LOSCHMIDT LABORATORIES

# Enzyme Kinetics

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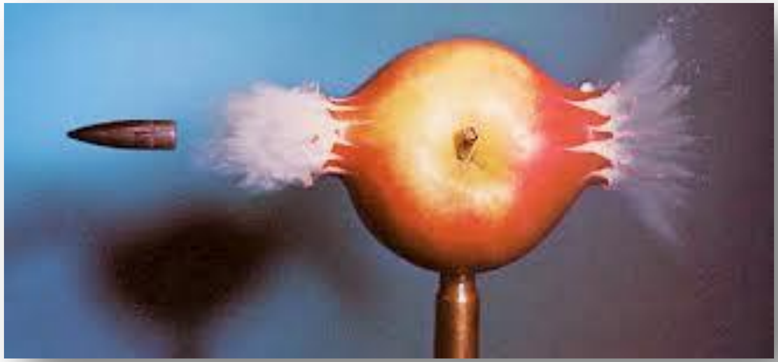
# OUTLINE

- ENZYME KINETICS
- STEADY-STATE vs. TRANSIENT KINETICS
- MATHEMATICS AND SOFTWARES
- RAPID MIXING TECHNIQUES
- KINETIC MECHANISMS
- KINETICS IN PROTEIN ENGINEERING

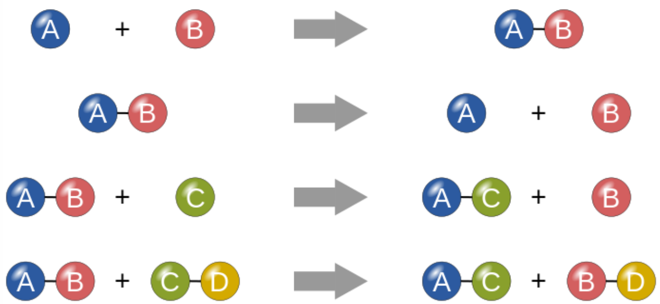
# ENZYME KINETICS

- **KINETICS** - study of motion and its causes

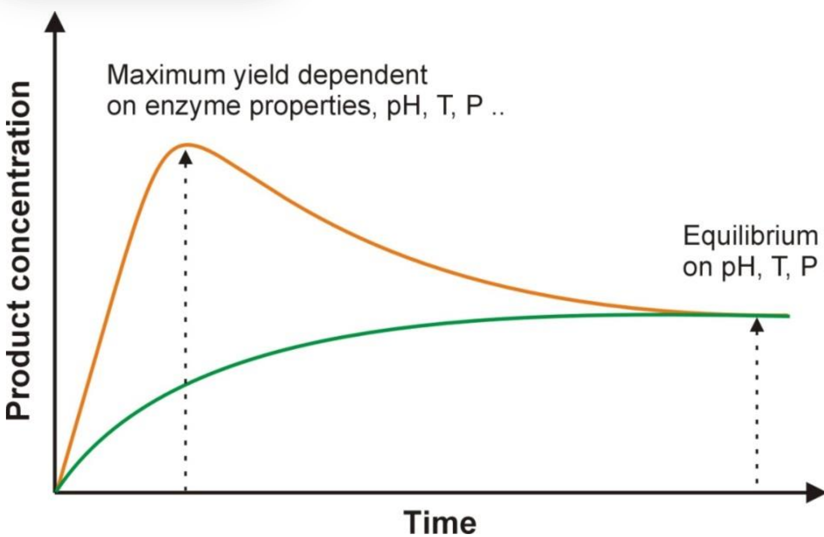


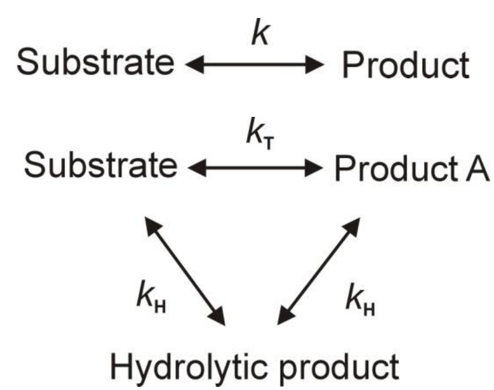
# ENZYME KINETICS

- **KINETICS** - study of motion and its causes
- **(BIO)CHEMICAL KINETICS** - rate of reactions/interactions

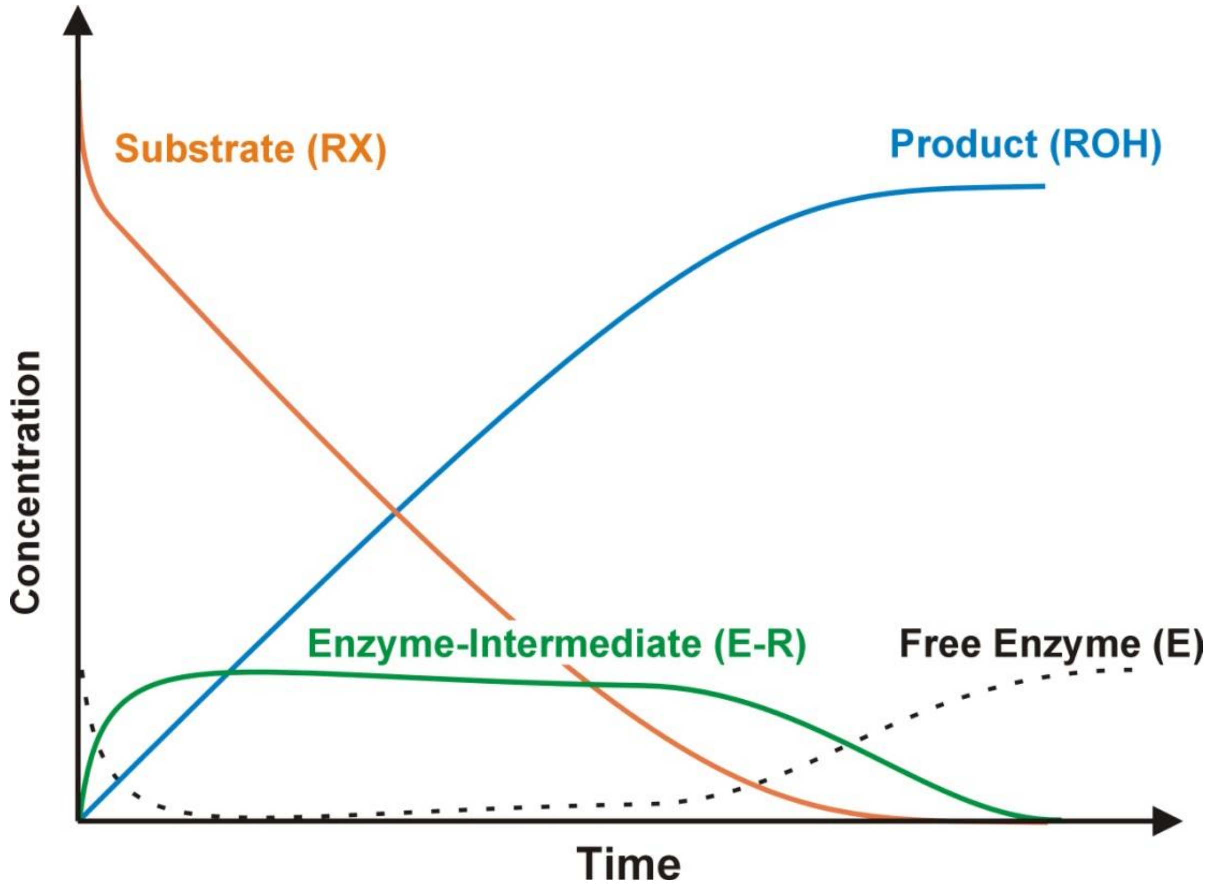



- chemical kinetics
- enzyme kinetics
- binding kinetics
- (un)folding kinetics
- pharmacokinetics



# ENZYME REACTION



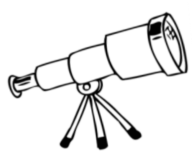
# STEADY-STATE vs. TRANSIENT

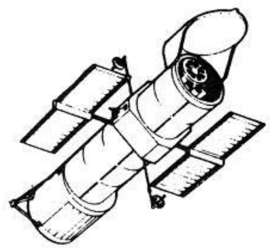
- ACTIVITY ANALYSIS
- STEADY-STATE KINETICS
- TRANSIENT KINETICS

$$E + S \xrightarrow{v} E + P$$

$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} E.S \xrightarrow{k_{cat}} E + P$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E.X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E.P \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P$$



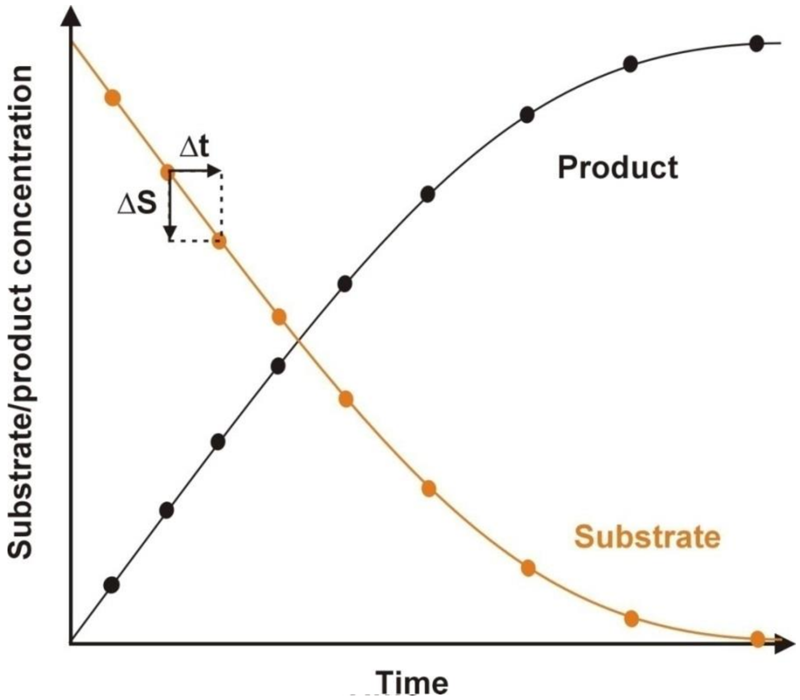


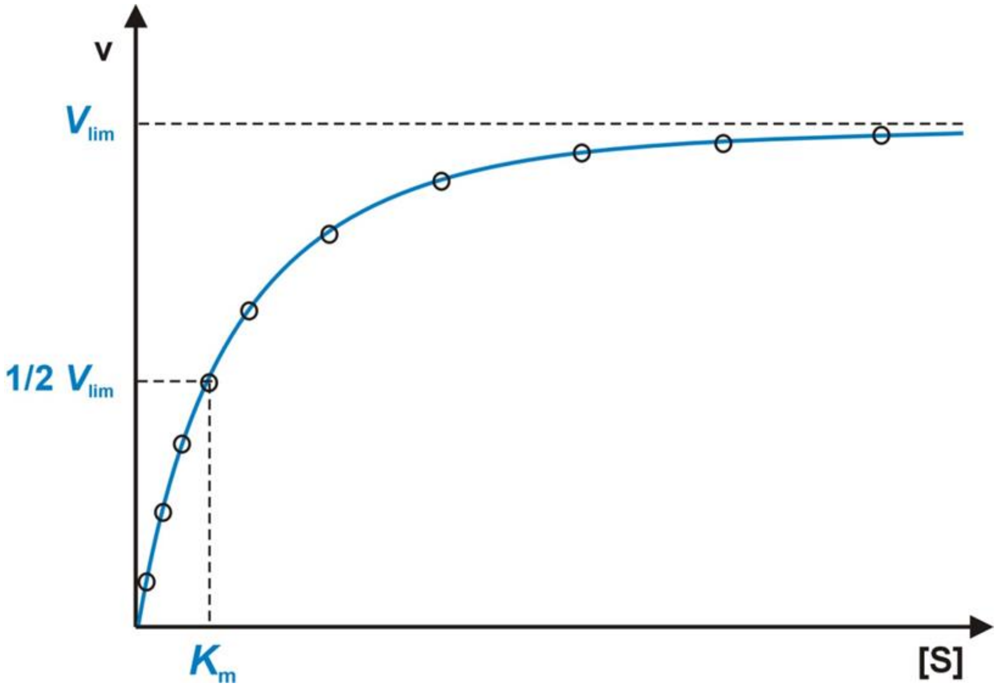
$$K_m = \frac{k_{-1}.(k_{-2}.(k_{-3} + k_4) + k_3.k_4) + k_2.k_3.k_4}{k_1.((k_2 + k_{-2}).(k_{-3} + k_4) + k_2.k_3 + k_3.k_4)}$$

$$k_{cat} = \frac{k_2.k_3.k_4}{(k_2 + k_{-2}).(k_{-3} + k_4) + k_2.k_3 + k_3.k_4}$$

# STEADY-STATE KINETICS

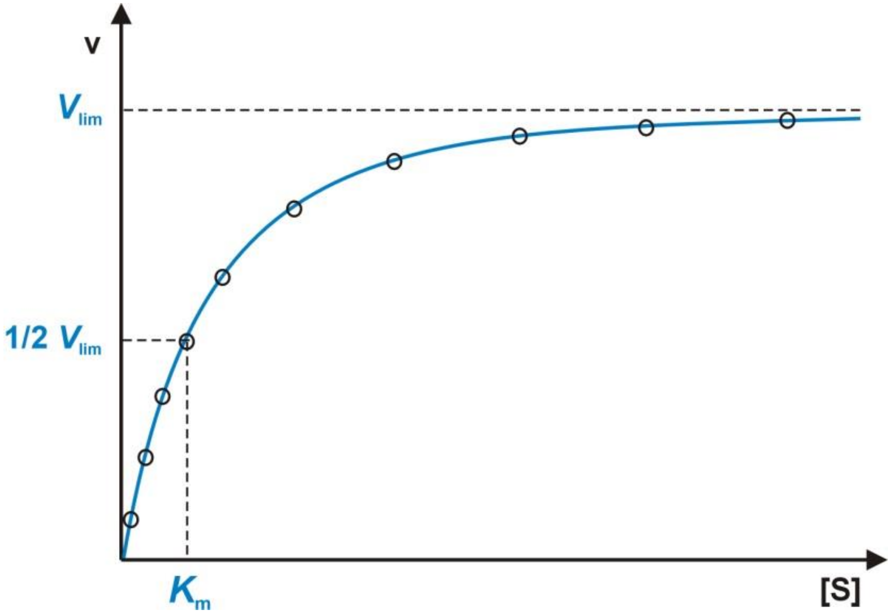
- ACTIVITY ANALYSIS
- MICHAELIS-MENTEN KINETICS

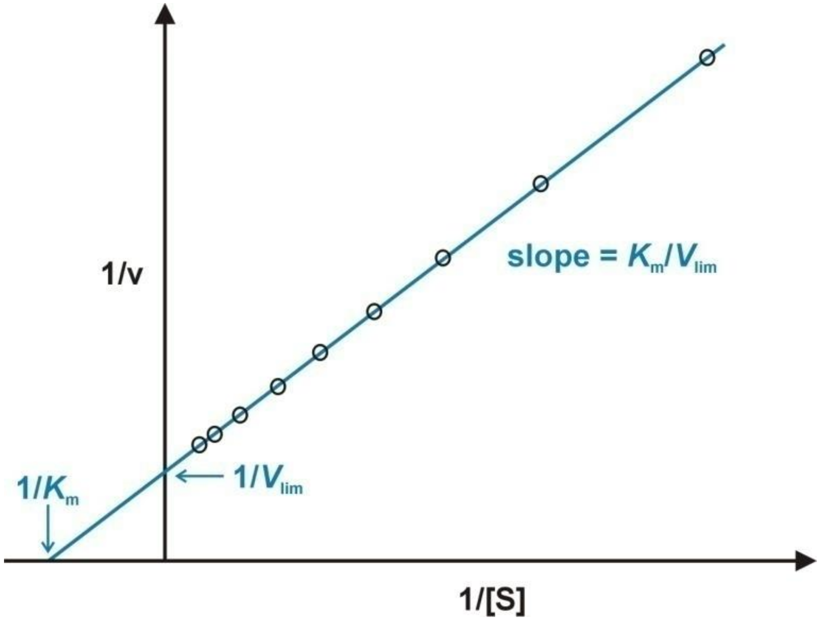


- linearization

$$v = \frac{k_{cat} \,.\, [S]}{K_m + [S]} \quad \rightarrow \quad \frac{1}{v} = \frac{1}{V_{lim}} + \frac{K_m}{V_{lim}} \cdot \frac{1}{[S]}$$

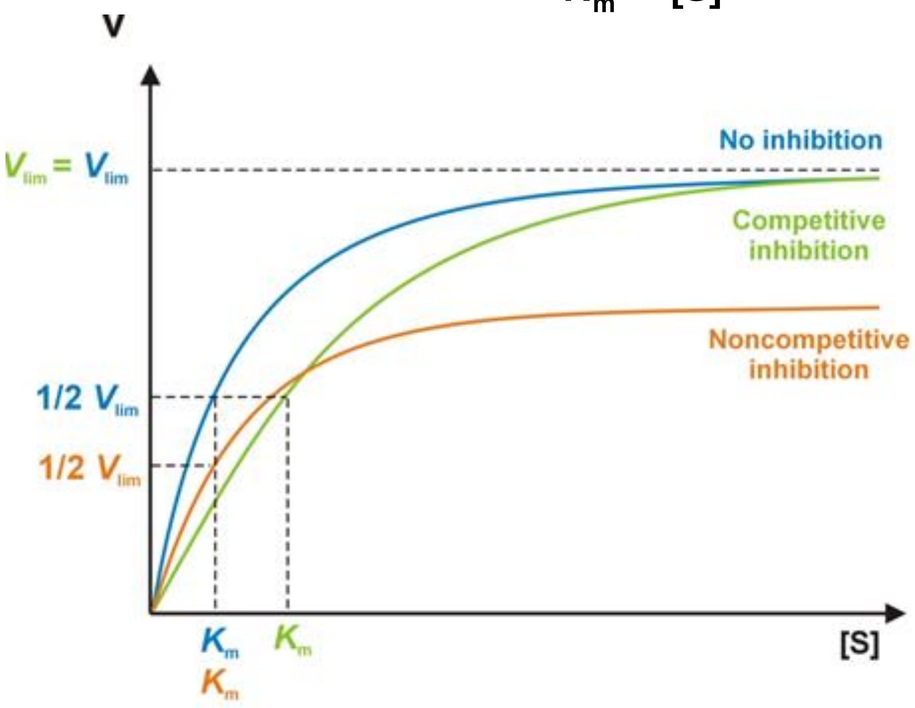


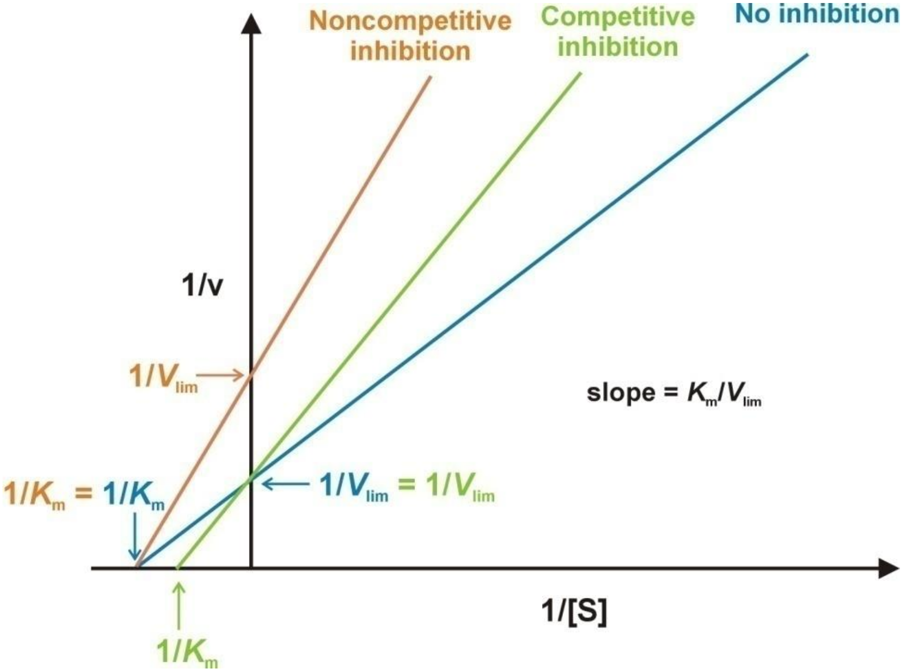



# STEADY-STATE KINETICS

## ❑ linearization

$$v = \frac{k_{cat} \cdot [S]}{K_m + [S]} \quad \Rightarrow \quad \frac{1}{v} = \frac{1}{V_{lim}} + \frac{K_m}{V_{lim}} \cdot \frac{1}{[S]}$$



# STEADY-STATE KINETICS

- **linearization**
- **nonlinear regression** (e.g., SigmaPlot, Origin, EnzFiter)
- **numerical simulation** (e.g., Gepasy, DynaFit, Kintek Explorer)

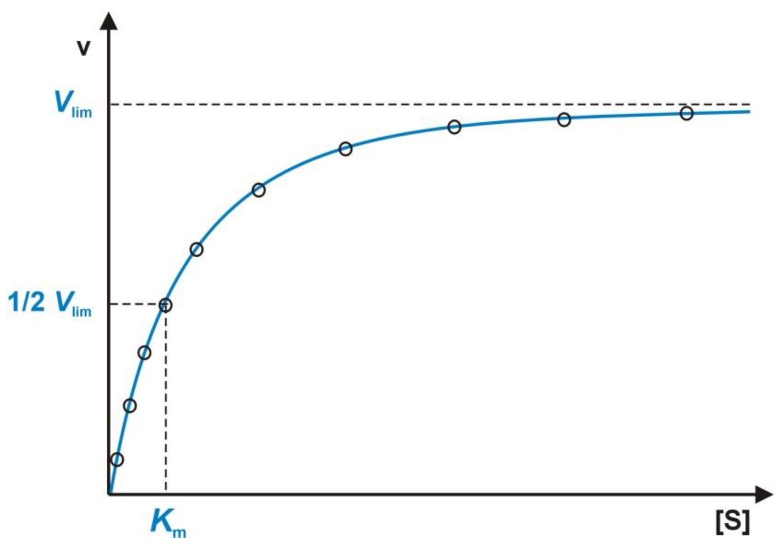
$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} E.S \xrightarrow{k_{cat}} E + P$$

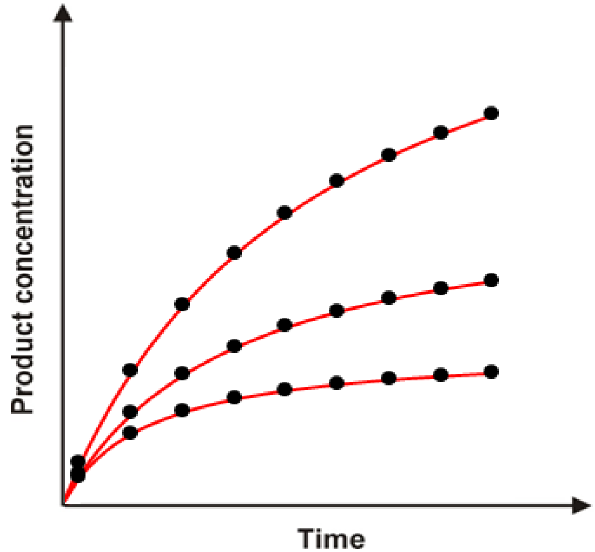
$$v = \frac{k_{cat} \cdot [S]}{K_m + [S]}$$

$$\frac{d[S]}{dt} = - k_1.[E].[S] + k_{-1}.[ES]$$

$$\frac{d[ES]}{dt} = k_1.[E].[S] - k_{-1}.[ES]$$

$$\frac{d[P]}{dt} = k_2.[ES]$$



# STEADY-STATE KINETICS

$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} E.S \xrightarrow{k_{cat}} E + P$$

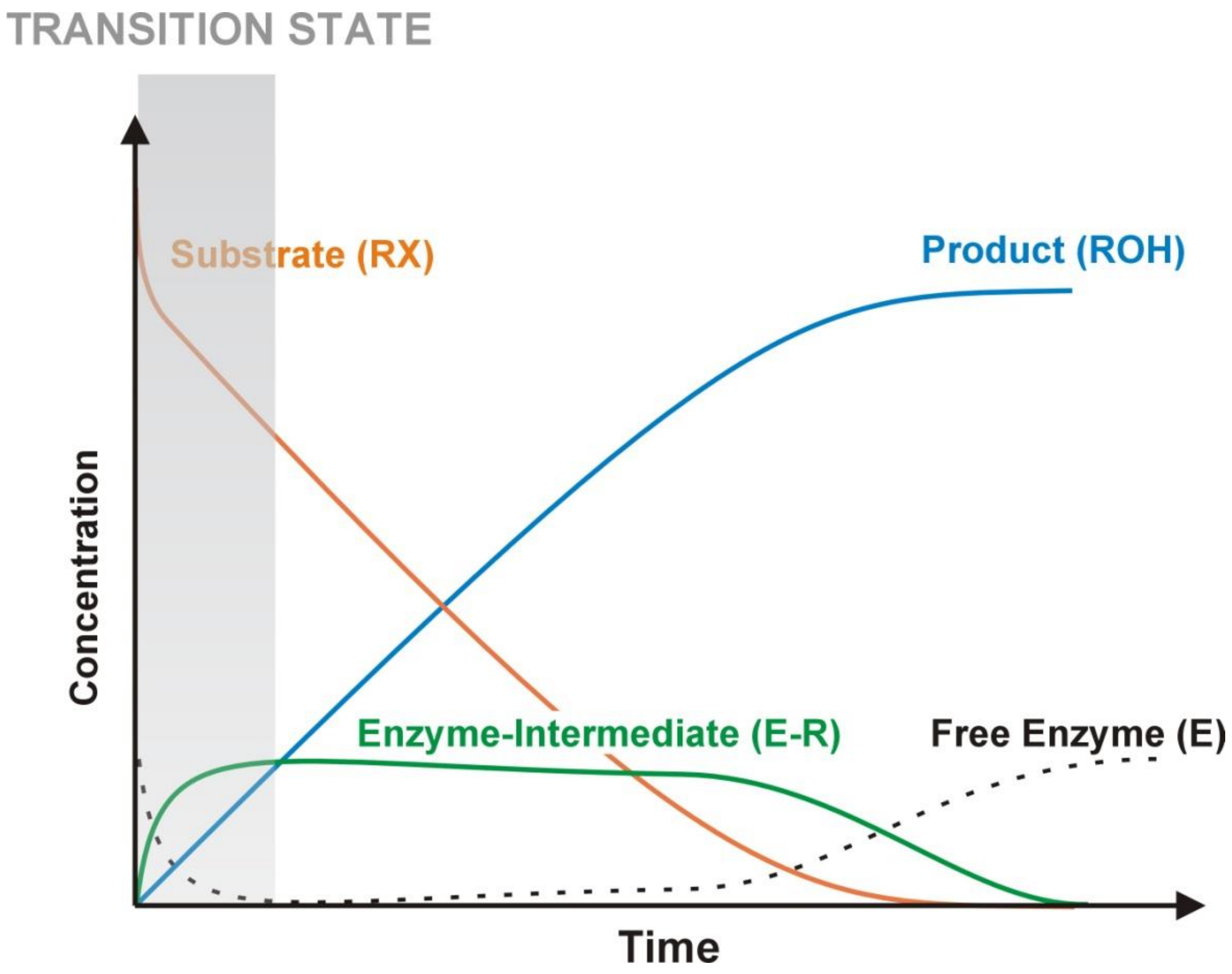
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E.X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E.P \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P$$

$$K_m = \frac{k_{-1}.(k_{-2}.(k_{-3} + k_4) + k_3.k_4) + k_2.k_3.k_4}{k_1.((k_2 + k_{-2}).(k_{-3} + k_4) + k_2.k_3 + k_3.k_4)}$$

$$k_{cat} = \frac{k_2.k_3.k_4}{(k_2 + k_{-2}).(k_{-3} + k_4) + k_2.k_3 + k_3.k_4}$$

- **Michaelis-Menten constant, $K_m$ (mol.l$^{-1}$)**
  - apparent equilibrium constant
  - determined by true binding constant and rates of catalytic steps
- **Turnover number, $k_{cat}$ (s$^{-1}$)**
  - number of molecules converted per second by one enzyme molecule under saturation
  - influenced only by rates of catalytic steps (limiting step)
- **Specificity constant, $k_{cat}/K_m$ (mol.l$^{-1}$.s$^{-1}$)**
  - apparent second order rate constant

# TRANSIENT KINETICS



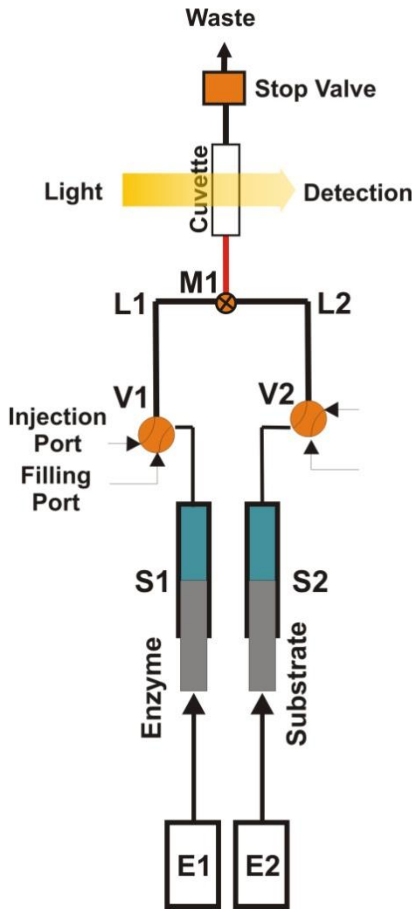
# RAPID MIXING TECHNIQUES

## STOPED-FLOW

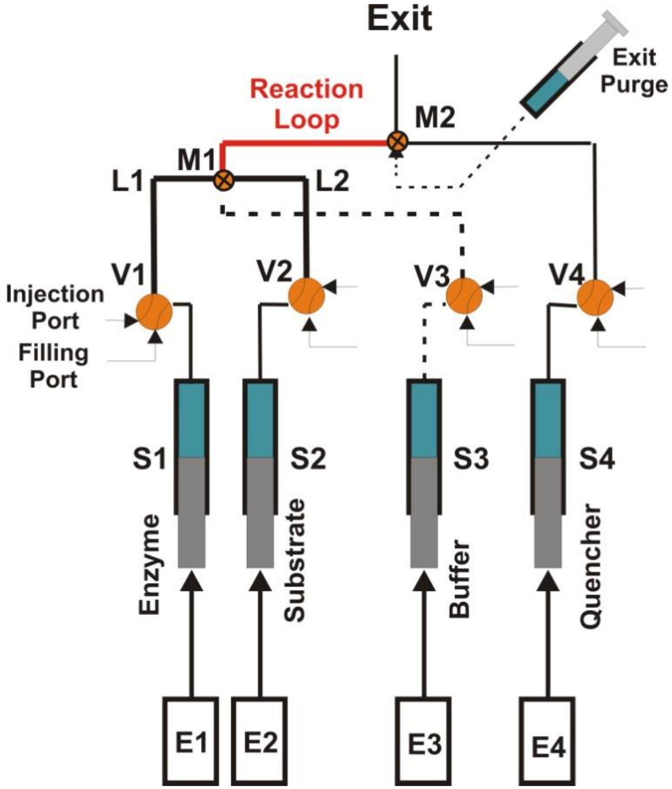
## RAPID QUENCH-FLOW

# RAPID MIXING TECHNIQUES

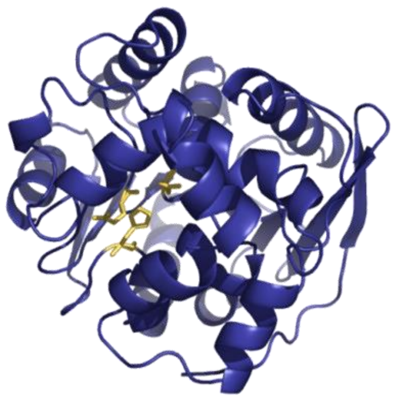
## STOPED-FLOW



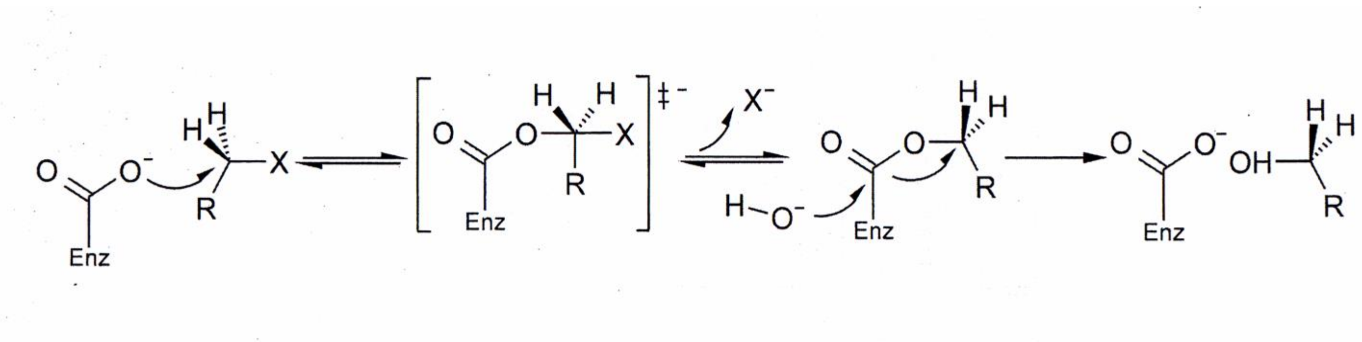

## RAPID QUENCH-FLOW



# KINETIC MECHANISMS



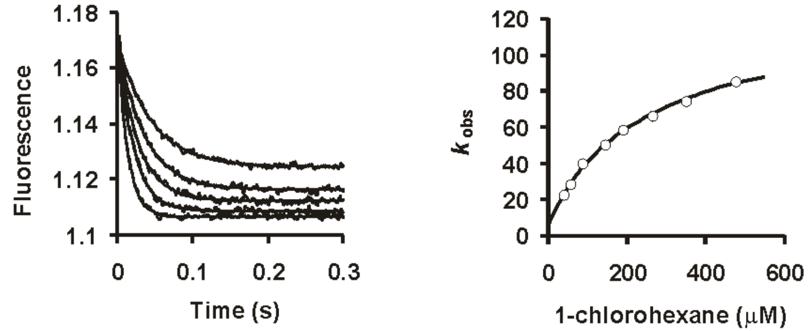
- **dehalogenase** (EC 3.8.1.5)
- **globular** proteins
- **monomeric**/oligomeric
- **no cofactor** requirement
- cleavage **carbon-halogen** bond



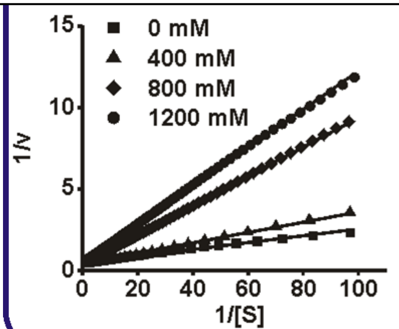

$$E + RX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.RX \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\text{-}R.X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E.X.ROH \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + X + ROH$$

# KINETIC MECHANISMS

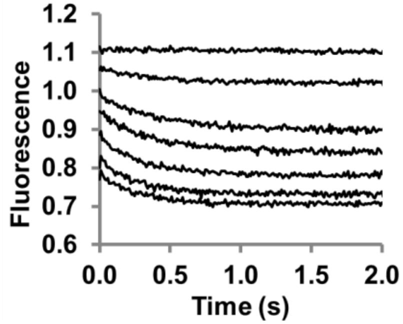
$$k_{obs} = \frac{k_2.[S]}{[S] + K_s} + k_{-2} + k_3$$




$$v = \frac{[S].k_{cat}}{K_m.(1 + [P]/K_{p1}) + [S].(1 + [P]/K_{p2})}$$

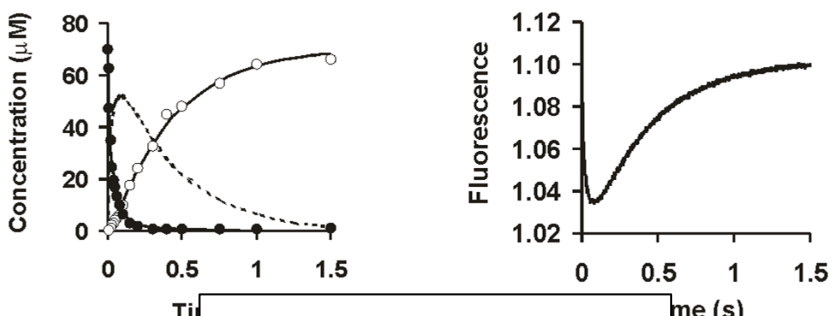



$$k_{obs} = \frac{[P] . k_{4b}}{K_{4a} + [P]} + k_{-4b}$$



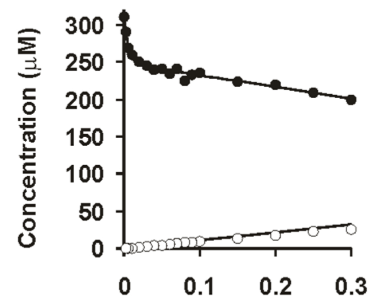

$$E + RX \underset{k_{-1}}{\overset{K_1}{\rightleftharpoons}} E.RX \underset{k_{-2}}{\overset{K_2}{\rightleftharpoons}} E\text{-}R.X \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E.X.ROH \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + X + ROH$$

## SINGLE TURNOVER KINETICS




$$A = A_1.e^{-k1.t} + A_2.e^{-k2.t}$$

## REACTION BURST



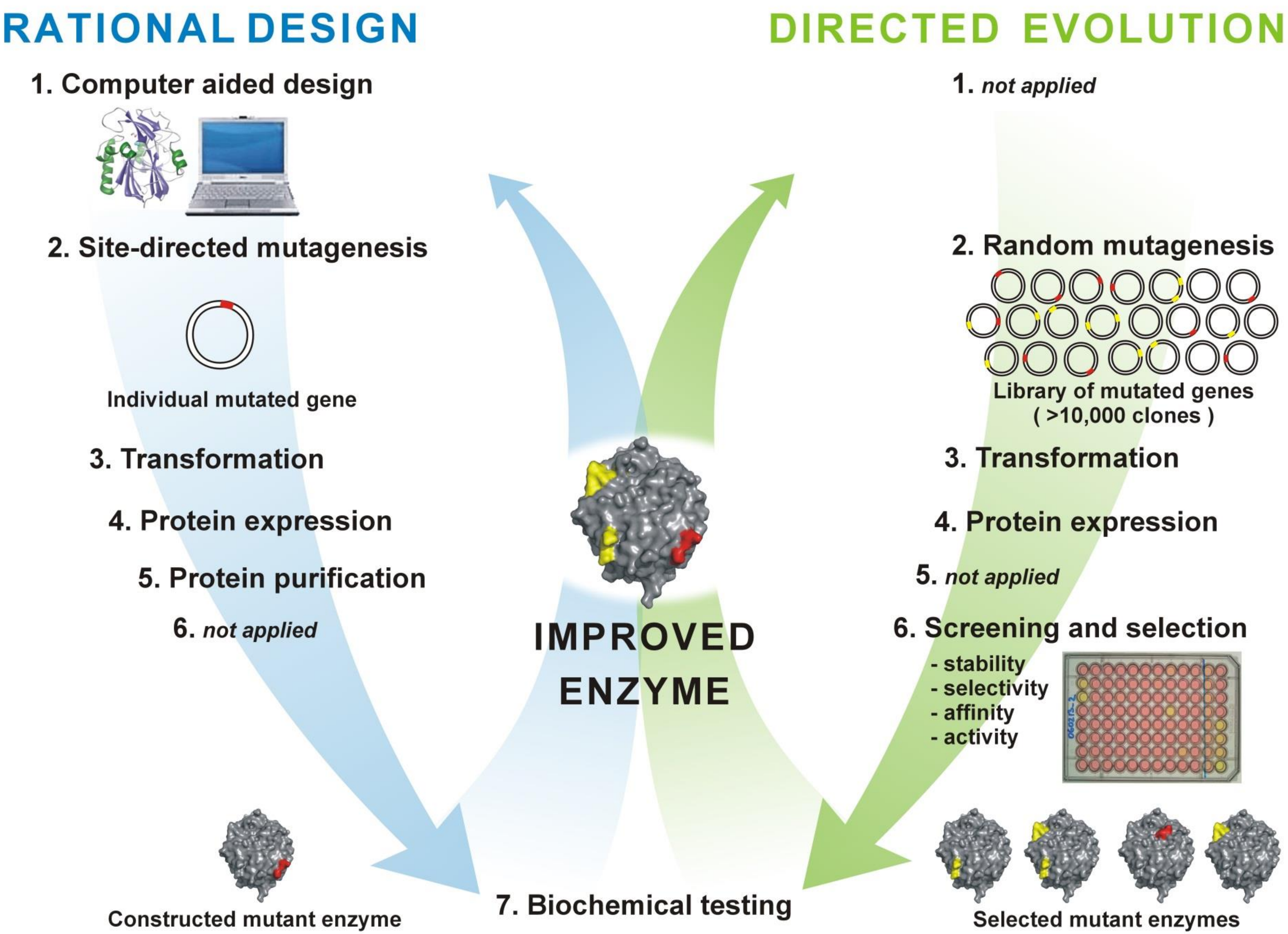

$$A = A_0.e^{-k.t} + k_2.t$$

# KINETIC MECHANISMS

| | Substrat | $K_s$ (μM) | $k_2$ ($s^{-1}$) | $k_{-2}$ ($s^{-1}$) | $k_3$ ($s^{-1}$) | $k_4$ ($s^{-1}$) | $K_m$ (μM) | $k_{cat}$ ($s^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| LinB | 1-chlorohexane | 240 | 117 | 0.4 | 3.2 | - | 16 | 2.6 |
| | bromocyclohexane | > 450 | > 200 | 1.1 | 2.5 | - | 23 | 1.8 |
| | chlorocyclohexane | > 500 | > 40 | 12.5 | 0.075 | - | 221 | 0.1 |
| DhlA | 1,2-dibromeethane[1] | > 27 | > 130 | - | 10 | 4 | 10 | 3 |
| | 1,2-dichloroethane[1] | 2 200 | 50 | - | 14 | 8 | 530 | 3.3 |
| DhaA | 1,3-dibromopropane[2] | 60 - 300 | 300 | - | 14.8 | 3.9 | 5 | 2.7 |

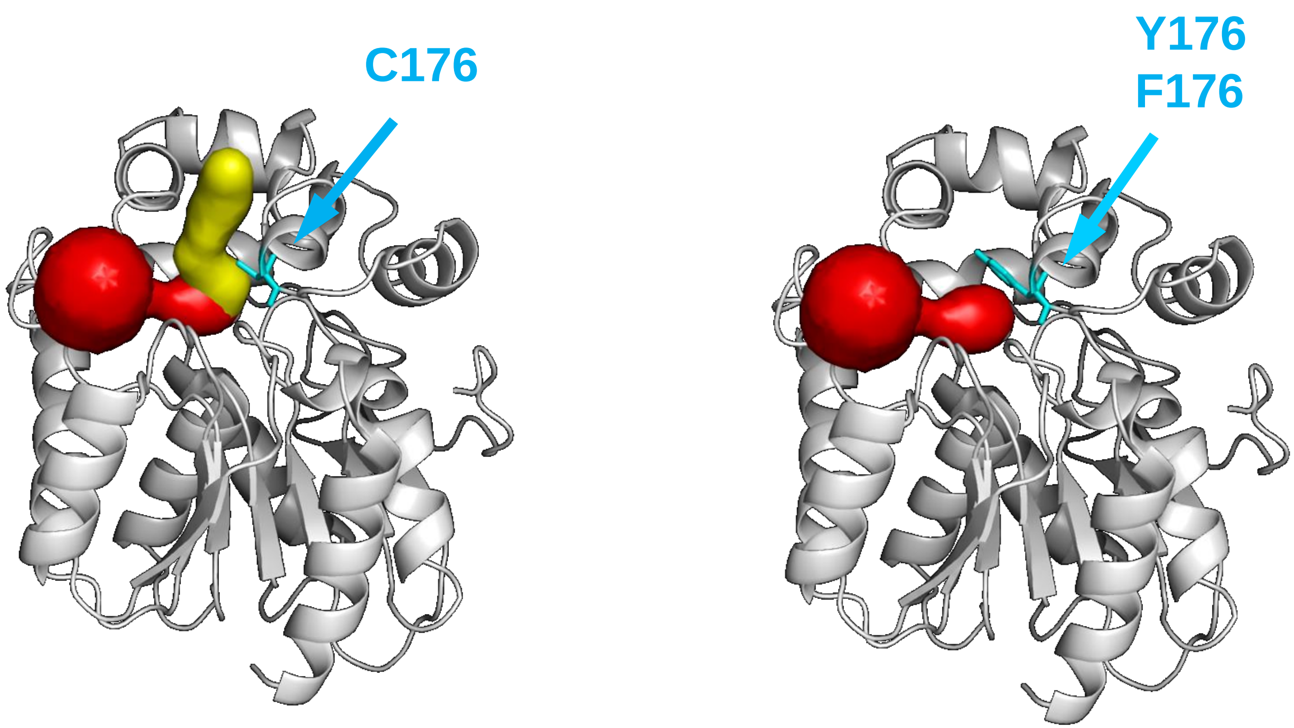
**distinct rate-limiting steps**
**for relative enzymes**

# PROTEIN ENGINEERING



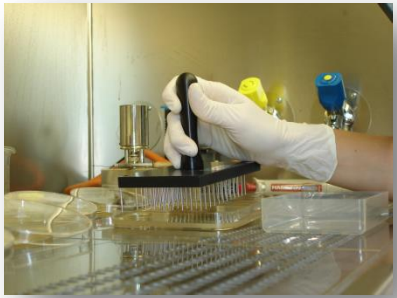
# PROTEIN ENGINEERING

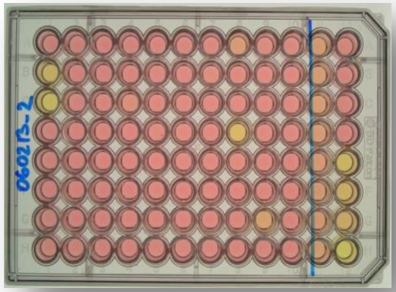
- **conversion of 1,2,3-trichloropropane** by dehalogenase from *Rhodococcus sp.*
- importance of access pathways



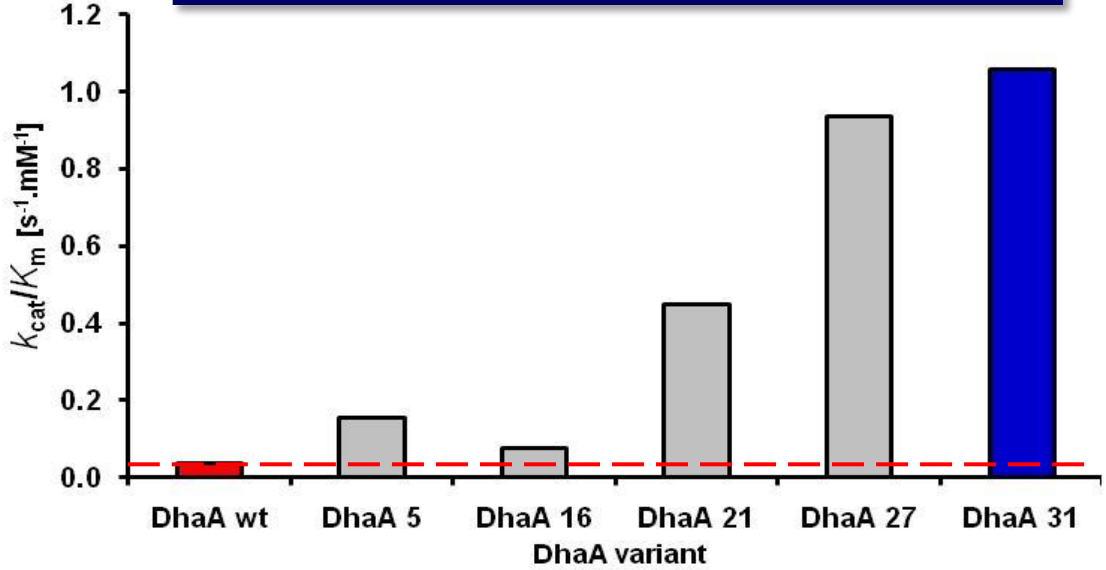
# PROTEIN ENGINEERING

- library of 5,300 clones
- **stady-state kinetics**





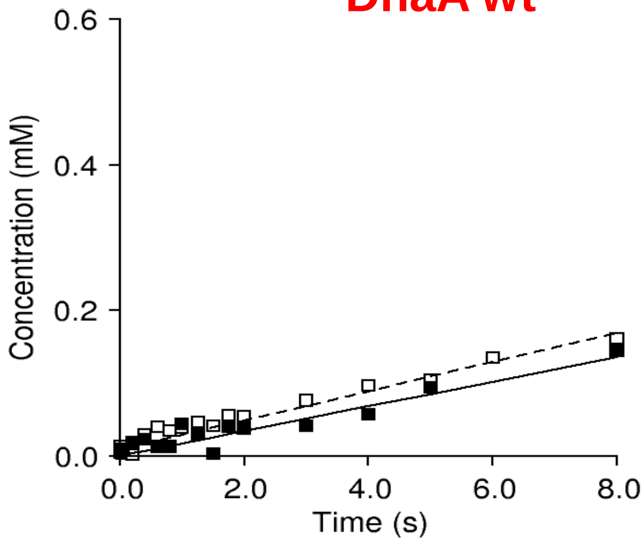
**steady-state catalysis improved 26x**



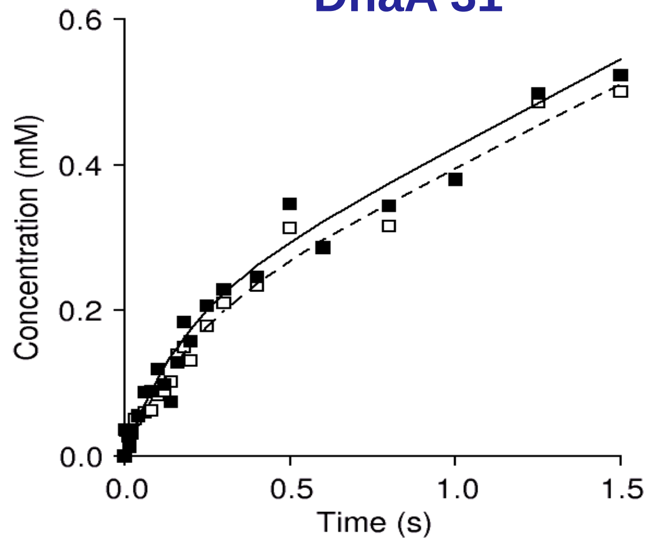

| | $K_m$ (mM) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ |
|---|---|---|---|
| DhaA wt | 0.98 ± 0.17 | 0.035 ± 0.002 | 0.04 |
| DhaA 31 | 1.19 ± 0.15 | 1.26 ± 0.05 | 1.06 |

## ❑ transient kinetics

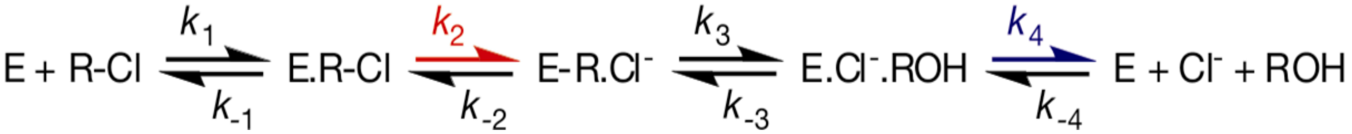
**DhaA wt**




**DhaA 31**

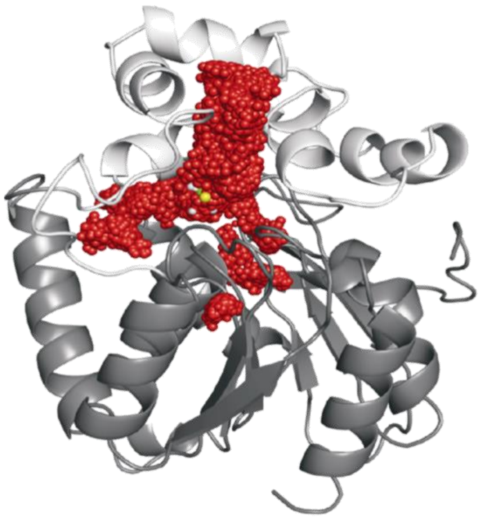



**Chemical reaction** **Product release**

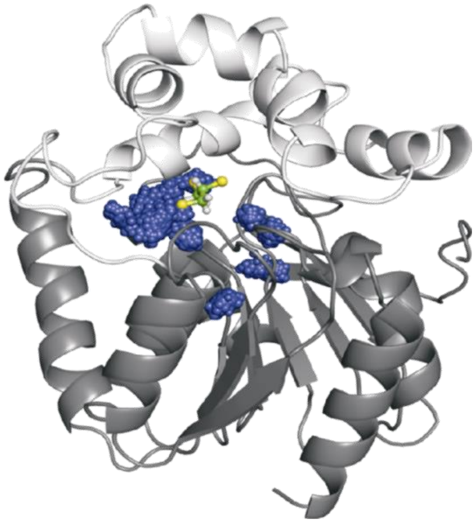


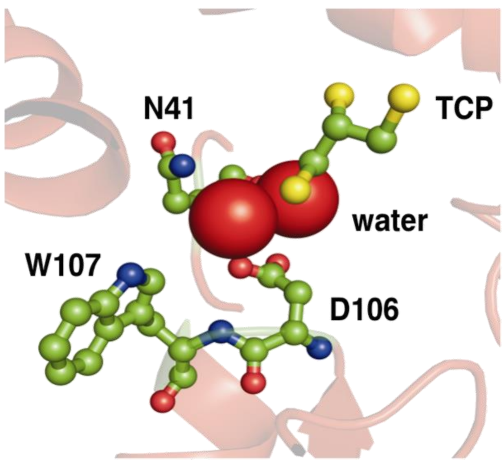
# PROTEIN ENGINEERING

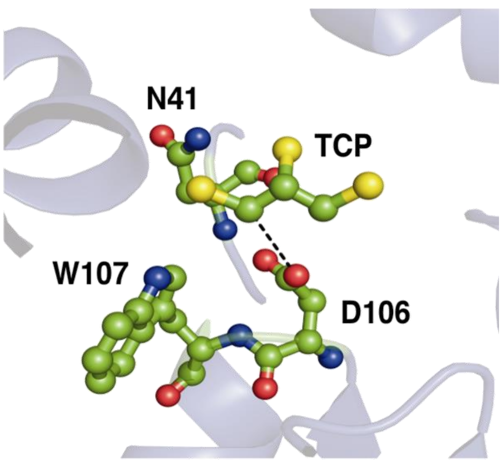
**DhaA wt**



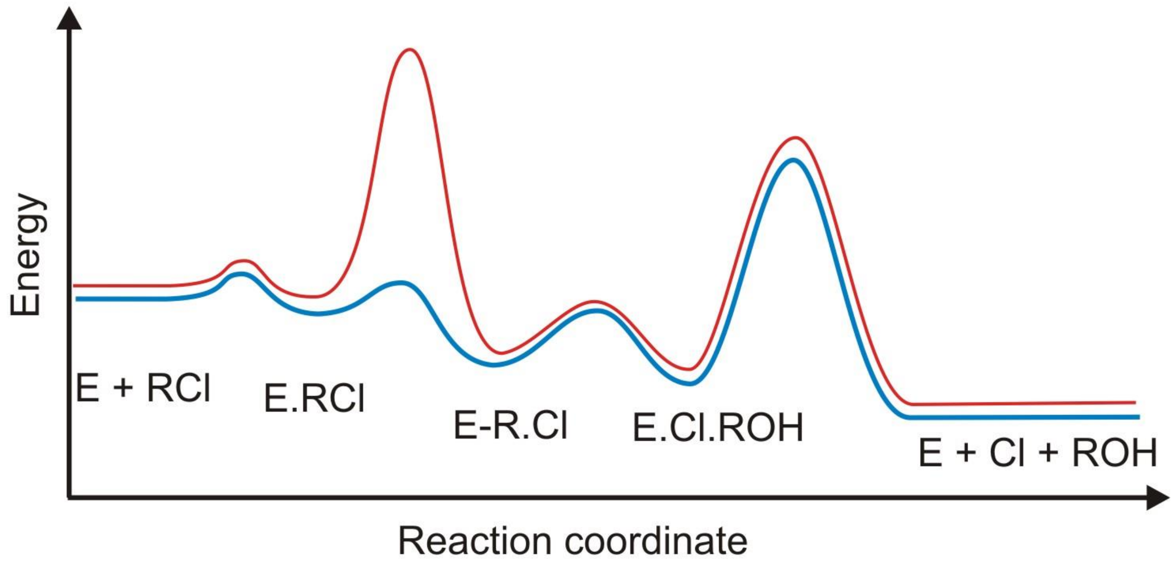
**DhaA 31**

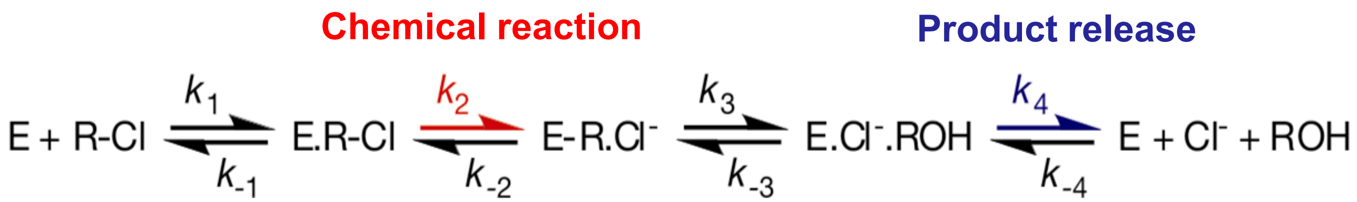




## transient kinetics




**chemical step improved 450x**