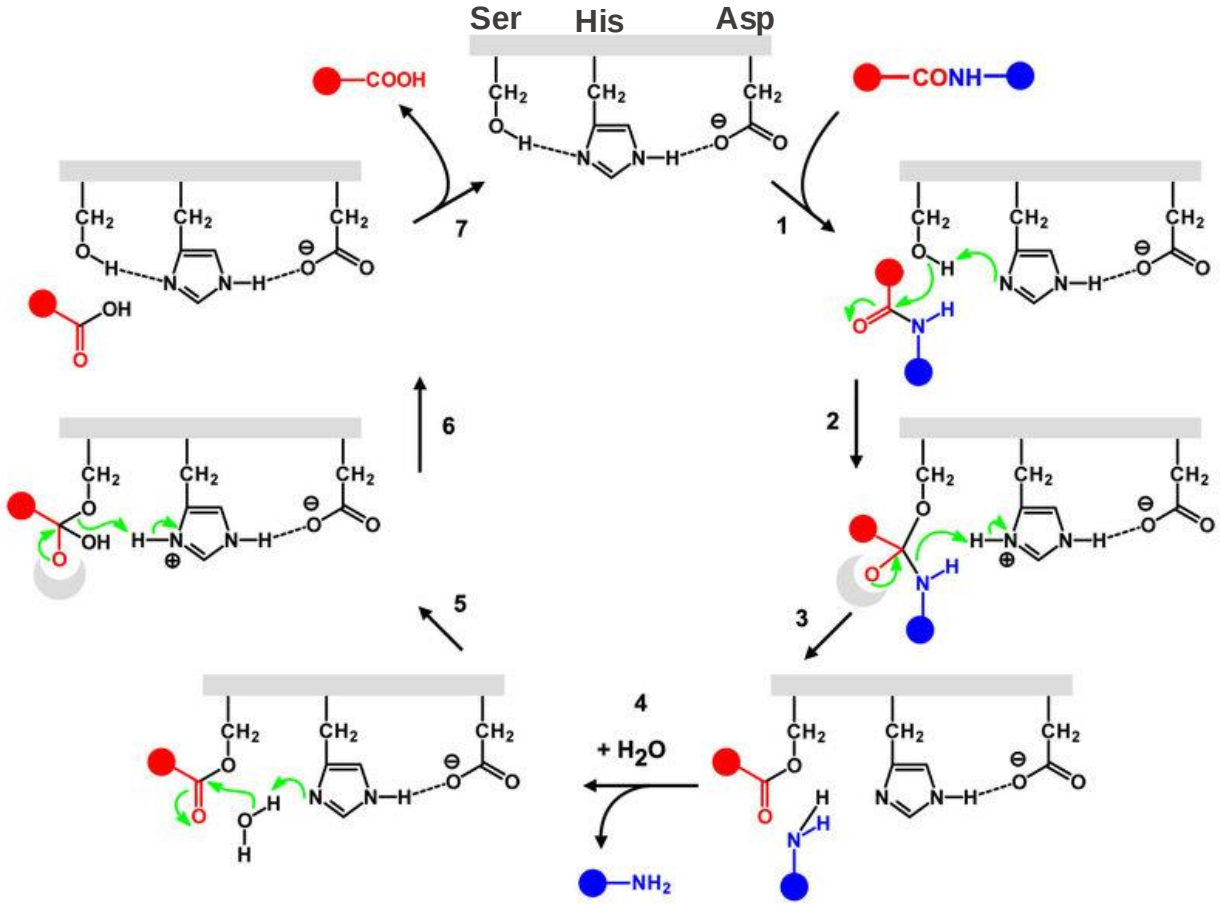
• Many enzymatic mechanisms involve transfer of protons (general acid-base catalysis) or the formation of transient covalent bonds in the active site via amino-acids or cofactors

Serine protease
(e.g., trypsin)



Active site

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



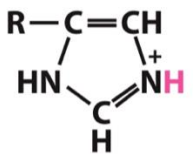
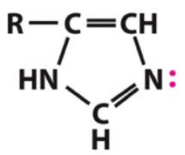
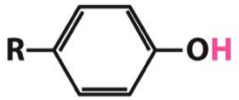
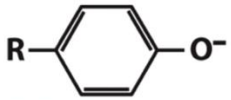
Green arrows indicate the nucleophilic attacks (electron transfer)

Ser + His + Asp
=
Catalytic triad

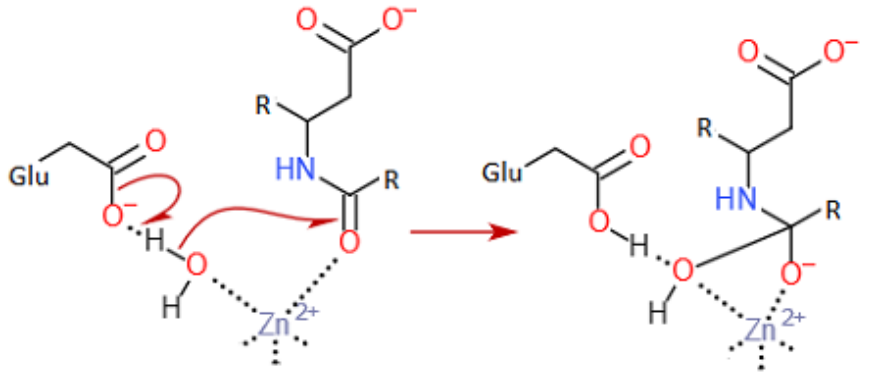
Groups commonly involved in catalysis

- 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—NH₃⁺	R—NH₂
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

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



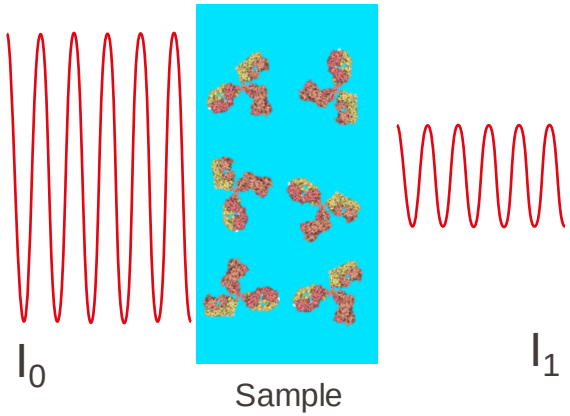
Enzyme substrate complex Tetrahedral intermediate

Enzyme kinetics

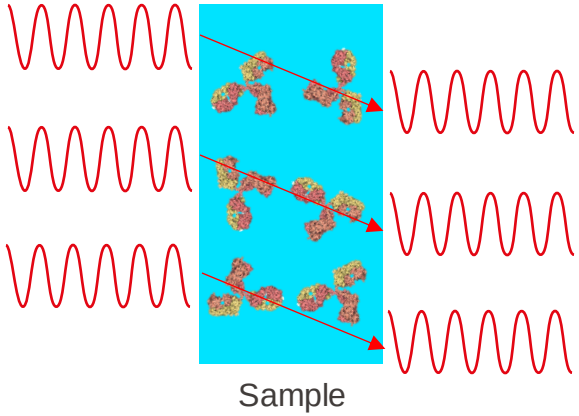
Biophysics is applied to measure reaction kinetics

- 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

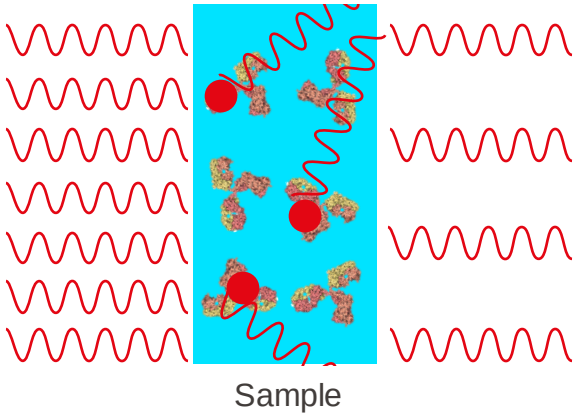
Absorption:



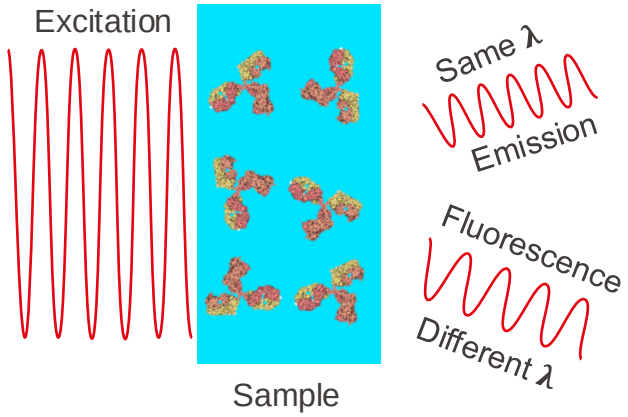
Refraction:



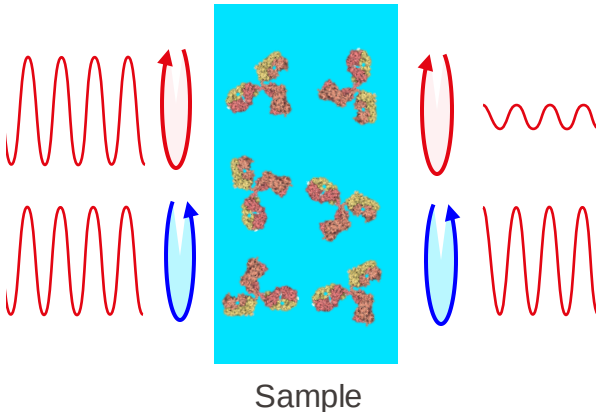
Scattering:



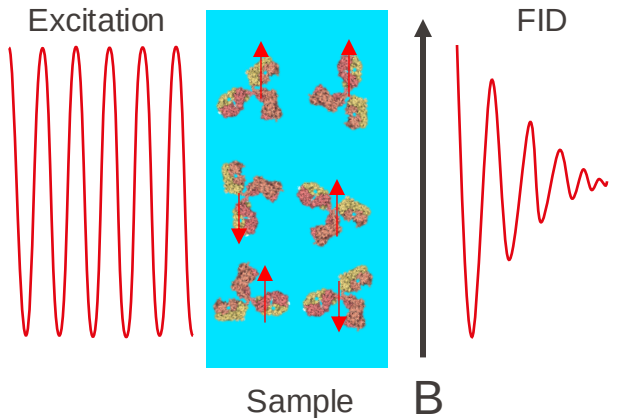
Emmission/Fluorescence:



Polarization:

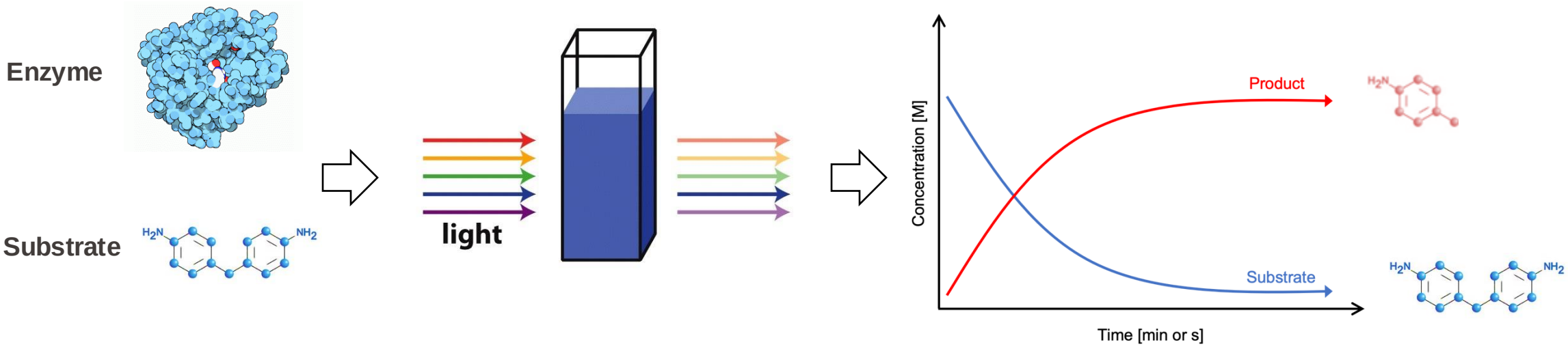


Resonance:



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**.

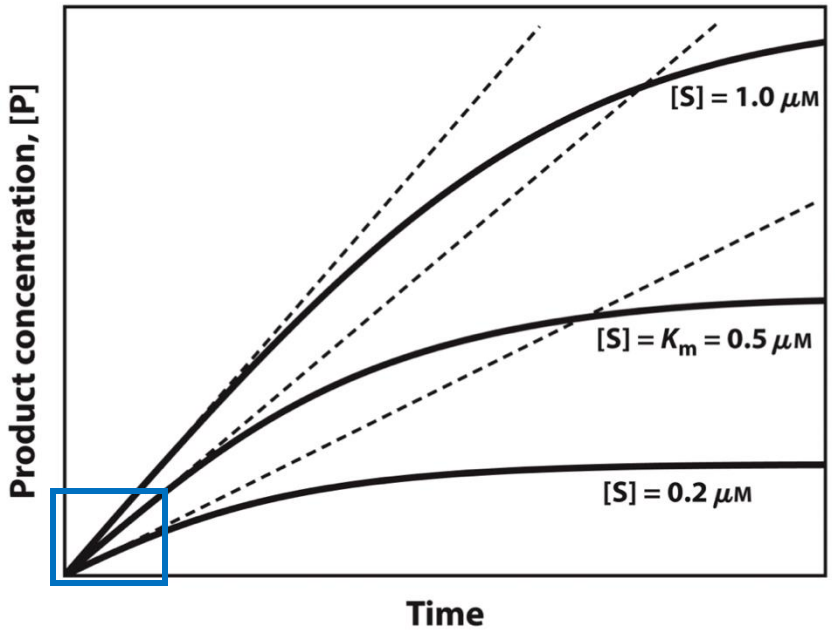
Measuring kinetic parameters of enzymes

- Studying the effects of substrate [S] or product [P] concentration is complicated as it changes with time
- The velocity (V) of the reaction will change proportionally with substrate concentration

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

$$V_{(t)} = k\,[S_{(t)}]$$

$V_{(t)}$ - reaction velocity at time t
k - rate constant (Arrhenius equation)
$[S_{(t)}]$ - Substrate concentration at time t



$V_{(t)} = k\,[S_{(t)}]$

$[S_{(t)}] = [S_0] - [P_{(t)}]$

$[P_0] = 0$

$V_0 = k\,[S_0 - 0]$

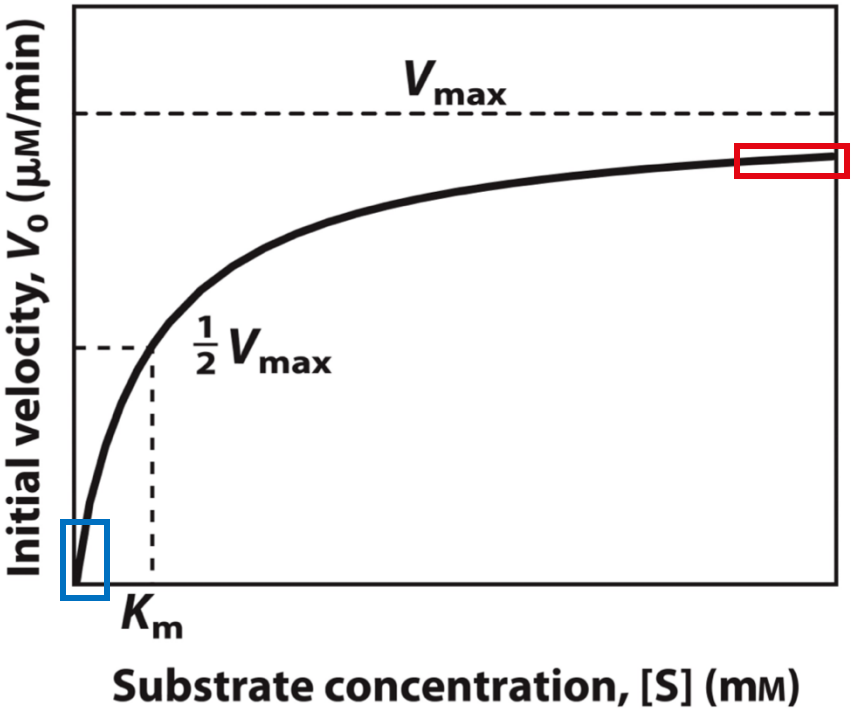
$V_0 = k\,[S_0]$

- To simplify the problem, we only measure the **initial velocity of the reaction** (designated V_0)

Measuring kinetic parameters of enzymes

- The initial velocity (V_0) expressed as a function of starting substrate concentration [S] represents a 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].

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



- 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].

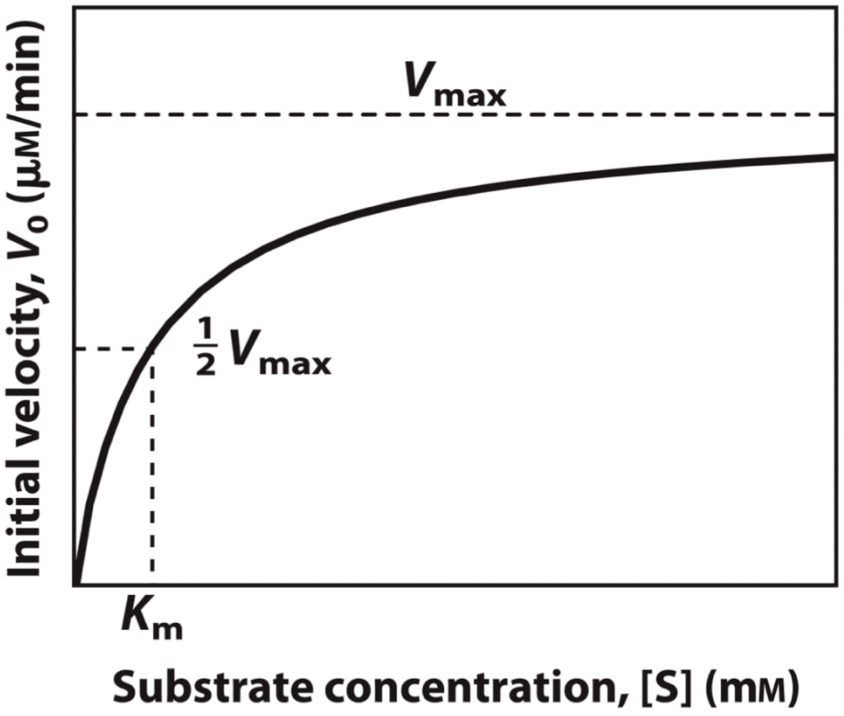
If [S] >> [E] then all E is **saturated** with S

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

Michaelis-Menten equation

• If we assume that (i) enzymatic reaction moves strictly towards product generation (ii) the EP complex dissociation is a very fast process and that (iii) the V_0 reflects a condition where [ES] is at a steady state, then the reaction can be written as:

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



• From this reaction we can easily derive the following equation that describes dependence of V_0 on initial substrate concentration

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

Where:

• **Vmax** = $k_2[E]$ (sometimes labeled as $[E]_{tot}$)

• $\mathbf{K_m} = \frac{k_{-1} + k_2}{k_1}$ Michaelis constant

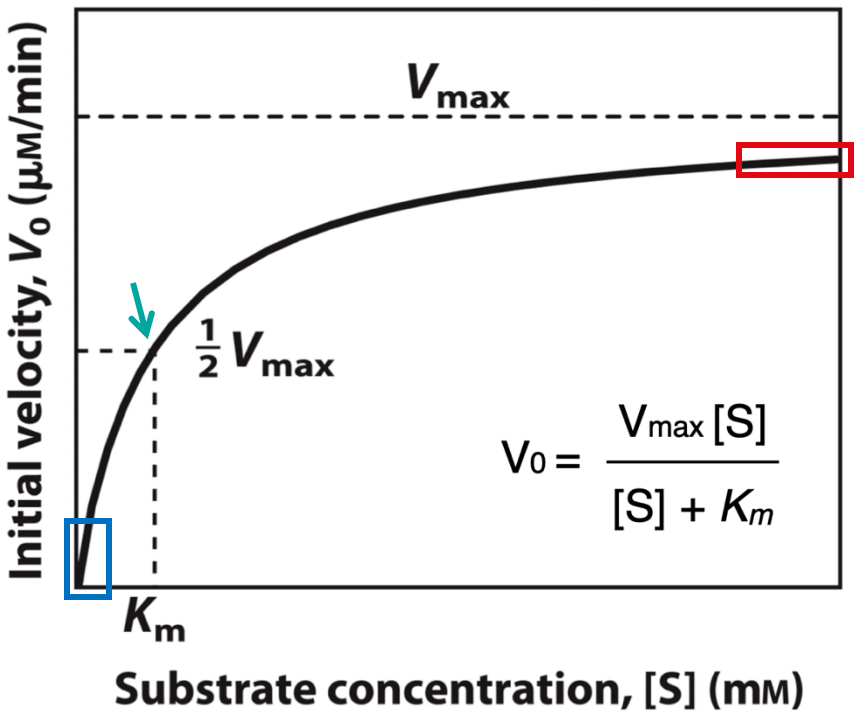
• This is a Michaelis-Menten equation that is widely used to describe the kinetic behavior of all enzymes that exhibit a hyperbolic dependence of V_0 on [S]

• This equation holds true for many enzymes under the steady-state conditions.

Michaelis-Menten equation

- The equation can be used to fit experimental data and calculate K_m, Vmax or k_2 parameters for any enzyme
- Different sections of the curve can be rationalized through this equation

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



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

$$V_0 = \frac{V_{max}[S]}{K_m} \qquad V_0 = k[S]$$

Linear increase of V_0 with [S]

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

$$V_0 = \frac{V_{max}[S]}{[S]} \qquad V_0 = V_{max}$$

Plateau at high [S]

- When [S] = K_m

$$V_0 = \frac{V_{max} K_m}{2 K_m} \qquad V_0 = \frac{V_{max}}{2}$$

[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 or K_d units but the values vary greatly even for different substrates of the same enzyme

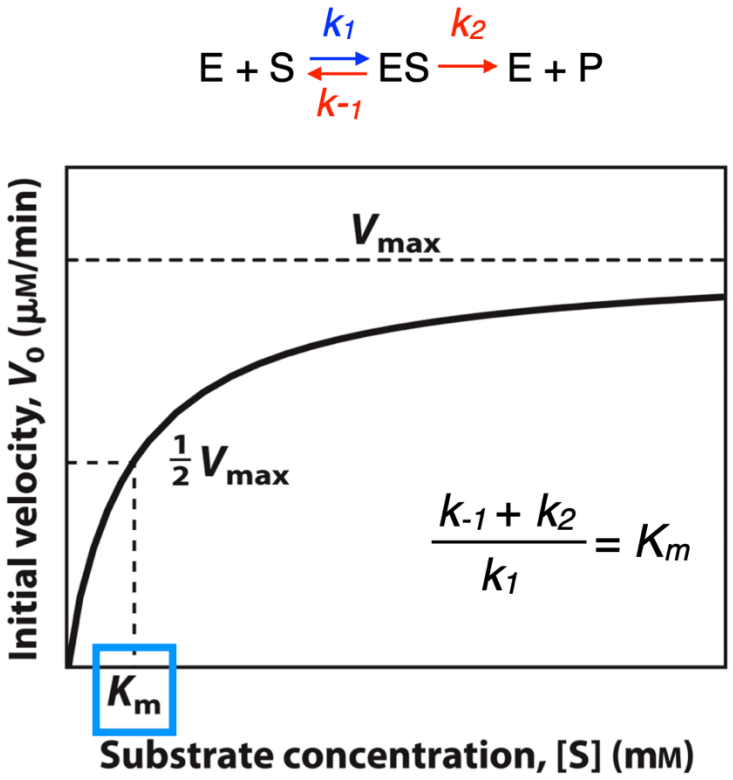


TABLE 6–6 K_m for Some Enzymes and Substrates

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

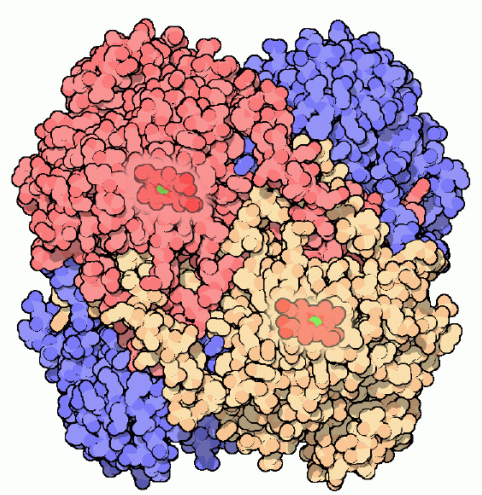
V_{max} and k_{cat}

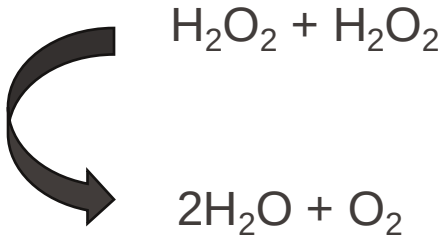
- V_{max} and k_{cat} are connected through the equation: $V_{max} = k_{cat}$ [E]
- Both constants are used to depict enzyme efficacy when converting substrate into product
- For simple 2-step MM mechanism, $k_{cat} \equiv k_2$, but it can also be used more generally to depict the overall rate constant of a multi-step reaction.

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



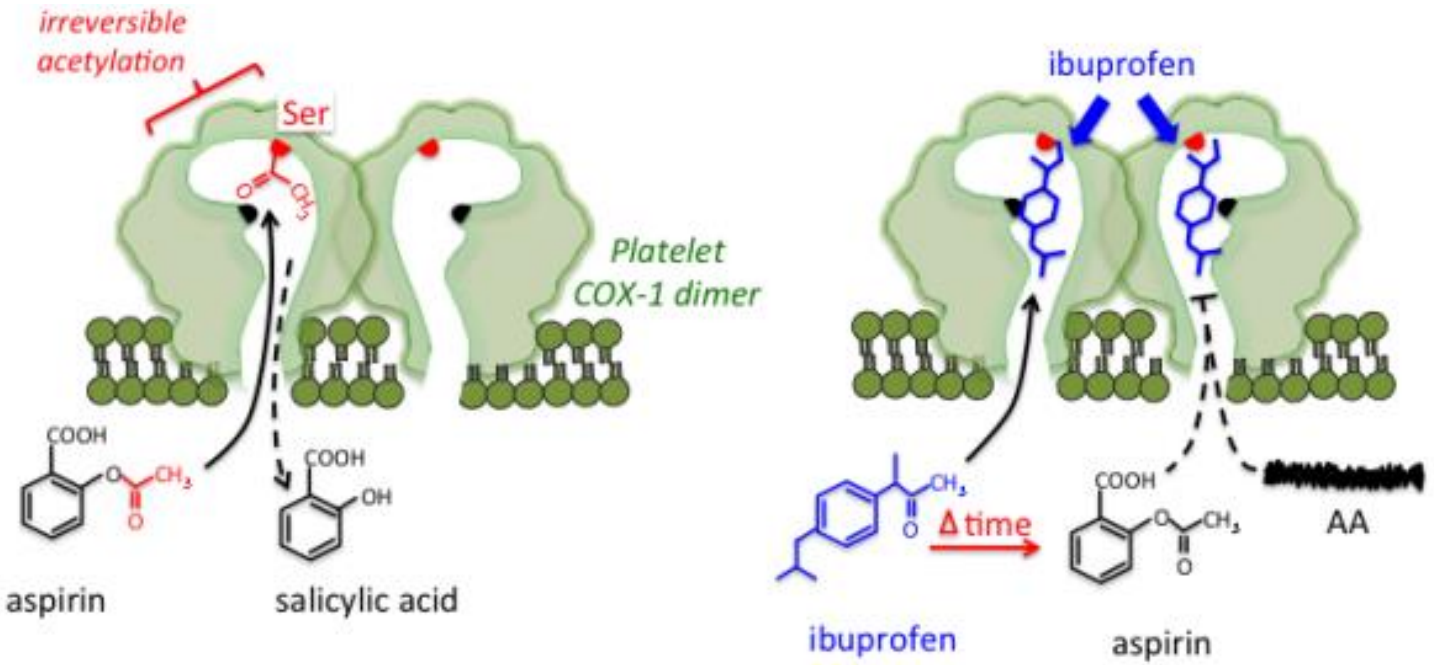


- k_{cat} is a 1st order rate constant and has units of s^{-1}.
- It is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule. So sometimes it is referred to as “turnover number”

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



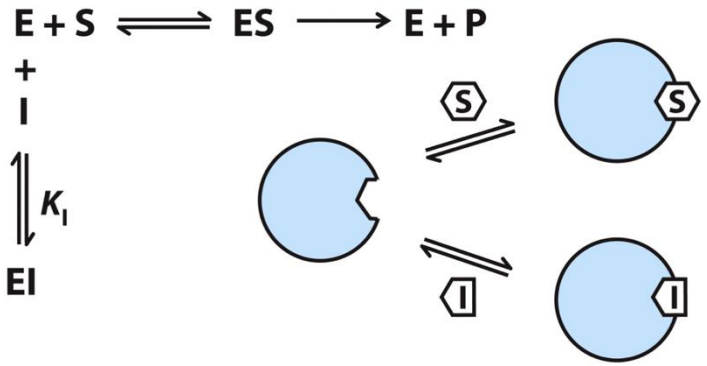
Transient binding
=
Reversible

- 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

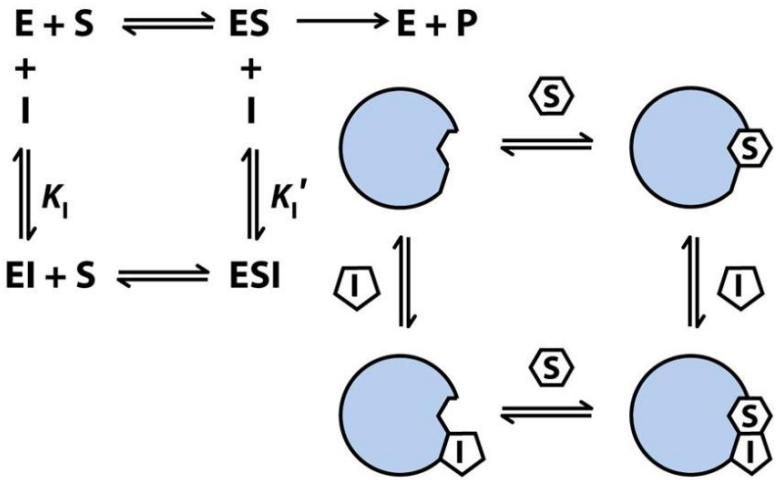
- Reversible enzyme inhibitors can act by **competitive, non-competitive** and **uncompetitive** mechanisms
- An inhibition constant (K_I) quantifies the strength of inhibitor in blocking the activity of an enzyme

Competitive



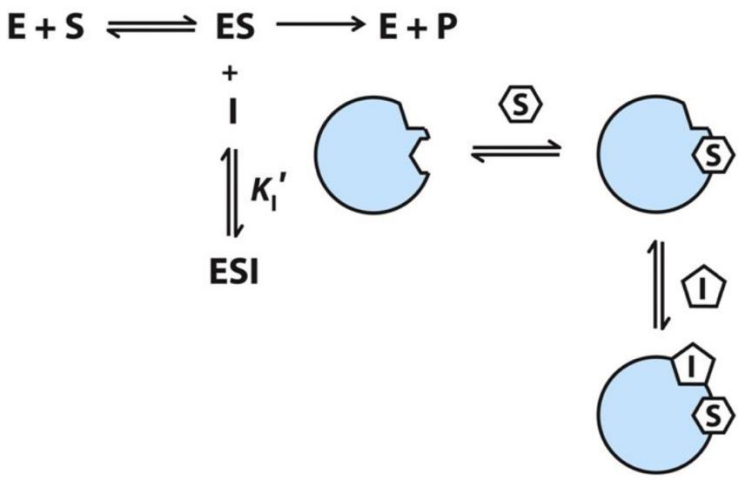
A competitive inhibitor (I) directly competes with the substrate for binding to the active site of an enzyme. They cannot bind at the same time.

Non-competitive



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.

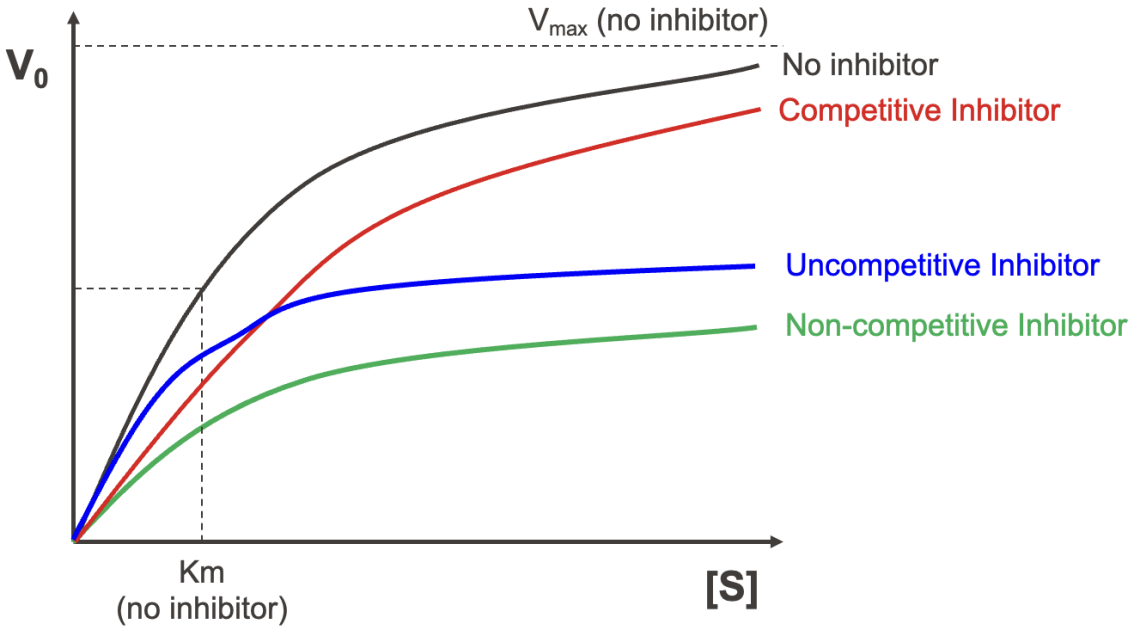
Uncompetitive



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: Km is higher V_{max} stays the same
Uncompetitive: Km is lower V_{max} is lower
Non-competitive: Km 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:

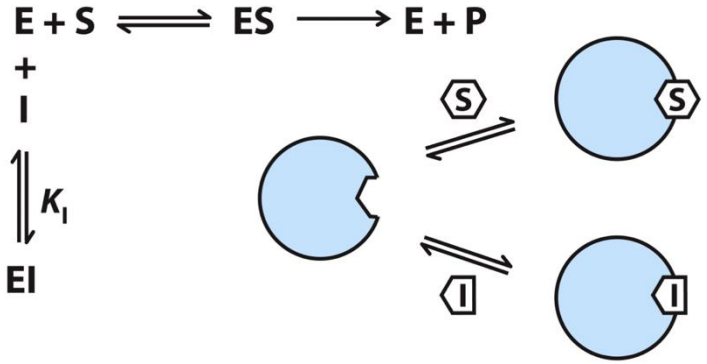
$$\alpha = 1 + \frac{[I]}{k_I} \qquad V_0 = \frac{V_{max}[S]}{[S] + \alpha K_m}$$

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

Reversible enzyme inhibition

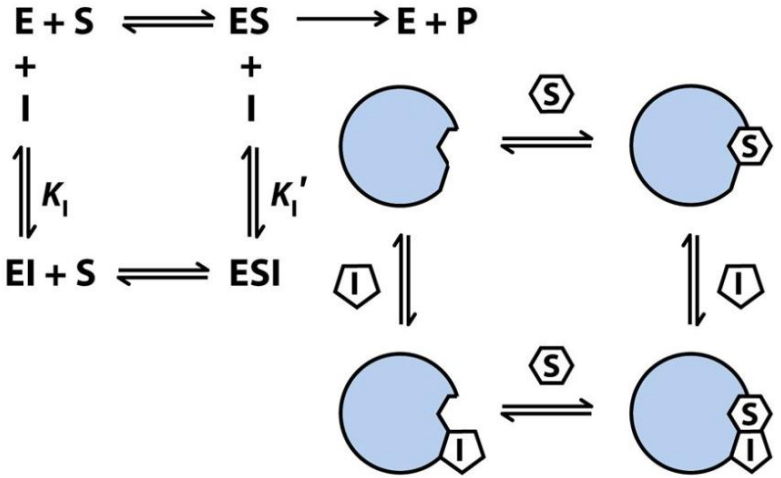
- Equations used to describe enzyme kinetics in the presence of different types of inhibitors

Competitive



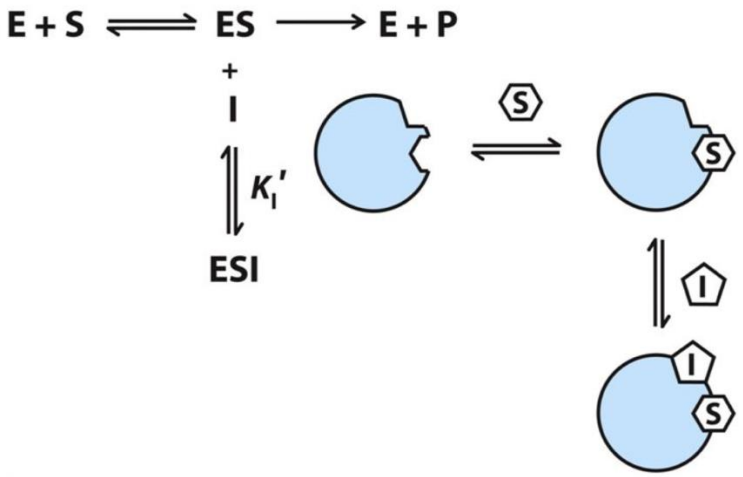
$$V_0 = \frac{V_{max}[S]}{[S] + \alpha K_m}$$

Non-competitive



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

Uncompetitive



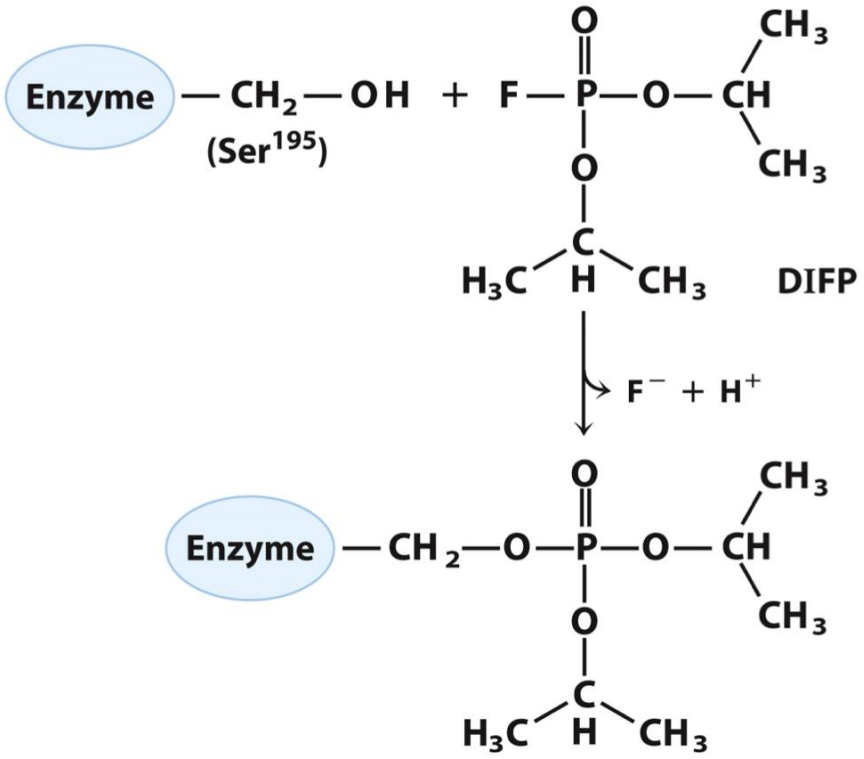
$$V_0 = \frac{V_{max}[S]}{\alpha'[S] + K_m}$$

- 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 or destroy a functional group on an enzyme that is essential for the enzyme's activity.

• An example of irreversible covalent inhibitor of the serine protease, chymotrypsin, is diisopropylfluorophosphate (DIFP) which covalently attaches to the catalytic serine residue (part of the catalytic triad)



- A special class of irreversible inhibitors are the **mechanism-based (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
- 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 describe how reactions change in response to changes in experimental parameters
- Concepts of Vo, V_{max} K_m, k_{cat} and specificity constant (k_{cat} / K_m)
- The principle of reversible and irreversible Enzyme Inhibition