

# Enzymes II

**Mechanism of enzyme reaction, metalloenzymes,  
kinetics, activity, enzymes in medicine**

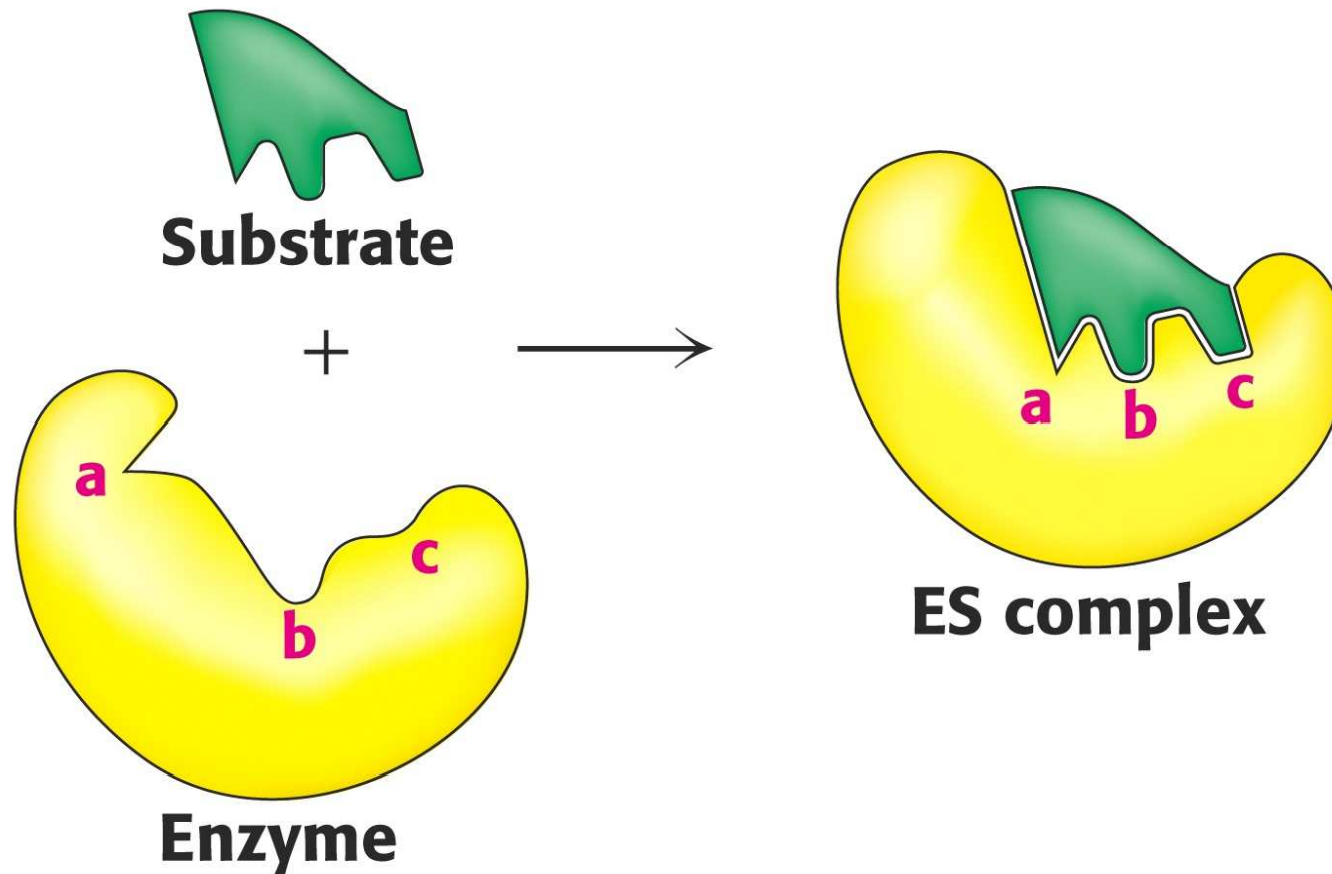
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# Active site of enzyme

- small part of molecule, deep cleft or pocket on the surface
- three-dimensional environment shields the substrate from water
- in oligomeric enzymes - interface between subunits
- made by side chains of AA which are distant in primary structure
- the site is really *active* - protein flexibility facilitates the conformation changes fitting to substrate
- substrate is bound relatively weakly

# Binding substrate to enzyme

- binding a substrate induces the conformational changes in enzyme molecule (induced fit model)
- an enzyme-substrate (ES) complex is formed



# Example: The active sites of three proteinases

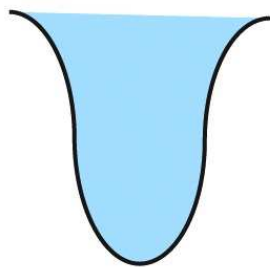
Certain AA residues determine the substrate specificity of these enzymes.

## The peptide bonds preferably hydrolyzed:

by **chymotrypsin** - near residues with large, hydrophobic, uncharged side chains (Phe, Trp),

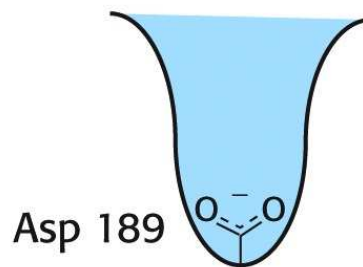
by **trypsin** – near residues with long, positively charged side chains (Arg, Lys),

by **elastase** - near residues with small hydrophobic side chains (Gly, Ala).



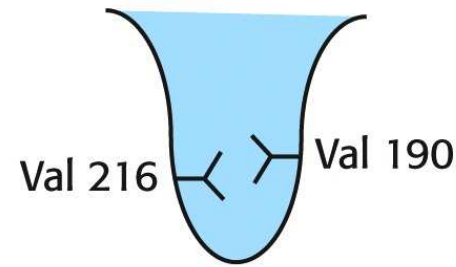
**Chymotrypsin**

A deep, relatively hydrophobic pocket



**Trypsin**

At the bottom of the deep pocket there is an acidic residue (Asp)



**Elastase**

Two residues of valine close off the mouth of the small hydrophobic pocket

# Catalytic mechanism depends on the number of substrates

- **Monosubstrate reactions**



- **Bisubstrate reactions (more common)**

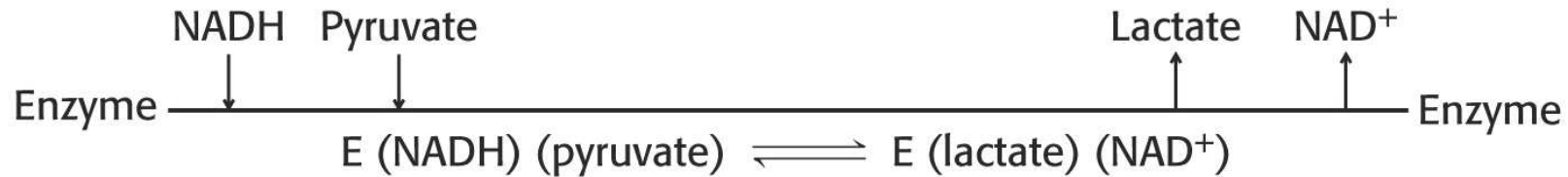


**Sequential reaction:** both substrates bind to enzyme, chemical conversion proceeds, and both products are released

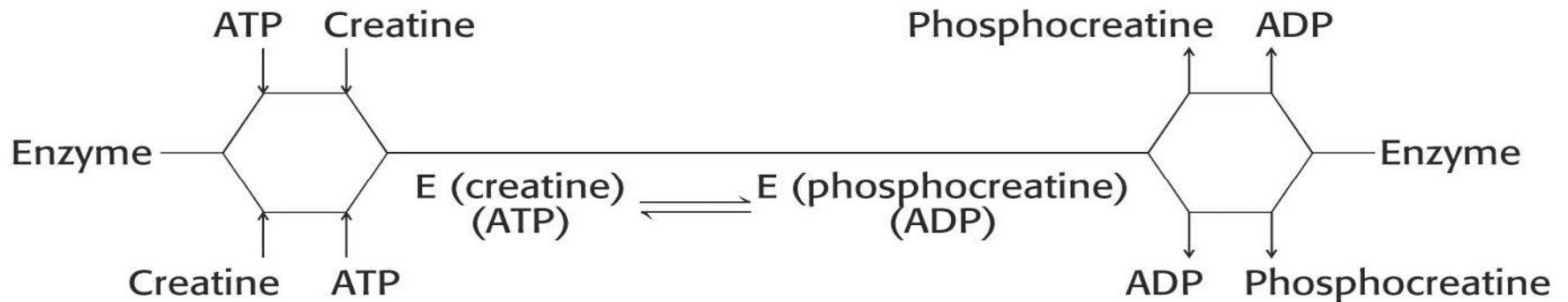
**Ping-pong reaction:** typical for aminotransferases, one substrate is attached, converted, and released, then the same events occur with the second substrate

## Sequential reaction:

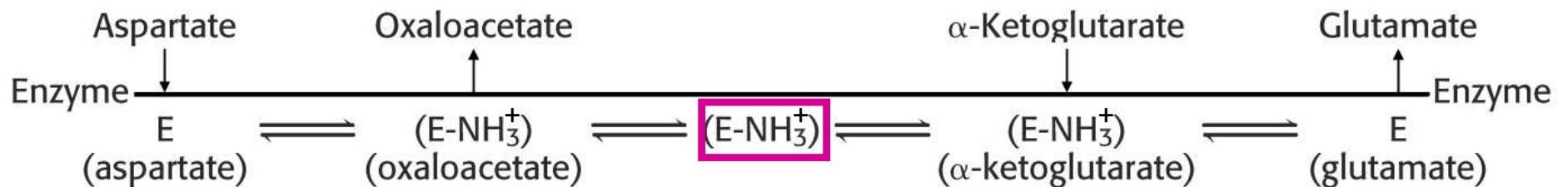
- type ordered - the substrates bind the enzyme in a defined sequence



- type random – the order of addition of substrates and release of products is random:



## Ping-pong reaction:



# Catalytic groups

- located in active site
- involved the chemical conversion of substrate
- nucleophilic (cysteine -SH, serine -OH)
- acidic (Asp, Glu), basic (His, Arg, Lys)

## **Examples of different types of catalytic mechanisms:**

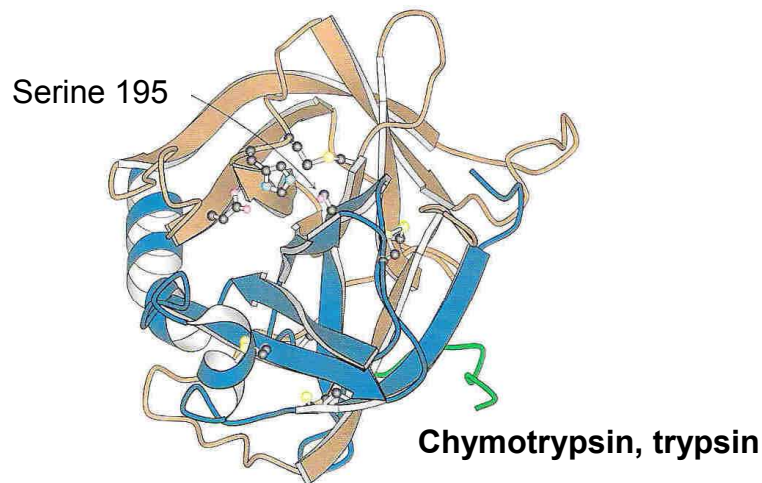
- Catalysis through proximity and orientation effects (strained reactants)
- Covalent catalysis – formation of transitory covalent bonds between enzyme and substrate
- Acid-base catalysis – protonization of substrates or catalytic groups of enzyme
- Metal ion catalysis mediating redox reactions or shielding negative el. charges
- Electrostatic catalysis (after excluding water from the active site by binding a substrate)

# Examples of proteinases

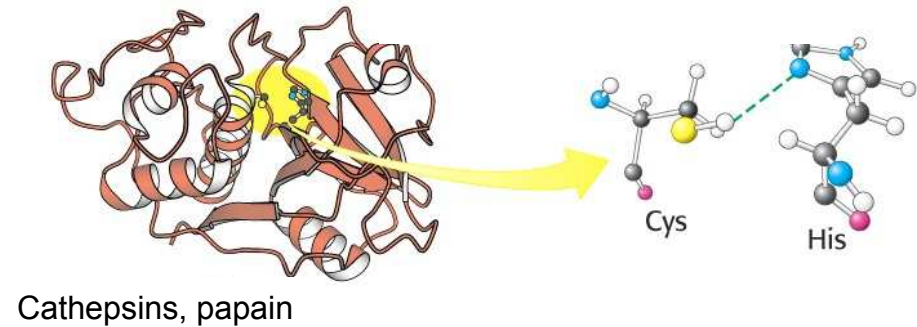
Due to the different arrangement of active sites, they exhibit

- different substrate specificities
- different catalytic mechanisms

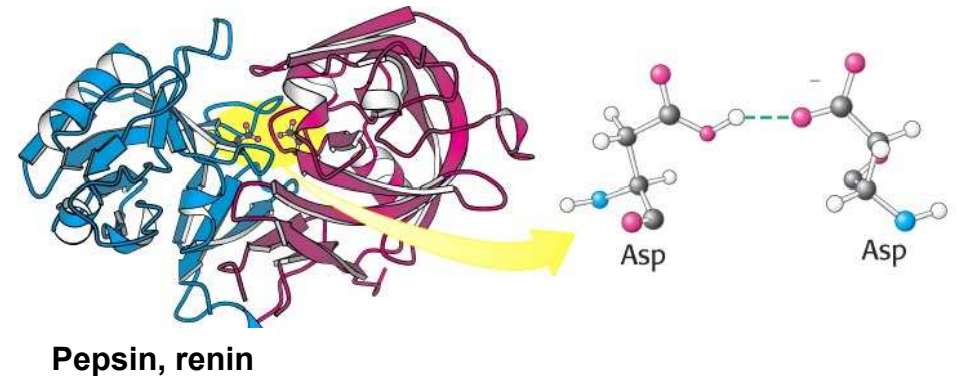
## SERINE PROTEASES



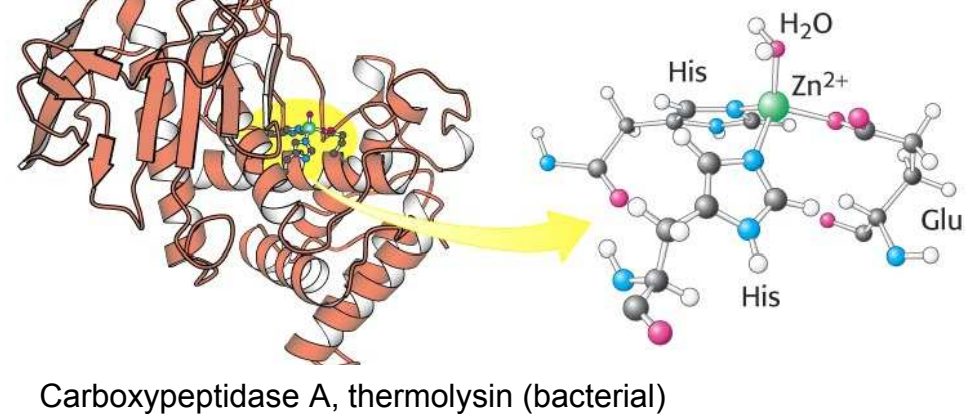
## CYSTEINE PROTEINASES



## ASPARTATE PROTEINASES



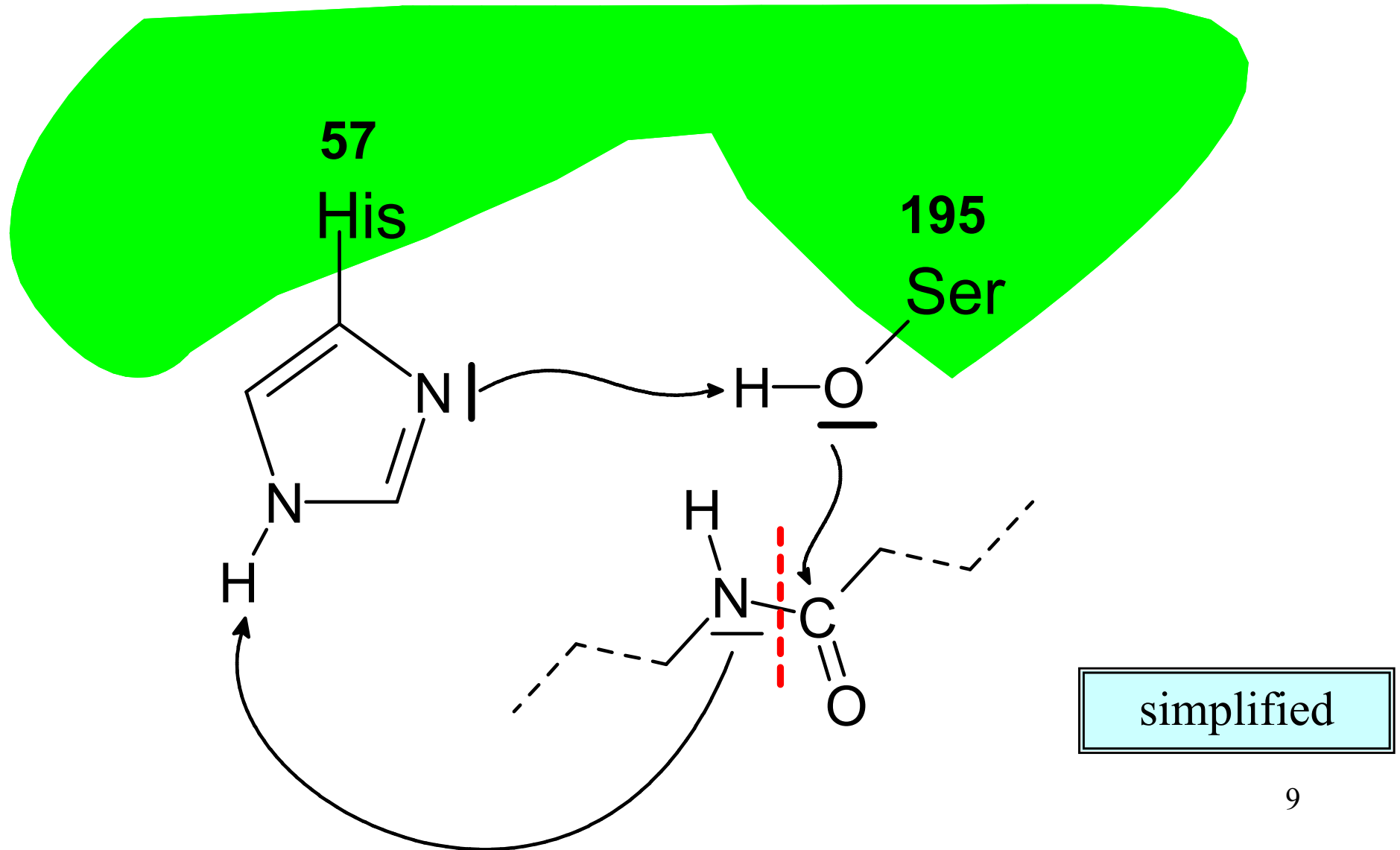
## METALLOPROTEINASES



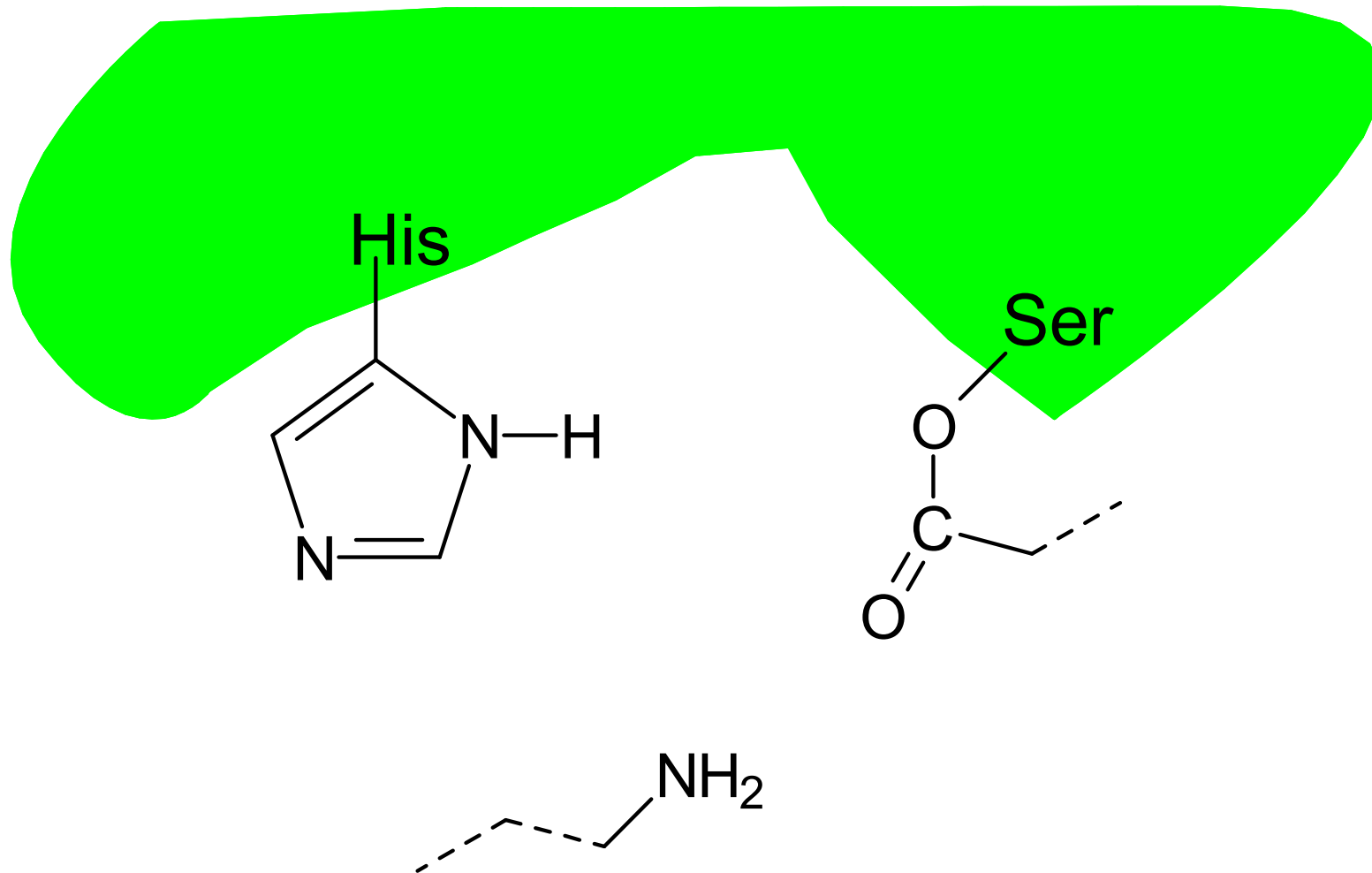


# Example: active site of chymotrypsin

Nucleophilic attack of serine -OH to carbonyl carbon of peptide bond  
⇒ serine protease



# Active site of chymotrypsin: the cleavage of peptide bond

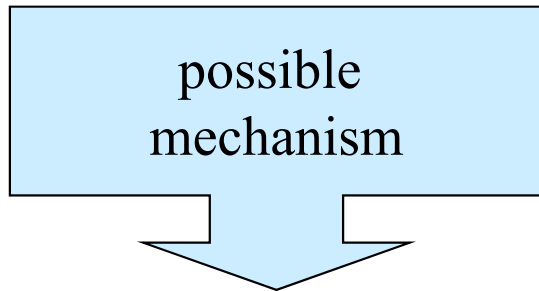


# Metalloenzymes

- contain **functioning metal cations** (prosthetic groups) directly involved in catalyzed reaction, metal ions attached tightly (Enz-M)
- some enzymes need metal ions just for **activation**, they are associated relatively weakly (Enz ...M), e.g.  $\text{Ca}^{2+}$  (coagulation factors),  $\text{Mg}^{2+}$  (kinases)

# Metal ion is a part of ternary complex

- three components make a ternary complex:  
enzyme (Enz), substrate (S), and metal ion (M)
- different types of complexes:  
Enz-S-M, Enz-M-S, or cyclic complexes



- vacant metal orbitals accept electron pair of nucleophile to make  $\sigma$ -bond
- metal ions make chelates with some groups of enzyme/substrate  $\Rightarrow$   
deformation of structure  $\Rightarrow$  strain  $\Rightarrow$  facilitates chemical conversion
- coordination sphere of metal creates a three-dimensional template  $\Rightarrow$   
stereospecific control of reaction

# Molybdenum (Mo)

- Some oxidoreductases
- cofactor – molybdopterin
- **Xanthine oxidase** (xanthine → uric acid)
- **Sulfite oxidase** (sulfite  $\text{HSO}_3^-$  → sulfate  $\text{SO}_4^{2-}$ )

Mo in food: legumes, wholemeal cereals

# Zinc (Zn)

- Many enzymes
- **Alcohol dehydrogenase** (ethanol  $\rightarrow$  acetaldehyde)
- **Carbonic anhydrase** ( $\text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3$ )
- **Carboxypeptidases** (cleavage of polypeptides from C-terminal)
- **Cu, Zn-superoxide dismutase** (cytosolic isoform)  
( $2 \cdot\text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$ )

Zn in food: red meat, lobsters, legumes, seeds, wholemeal cereals

# Copper (Cu)

- many oxidoreductases
- **Ceruloplasmin (ferroxidase)** ( $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ )
- **Cytochrome-*c*-oxidase** (R.Ch., electron transfer to  $\text{O}_2$ )
- **Monoamine oxidases** (MAO, inactivation of biogenic amines, side product  $\text{H}_2\text{O}_2$ , see Med. Chem. II, p. 60)
- **Dopamine hydroxylase** (dopamine  $\rightarrow$  noradrenaline)
- **Lysyl oxidase** (collagen maturation, Lys  $\rightarrow$  alLys)

Cu in food: liver, meat, cocoa, legumes, nuts

# Manganese (Mn)

- some hydrolases, decarboxylases, transferases
- **Mn-superoxide dismutase** (mitochondrial isoform)
- **Arginase** (arginine → urea + ornithine)
- Synthesis of proteoglycans + glycoproteins

Mn in food: legumes, nuts, wholemeal cereals



# Iron (Fe)

- heme and non-heme iron enzymes
- **Catalase** (heme,  $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \frac{1}{2}\text{O}_2$ )
- **Myeloperoxidase** (heme, neutrophils)  
 $\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HClO} + \text{H}_2\text{O}$
- **Cytochromes** (heme, transfer of electrons in R.Ch.)
- **Fe-S proteins** (non-heme, transfer of electrons in R.Ch.)

Fe in food: animal blood, red meat, liver, egg yolk, nuts, broccoli

# Selenium (Se)

- few enzymes (redox reactions), Se in selenocysteine
- **Glutathione peroxidase**  
( $2 \text{ GSH} + \text{H}_2\text{O}_2 \rightarrow 2 \text{ H}_2\text{O} + \text{G-S-S-G}$ )
- **Deiodases of thyronines** (thyroxine T4  $\rightarrow$  triiodothyronine T3)
- **Thioredoxin reductase** (ribose  $\rightarrow$  deoxyribose)

Se in food: sea products, legumes

# Factors determining the rate of enzymatic reaction

|                                |   |
|--------------------------------|---|
| <b>Temperature</b>             | Reaction rate increases with temperature, optimal around 40 °C,<br>at higher temperatures the rate decreases due to enzyme denaturation   |
| <b>pH</b>                      | pH value determines ionization of groups in active site and its surroundings,<br>Constant pH of body fluids is maintained by buffer systems<br>Each enzyme has its pH optimum, intracellular enzymes around pH 7<br>Digestion enzymes differ: pepsin pH 2 |
| <b>Activators</b>              | They accelerate enzyme reaction<br>Often divalent metal cations: $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Mn}^{2+}$  |
| <b>Inhibitors</b>              | Slow down or stop enzyme reaction<br>competitive: similar to substrate, they compete for active site<br>Other, e.g. heavy metal ions, strongly bind to important enzyme groups  |
| <b>Substrate concentration</b> | At high substrate concentration, enzyme is saturated, so the reactions proceeds<br>with maximal velocity, graphically expressed as saturation curve   |

# Basic kinetic terms

- reaction:  $S \rightarrow P$  (S = substrate, P = product)
- definition of reaction velocity (rate):

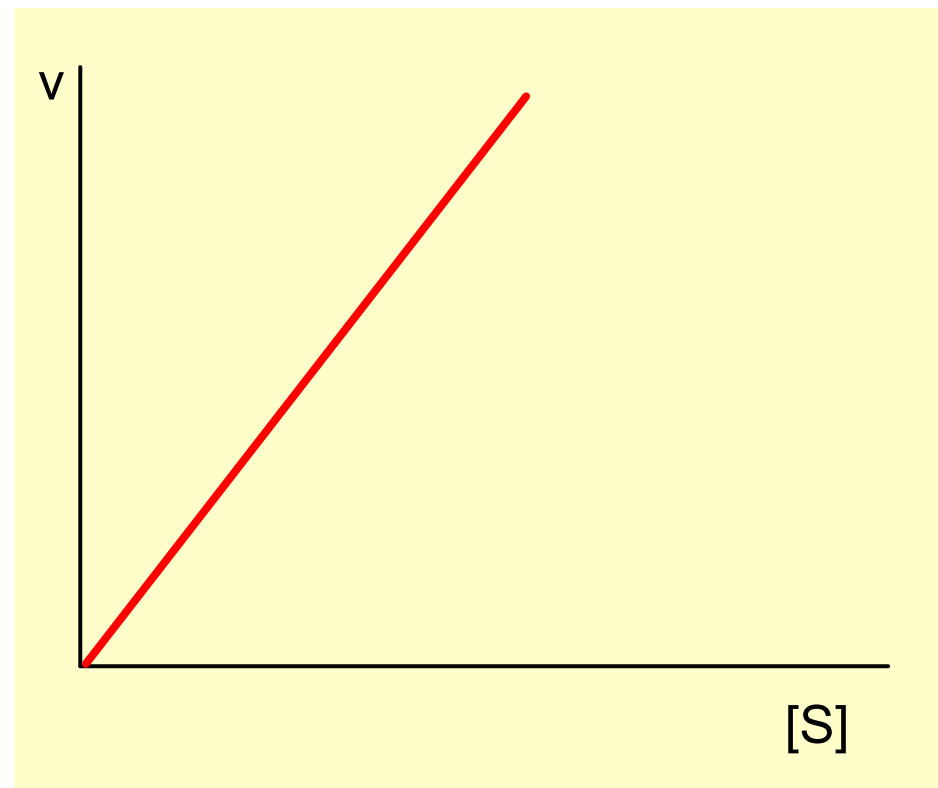
$$v = -\frac{\Delta[S]}{\Delta t} = \frac{\Delta[P]}{\Delta t} > 0 \quad \left[ \frac{\text{mol}}{\text{l.s}} \right]$$

this definition is for average rate,  
instantaneous rate:  $d[S]/dt$

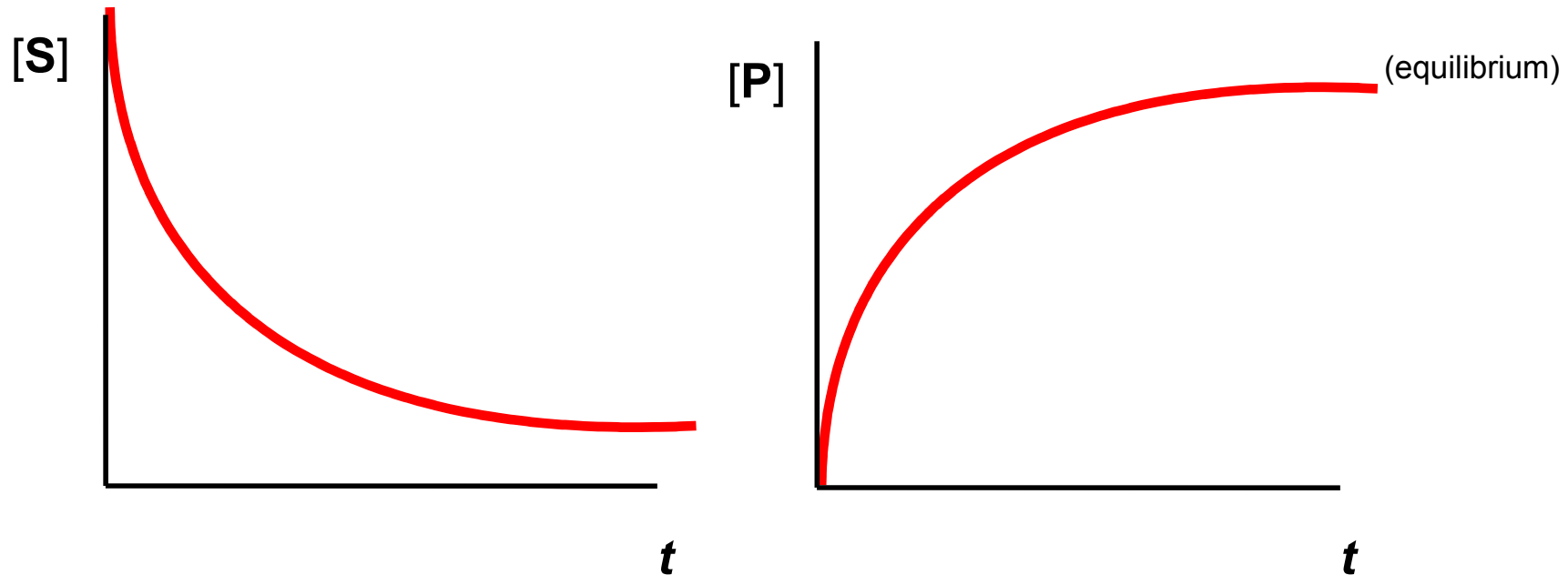
# Kinetic equation for reaction $S \rightarrow P$

$$v = k [S] = k [S]^1 \Rightarrow \text{reaction of 1. order}$$

$k$  = rate constant



# Kinetic (progress) curves for substrate and product



during reaction:  
concentration of substrate decreases  
concentration of product increases

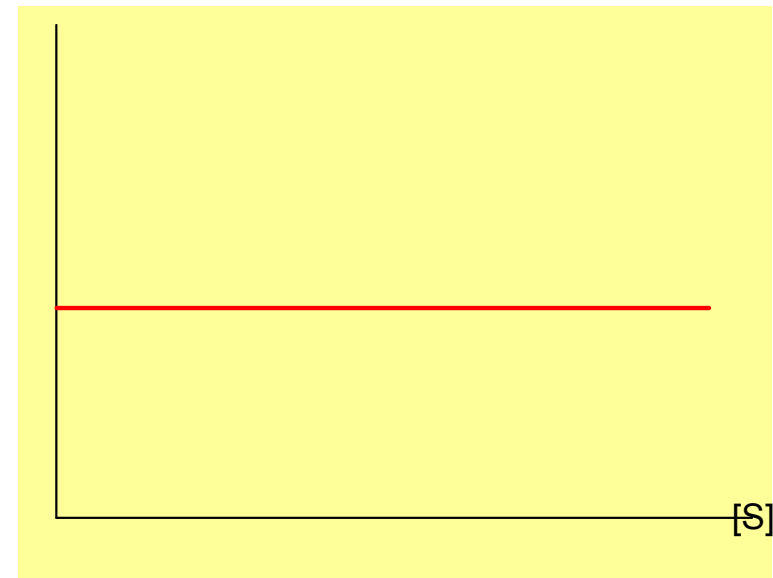
reaction rate is determined  
from kinetic curves

The instantaneous velocity  $v_x$  at any particular time  $t_x$  is given by the slope of the tangent to the curve at that time.

# Reaction of 0. order

- Reaction rate does not depend on the substrate concentration
- $v = k [S]^0 = k \times 1 = k = \text{constant}$
- At great excess of substrate S

in enzyme reactions only in laboratory conditions



# Initial velocity $v_0$

- The highest value of velocity
- It is not influenced by the decrease of substrate nor the reverse change of product
- **Determined from kinetic curves at the time  $t = 0$**



## Initial velocity $v_o$ depends on substrate concentration

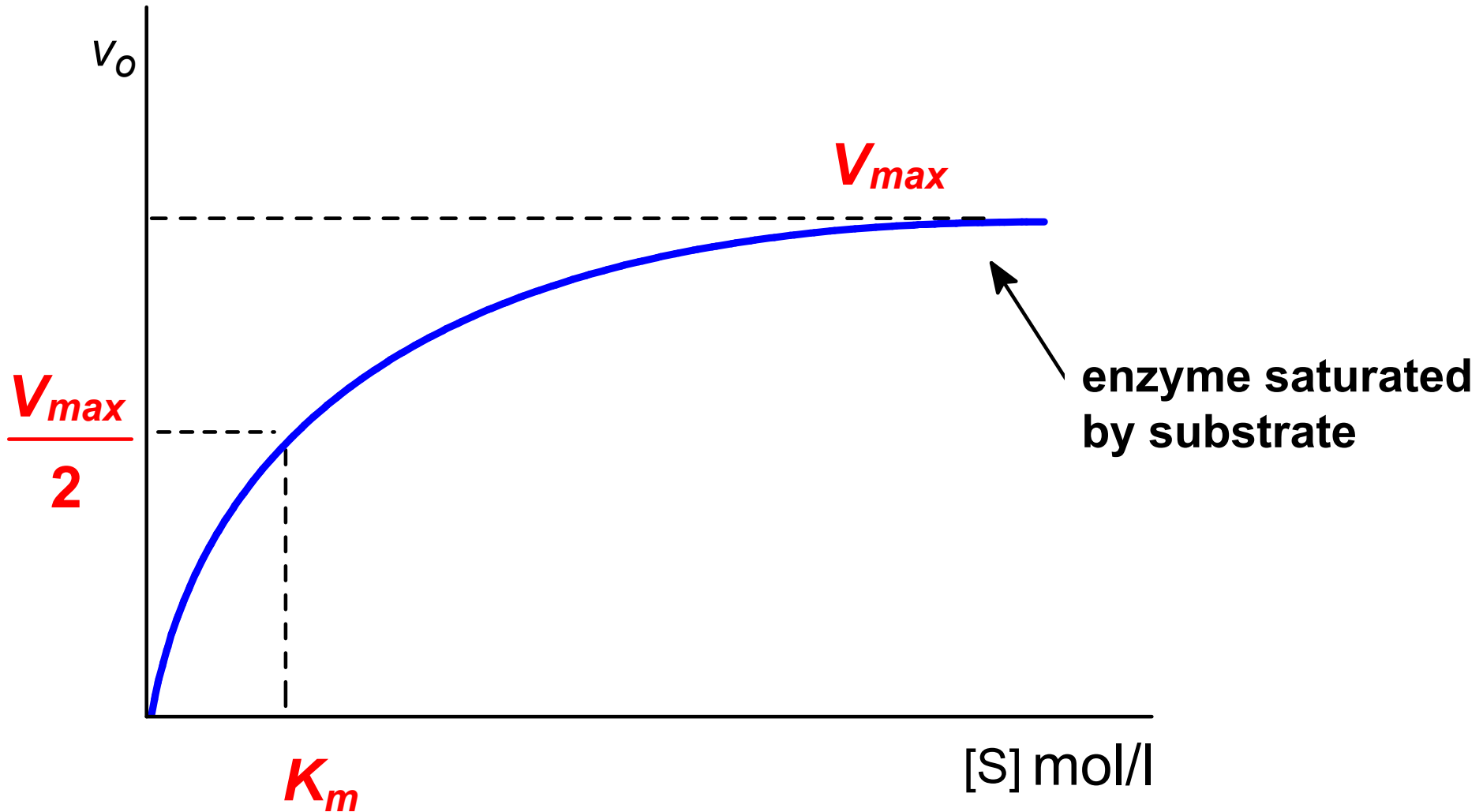
- Michaelis-Menten equation
- for one-substrate reactions

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

$V_{\max}$  = maximal velocity (for the given concentration of enzyme)

$K_m$  = Michaelis constant

The graph of previous equation is saturation curve



If  $[S] \ll K_m$

$$v_o = V_{\max} \frac{[S]}{\cancel{[S]} + K_m} = \frac{V_{\max}}{K_m} [S] = k[S]^1$$

at low substrate concentration,

the reaction proceeds by the **1<sup>st</sup> order kinetics**

If  $[S] \gg K_m$

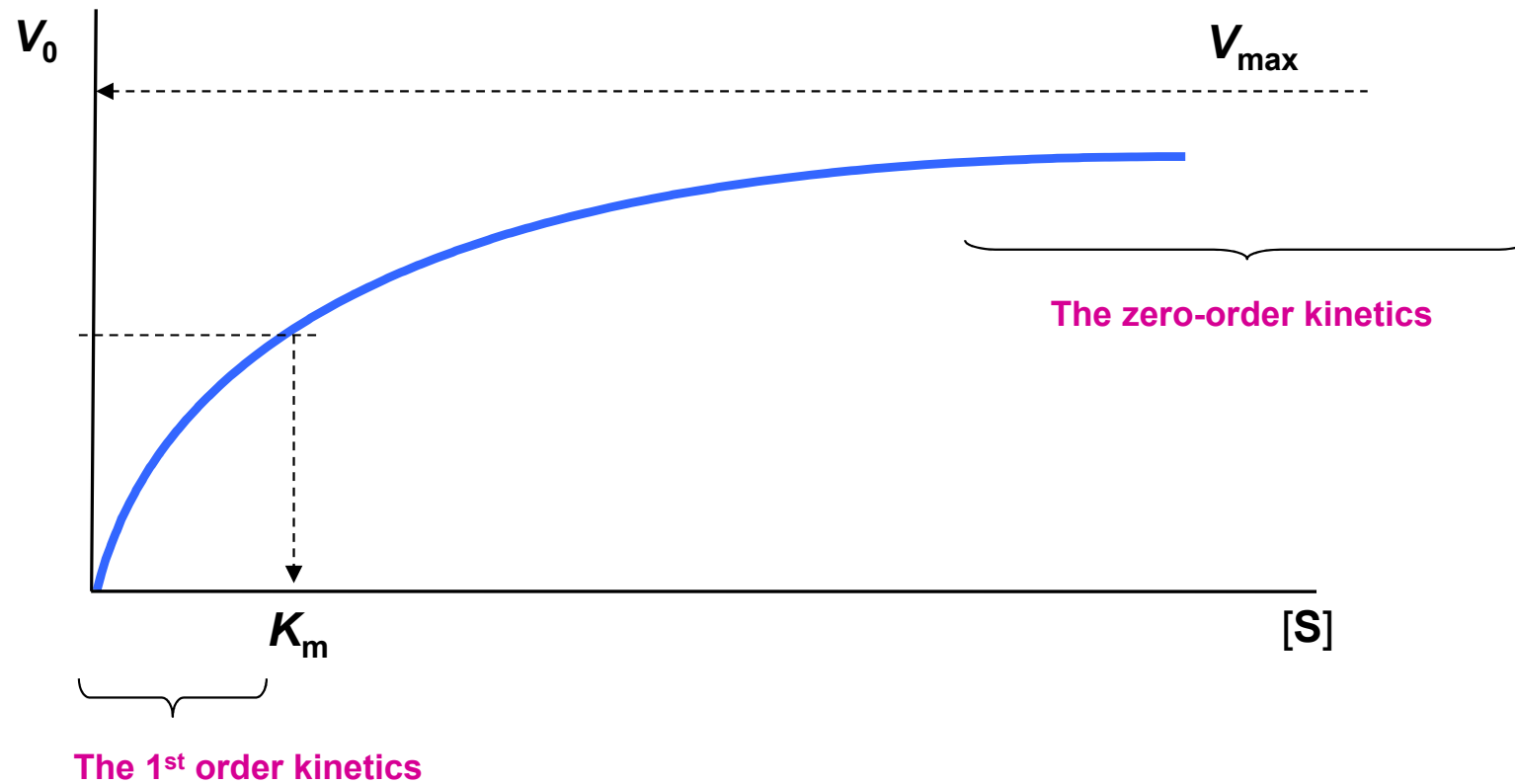
$$v_o = V_{\max} \frac{[S]}{[S] + \cancel{K_m}} = V_{\max} \frac{[S]}{[S]} = V_{\max} = k[S]^0$$

at high substrate concentration,

the reaction proceeds by the **0. order kinetics**

# Two parts of saturation curve

compare with pages 21 and 23



**If  $[S] = K_m$**

$$v_o = V_{\max} \frac{[S]}{[S] + [S]} = V_{\max} \frac{[S]}{2[S]} = \frac{V_{\max}}{2}$$

# Significance of $K_m$ and $V_{max}$

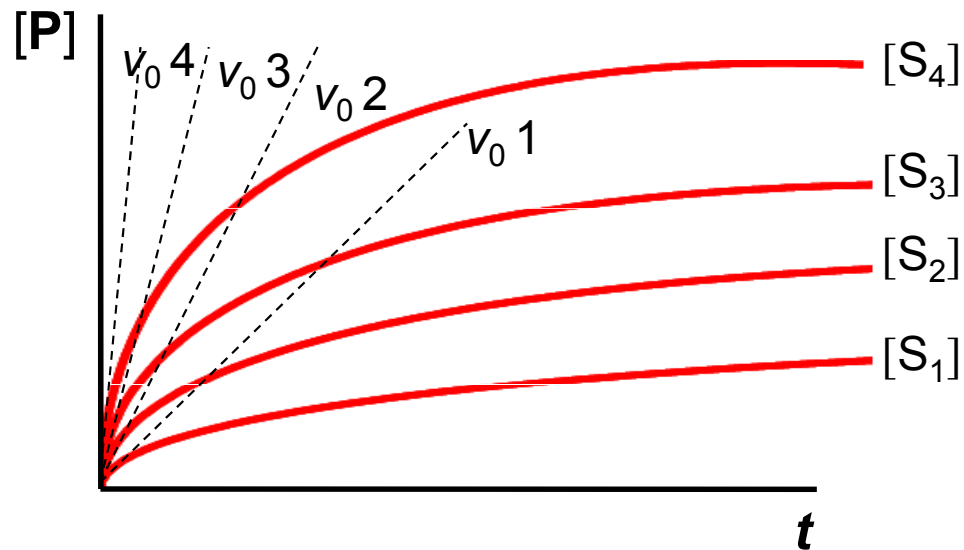
- the Michaelis constant  $K_m$  is the concentration of substrate [S] which gives half the maximal velocity  $V_{max}$  (50 % saturation of enzyme)
- the  $K_m$  has the dimension of concentration (mol/l)
- $K_m$  is inversely related to the affinity of enzyme for its substrate.

If more substrates with similar structure exist, then the best natural substrate is one with the least value of  $K_m$

- if there is a need to measure the activity of enzyme, the substrate concentration has to be at least several times higher than the  $K_m$  value.

# How to get a saturation curve?

A series of measurements of initial velocity is arranged at a constant enzyme concentration  $[E]$  and different substrate concentrations  $[S]$  (in the range of 2 - 3 orders).



**$[E] = \text{const.}$**

From the obtained progress curves, the values  $v_0$  are estimated and plotted against the corresponding  $[S]$ .

The velocity  $v_0$  rises linearly as substrate concentration increases, and then begins to level till it reaches a limit value at high substrate concentrations.



# Distinguish

## Kinetic curve

- time record  
of **one** reaction
- $[S] = f(t)$  or  $[P] = f(t)$

## Saturation curve

- dependence obtained  
from **many** reactions  
(see previous page)
- $v_o = f([S])$

[S] ..... substrate concentration

[P] ..... product concentration

f ..... function

$t$  ..... time

$v_o$  ..... initial velocity

# $V_{\max}$ and $K_m$ describe the kinetic properties of enzyme

- are hardly obtained from saturation curve
- easily obtained from linear double reciprocal plot
- Lineweaver-Burk:  $1/v_0$  is plotted against  $1/[S]$

# Reciprocal equation

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

$$\frac{1}{v_o} = \frac{1}{V_{\max}} \cdot \frac{[S] + K_m}{[S]} = \frac{1}{V_{\max}} \left( \frac{[S]}{[S]} + \frac{K_m}{[S]} \right)$$

$$\frac{1}{v_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]}$$

# Reciprocal form is the equation of a line

$$(y = a x + b)$$

$$\frac{1}{v_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]}$$

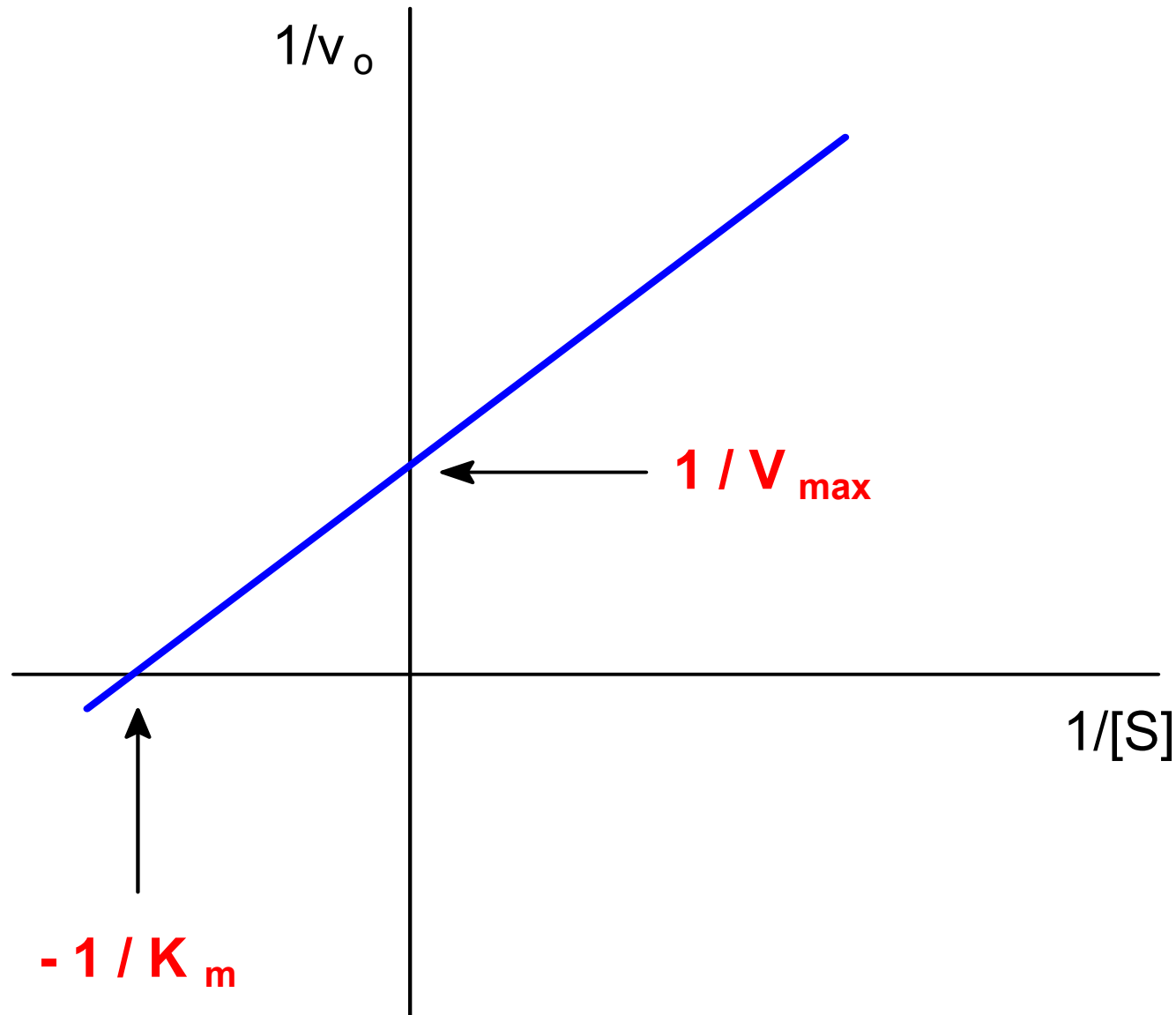
**1/v<sub>o</sub>** ..... dependent variable (y)

**1/[S]** ..... independent variable (x)

1/K<sub>m</sub> .....  
1/V<sub>max</sub> .....

easily determined  
from the graph

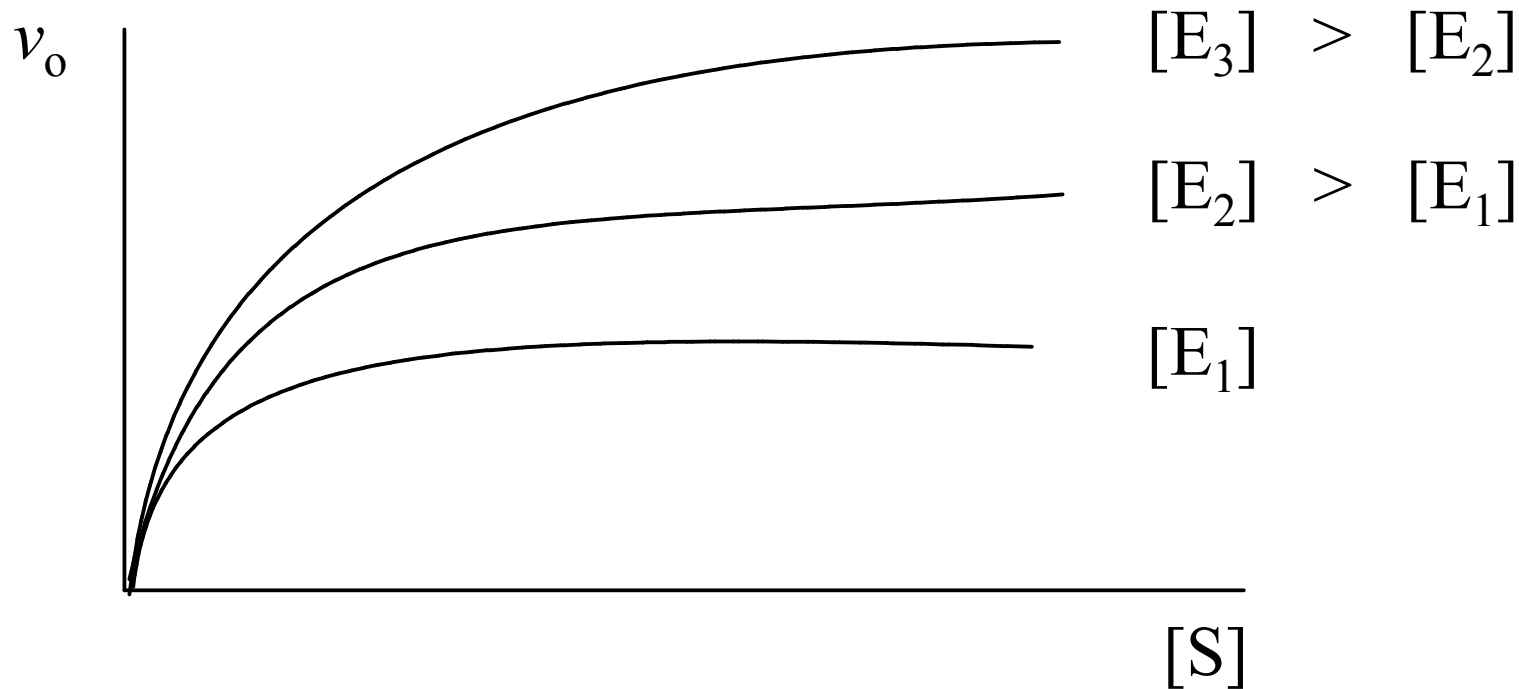
# Linear reciprocal plot: $1/v_0$ is the function of $1/[S]$



# Initial velocity depends on enzyme concentration [E]

saturated enzyme:  $v_o = k [E]_t$

$[E]_t$  is total concentration of enzyme



$K_M$  does not change,  $V_{\max}$  increases with increased [E]

# How to quantify enzymes in biological material?

- very low concentrations of enzymes
- in very complex mixtures
- in the presence of many other proteins
- simple chemical reactions cannot be used, they are not specific enough to distinguish individual enzymes

# Enzyme quantity in a biological sample can be determined by two ways

- **catalytic concentration**
- **$\mu\text{kat/l}$**
- the product of enzyme reaction is determined
- **mass concentration**
- **$\mu\text{g/l}$**
- enzyme itself is determined as antigen (immunochemical assay)



# Catalytic activity of enzyme

- unit **katal**, 1 kat = mol/s
- such amount of enzyme that catalyzes the conversion of one mole of substrate per one second

IU (international unit), 1 IU =  $\mu\text{mol}/\text{min}$   
1  $\mu\text{kat}$  = 60 IU, 1 IU = 16.6 nkat

# Catalytic concentration of enzyme

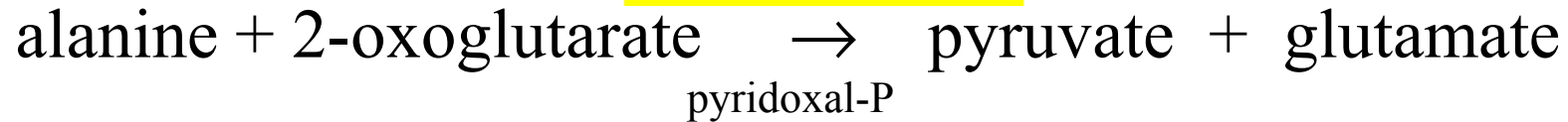
- activity related to the **volume of body fluid** (blood serum)
- typical units: mkat/l,  $\mu\text{kat}/\text{l}$

# Distinguish: unit and its dimension

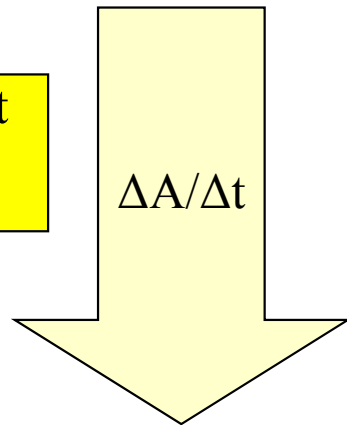
| Quantity                | Unit  | Dimension |
|-------------------------|-------|-----------|
| Catalytic activity      | kat   | mol/s     |
| Catalytic concentration | kat/l | mol/l.s   |

# Determination of catalytic activity of ALT: two coupled enzyme reactions

blood serum sample  
(ALT)



optical (UV) test  
Seminars, p.18



the decrease of absorbance at 340 nm is followed in time

# Determination of catalytic activity *in vitro*

- optimal conditions: temperature, pH  
the presence of all necessary cofactors  
the absence of all (known) inhibitors
- **excess of substrate**  $\Rightarrow$  0. order kinetics:  **$[S] \gg K_m$**   $\Rightarrow$   
saturated enzyme, reaction rate is constant and close to  $V_{\max}$
- $\Delta[S]$  or  $\Delta[P]$  is followed during time

# Two methods for catalytic concentration

| Feature              | Kinetic method                      | Constant-time method   |
|----------------------|-------------------------------------|--|
| What is measured     | [S] or [P]                          | [P]  |
| How                  | continually<br>(e.g. in 10 seconds) | after some time (e.g. 10 min)<br>the reaction is stopped<br>by enzyme inactivation |
| Kinetic curve needed | yes                                 | no   |
| What is determined   | initial velocity $v_0$              | average velocity   |
| Evaluation of method | exact                               | less exact   |

# Problem 1

Enzyme sample (0.1 ml) was added to substrate solution.  
After 5 min, 0.2 mmol of product was determined.

What is catalytic concentration of enzyme?

$$\frac{\text{amount of product (mmol)}}{\text{time (s)} \times \text{volume (l)}}$$

# Problem 1 - Solution

$$\frac{0,2 \text{ (mmol)}}{300 \text{ (s)} \times 0,0001 \text{ (l)}} = \mathbf{6,7 \text{ mkat/l}}$$

# Problem 2

Reaction mixture contains:

2.5 ml buffer

0.2 ml solution of NADH (optical UV test)

0.1 ml blood serum

0.2 ml substrate solution

After 60 s, the decrease of NADH absorbance is  $\Delta A = 0.03$

$\epsilon_{\text{NADH}} = 6220 \text{ l/mol.cm}$ , cuvette width  $l = 1 \text{ cm}$ .

What is catalytic concentration of enzyme?



## Problem 2 - Solution

Serum sample was diluted:  $V_{\text{final}}/V_{\text{initial}} = 3,0 / 0,1 = 30$

Lambert-Beer law:  $\Delta A = \varepsilon \Delta c l$

changes of absorbance and concentration expressed per time  $\Delta t \Rightarrow$

$$\Delta A/\Delta t = \varepsilon \Delta c l/\Delta t \quad \Delta t = 60 \text{ s}$$

$$\frac{\Delta c}{\Delta t} = \frac{\Delta A}{\varepsilon \times l \times \Delta t} = \frac{0,03}{6220 \times 1 \times 60} = 8 \cdot 10^{-8} \text{ mol/l.s}$$

Multiplied by dilution:  $30 \times 8 \times 10^{-8} = 2,4 \times 10^{-6} \text{ mol/l.s} =$

$$2,4 \times 10^{-6} \text{ kat/l} = \mathbf{2,4 \mu\text{kat/l}}$$

# Consider that

catalytic concentration = rate of chemical reaction

$$\text{kat/l} = \text{mol/l.s}$$

# Inhibitors reduce enzyme activity

## Irreversible inhibitors

Irreversible inhibitors are usually compounds not of biological origin, bind onto enzyme mostly **covalently** and make substrate binding impossible.

### **Heavy metal ions, organophosphates, cyanides**

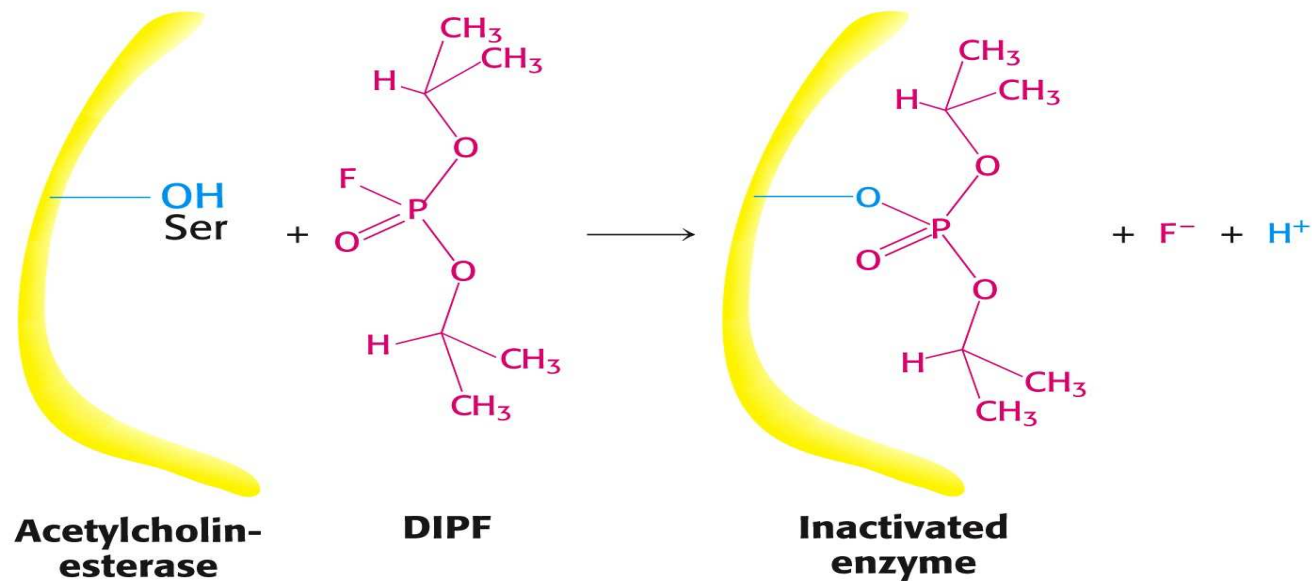
bind and inhibit irreversibly enzymes during isolation.

## Reversible inhibitors

In contrast with irreversible inhibitors, reversible inhibitors bind to the enzyme loosely and can rapidly dissociate from the enzyme-inhibitor complex.

These inhibitors are classified as **competitive, non-competitive**.

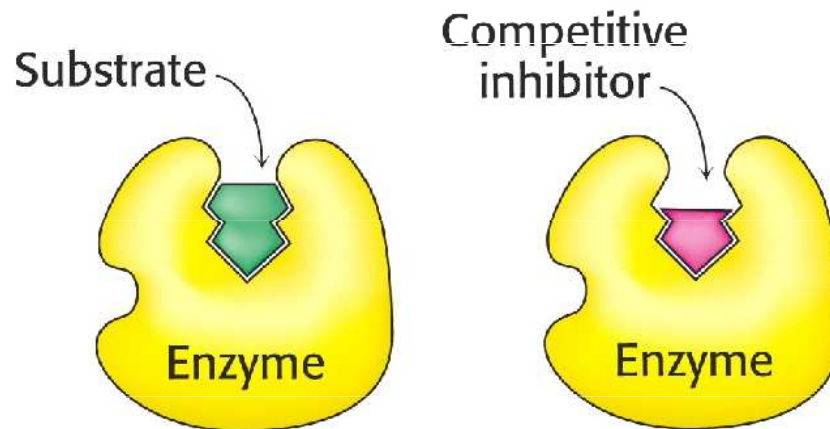
# Example of irreversible inhibition



Diisopropyl fluorophosphate (and similar pesticides and nerve gases) inhibits acetylcholine esterase by phosphorylation of a crucial serine residue.

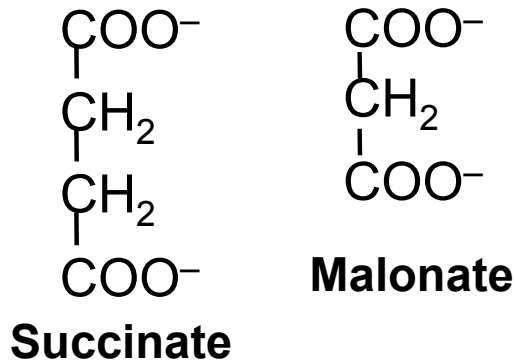
# Competitive inhibitors

- resemble the substrates (similar shape of molecule)
- bind to the active sites, but the complex is non-reactive
- they compete with normal substrates for the active sites

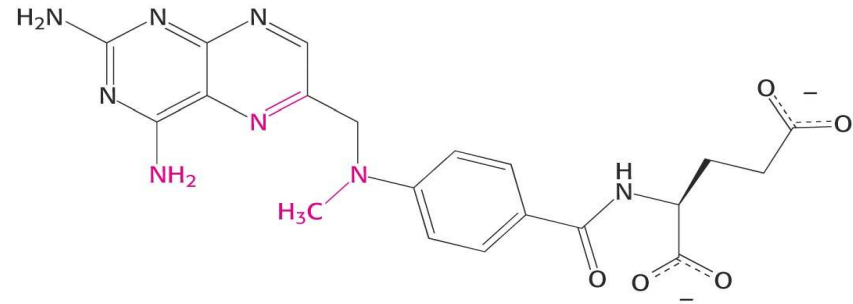


# Examples

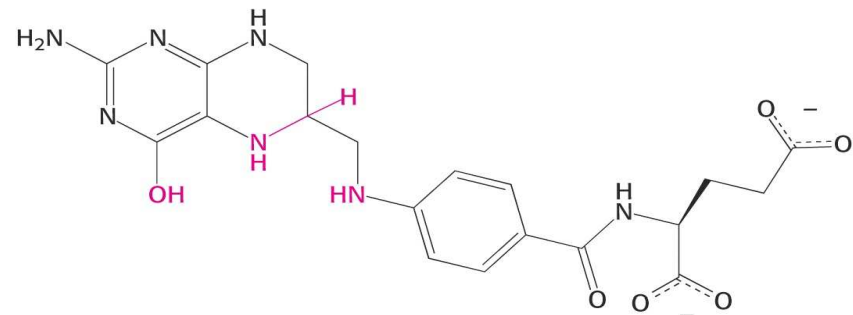
Malonate competitively inhibit succinate dehydrogenase



Methotrexate competitively inhibits active sites for tetrahydrofolate of the dihydrofolate reductase in the synthesis of purine and pyrimidine bases of nucleic acids. It is used to treat cancer.

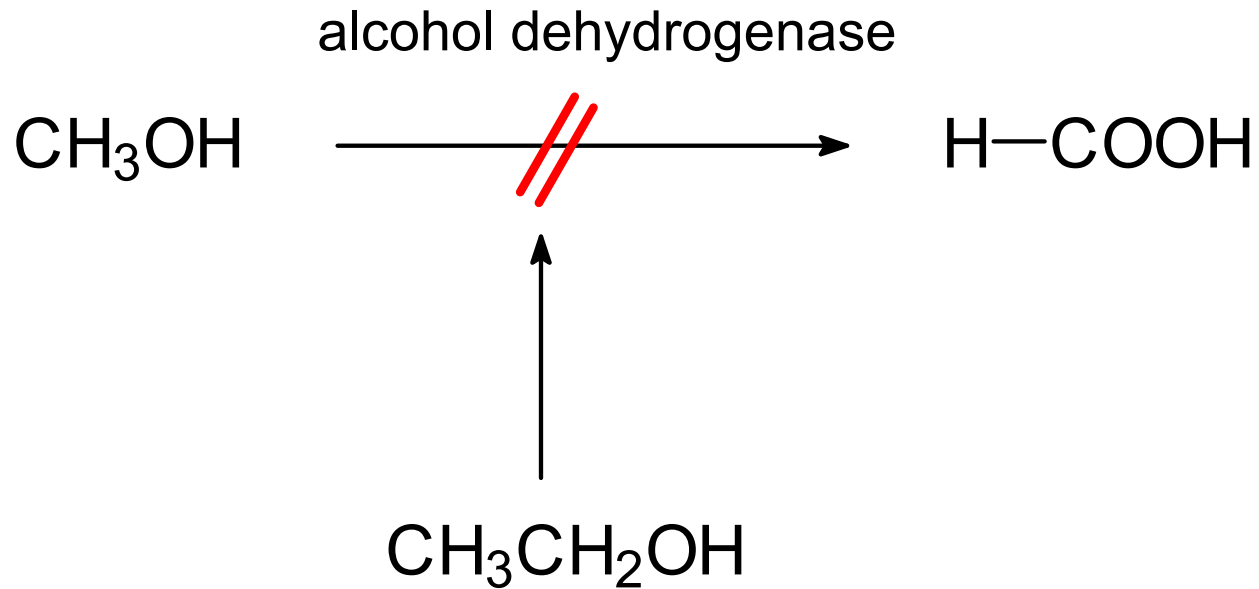


**Methotrexate**



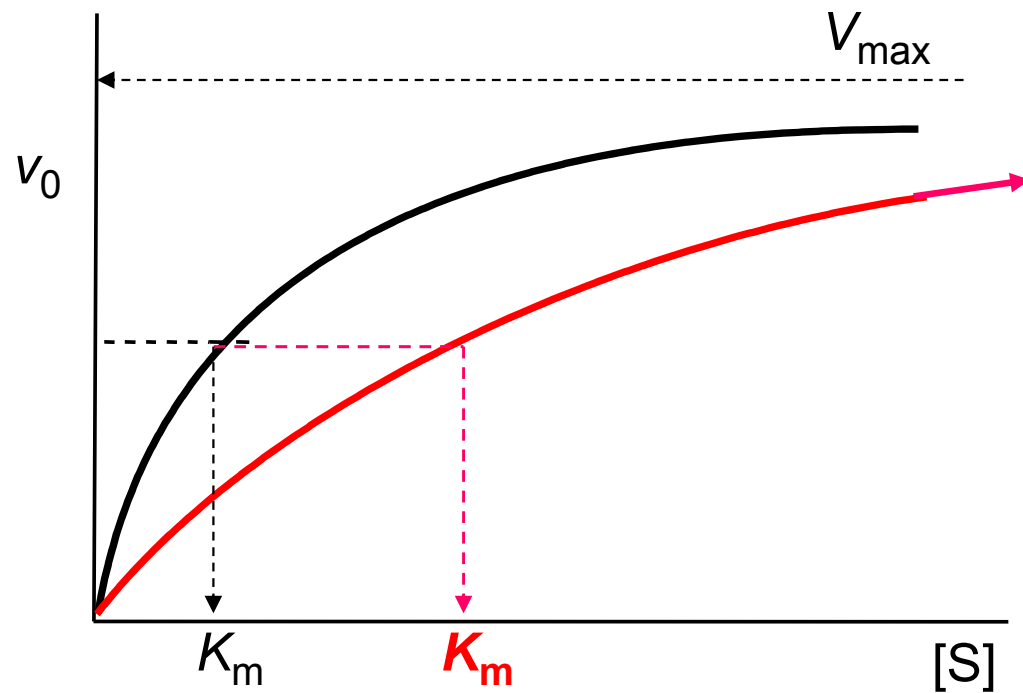
**Tetrahydrofolate**

# Methanol poisoning is treated by ethanol

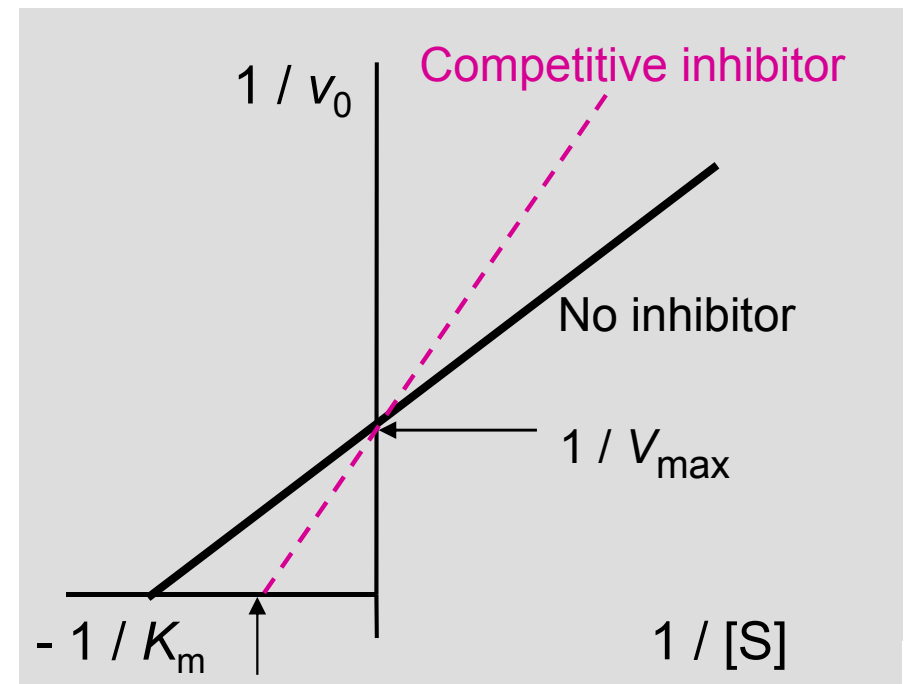


ethanol and methanol are similar molecules  
they compete for active site in enzyme

## Competitive inhibitors increase $K_m$ without any change in $V_{max}$



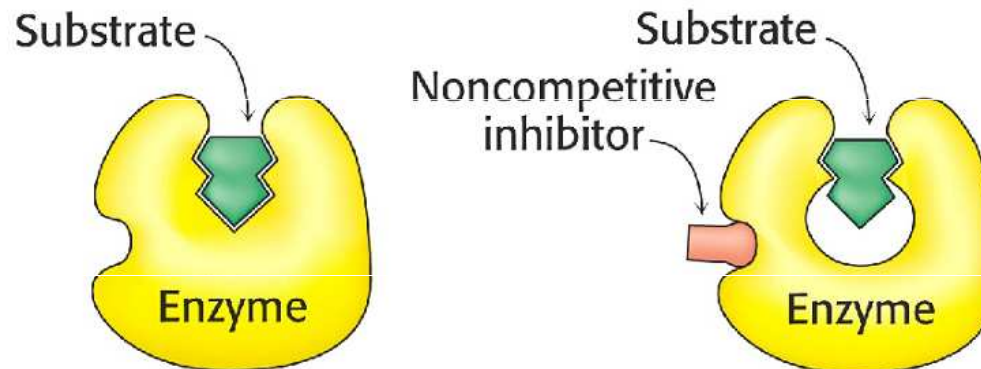
The  $V_{max}$  can be reached even in the presence of inhibitor, but at much higher concentrations of  $[S]$  that have to overcome the competing inhibitor concentration.





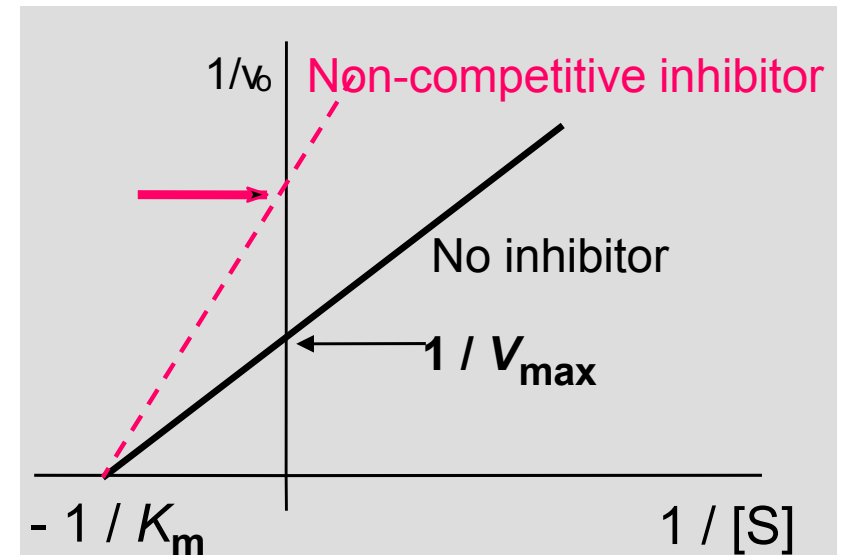
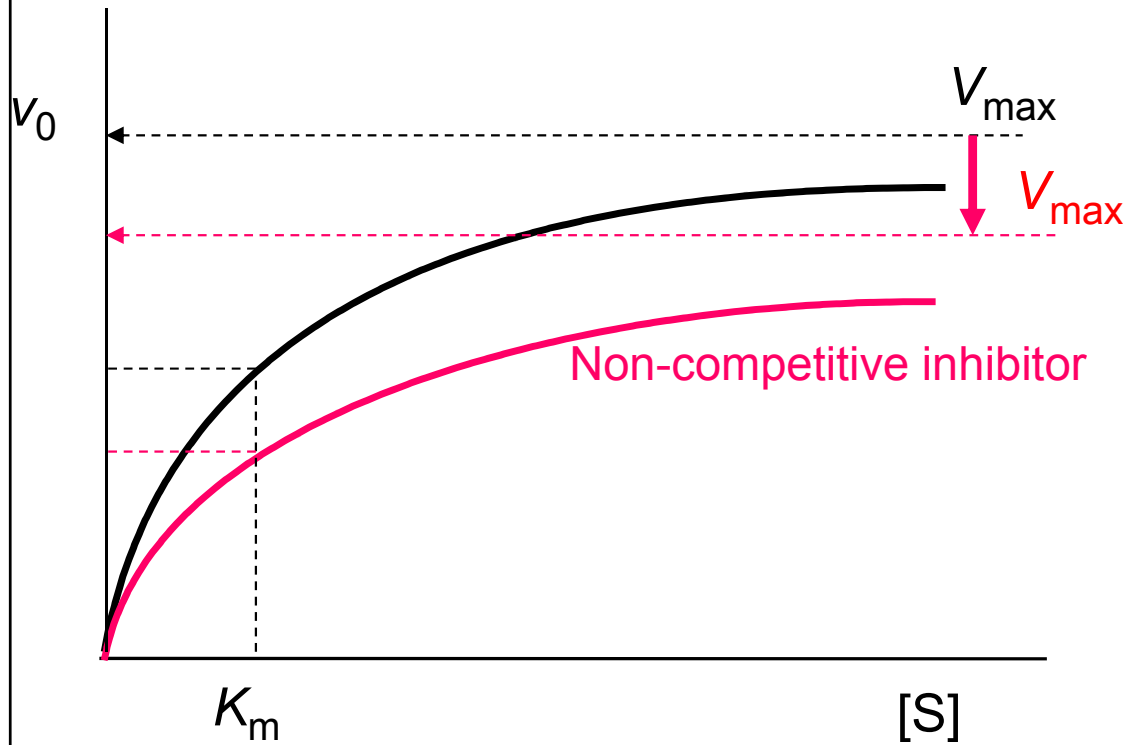
# Non-competitive inhibition

Non-competitive inhibitors bind to both free enzyme and enzyme-substrate complex, but in contrast to competitive inhibitors, **not in the active site** (the structure of inhibitor is distinct from that of substrate).



Non-competitive inhibition cannot be overcome by increasing the substrate concentration. The non-inhibited remaining molecules of the enzyme behave like a **more diluted solution of the enzyme.**

# Non-competitive inhibitors decrease $V_{\max}$ without any change in $K_m$



# Many drugs are inhibitors of enzymes

- Acetylsalicylic acid, ibuprofen (cyclooxygenase) - see Seminars, p. 67
- Statins (HMG-CoA reductase) inhibit cholesterol synthesis (e.g. lovastatin)
- ACE Inhibitors (angiotensin-converting enzyme) – treatment of hypertension
- Reversible acetylcholinesterase inhibitors (e.g. neostigmine) – myasthenia gravis, post-surgical atonia,
- Brain acetylcholinesterase inhibitors (rivastigmine, galantamin) – Alzheimer d.
- Many antibiotics inhibit bacterial enzymes:

Penicillins – inhibit bacterial transpeptidases (formation of bacterial cell-wall)

Tetracyclins, macrolides, chloramphenicol – inhibit bacterial proteosynthesis

Fluoroquinolones (ciprofloxacin) – inhibit bacterial topoisomerase

# Regulation principles in enzyme reactions

(three general aspects)

1. Regulation of enzyme quantity
2. Regulation of enzyme activity
3. Availability and concentration of substrate and/or cofactor  
(*in vivo* less important, *in vitro* critical condition)

# Regulation of enzyme quantity

- **Controlled enzyme proteosynthesis**  
constitutive and induced gene expression,  
regulation of transcription rate,  
posttranscriptional RNA processing,  
regulation of translation rate and posttranslational  
modifications
- **Controlled enzyme degradation**  
specific intracellular proteinases determine  
biological half-life of enzymes

# Regulation of enzyme activity

- Activation by partial and **irreversible** proteolysis
- **Reversible** covalent modification
- Allosteric regulation

# Enzyme activation by partial proteolysis

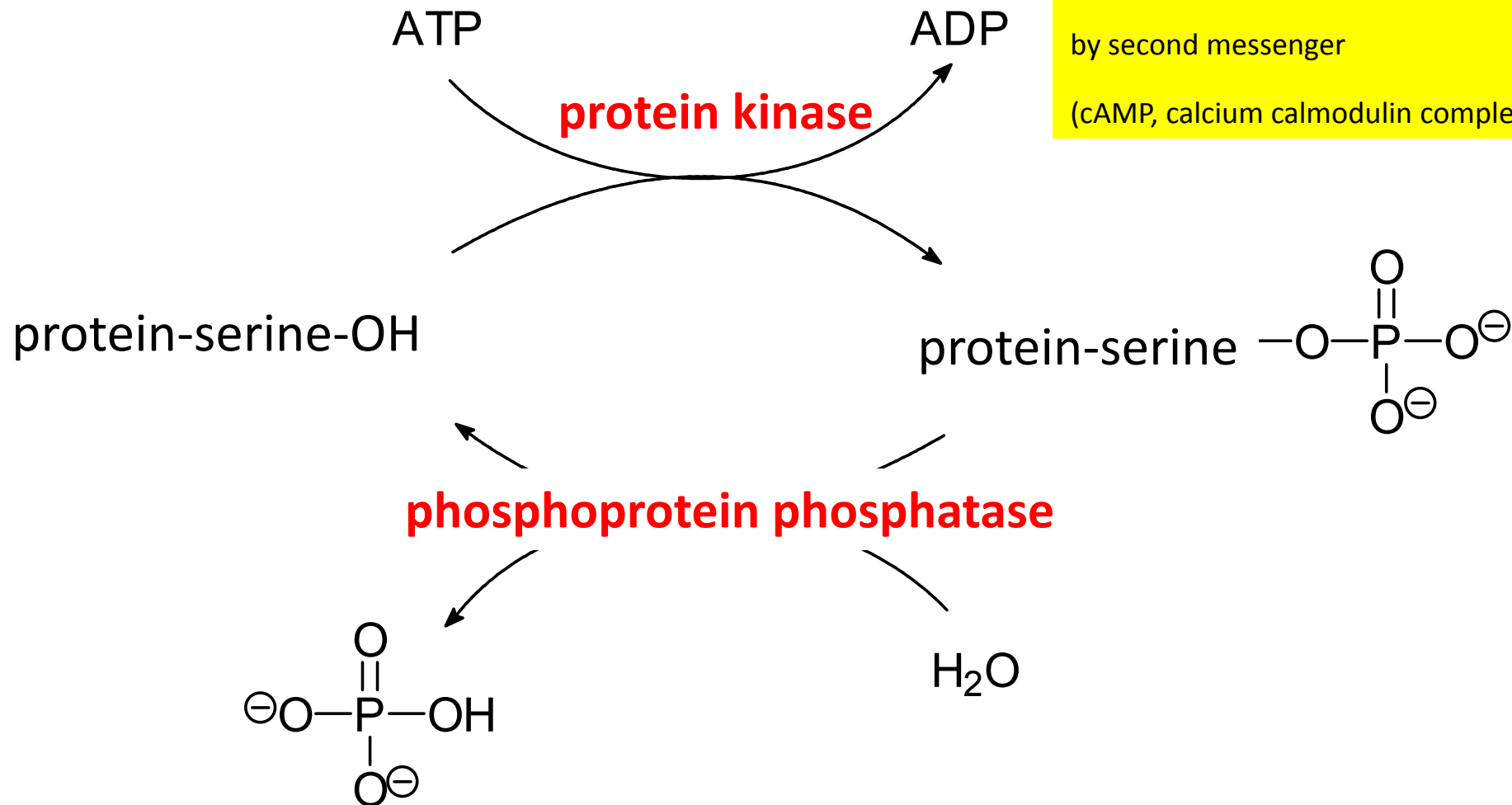
- Active enzyme is formed by **irreversible** cleavage of certain sequence from **proenzyme** (zymogen) molecule
- Proteinases in digestion (pepsinogen → pepsin)
- Factors of blood coagulation
- Proteinases caspases in apoptosis

# Reversible covalent enzyme modification

- **phosphorylation**, catalyzed by kinases
- transfer of phosphoryl  $-\text{PO}_3^{2-}$  from ATP to  $-\text{OH}$  group of enzyme  
(Ser, Thr, Tyr)
- reversible process, dephosphorylation catalyzed by **phosphatase**, hydrolysis of phosphoester
- Other modifications: carboxylation, acetylation, prenylation ...



# Phosphorylation and dephosphorylation of enzyme alters its activity



protein kinases are activated  
by second messenger  
(cAMP, calcium calmodulin complex)

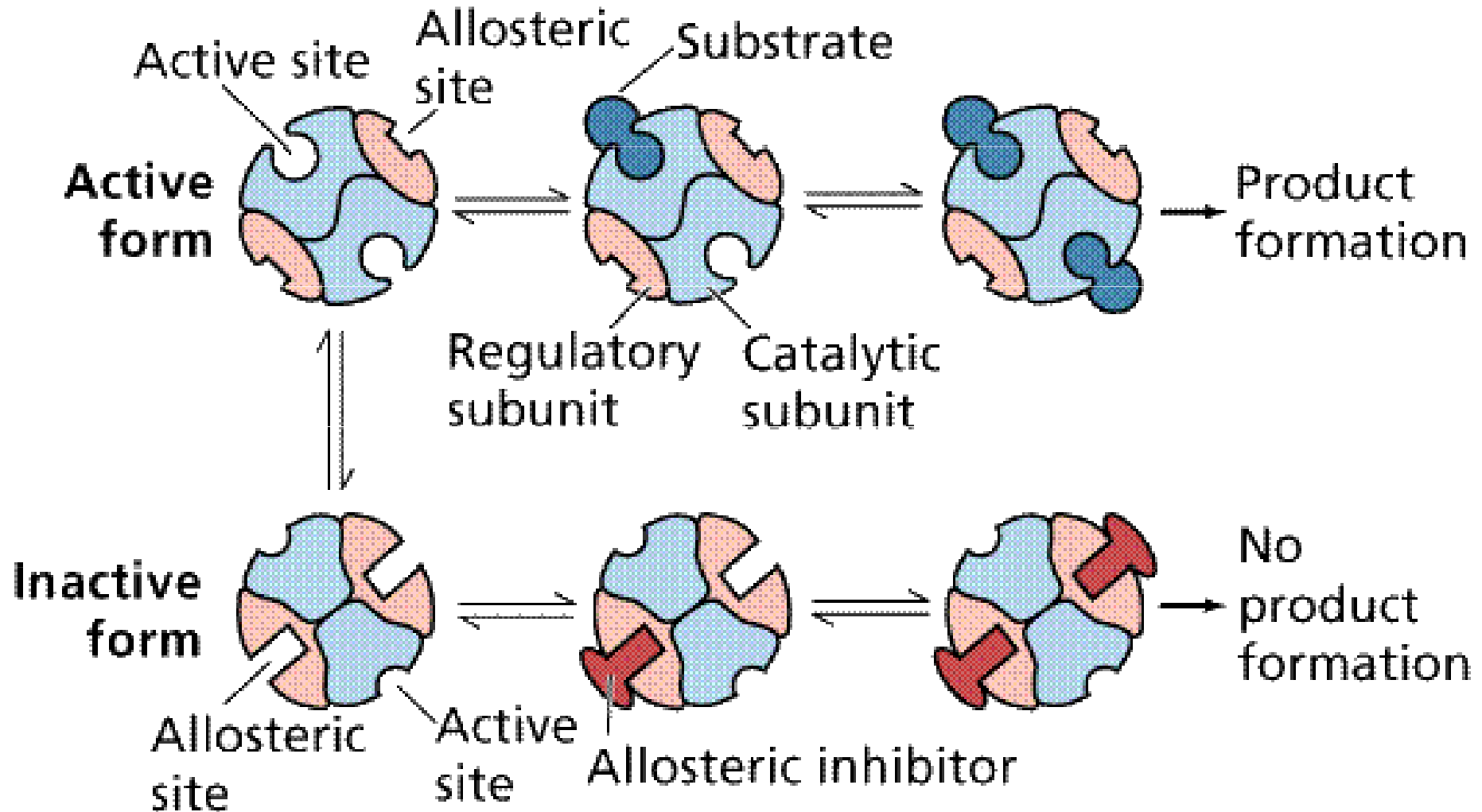
# The consequences of phosphorylation/dephosphorylation in two antagonistic enzymes

| Feature                         | Glycogen phosphorylase                                  | Glycogen synthase                               |
|---------------------------------|---|---|
| Enzyme catalyzes                | glycogen degradation by energy-poor phosphate ( $P_i$ ) | glycogen synthesis from energy-rich UDP-glucose |
| By phosphorylation, enzyme is   | <b>activated</b>  | <b>inhibited</b>                                |
| By dephosphorylation, enzyme is | <b>inhibited</b>  | <b>activated</b>                                |

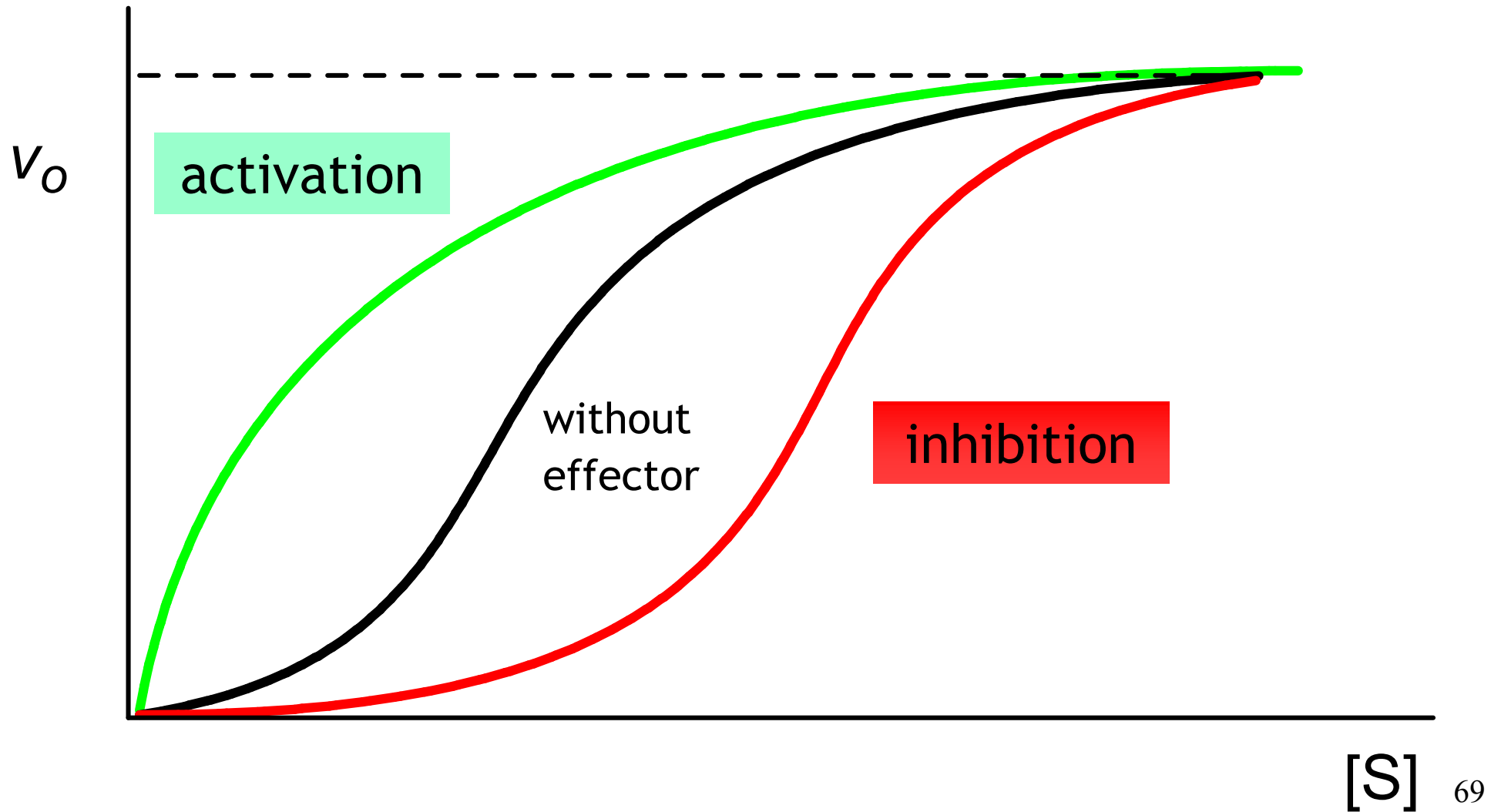
# Allosteric enzymes are oligomeric

- more subunits, often regulatory and catalytic
- **effector**, structurally different from substrate - often product, binds to enzyme to **allosteric site** (other than active site)
- binding effector triggers the changes in conformation and activity → allosteric activation / inhibition

# Allosteric enzymes are oligomeric



# Saturation curve of allosteric enzymes is sigmoidal

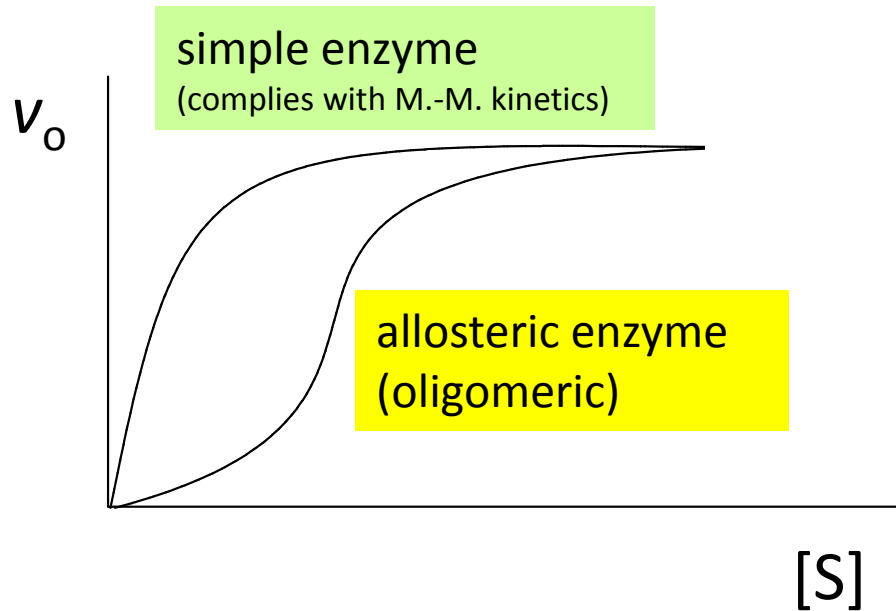


# Cooperative effect

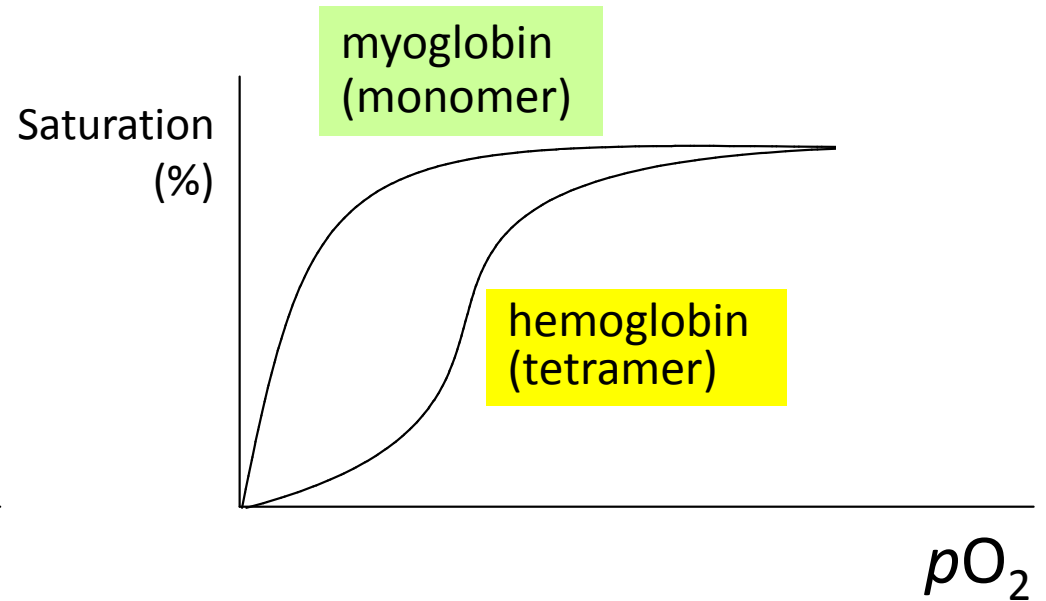
- in oligomeric enzymes and non-catalyzing proteins (e.g. Hb)
- more subunits = more active sites
- binding substrate (or  $O_2$  to Hb) to one subunit/active site induces conformation changes in other subunits/active sites so that other substrate (or  $O_2$ ) molecules bind more easily (or more hardly)
- example: hemoglobin (tetramer)  $\times$  myoglobin (monomer)

# Compare

Saturation of enzyme by substrate



Saturation of hemoglobin by oxygen



# Three utilizations of enzymes in medicine

1. enzymes as **indicators** of pathological condition
2. enzymes as **analytic reagents** in clinical chemistry
3. enzymes as **drugs**



# Examples of enzymes in clinical diagnostics

In cell damages, activity of intracellular enzymes in extracellular fluid (blood serum) is elevated

| Enzyme | Reference values            | Elevation in serum indicates     |
|--------|-----------------------------|----------------------------------|
| ALT    | up to 0,9 $\mu\text{kat/l}$ | hepatopaties                     |
| CK     | up to 4 $\mu\text{kat/l}$   | myopaties, myocardial infarction |
| PSA    | up to 4 $\mu\text{g/l}$     | prostate cancer                  |

ALT alanine aminotransferase, CK creatine kinase,

PSA prostate specific antigen

# Isoenzymes/Isoforms

- catalyze the same reaction
- differ in primary structure, physical, and kinetic properties
- often have different tissue distribution
- determined mostly by electrophoresis and/or by immunochemical assays

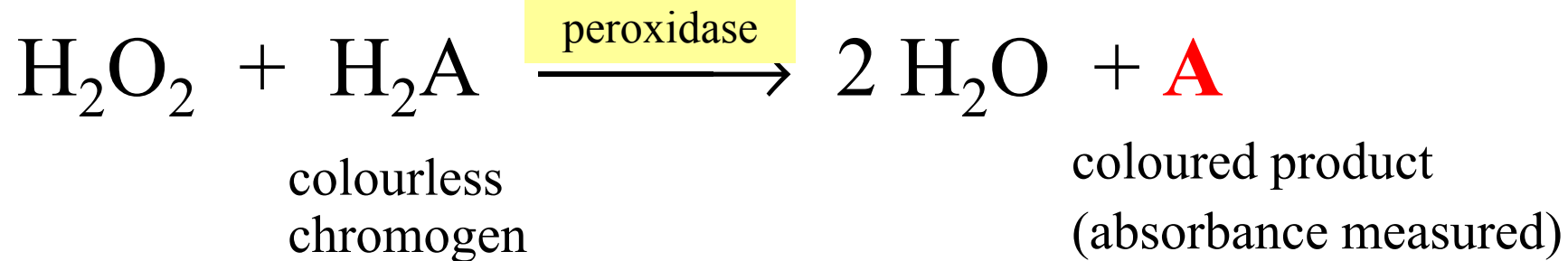
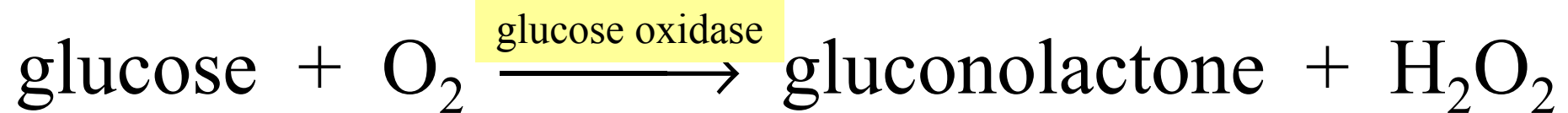
**Creatine kinase (CK) is a dimer and makes three isoenzymes**

| <b>Isoenzyme</b> | <b>Organ</b> | <b>% of total activity in blood plasma</b> | <b>Elevated value</b> |
|------------------|--------------|--|-----------------------|
| CK-MM            | muscles      | 94-96 %                                    | muscle trauma         |
| CK-MB            | heart        | up to 6 %                                  | infarction            |
| CK-BB            | brain        | traces                                     | brain damage          |

# Enzymes as analytic reagents

| Enzyme                | Enzyme origin                        | Assay for        |
|-----------------------|--------------------------------------|------------------|
| Glucose oxidase       | <i>Aspergillus niger</i>             | glucose          |
| Peroxidase            | horse radish ( <i>Armoracia</i> sp.) | glucose          |
| Lipase                | <i>Candida</i> sp.                   | triacylglycerols |
| Cholesterol oxidase   | <i>Pseudomonas</i> sp.               | cholesterol      |
| Uricase               | <i>Candida</i> sp.                   | uric acid        |
| Bilirubin oxidase     | <i>Myrothecium</i> sp.               | bilirubin        |
| Urease                | <i>Canavalia</i> sp.                 | urea             |
| Lactate dehydrogenase | <i>Pediococcus</i> sp.               | ALT, AST         |
| <i>Taq</i> polymerase | <i>Thermus aquaticus</i>             | PCR method       |

# Enzymatic determination of glucose



The principle of glucose determination in biochemical analyzers and in personal glucometers

# Personal glucometer

(Practice in 4. semester)



# Pancreatic enzymes in therapy

- enzyme mixtures (lipase, amylase, proteinases) of animal origin
- indication: insufficient secretion of pancreas, cystic fibrosis
- 3 × times daily a capsule during meal

gastro-resistant capsules, they survive the passage through stomach, soluble and active in duodenum

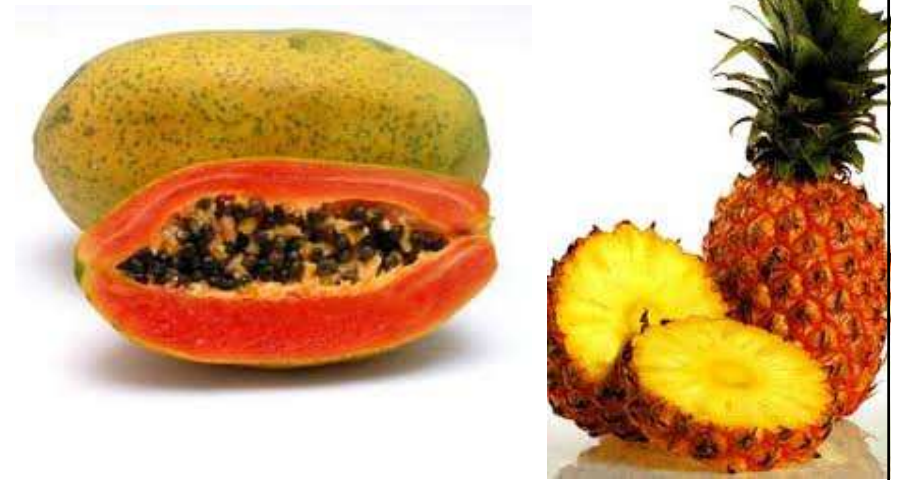
# Asparaginase in leukemia treatment

- Catalyzes the hydrolysis of asparagine amide group (deamidation)
- $\text{Asn} + \text{H}_2\text{O} \rightarrow \text{Asp} + \text{NH}_3$
- L-asparagine is necessary for the proteosynthesis of some cancer cells
- Hydrolysis of Asp reduces the cell proliferation (see also Seminars, p. 19)

## Enzyme fibrinolytics

- thrombolytic drugs, dissolve blood clots in veins
- **urokinase** (human enzyme, serine protease)
- converts plasminogen to plasmin  $\rightarrow$  which degrades fibrin  $\rightarrow$  thrombolysis
- venous thrombosis, pulmonary embolism, acute myocardial infarction

# Proteases in enzyme therapy



## Local treatment

- fibrinolysin, chymotrypsin, collagenase and other
- degrade necrotic tissue, clean wounds, decubital ulcers etc.

## Systemic treatment

- trypsin, chymotrypsin, papain (papaya), bromelain (pineapple)
- anti-inflammatory agents
- sports injury, trauma, arthritis, other kinds of swelling, arthritis etc.
- Wobenzym, Phlogenzym and other