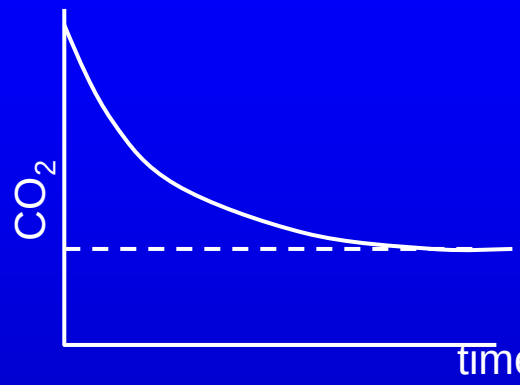
Methods of Biomaterials Testing
Lesson 3-5

Biochemical Methods

- Enzymatic Reactions -

Chemical Reactions
The Mass-Action Law (I)

$$CO_2 + H_2O \rightarrow H_2CO_3^- + H^+$$
$$H_2CO_3^- + H^+ \rightarrow CO_2 + H_2O$$



Substrate 1 + Substrate 2 +...+ Substrate n $\rightleftharpoons$ Product 1 + Product 2 +...+ Product n

In the steady state situation:

$$\frac{[\text{Product 1}]x[\text{Product 2}]x...x[\text{Product 3}]}{[\text{Substrate 1}]x[\text{Substrate 2}]x...x[\text{Substrate n}]} = K$$

The Mass Action Law (II)

Substrate 1 + Substrate 2 +...+ Substrate n $\rightleftharpoons$ Product 1 + Product 2 +...+ Product n

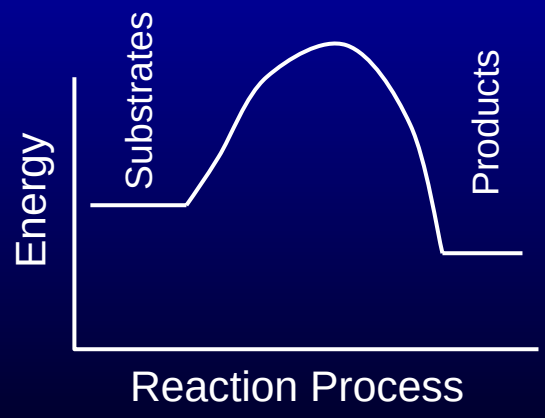
$$\frac{[\text{Product 1}]x[\text{Product 2}]x...x[\text{Product 3}]}{[\text{Substrate 1}]x[\text{Substrate 2}]x...x[\text{Substrate n}]} = K$$

Consequences

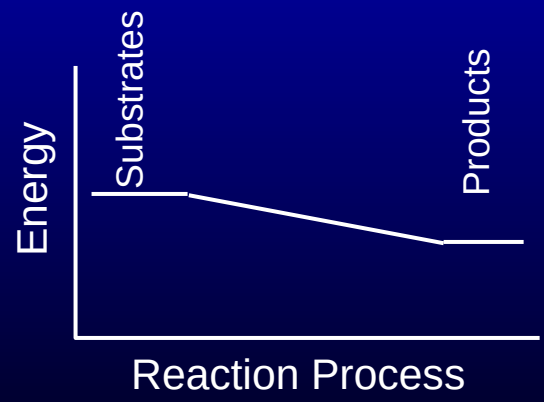
- The mass action law gives no information about the speed of a reaction
- The increase of **any** substrate concentration will increase the concentration of all products in the reaction
- Removal of any single product will increase the formation of the other products
- Addition of one or more of the products can reverse the reaction and lead to the formation of more substrates.

Enzymes I

- Enzymes are biological catalysts: They facilitate a chemical reaction
- They **do not change** the mass action law
- They **do not change** the K
- They **do not prefer** one of the reaction directions
- They act by reducing the activation energy of the reaction or the energy of an intermediate step
- Terminology: Name: Substrate + Type of reaction + '-ase'
  'Activity' (measured in 'Units' U) instead of 'Concentration'

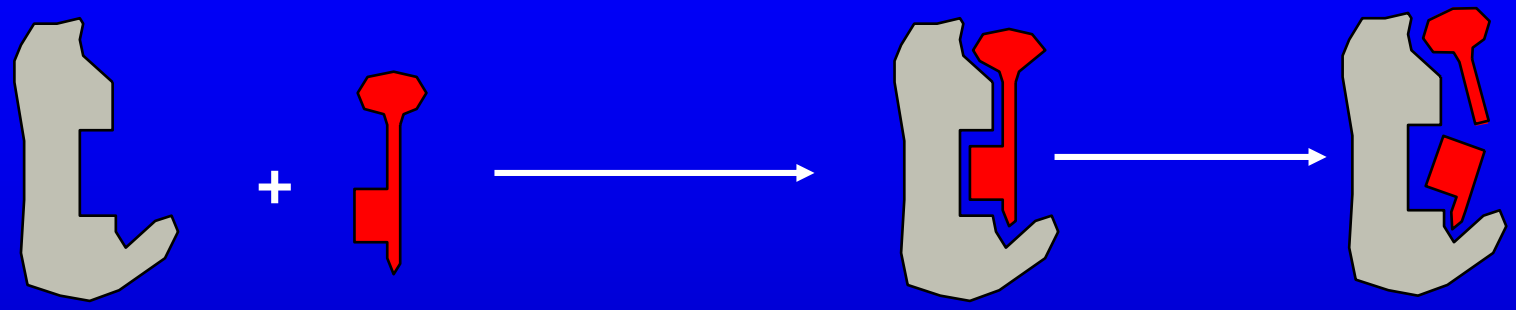


Reaction without Enzyme



Reaction with Enzyme

Enzyme-Substrate Complex



Enzyme **Substrate** **Enzyme-Substrate Komplex** **Reaction/ release of the products**

By random (Brownian) movement enzyme and substrate connect to the enzyme substrate complex, where the reaction happens

Consequences:

- **<u>Very high substrate concentration</u> → The enzyme is the limiting step. Reaction speed is proportional to the enzyme activity**
- **<u>Very high enzyme concentration</u> → The substrate is the limiting step. Reaction speed is proportional to the substrate concentration.**

  In this case: Michaelis-Menten Equation:

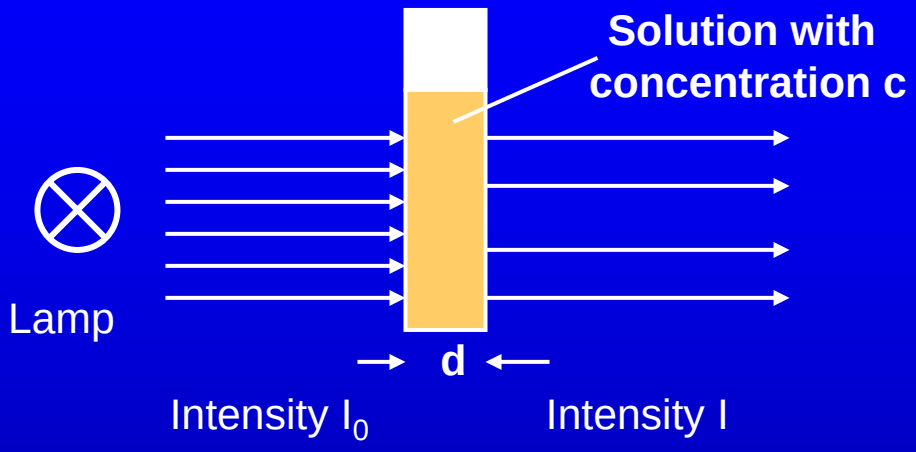
$$v = \frac{V_{max} \times [S]}{K_m + [S]}$$ **; K_m: Michaelis constant**

Enzymes III

- Enzymes are proteins
- Enzymes mostly have strict requirements to their environment (temperature, pH, salt concentration...)
- Enzymes can be ‘intoxicated’ by simulators of their substrate
  - They cannot be processed at all
  - They are processed much slower
  - The simulator or the products react chemically with the enzyme

  →This can be used for selective testing of specific pathways
- Many enzymes are produced in inactive form, which get activated on demand: **pro-enzymes**
- Enzymes become degraded/ inactivated after some time
- According to their type and function enzymes can be located inside the cell/ in special compartments of the cell/ on the cell surface or freely outside the cell.

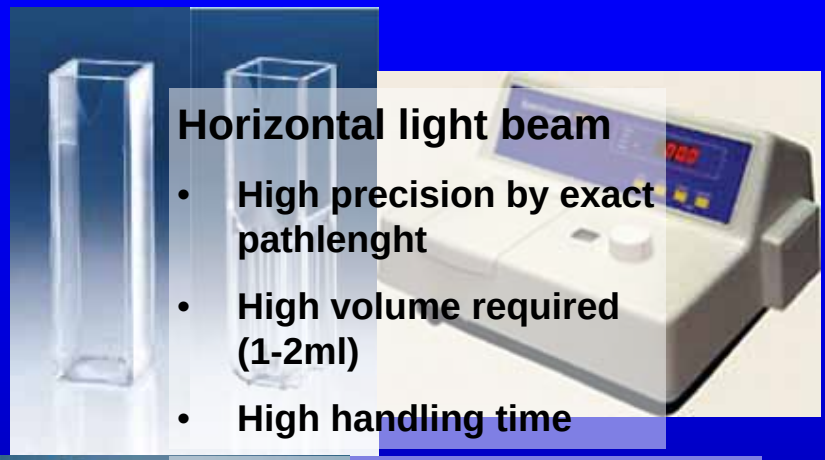
The Lambert-Beer‘s Law



$$I = I_0 \, e^{-\kappa cd}$$

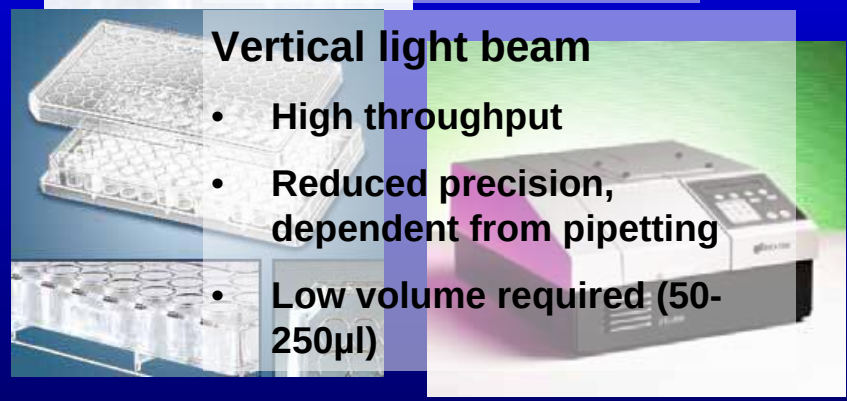
$$Ln(I/I_0) = -\kappa cd$$

$$E = \log(I_0/I) = \varepsilon cd;$$ **E: Extinction**



Horizontal light beam

- High precision by exact pathlenght
- High volume required (1-2ml)
- High handling time



Vertical light beam

- High throughput
- Reduced precision, dependent from pipetting
- Low volume required (50-250µl)

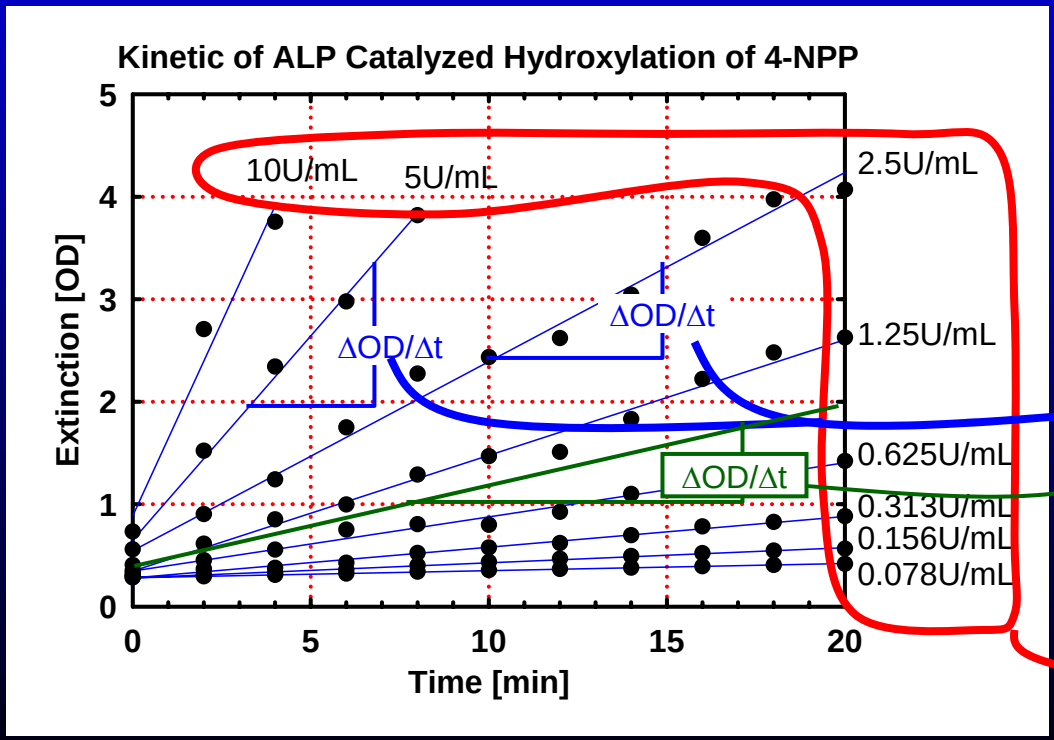
- The extinction E (Absorbance A) is directly given from the photometer
- ***The extinction/absorbance is proportional to the concentration c***
- The extinction is proportional to the pathlengh d
  - Take care of pipetting errors at vertical set-ups (microplates)

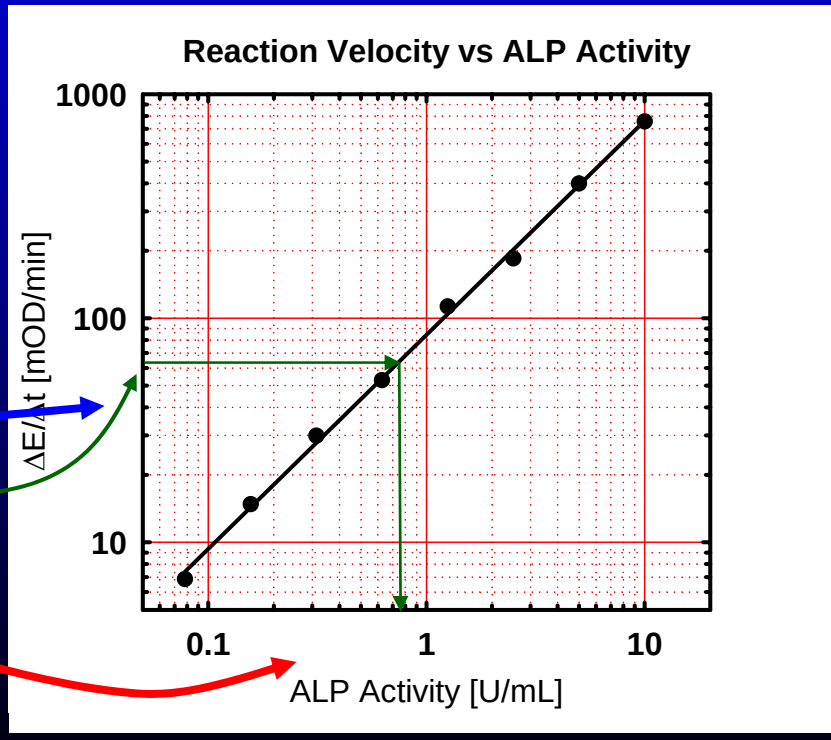
Alkaline Phosphatase

- Low Substrate Specifity: R-OPO(OH)$_2$
- Reaction:

$$\text{R-O-PO}_3^{2-} + \text{H}_2\text{O} \xrightleftharpoons{\text{ALP}} \text{R-OH} + \text{PO}_4^{3-} + \text{H}^+$$

- For Analysis frequently 4-Nitrophenly Phosphate as substrate: The Product has yellow color: $\lambda(E_{max})$=405nm





Examples of Enzymes: LDH

- Lactate Dehydrogenase (LDH)
  - Necessary for the energy metabolism
  - Very constant activity in all cells
  - Only inside the cell
  - Presence outside of a cell indicates cell damage (leakage)
- Reaction

$$\text{Lacatate} + NAD^+ \xrightleftharpoons{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

LDH-Measurement Protocol

- Tris Buffer: pH 7.2
- Reagent solution:
  - NADH
  - Pyruvate
- Start reaction by addition of LDH or sample
- Measure absorption (kinetic) at λ=340nm (this is an absorption maximum of NADH)

Lacatate + NAD^+ ⇌ Pyruvate + NADH + H^+

→ **Obviously the test measures the reverse reaction**