Kinetika reaksi enzimatis

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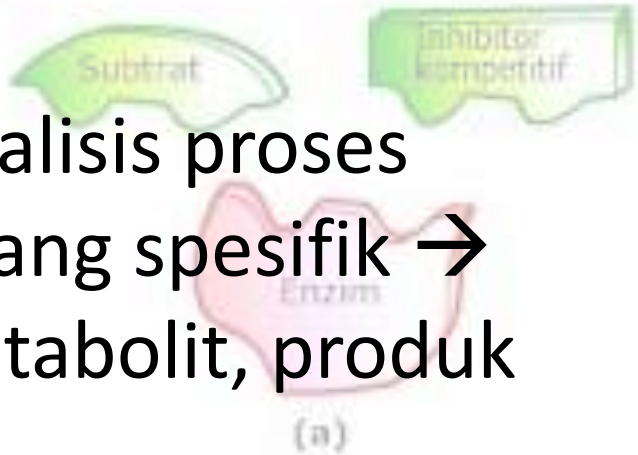
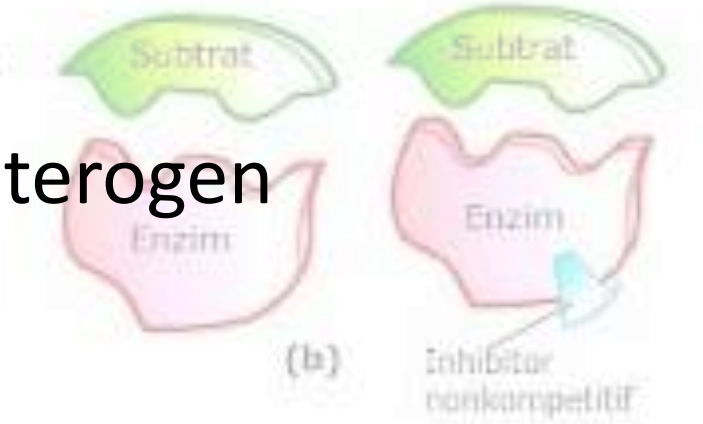


- Enzim : protein yg mengkatalisis proses biokimia dalam sel hayati yang spesifik → produksi biomassa atau metabolit, produk pangan, dll
- Reaksi enzimatis :
  - Sistem homogen atau heterogen
  - Satu substrat atau lebih



Dasar kinetika reaksi enzimatis

- Michaelis –Menten →laju awal reaksi enzimatis dapat ditentukan berdasarkan fungsi terhadap konsentrasi substrat dan parameter yg berpengaruh dalam enzim
- Henri → pembentukan kompleks enzim substrat reversibel selama reaksi pembentukan produk dari substrat → laju reaksi berbanding lurus dengan konsentrasi kompleks

Reaksi reversibel

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

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

$$v = \frac{d[ES]}{dt} = k_1[E][S] - [k_{-1} + k_2][ES]$$

laju pembentukan (kekanan) = laju penguraian (kekiri)

$$k_1[E][S] - [k_{-1} + k_2][ES] = 0$$

$$k_1[E][S] = [k_{-1} + k_2][ES]$$

$$[E][S] = [ES]\left\{\frac{k_{-1} + k_2}{k_1}\right\}$$

$$Km = \frac{k_{-1} + k_2}{k_1}$$

$$[E][S] = [ES]Km$$

$$[E_T] = [E] + [ES]$$

$$\{[E_T] - [ES]\}[S] = [ES]Km$$

$$[E_T][S] - [ES][S] = [ES]Km$$

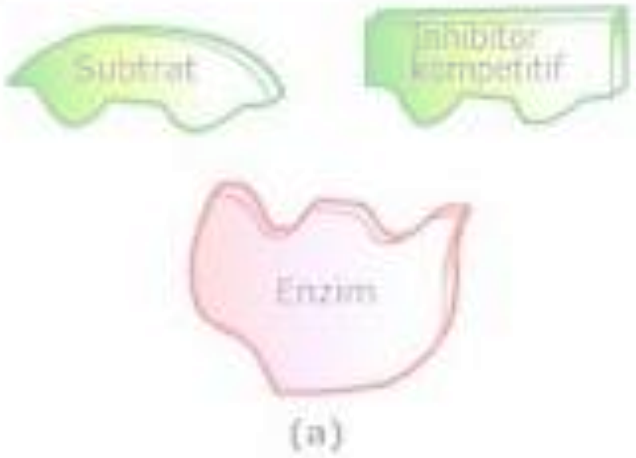
$$[E_T][S] = [ES]\{Km + [S]\}$$

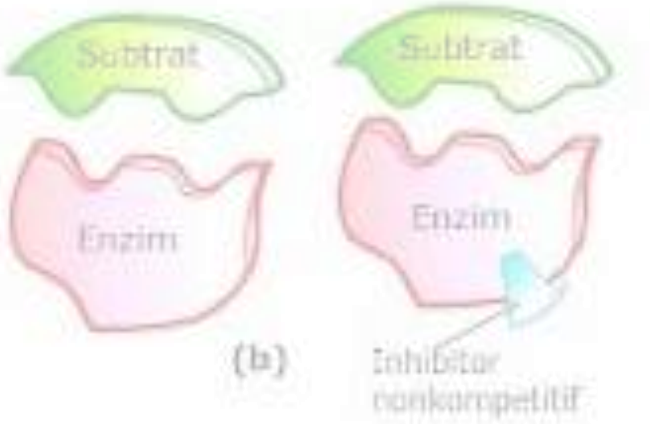
$$[ES] = \frac{[E_T][S]}{Km + [S]}$$

$$v = k_2[ES] = k_2 \frac{[E_T][S]}{Km + [S]}$$

$$v_{max} = k_2[E_T]$$

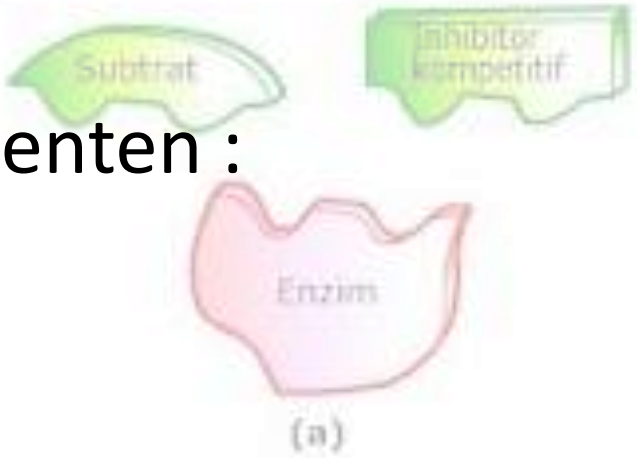
$$v = \frac{v_{max}[S]}{Km + [S]}$$

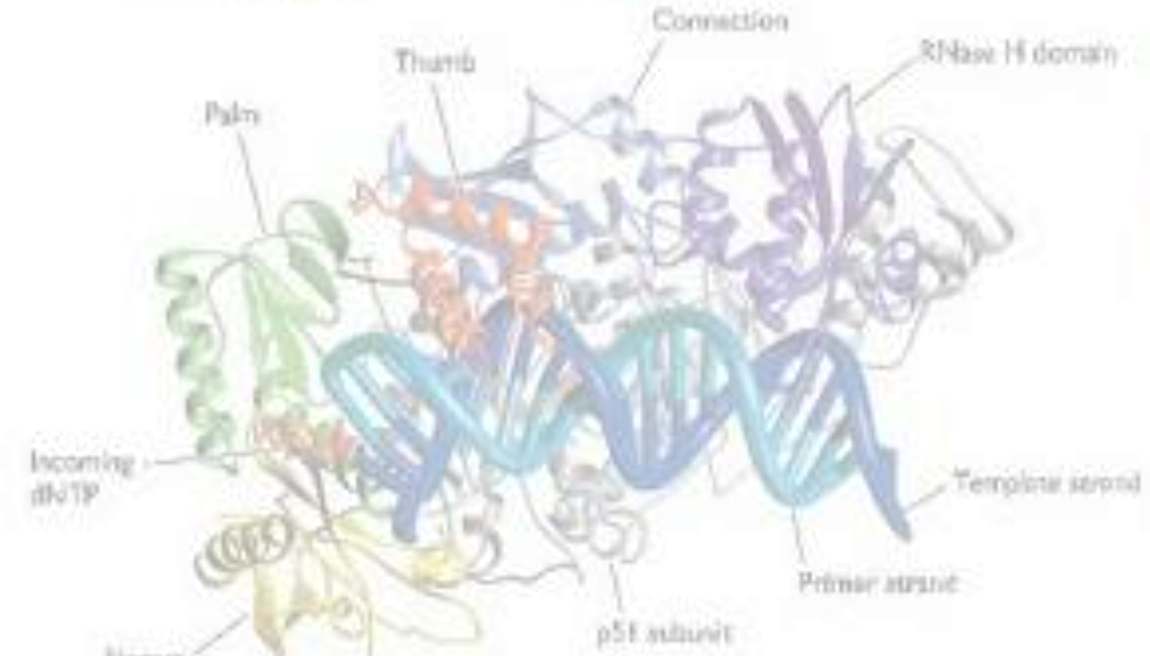


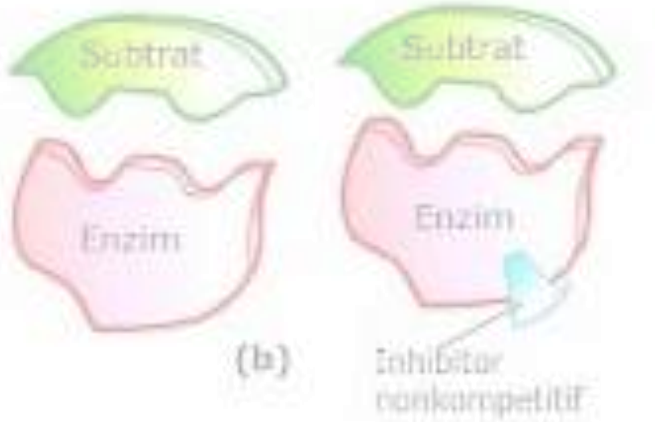


- Kinetika reaksi Michaelis-Menten :

$$v = \frac{v_{max}[S]}{Km + [S]}$$







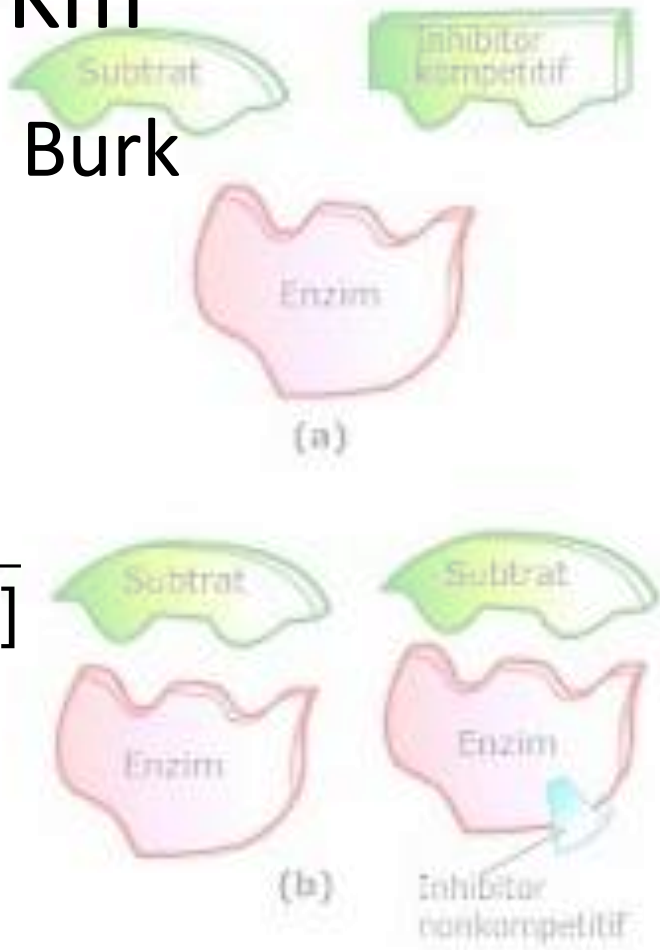
Kinetika reaksi homogen : penentuan Km

- Persamaan Lineweaver dan Burk

$$v = \frac{v_{max}[S]}{Km + [S]}$$

$$\frac{1}{v} = \frac{Km + [S]}{v_{max}[S]} = \frac{Km}{v_{max}[S]} + \frac{[S]}{v_{max}[S]}$$

$$\frac{1}{v} = \frac{Km}{v_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$



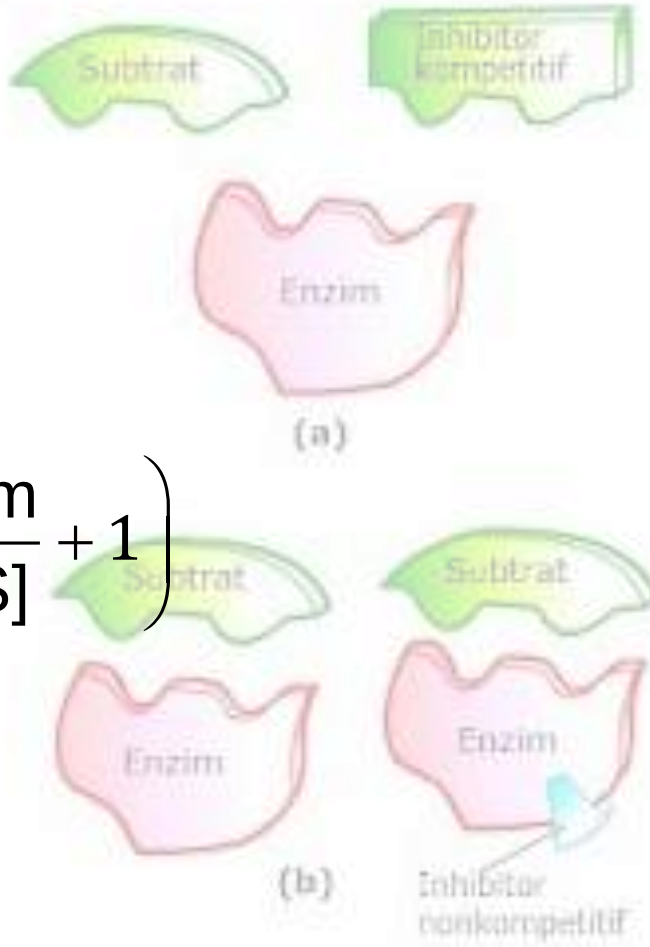
- Persamaan Eadie Hofstee

$$v = \frac{v_{max}[S]}{Km + [S]}$$

$$v = \frac{v_{max}}{\frac{Km}{[S]} + 1} \text{ sehingga } v_{max} = v\left(\frac{Km}{[S]} + 1\right)$$

$$v_{max} = \frac{v}{[S]} Km + v$$

$$v = -Km\frac{v}{[S]} + v_{max}$$



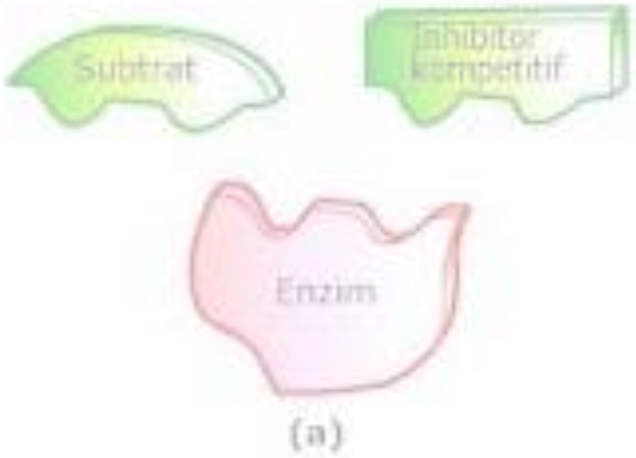
- Persamaan Hanes

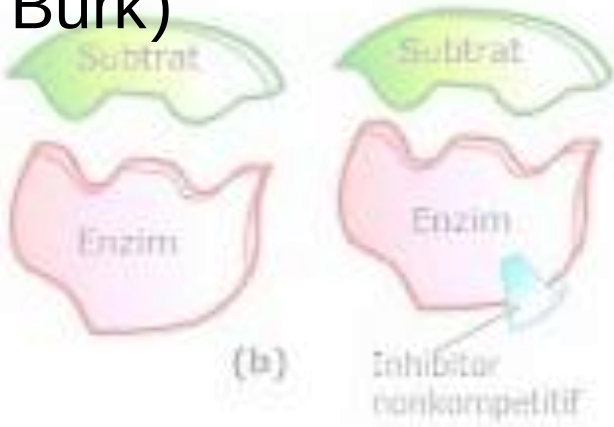
$$v = \frac{v_{max}[S]}{Km + [S]}$$

$$\frac{1}{v} = \frac{Km}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}} \text{(Lineweaver – Burk)}$$

$$\frac{1}{v}[S] = \frac{Km}{v_{max}}\frac{1}{[S]}[S] + \frac{1}{v_{max}}[S]$$

$$\frac{[S]}{v} = \frac{Km}{v_{max}} + \frac{1}{v_{max}}[S]$$





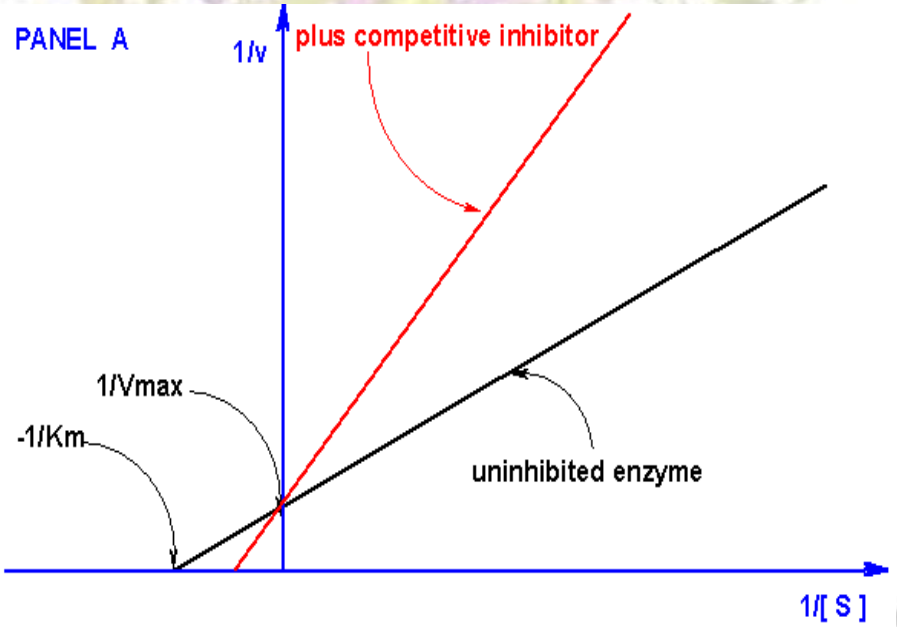
Inhibisi

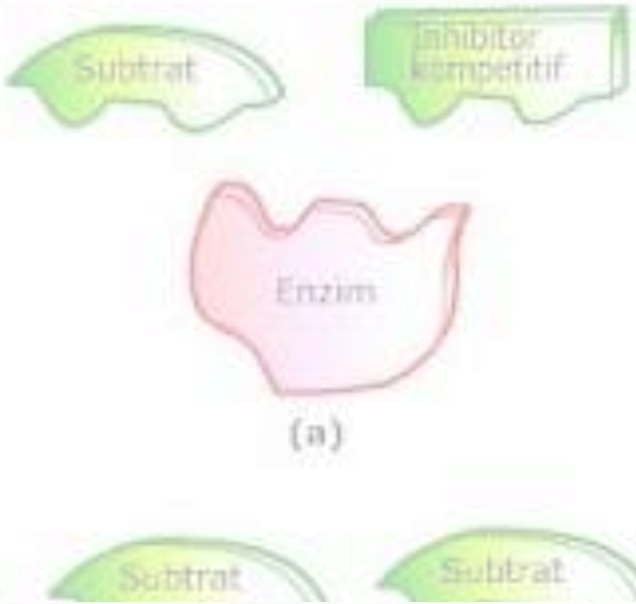
- Kompetitif : inhibitor berkompetisi dengan substrat untuk berinteraksi dengan sisi aktif enzim
- uncompetitive: inhibitor menghambat kerja enzim di sisi yg berbeda dari interaksi substrat-enzim → terikat pada ES
- Non- kompetitif : inhibitor menghambat di sisi berbeda, tidak menghasilkan produk → terikat pada E atau ES

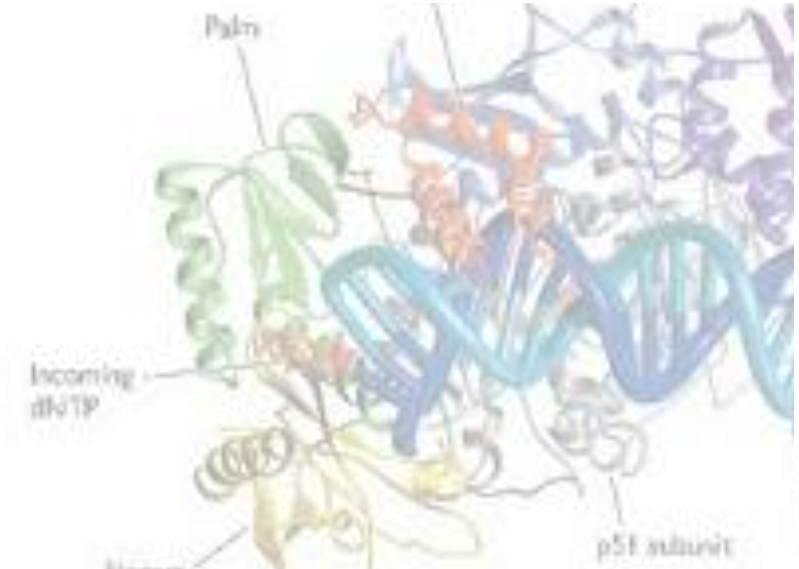
Inhibisi

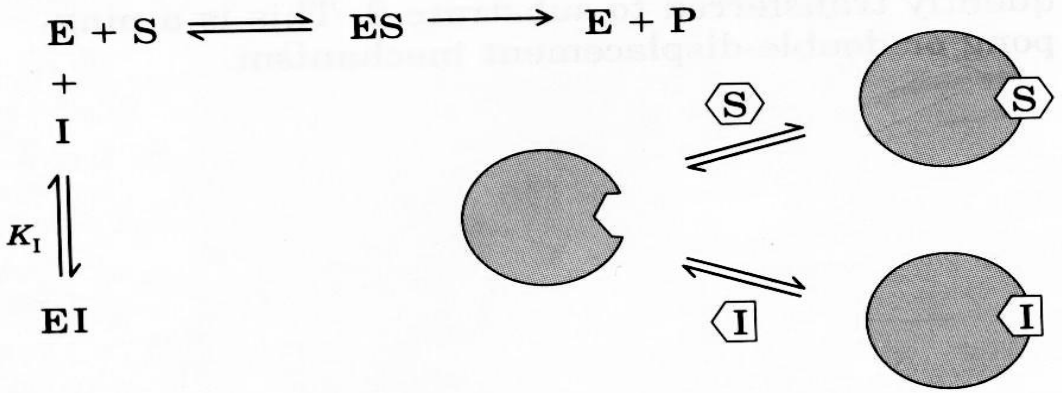
- Oleh produk → produk yang dihasilkan dapat menghambat kerja enzim → kompetitif atau tidak kompetitif
- Oleh substrat → substrat menghambat kerja enzim (konsentrasi substrat terlalu tinggi) → membentuk kompleks → tidak kompetitif

Inhibisi kompetitif









Inhibisi kompetitif

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

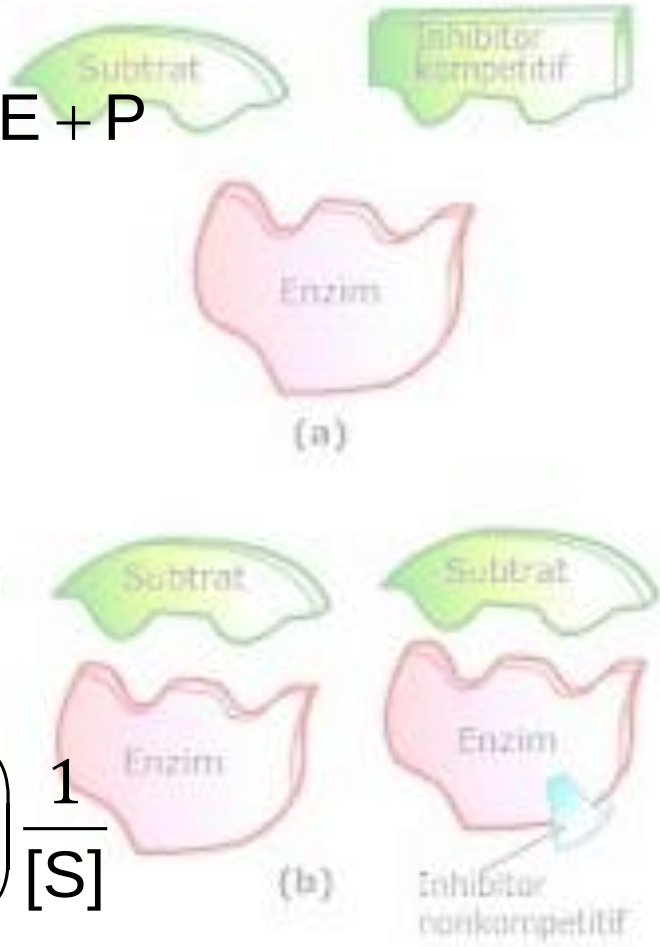
$$E + I \underset{}{\overset{ki}{\rightleftarrows}} EI$$

$$[ET] = [ES] + [E] + [EI]$$

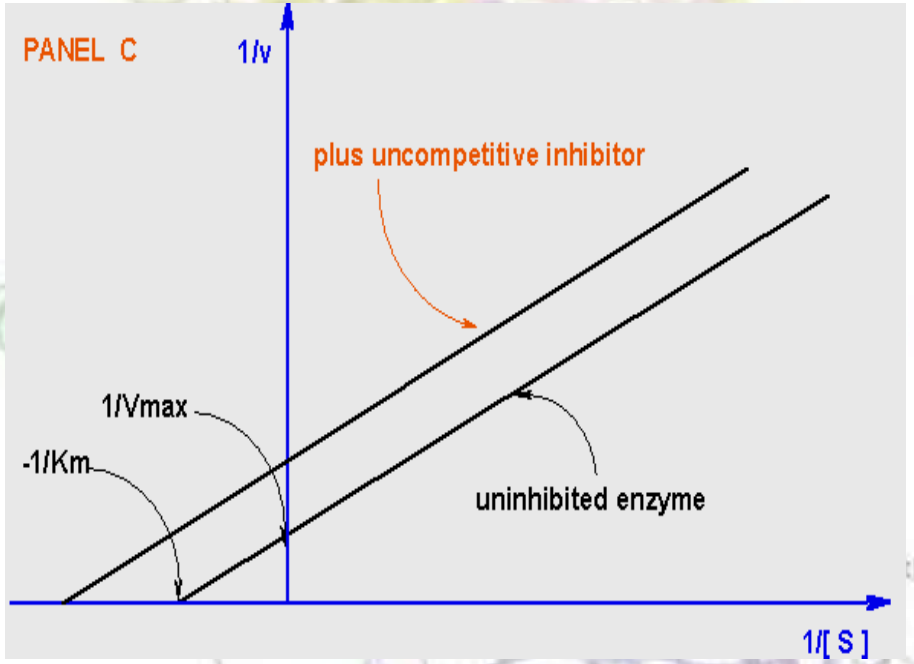
$$v = \frac{v_{max}[S]}{Km\left(1 + \frac{[I]}{Ki}\right) + [S]}$$

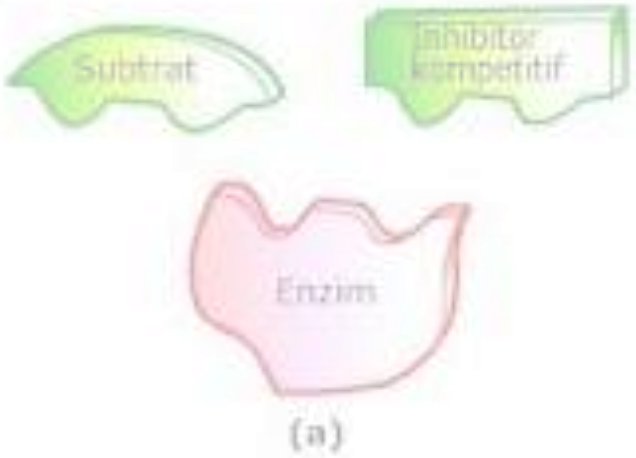
$$\frac{1}{v} = \frac{1}{v_{max}} + \frac{Km}{v_{max}}\left(1 + \frac{[I]}{Ki}\right)\frac{1}{[S]}$$

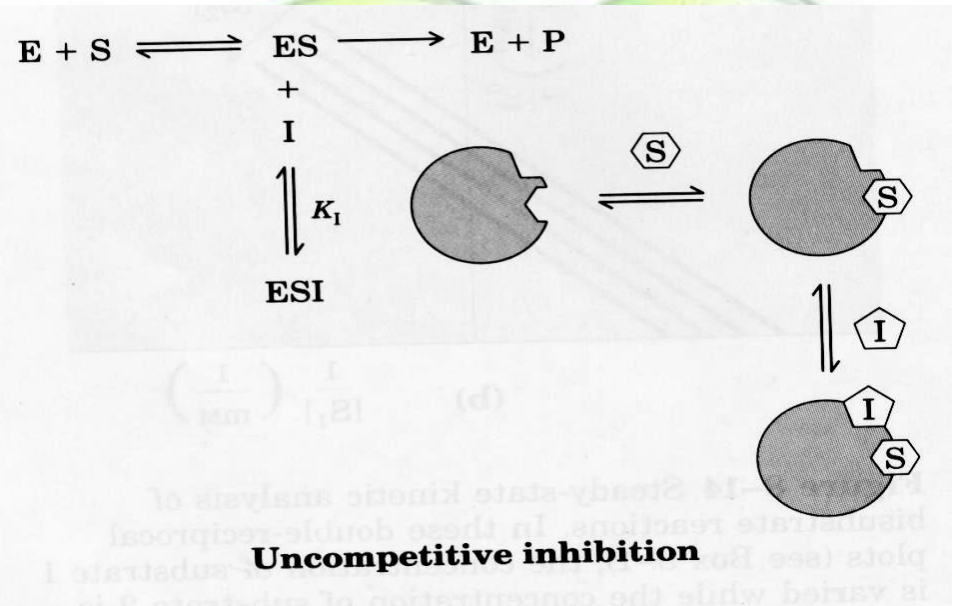
$$K'm = Km\left(1 + \frac{[I]}{Ki}\right)$$



Inhibisi uncompetitive







Inhibisi uncompetitive

$$E + S + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES + I \overset{k_1 i}{\rightleftharpoons} ESI$$

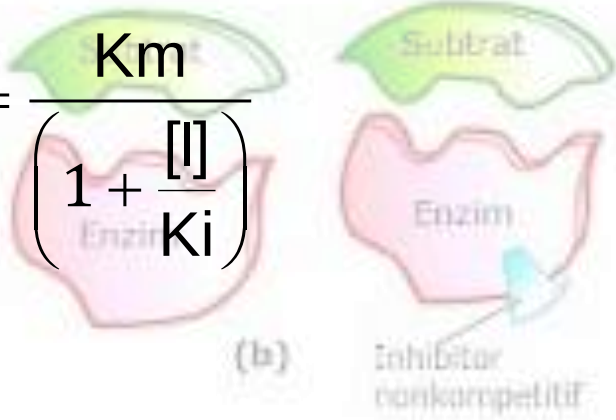
$$ES \longrightarrow E + P$$

$$[ET] = [ES] + [E] + [ESI]$$

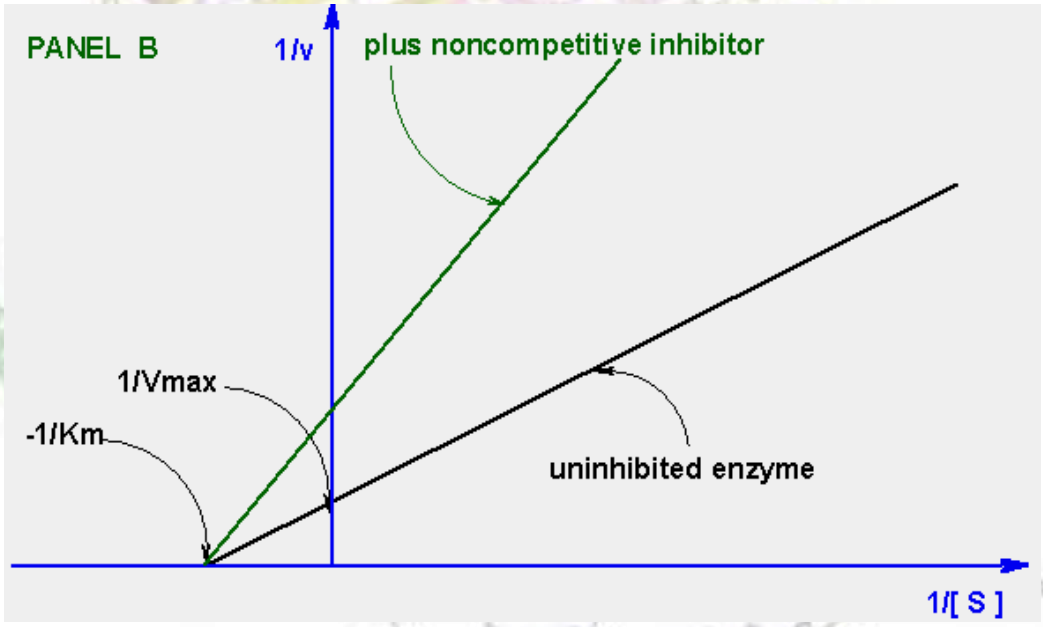
$$v = \frac{\frac{v_{max}}{\left(1 + \frac{[I]}{Ki}\right)}[S]}{\frac{Km}{\left(1 + \frac{[I]}{Ki}\right)} + [S]}$$

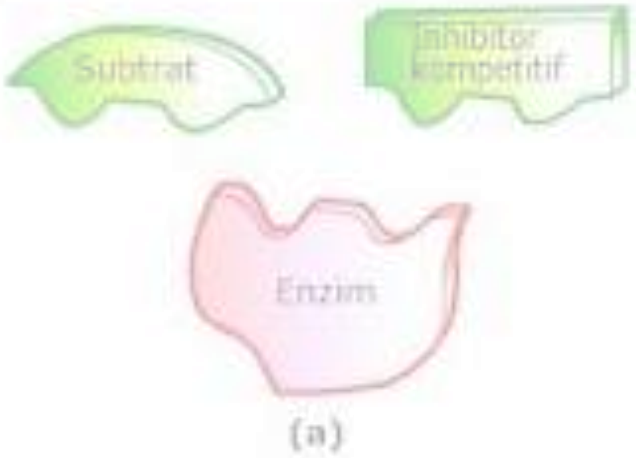
$$\frac{1}{v} = \frac{Km}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}}\left(1 + \frac{[I]}{Ki}\right)$$

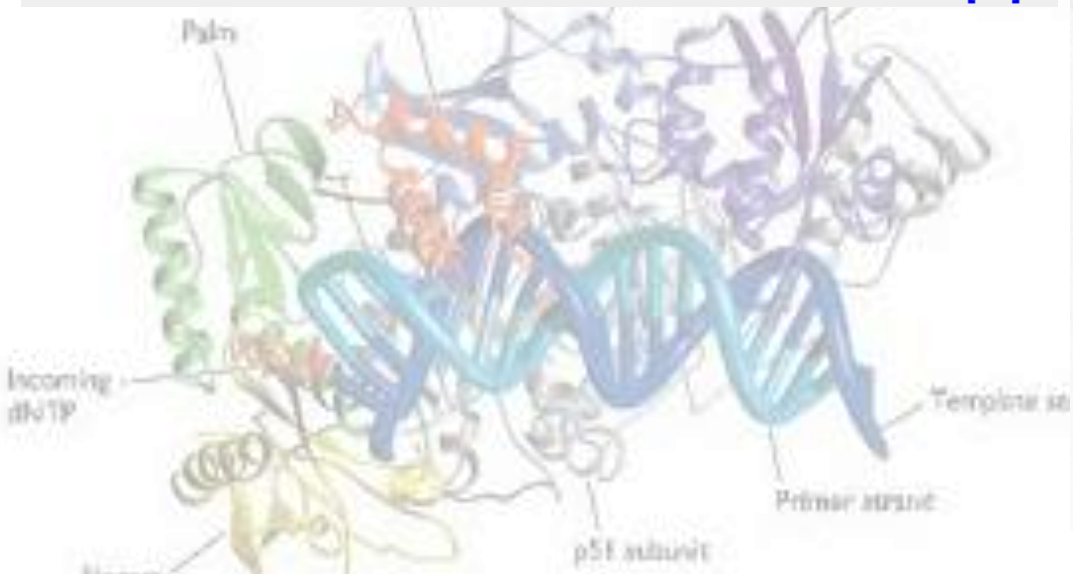
$$K'm = \frac{Km}{\left(1 + \frac{[I]}{Ki}\right)}$$

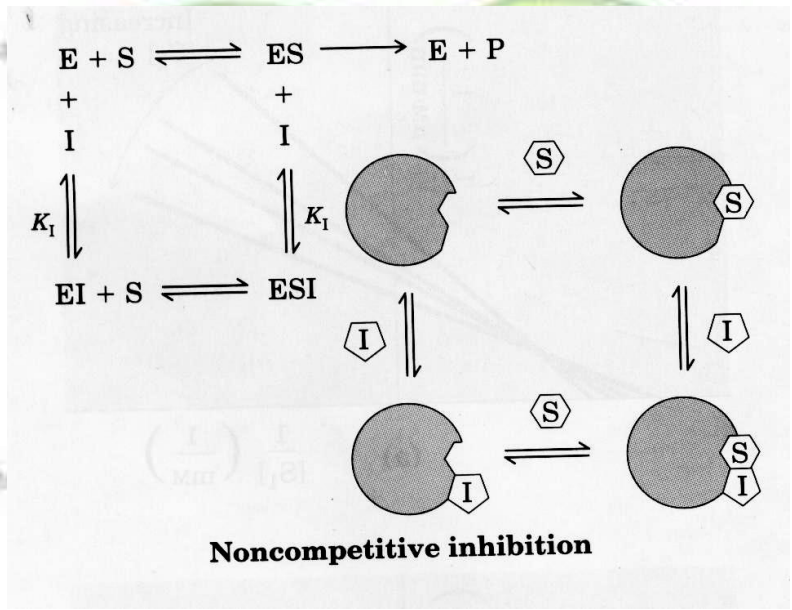


Inhibisi non-kompetitif









Inhibisi non-kompetitif

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

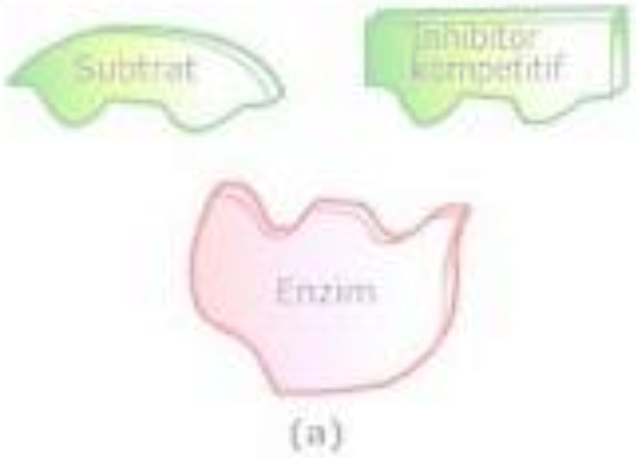
$$E + I \overset{ki}{\rightleftarrows} EI$$

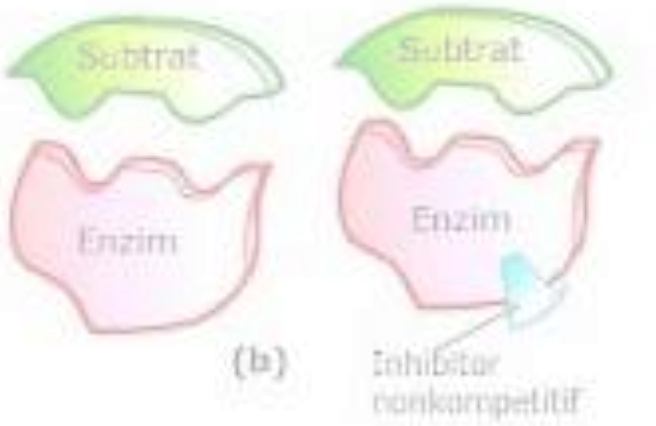
$$ES + I \overset{ki}{\rightleftarrows} ESI$$

$$[ET] = [ES] + [E] + [ESI]$$

$$v = \frac{v_{max}[S]}{Km + [S]} \frac{1}{1 + \frac{[I]}{Ki}}$$

$$\frac{1}{v} = \left(\frac{Km}{v_{max}} \frac{1}{[S]} + \frac{1}{v_{max}} \right) \left(1 + \frac{[I]}{Ki} \right)$$



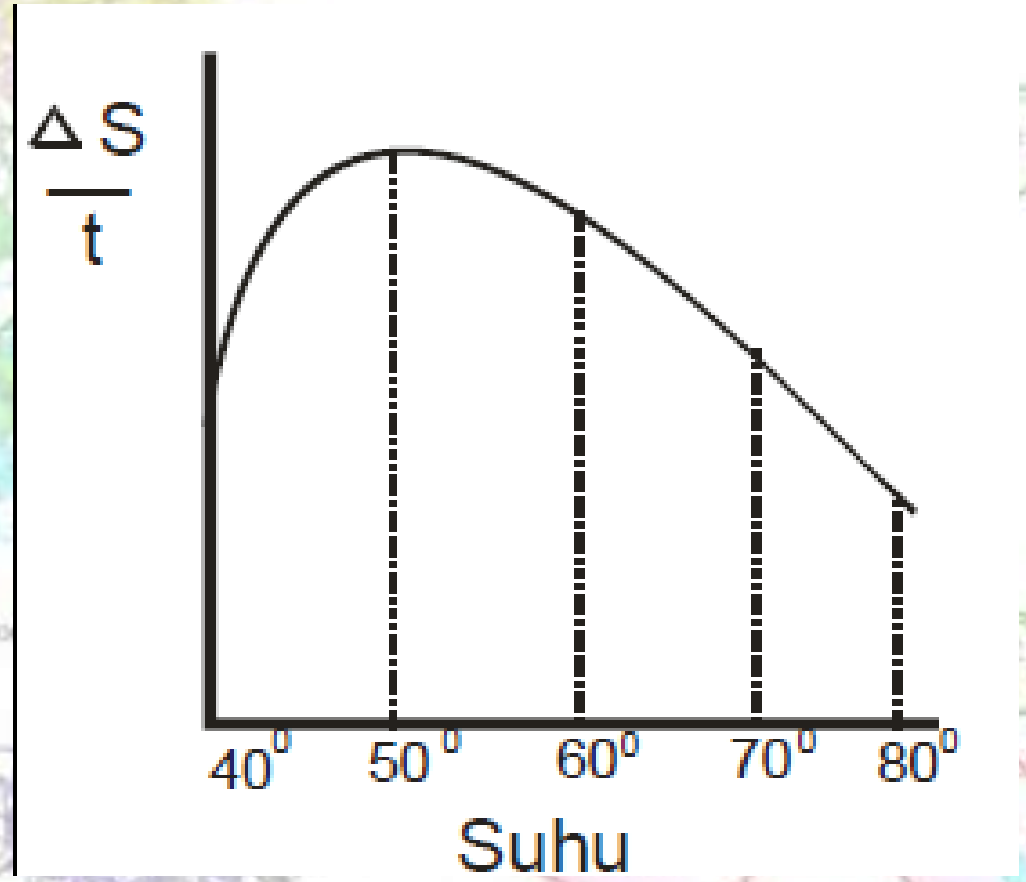


Faktor yang mempengaruhi reaksi enzimatis

- pH → perubahan struktur, ionisasi, pengikatan enzim
  - pH mempengaruhi muatan
  - Muatan mempengaruhi struktur enzim
  - Selanjutnya akan mempengaruhi reaksi ES

- Suhu

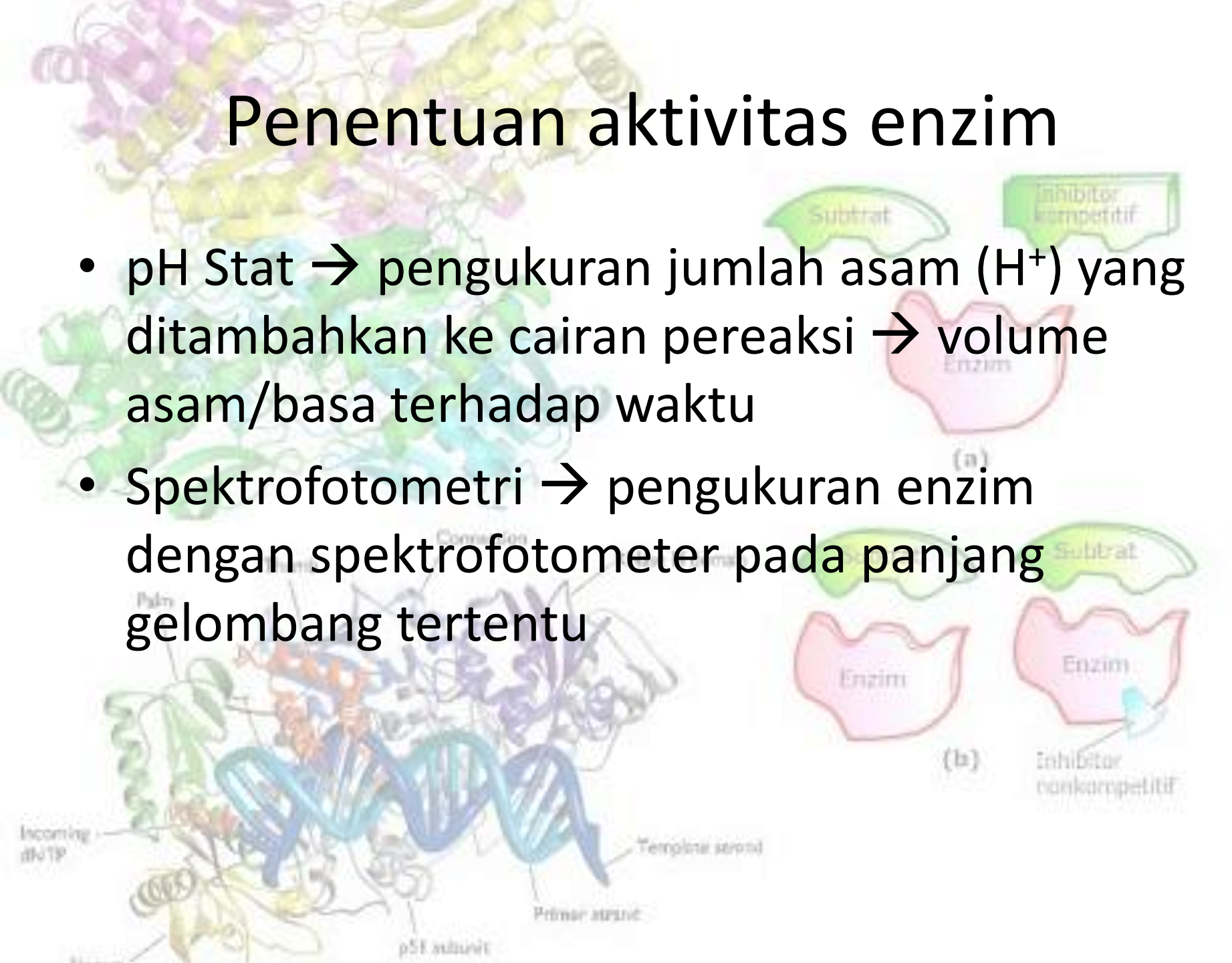
$$k = Ae^{-E/RT}$$



- Pereaksi kimia

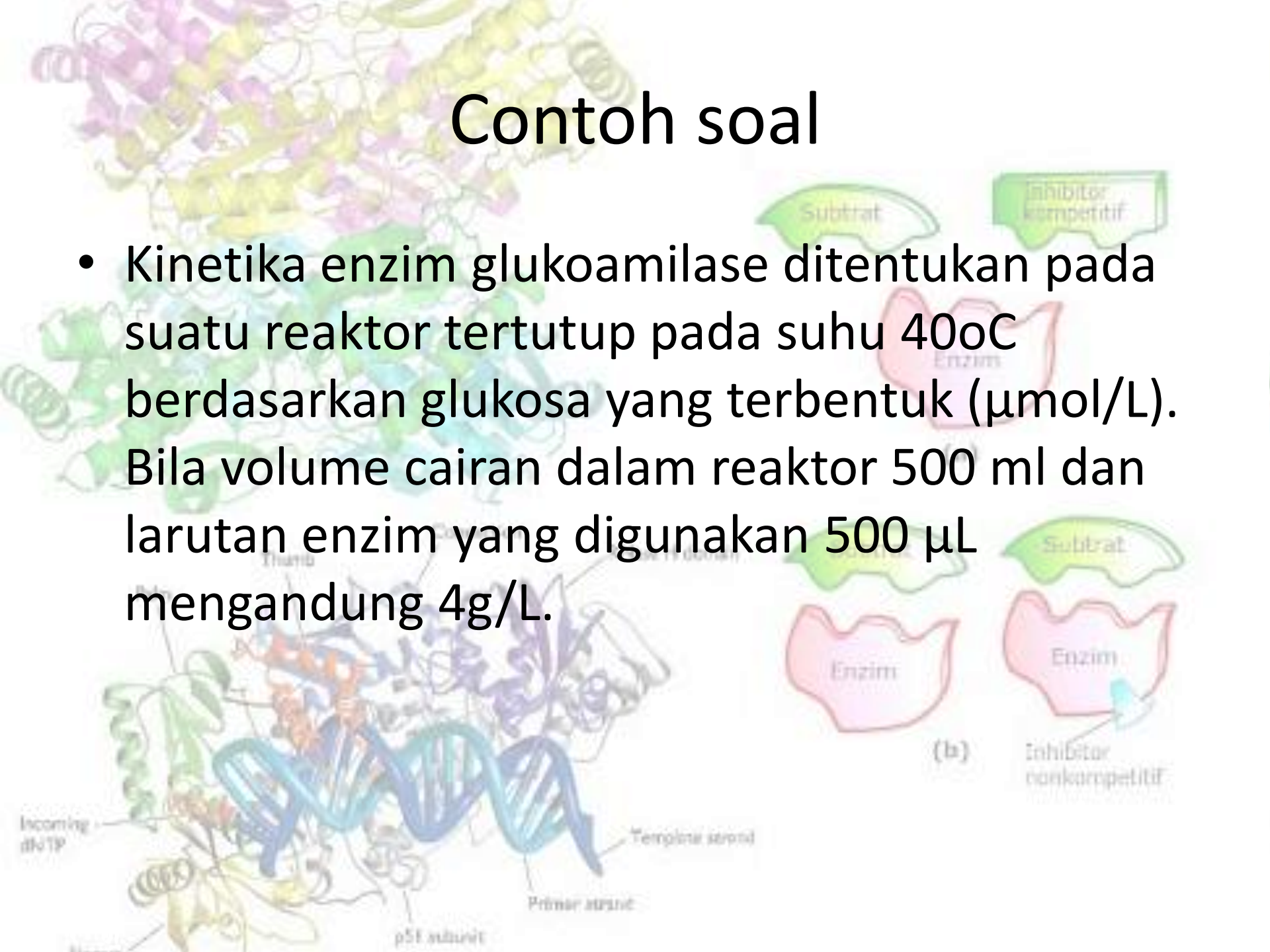
Penentuan aktivitas enzim

- pH Stat → pengukuran jumlah asam (H^+) yang ditambahkan ke cairan pereaksi → volume asam/basa terhadap waktu
- Spektrofotometri → pengukuran enzim dengan spektrofotometer pada panjang gelombang tertentu



Contoh soal

- Kinetika enzim glukoamilase ditentukan pada suatu reaktor tertutup pada suhu 40oC berdasarkan glukosa yang terbentuk (µmol/L). Bila volume cairan dalam reaktor 500 ml dan larutan enzim yang digunakan 500 µL mengandung 4g/L.



Waktu (menit)	Konsentrasi awal maltosa (mM)			
	50	**100**	**150**	**200**
2	95	110	130	140
5	230	290	320	340
10	440	570	640	670
15	700	860	960	1.000
30	1.400	1.700	1.900	2.000

- Bagaimana bentuk kinetika hidrolisis maltosa oleh enzim glukoamilase?
- Glukosa sebagai produk juga berfungsi sebagai inhibitor. Kinetika penghambatannya ditunjukkan pd tabel di bawah. Tentukan model kinetika penghambatan

Glukosa (mM)	Konsentrasi substrat maltosa (mM)			
	50	**100**	**150**	**200**
0	5,8	7,2	8	8,4
2,5	5,2	6,9	7.6	8,1
5	4,8	6,4	7,3	7,9
10	4,1	5,8	6,7	7,4

- TRK meeting 5.xlsx