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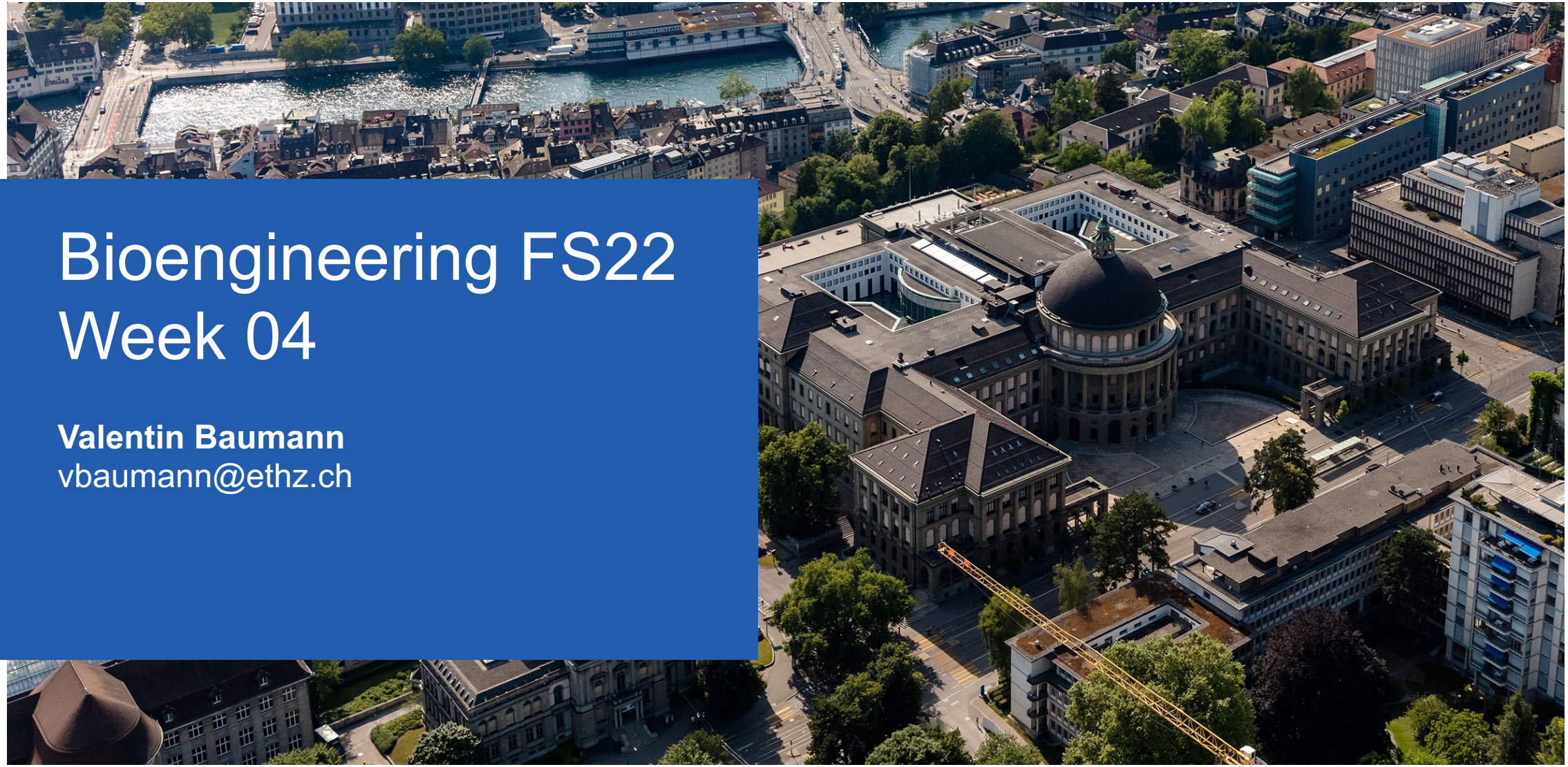

Bioengineering FS22
Week 04

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Agenda

1. **Cell metabolism II**
    1. Catalysis – Inlfuencing factors on enzyme reaction kinetics
    2. Enzyme specificity
    3. Enzyme nomenclature & classification
    4. Importance of enzymes
    5. Industrial applications of enzymes
2. **Exercise 3**

Catalysis– Influencing factors of enzyme reaction kinetics

- 1. Substrate concentration
- 2. Temperature
- 3. pH-value
- 4. Inhibition

Rate of reaction ”r”:

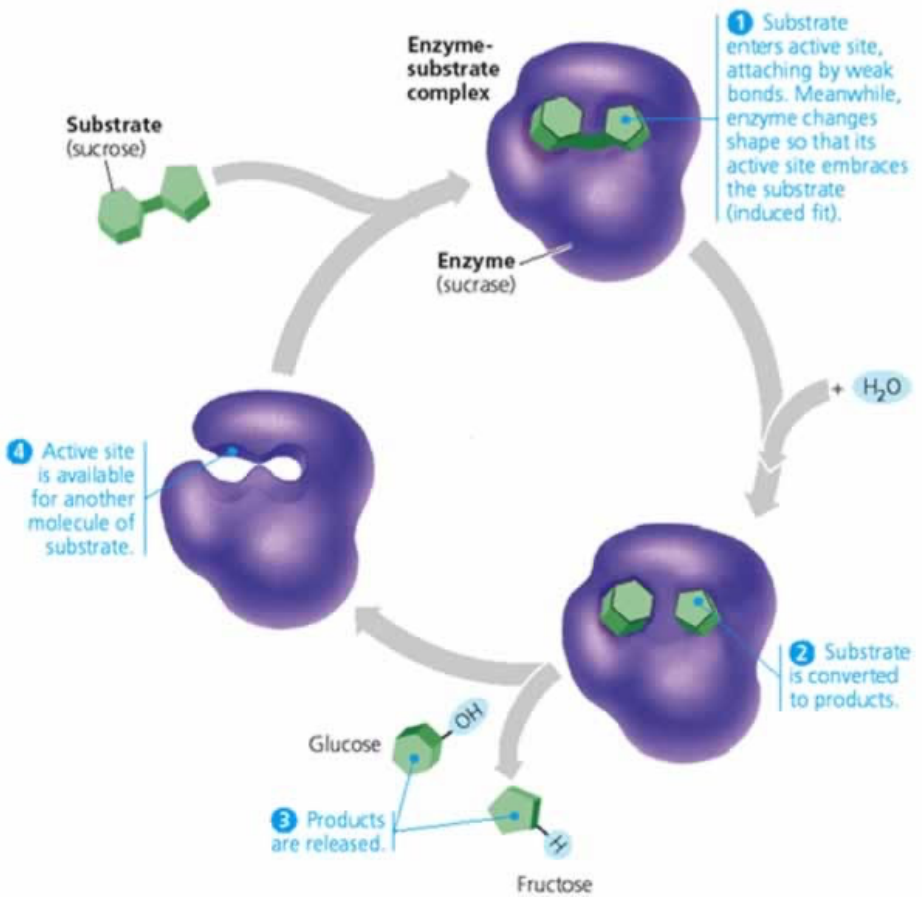
$$r = -\frac{dc_S}{dt} = \frac{dc_P}{dt}$$

c_S Substratkonzentration

c_P Produktkonzentration

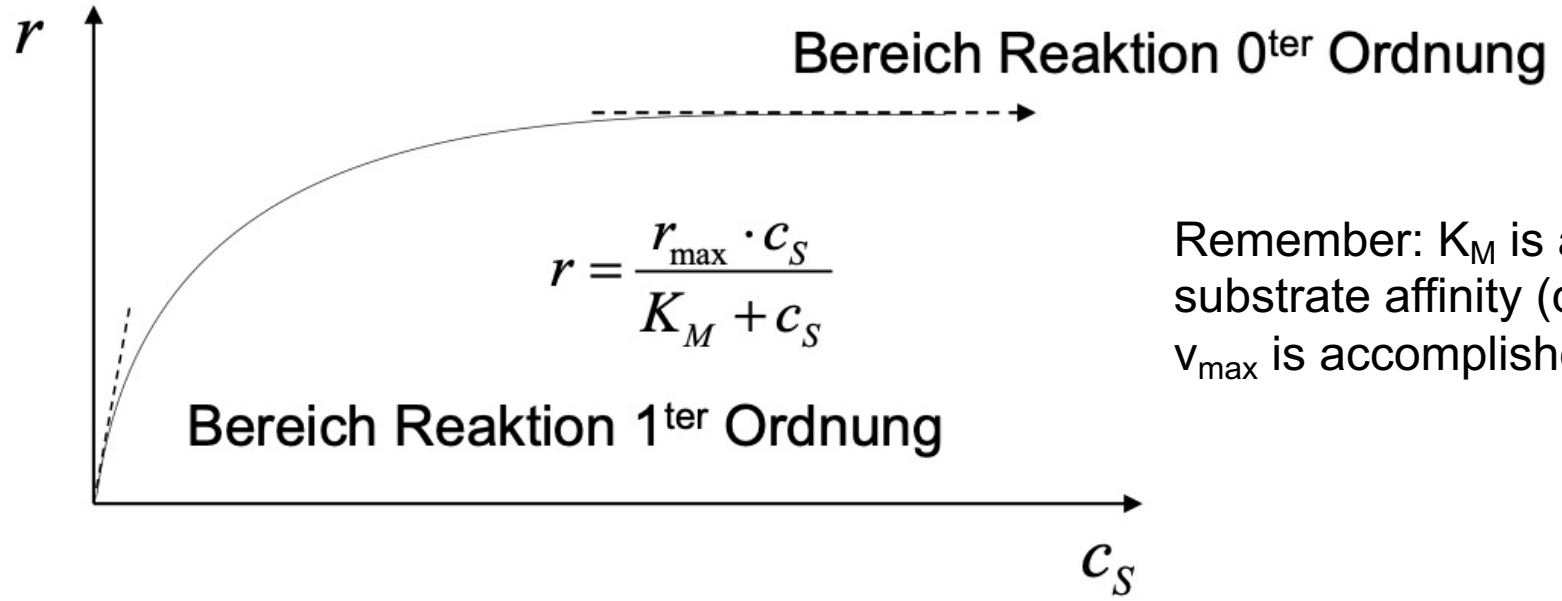
$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_S + [S]} = \frac{r_{max}[S]}{K_M + [S]}$$

Michaelis-Menten-Kinetik



Catalysis– Influencing factors of enzyme reaction kinetics

- **1. Substrate concentration**



Remember: K_M is a measure for substrate affinity (c_s where half of v_{max} is accomplished)

Special Cases:

$c_S >> K_M : \quad r = r_{max}$

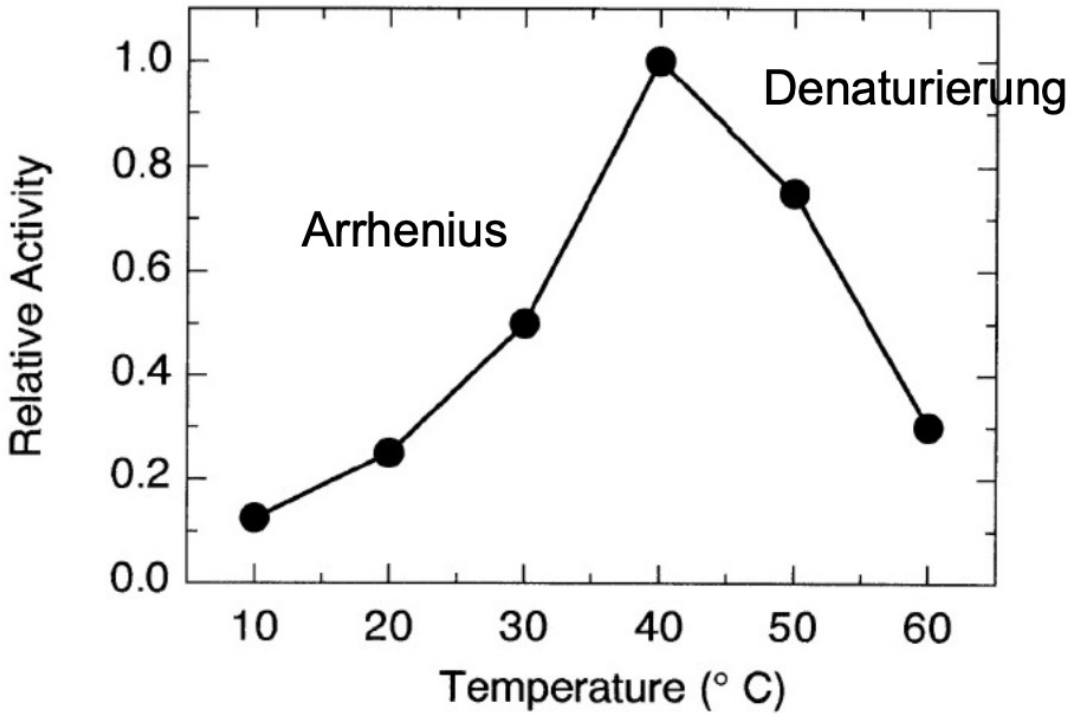
If c_s big → reaction rate independent of substate concentration (enzyme always satisfied

$c_S << K_M : \quad r = \frac{r_{max} \cdot c_S}{K_M}$

If c_s small → reaction rate increases linearly with the substrate concentration

Catalysis– Influencing factors of enzyme reaction kinetics

- **2. Temperature**



Optimal enzyme-reaction-temperature is organism dependent (for humans ca. 37°C → body temperature)

- <u>Below the optimal enzyme-reaction-temperature</u>
  Reaction rate increases with increasing temperature according to Arrhenius:

$$r_{\max} = k_{+2}(T) \cdot c_{E0} = (k_{+2})_0 \cdot e^{-E_a/RT} \cdot c_{E0}$$

- <u>Beyond the optimal enzyme-reaction-temperature</u>
  Enzyme denatures with increasing temperature:

- Secondary and tertiary structures of the protein are destroyed -> **protein loses biological function**
- Protein goes from its soluble state into an insoluble state:

$$E_a \longrightarrow E_d$$

$$\frac{d[E_a]}{dt} = -k_d[E_a]$$

K_d – reaction constant itself again temperature dependent (Arrhenius)

Catalysis– Influencing factors of enzyme reaction kinetics

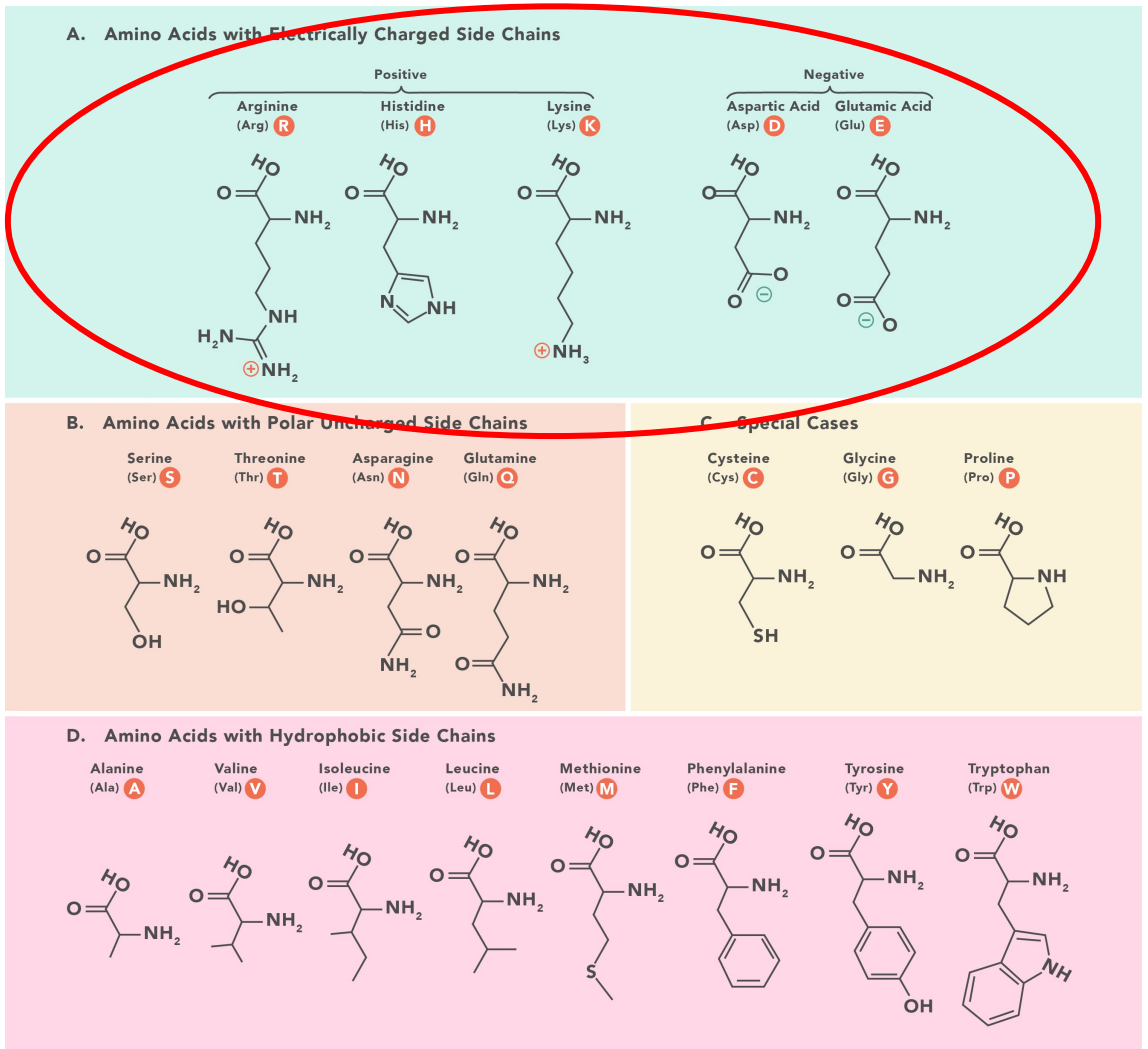
- **3. pH-value**

Why does pH have an effect on proteins?

$pH = - \log (c_{H3O+})$

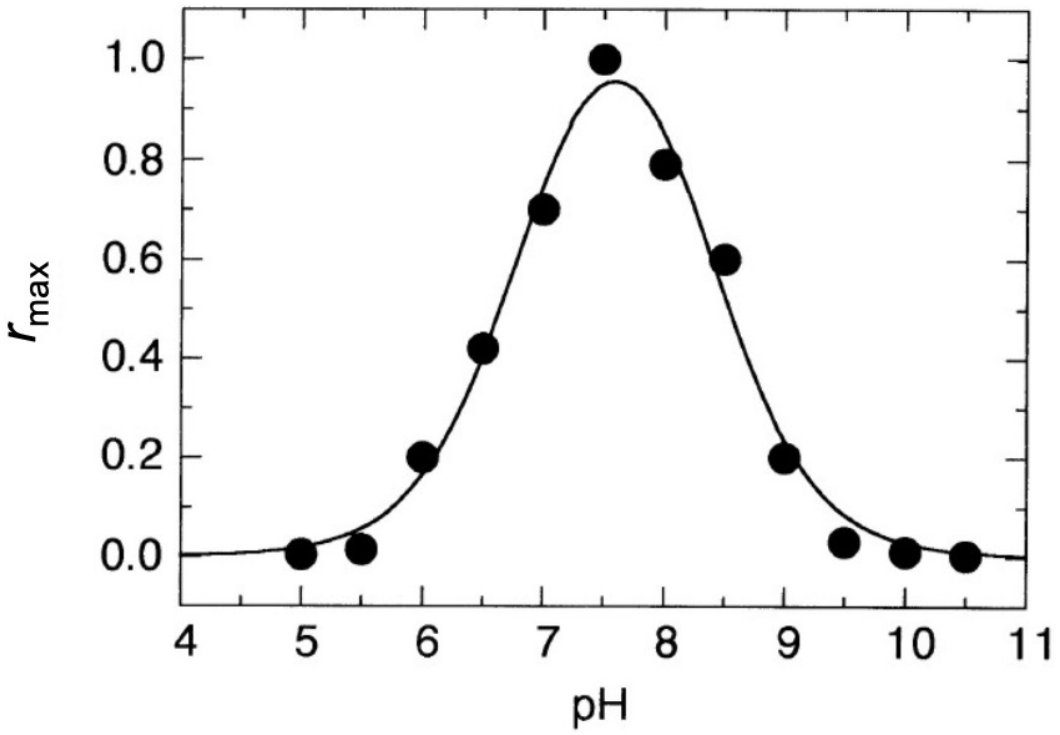
Proteins (Enzymes) are 3D folded chains of amino acids

→ There are acidic and basic amino acids that can, depending on the pH, be protonated or deprotonated
→ changes properties of amino acids and thus 3D folding of the protein and protein's function



Catalysis– Influencing factors of enzyme reaction kinetics

- **3. pH-value**



- pH-Optimum can be very different depending on organ or cell compartment.
  - Pepsin pH 2 (stomach)
  - Trypsin pH 8 (small intestine)

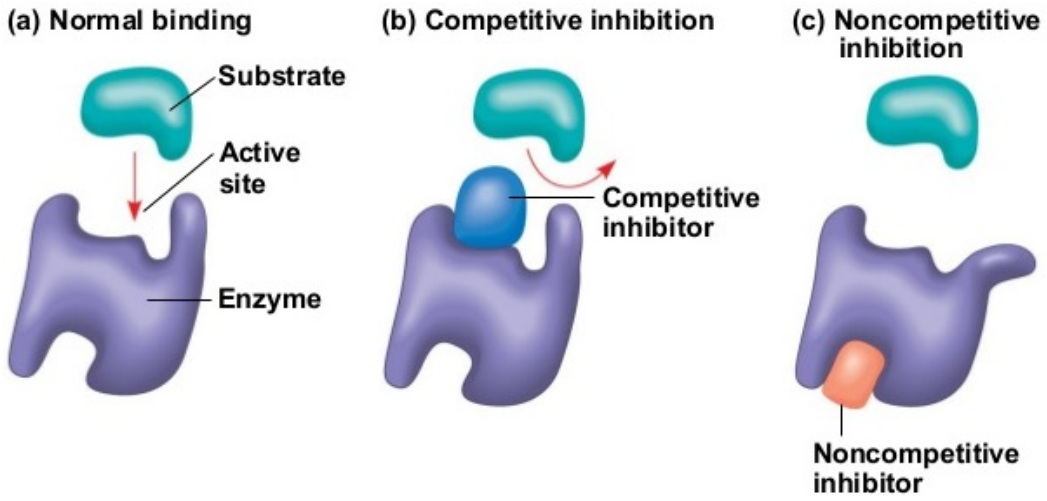
Impact of pH on enzyme reaction kinetics:

- Changes electrical charge of the enzyme
- Changes in pH can lead to changes in substrate structure → substrate affinity for the enzyme might be increased or decreased
- Big pH deviations from its optimum can lead to irreversible denaturation → 3D fold of enzyme irreversibly damaged

Catalysis– Influencing factors of enzyme reaction kinetics

- **4. Inhibition**

  - Enzymes can (despite high specificity) bind other substances than its substrates
  - Enzyme can either be inhibited, or its reaction slowed down or blocked
  - Inhibitors can bind the enzyme itself or the substrate-enzyme-complex



Competitive inhibition: Inhibitor structurally similar to substrate occupies the active site

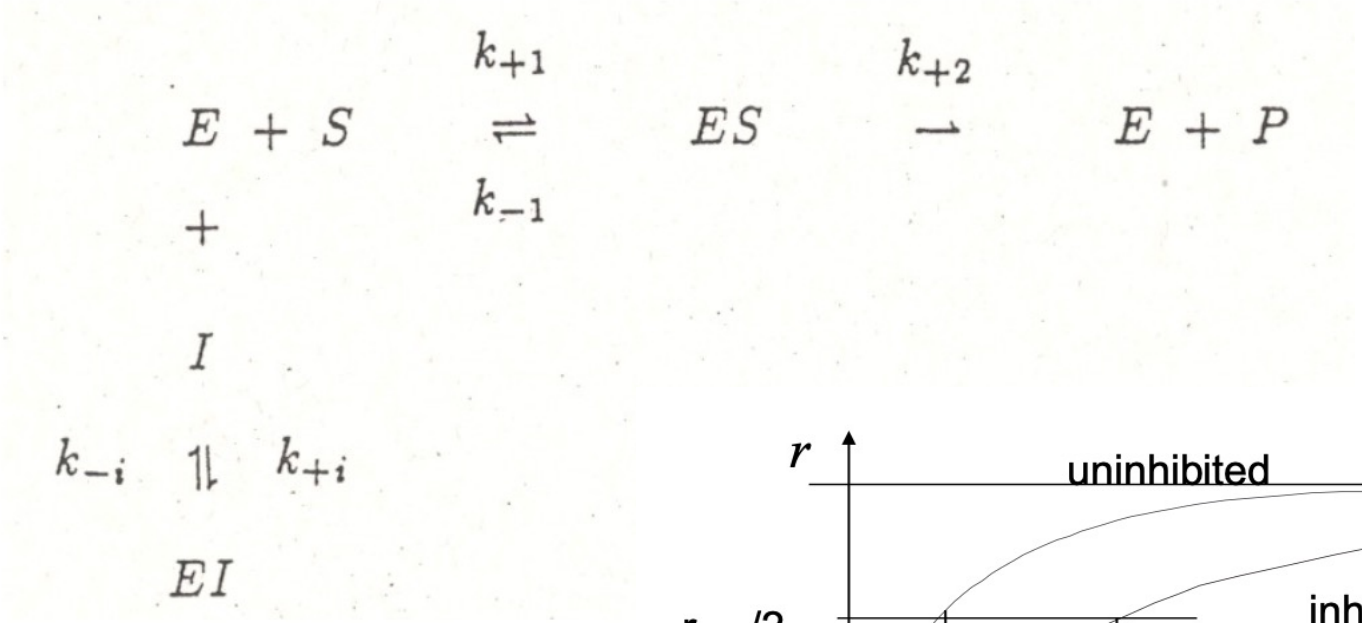
Noncompetitive inhibition: Inhibitor binds allosterically (at some other site of the enzyme) and thus leads to allosteric inhibition (ex. By changing enzyme structure to block active site)

Excess of substrate: ES → ES_2

Catalysis– Influencing factors of enzyme reaction kinetics

- **4. Inhibition**

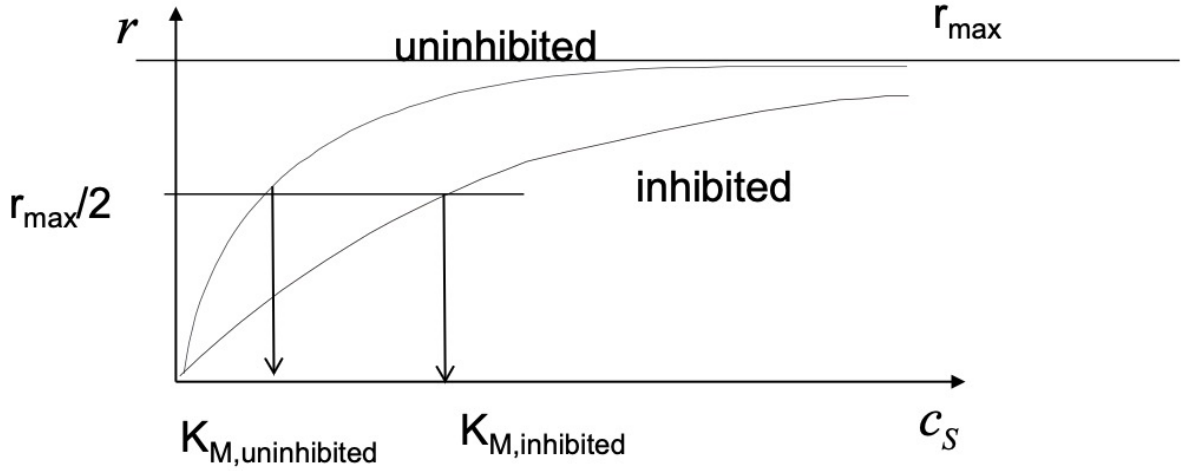
Competitive Inhibition:



r_{max} at substrate saturation does not change

Affinity of between substrate and enzyme is decreased → Higher substrate concentrations needed to reach r_{max}

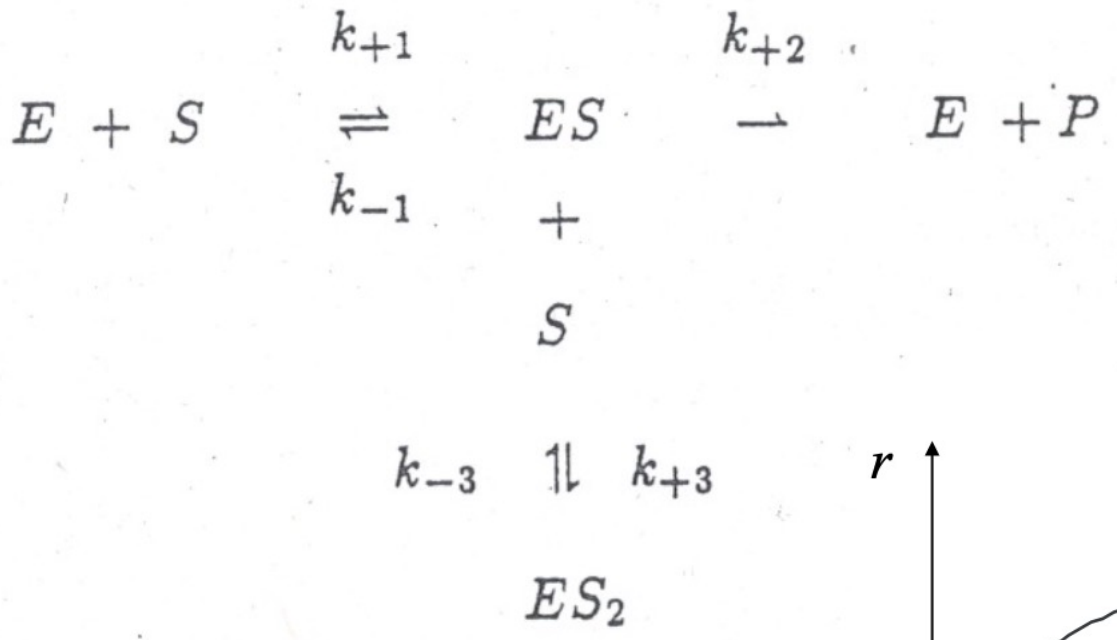
Michaelis-constant K_M is increased



Catalysis– Influencing factors of enzyme reaction kinetics

- **4. Inhibition**

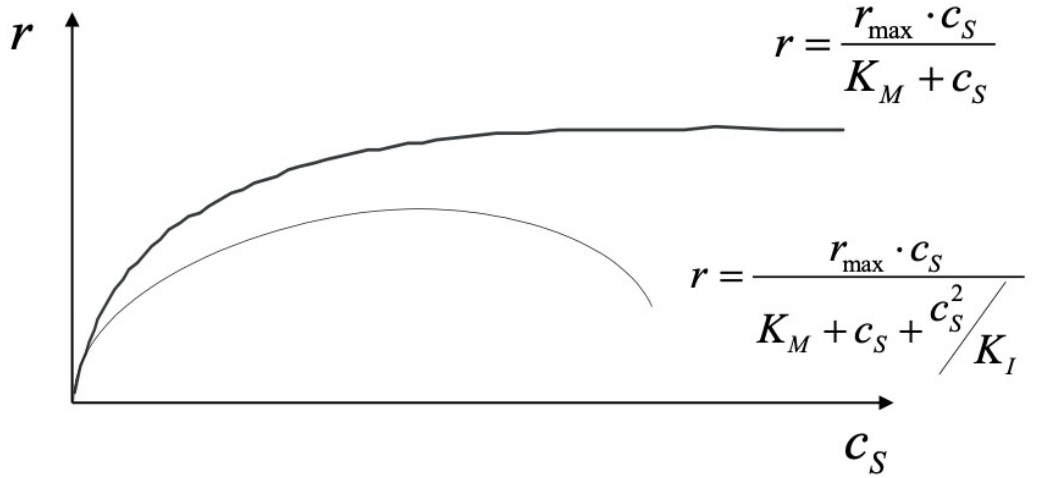
Excess of substrate:



If substrate concentration very high:

An enzyme-substrate-substrate-complex (ES_2 can be produced) → blocks the active site

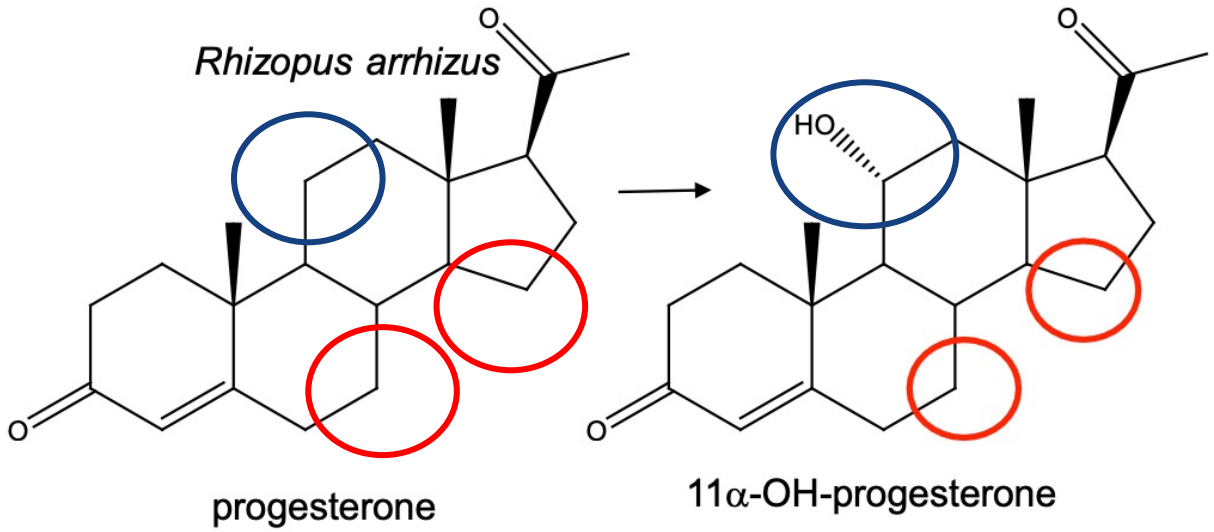
The bigger the excess of substrate the stronger the effect of inhibition



Enzymes specificity

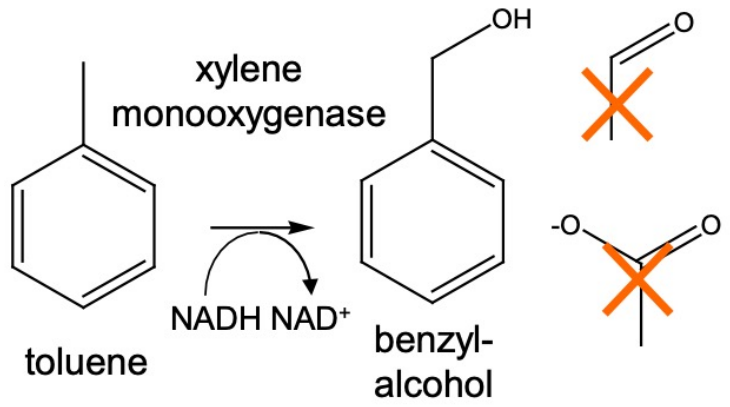
Substrate specificity – Normally enzymes only bind one specific substrate BUT extent of substrate specificity differs between enzymes.

Regiospecificity – Enzymes can distinguish between chemically similar groups within the same molecules/substrate and only manipulate one specific group (only blue circled, not red circled groups)



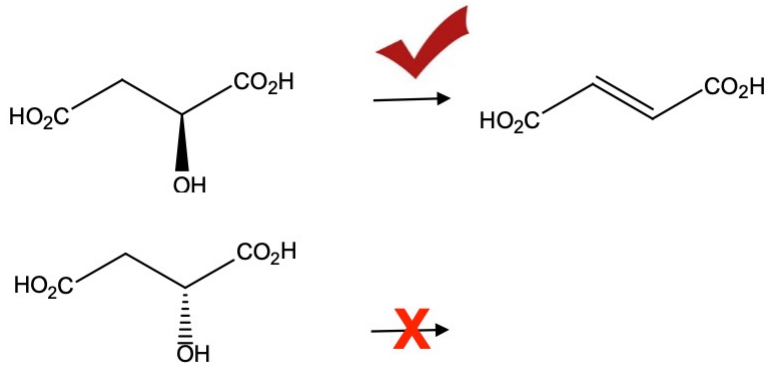
Enzymes specificity

Reaction specificity– The enzyme only catalyses one specific reaction, the substrate can potentially undergo.

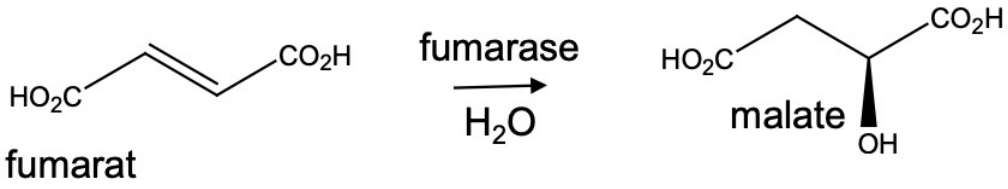


Enantiomer specificity & selectivity

Enantiomer specificity – Enzyme only reacts with one substrate enantiomer (form of substrate specificity)



Enantiomer selectivity – Enzyme only produces one of two possible enantiomers from a certain substrate



Enzyme nomenclature & classification

- Name of the substrate + ending “-ase”
  - Saccharose is cleaved by the enzyme Saccharase

- Enzymes with similar effects/catalysing reactions are grouped together. The group name is the description of that reaction + ending “-ase”
  - Transferases transfer molecular groups
  - Dehydrogenases have a dehydrogenating effect

- Oxidoreduktasen: katalysieren Redoxprozesse (übertragen Wasserstoff oder Elektronen)
- Transferasen: katalysieren Gruppenübertragungen (übertragen funktionelle Gruppen)
- Hydrolasen: katalysieren Hydrolysen (spalten Glykoside, Ester- und Peptidbindungen)
- Lyasen: knüpfen/spalten C-C, C-O, C-N, C-S-Bindungen ohne Beteiligung oxidativer oder hydrolytischer Schritte
- Isomerasen: katalysieren intramolekulare Umwandlungen isomerer Verbindungen
- Ligasen: katalysieren Zusammenlagerung von zwei Substratmolekülen unter ATP-Verbrauch

Importance of Enzymes

Table 1. Enzymes listed in order of decreasing catalytic proficiency.*

Enzyme	Nonenzymatic $t_{1/2}$*	k_{non}* (s^{-1})	k_{cat}† (s^{-1})	k_{cat}/K_m† (s^{-1} M^{-1})	Rate enhancement (k_{cat}/k_{non})	Catalytic proficiency $[(k_{cat}/K_m)/k_{non}]$ (M^{-1})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	5.6×10^{7}	1.4×10^{17}	2.0×10^{23}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	1.0×10^{7}	5.6×10^{14}	5.9×10^{19}
Adenosine deaminase	120 years	1.8×10^{-10}	370	1.4×10^{7}	2.1×10^{12}	7.8×10^{16}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	5.0×10^{5}	6.0×10^{12}	5.0×10^{16}
Cytidine deaminase	69 years	3.2×10^{-10}	299	2.9×10^{6}	1.2×10^{12}	9.1×10^{15}
Phosphotriesterase	2.9 years	7.5×10^{-9}	2100	4.0×10^{7}	2.8×10^{11}	5.3×10^{15}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	6.6×10^{6}	1.9×10^{11}	2.2×10^{15}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66000	3.0×10^{8}	3.9×10^{11}	1.8×10^{15}
Triosephosphate isomerase	1.9 days	4.3×10^{-6}	4300	2.4×10^{8}	1.0×10^{9}	5.6×10^{13}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.1×10^{6}	1.9×10^{6}	4.2×10^{10}
Carbonic anhydrase	5 s	1.3×10^{-1}	1×10^{6}	1.2×10^{8}	7.7×10^{6}	9.2×10^{8}
Cyclophilin, human	23 s	2.8×10^{-2}	13000	1.5×10^{7}	4.6×10^{5}	5.3×10^{8}

*Nonenzymatic reaction rate constants were obtained for OMP decarboxylase and staphylococcal nuclease from the present work, for adenosine and cytidine deaminases from (5), for AMP nucleosidase from (25), for phosphotriesterase from (26), for carboxypeptidase A from (3), for ketosteroid isomerase from (27), for triosephosphate isomerase from (28), for chorismate mutase from (4), for carbonic anhydrase from (2), and for cyclophilin from (3). †Enzyme reaction rate constants were obtained for OMP decarboxylase from (7), for staphylococcal nuclease from (29), for adenosine deaminase from (30), for AMP nucleosidase from (31), for phosphotriesterase from (26), for carboxypeptidase A from (32), for ketosteroid isomerase from (33), for triosephosphate isomerase from (34), for chorismate mutase from (4), for carbonic anhydrase from (35), and for cyclophilin from (36).

Industrial applications of Enzymes

- Lebensmittelindustrie zur Herstellung von Joghurt, Sauerteig und zur alkoholischen Gärung
- Waschmittelindustrie zur Hydrolyse von Proteinen
- Pharmazeutischen Industrie zur Herstellung enantiomerenreiner Wirkstoffe
- Amylasen kommen in der Textilindustrie zum Einsatz
- Lipasen und Proteasen werden in der Lederindustrie zur Reinigung von Häuten eingesetzt
- Hydrolasen und Redoxenzyme werden in der Feinchemie zur Produktion organischer Substanzen (Pharmaintermediate) eingesetzt (enantioselektiv!)