Jure Stojan
4. predavanje

Inhibicija - aktivacija - modulacija encimske katalize
hitre kinetične metode
matematično modeliranje - analiza podatkov

Inhibicija - aktivacija - modulacija

so pojavi pri katerih snovi spremenijo encimsko aktivnost. Običajno uporabljamo izraz “encimski inhibitorji”. Kadar snov aktivira delovanje encima to posebej povemo.

1 Reverzibilna

- kompetitivna
- nekompetitivna
- akompetitivna
- mešana

2 Ireverzibilna

1-2 Inhibicija z analogi prehodnega stanja

- počasna vezava
- tesna počasna vezava

3 Inhibicija - aktivacija - agregacija - inaktivacija s kovinskimi ioni

Kompetitivna inhibicija

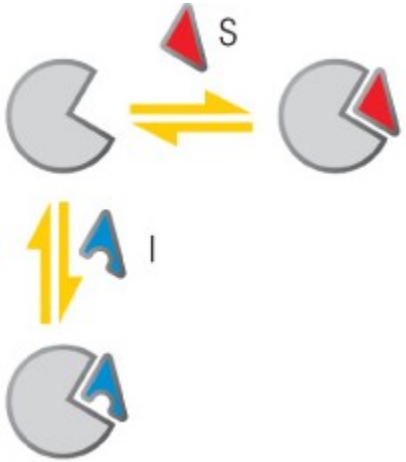
$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$+$$

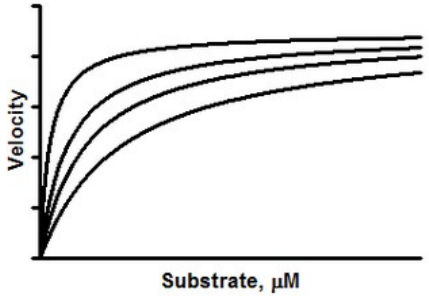
$$I$$

$$\Big\updownarrow K_i$$

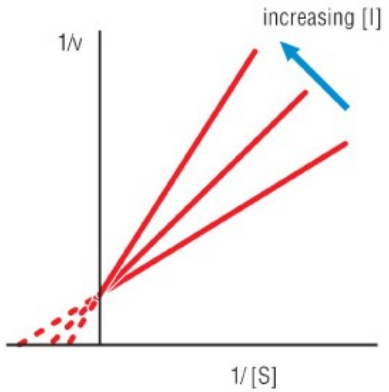
$$EI$$



$$v = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)}$$

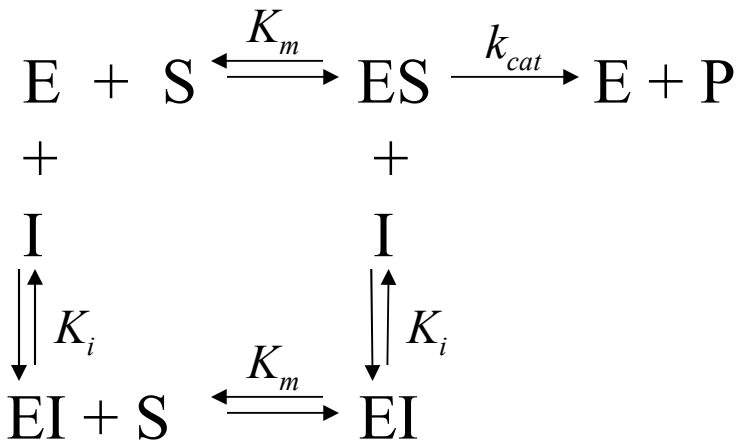


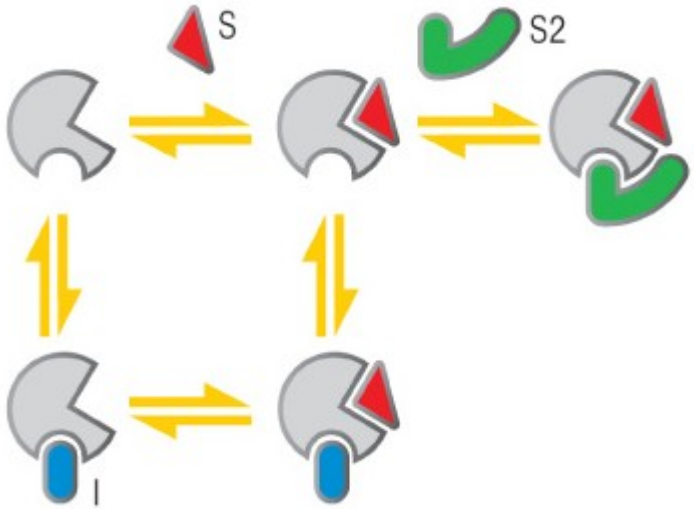
$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]}\left(1 + \frac{[I]}{K_i}\right) + \frac{1}{V_{max}}$$



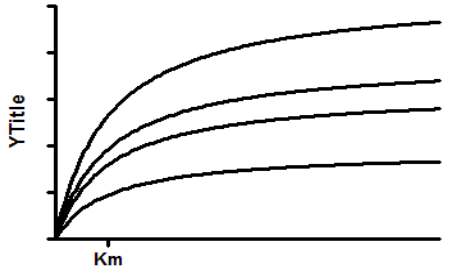
Afiniteta za inhibitor podobna tisti za substrat: mM

Nekompetitivna inhibicija

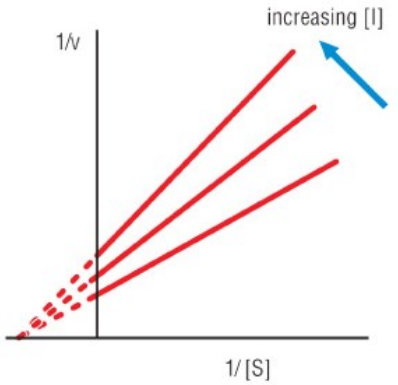




$$v = \frac{V_{max}[S]}{\left([S] + K_m\right)\left(1 + \frac{[I]}{K_i}\right)}$$

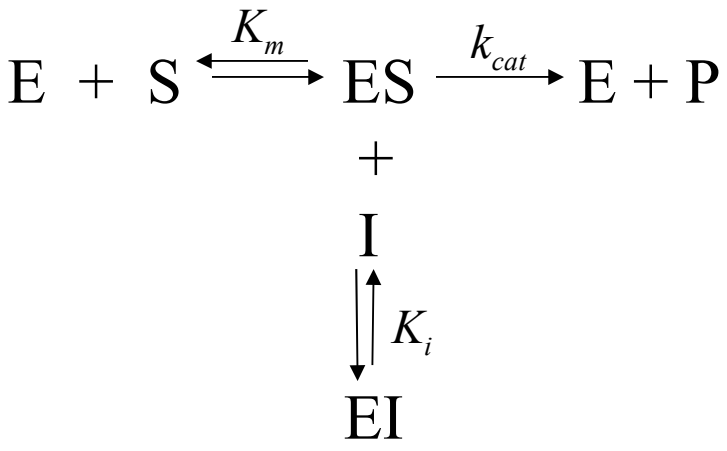


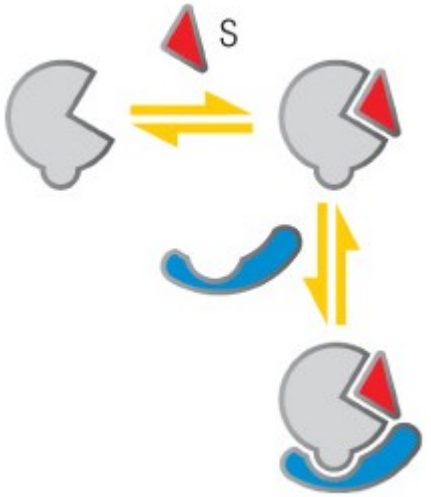
$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]}\left(1 + \frac{[I]}{K_i}\right) + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$



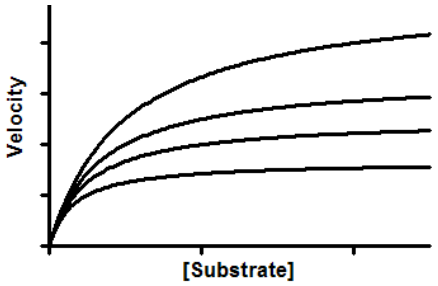
Afiniteta za inhibitor: µM

Akompetitivna inhibicija

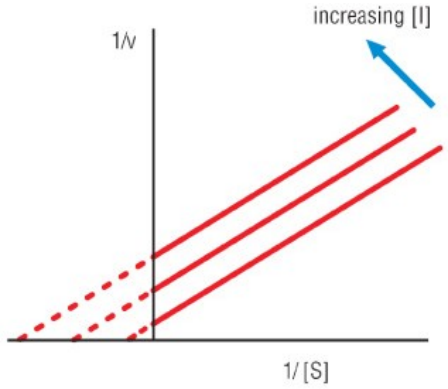




$$v = \frac{V_{max}[S]}{Km + [S]\left(1 + \frac{[I]}{K_i}\right)}$$

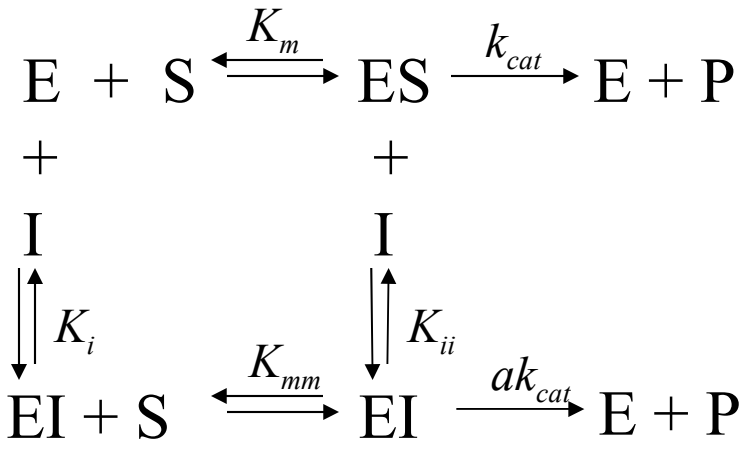


$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$



Afiniteta za inhibitor: μM

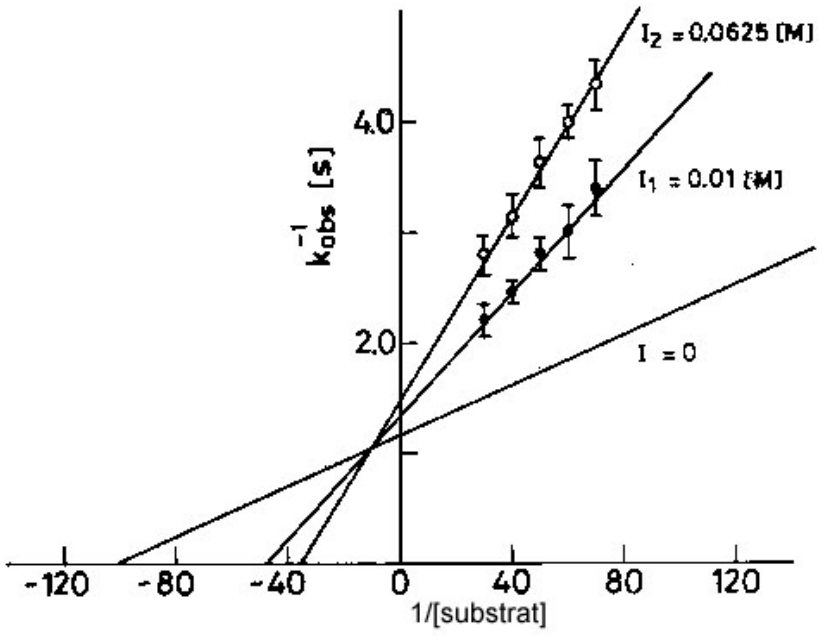
Mešana in parcialna inhibicija



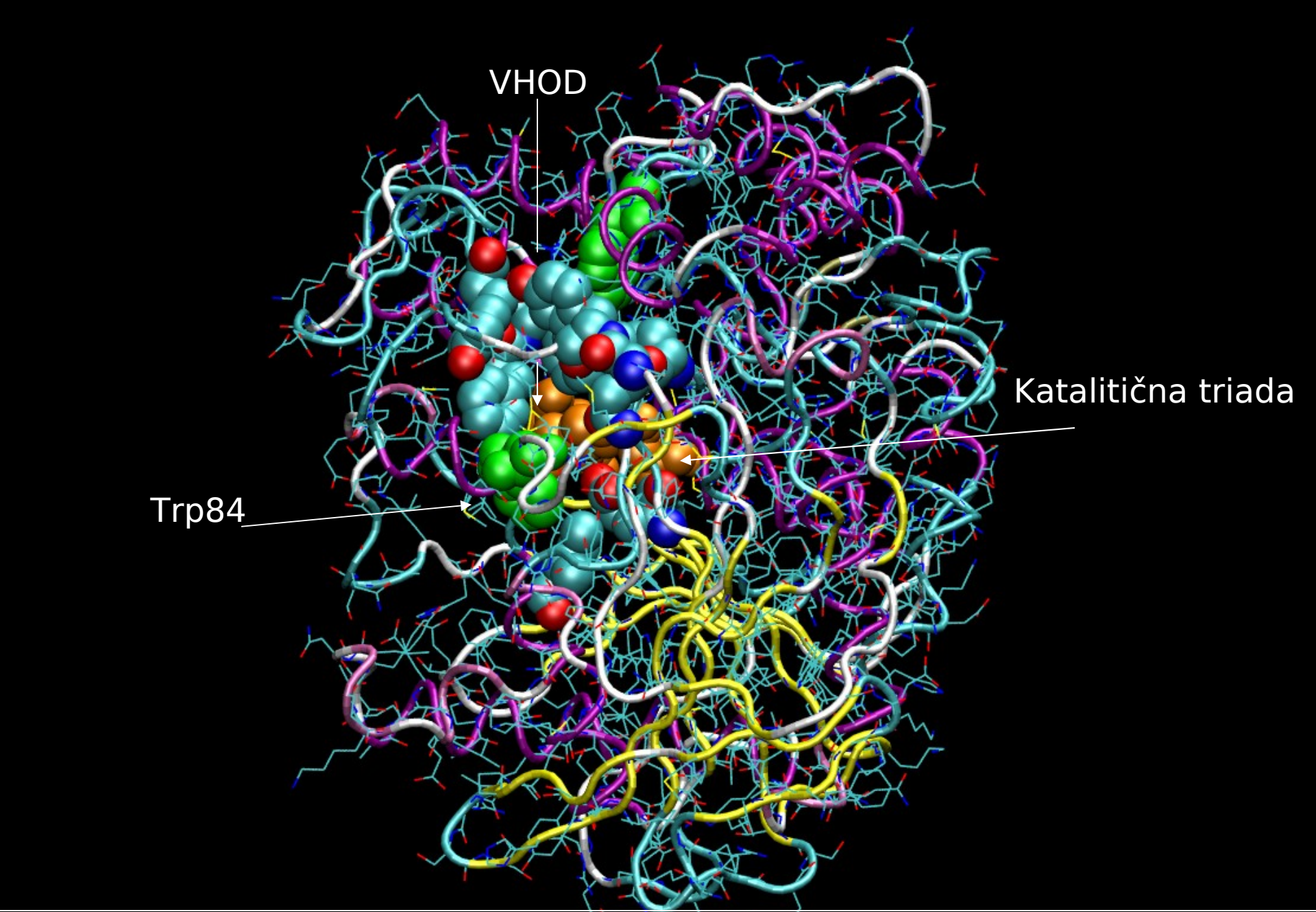
Mešana: $K_m \neq K_{mm}$

velja: $K_m/K_{mm} = K_i/K_{ii}$

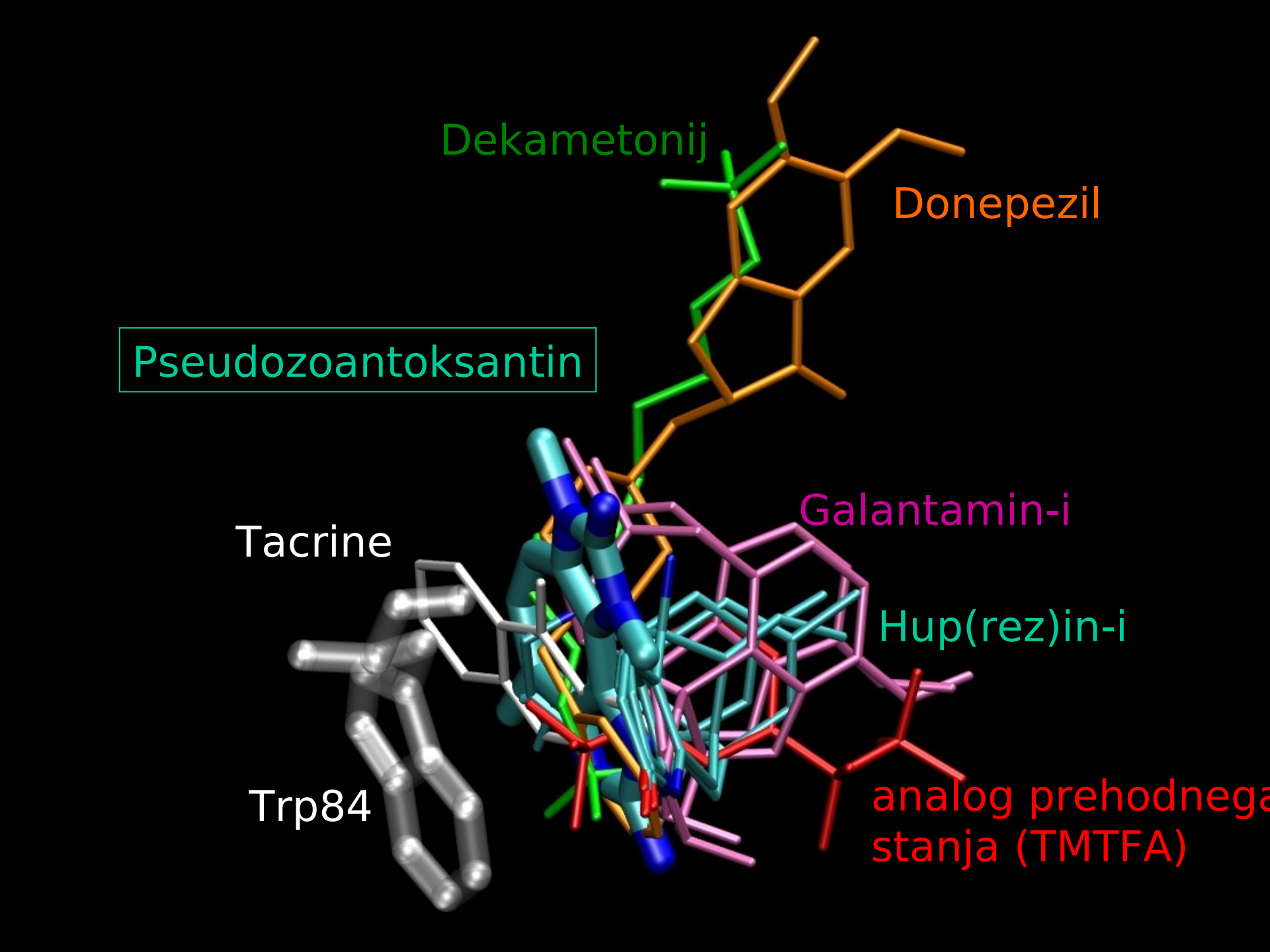
Parcialna: $a \neq 1$



Afiniteta za inhibitor: μM

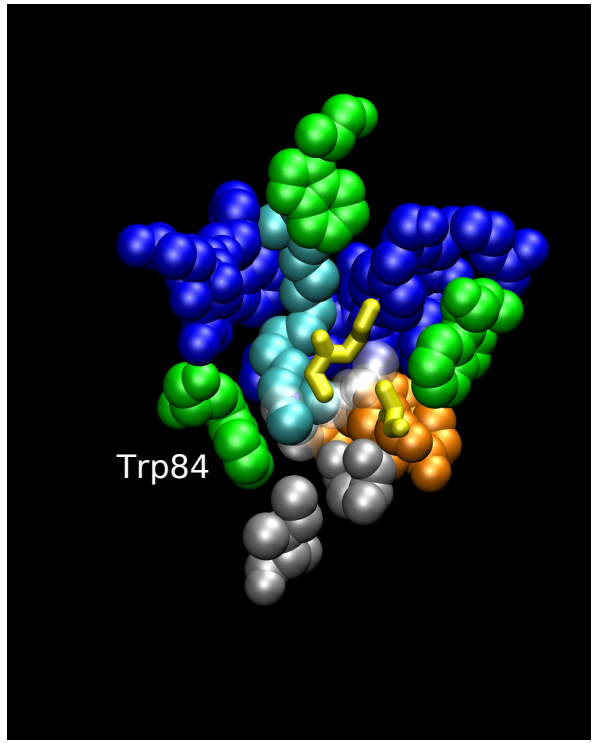


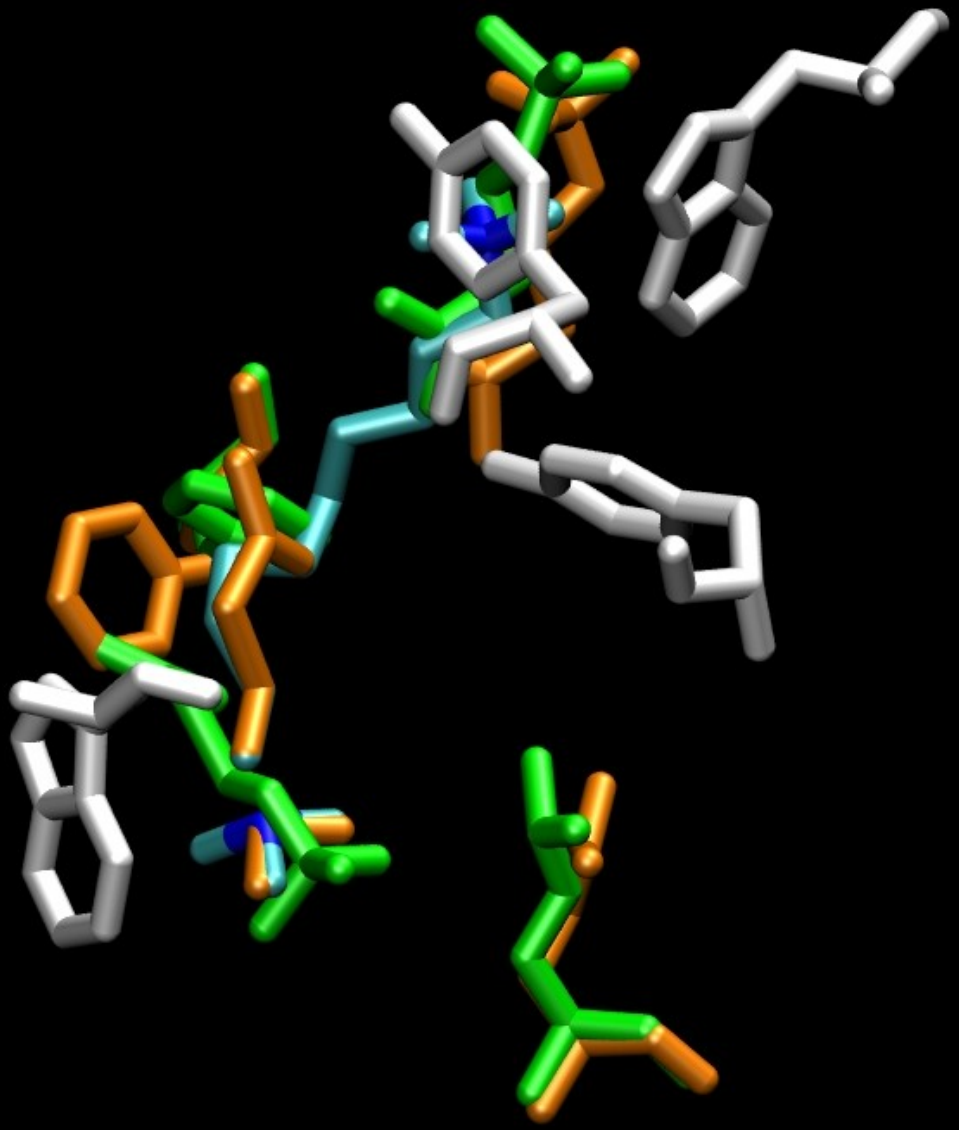
Acetilholinesteraza iz kalifornijskega električnega skata - TorcaAChE - 1


Dekametonij
Donepezil
Pseudozoantoksantin
Galantamin-i
Tacrine
Hup(rez)in-i
Trp84
analog prehodnega
stanja (TMTFA)

Dekametonij

Acetiltioholin





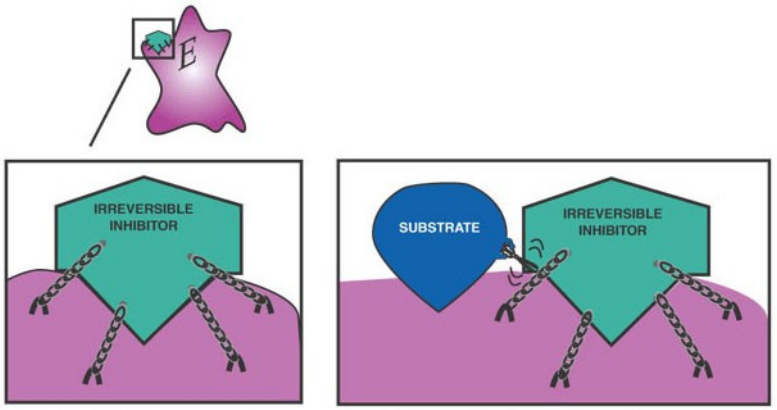
Ireverzibilna inhibicija

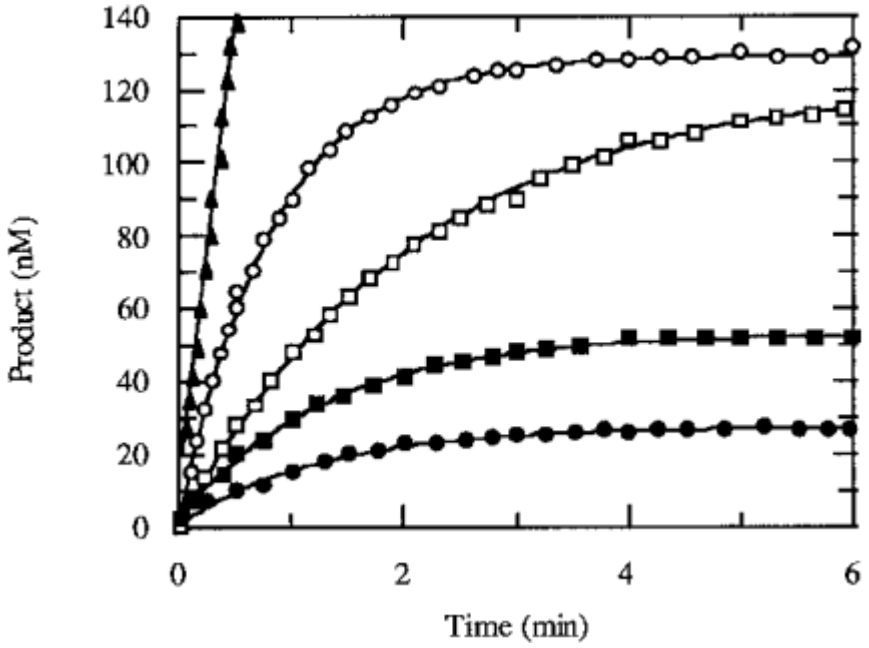
katalitični strupi, ki se kovalentno vežejo z amino kislinami v aktivnem mestu

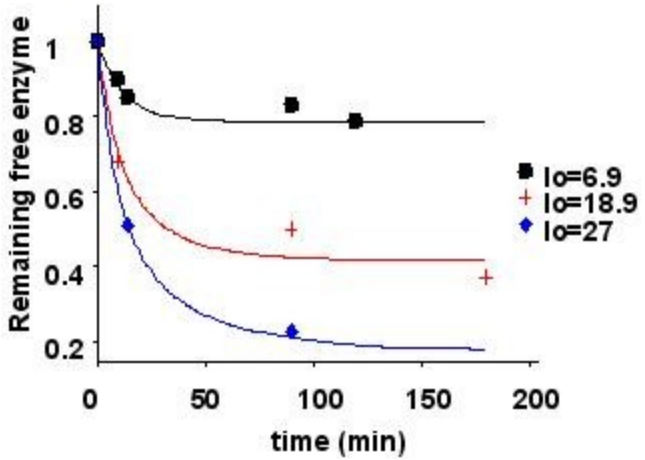
$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$+ \; I \xrightarrow{k_I} EI$$

Dializni poskus !

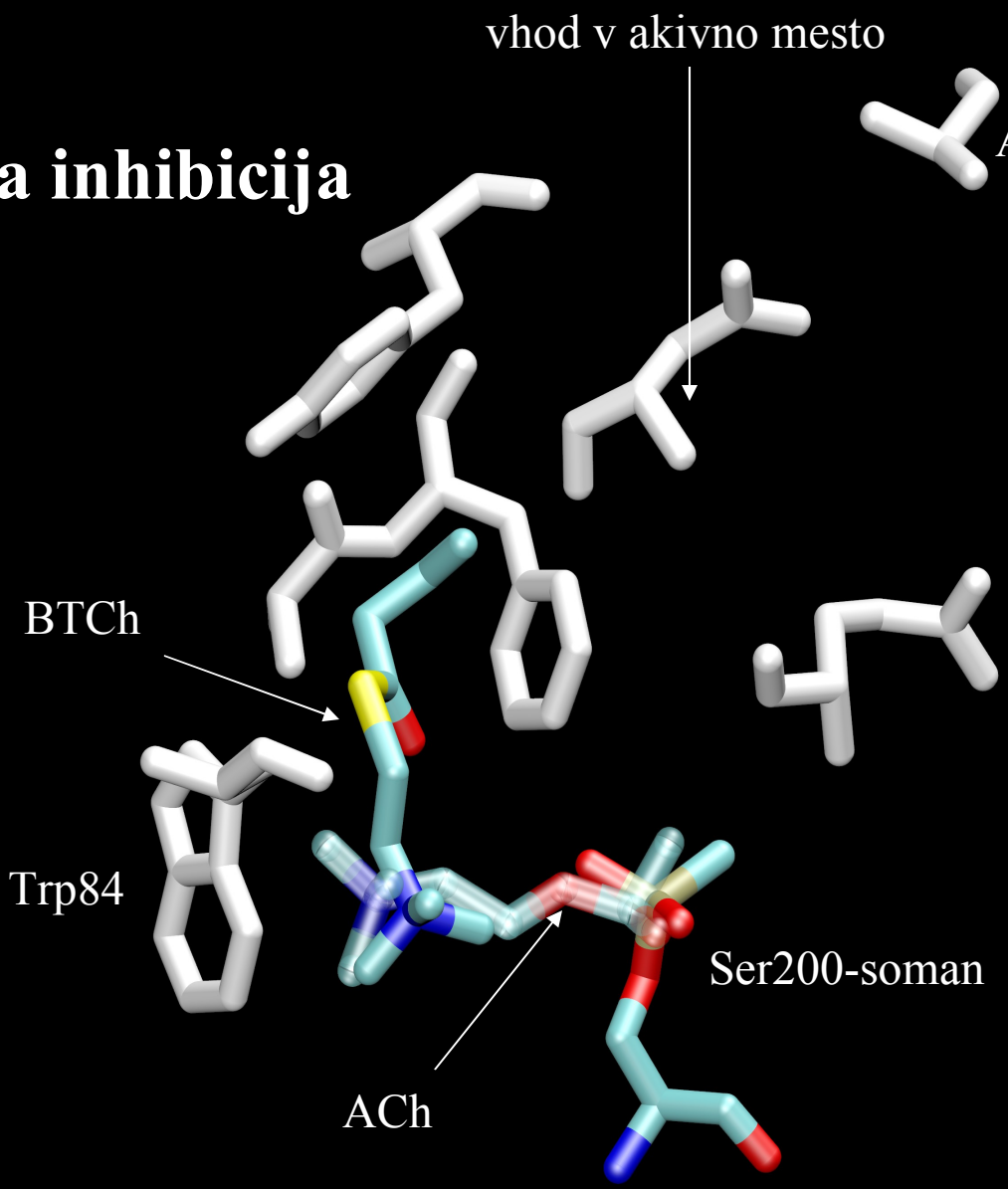






Afiniteta za inhibitor: neskončna

Ireverzibilna inhibicija



človeška butirilholinesteraza - HuBChE

Hitre kinetične metode in analiza podatkov

1. O hitrih kinetičnih metodah

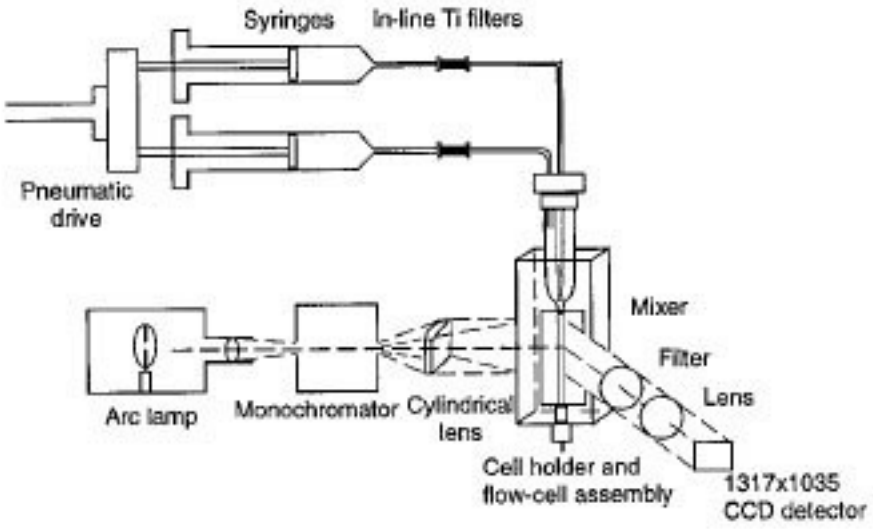
- mešalne-pretočne tehnike:
  continous flow, stopped flow

zmešamo, opazujemo nastajanje ravnotežnih ali stacionarnih stanj

- relaksacijske tehnike: temperaturni skok T-jump,
  fotoliza, pressure jump, ultrazvok, električno polje

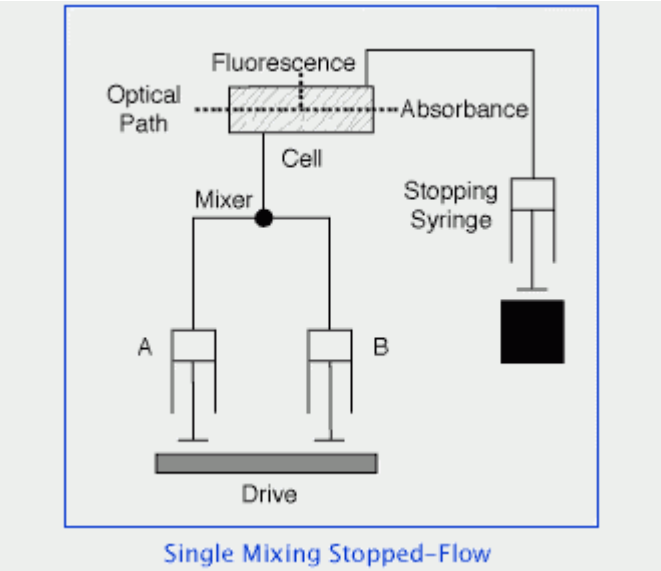
zmotimo, opazujemo vračanje sistema v ravnotežno stanje

Hitre kinetične metode in analiza podatkov

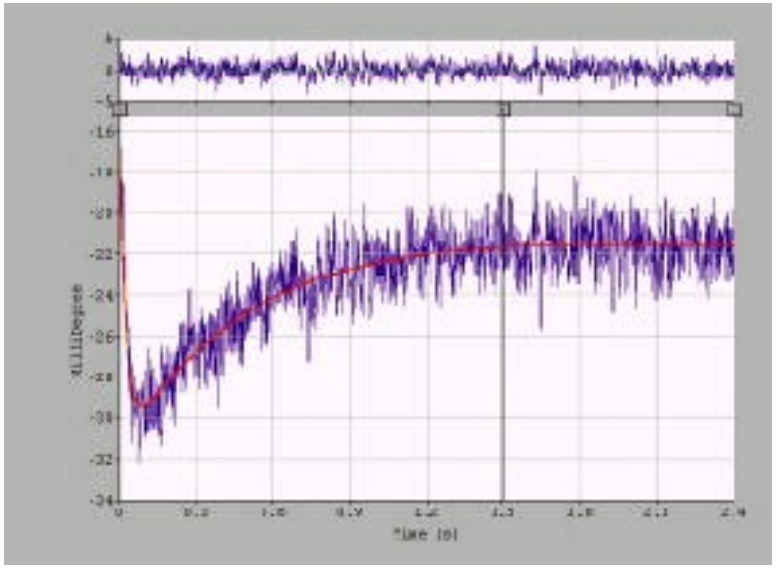


continous flow - metoda stalnega pretoka

Hitre kinetične metode in analiza podatkov

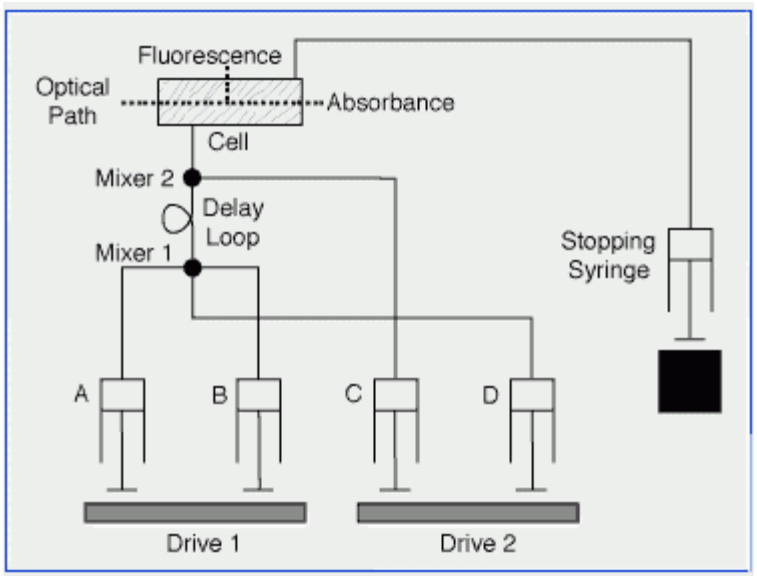


Single Mixing Stopped-Flow

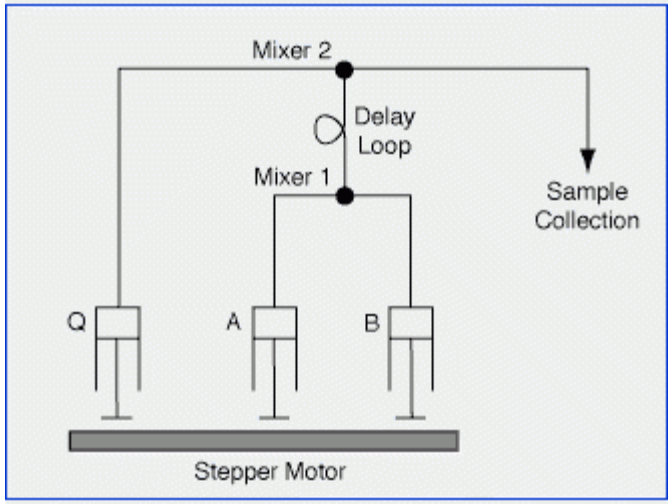


stopped flow - metoda nenadne prekinitve pretoka

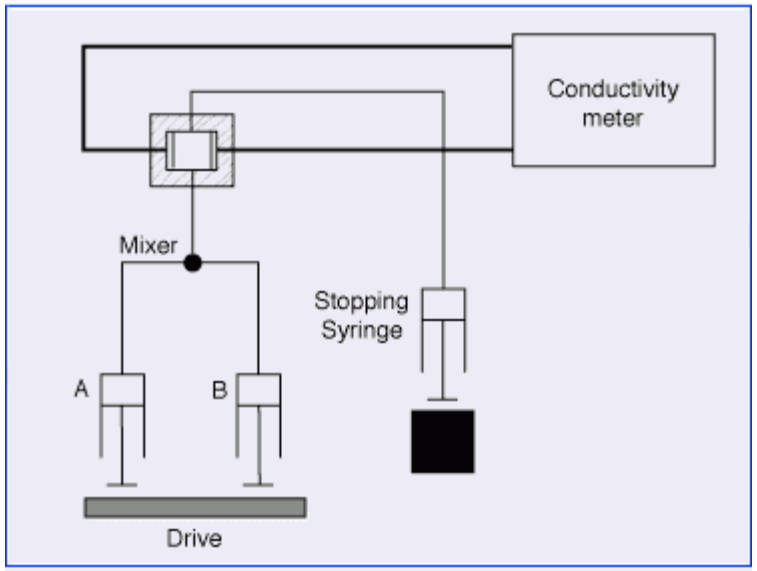
stopped flow - izpeljave - gašenje



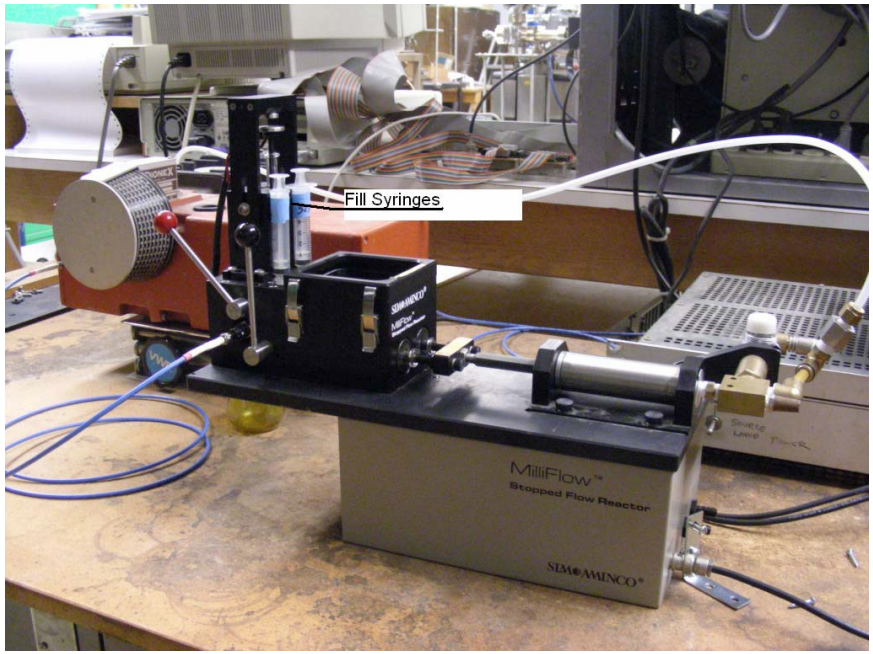
Double Mixing Stopped-Flow



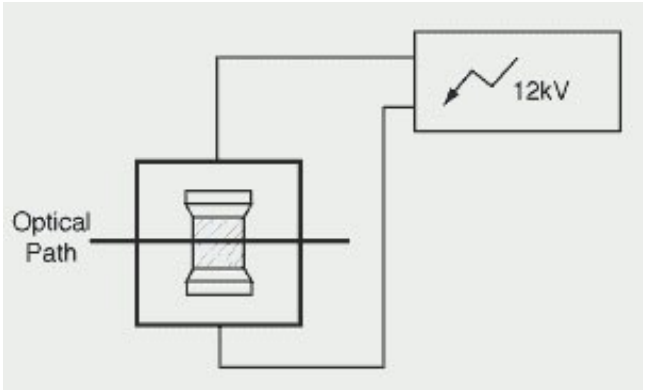
Quench Flow Plumbing (not to scale)

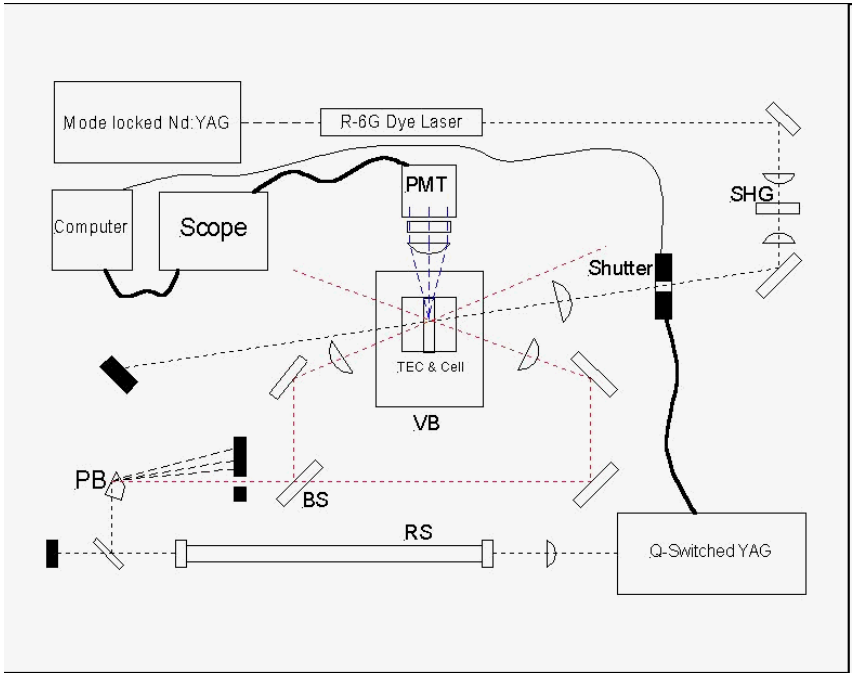


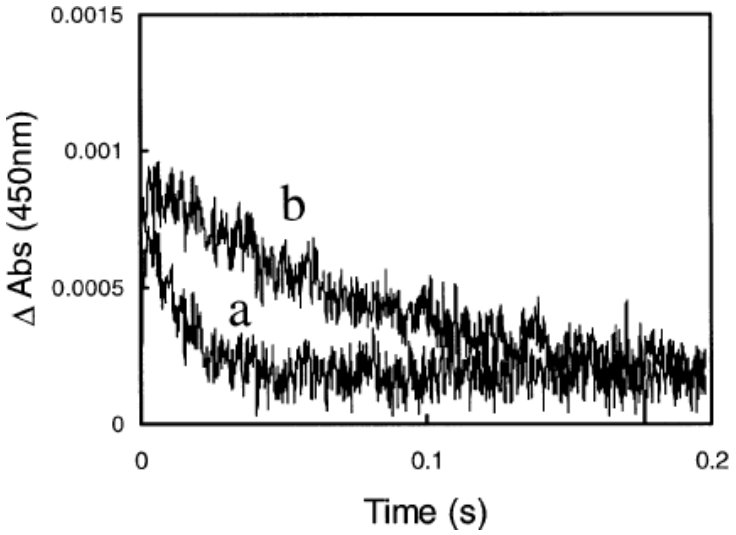
Conductivity Stopped-Flow (not to scale)



Hitre kinetične metode in analiza podatkov

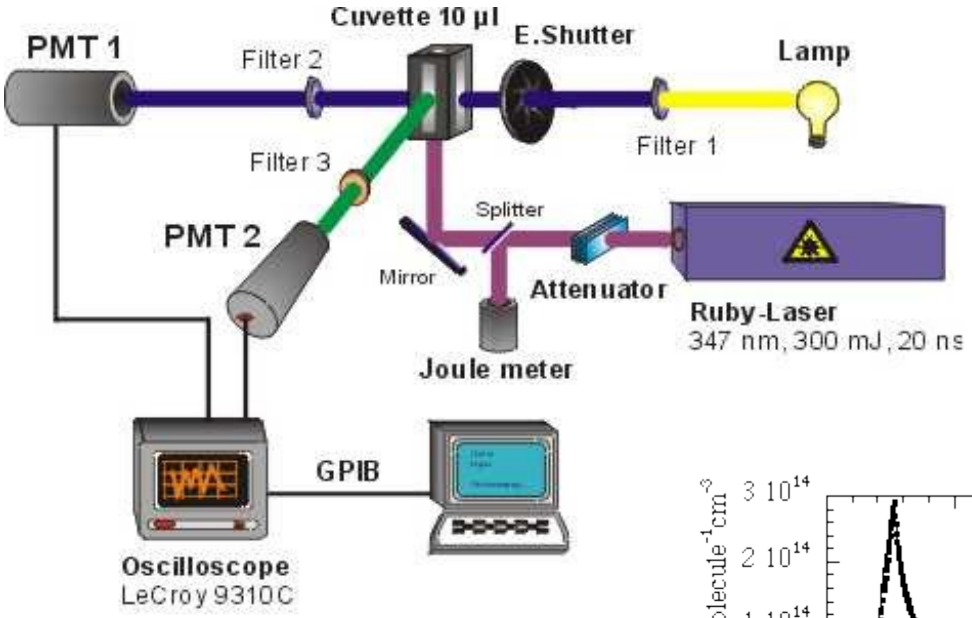




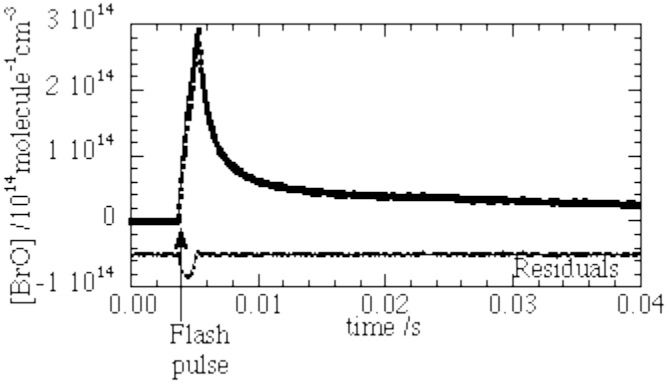


Temperaturni skok T - jump

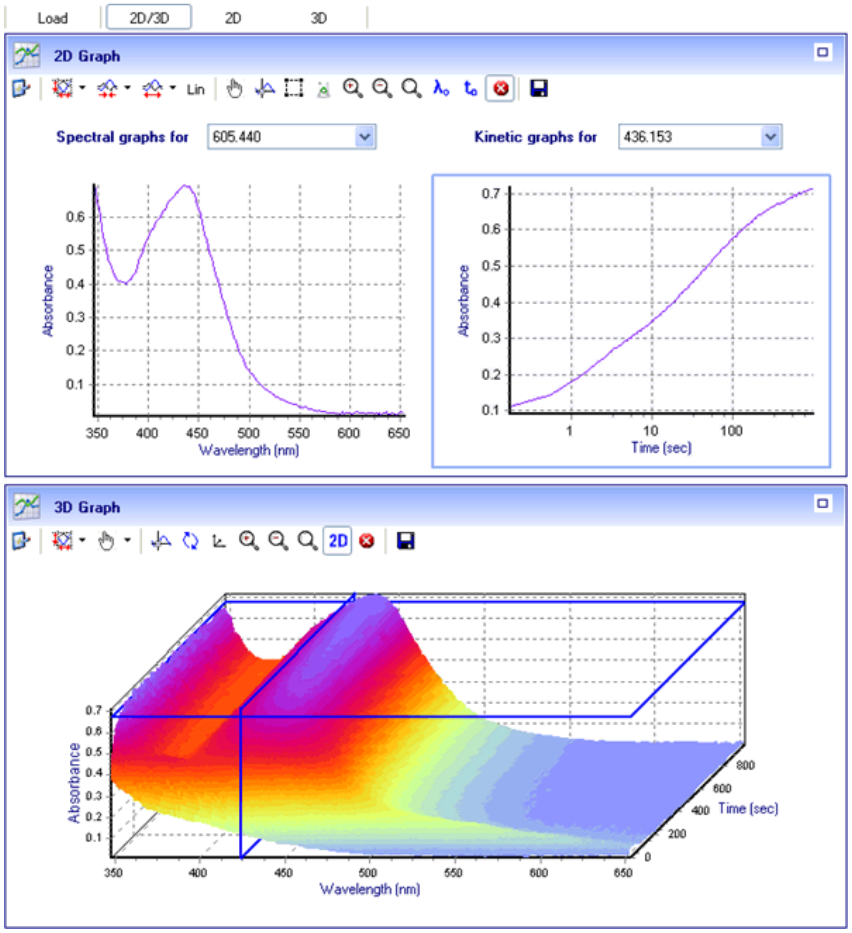
Hitre kinetične metode in analiza podatkov



Fotoliza z bliskavico - flash photolysis



Hitre kinetične metode in analiza podatkov



Hitre kinetične metode in analiza podatkov

2. Analiza podatkov - matematično modeliranje:
je večstopenjski postopek, s katerim želimo matematično formulirati zaporedje dogodkov v poteku biokemične reakcije ali zaporedja reakcij.

Rezultat so eksplicitne ali diferencialne enačbe,
skupaj z njihovimi parametri, ki hkrati popisujejo podatke iz različnih kinetičnih poskusov.

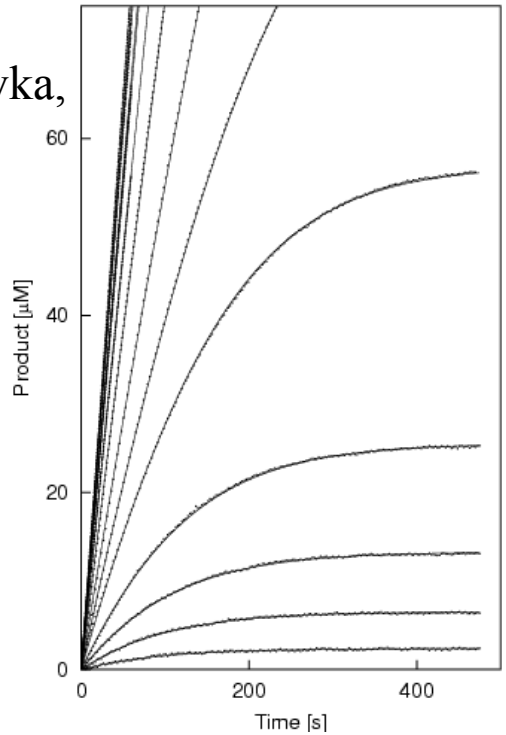
Hitre kinetične metode in analiza podatkov

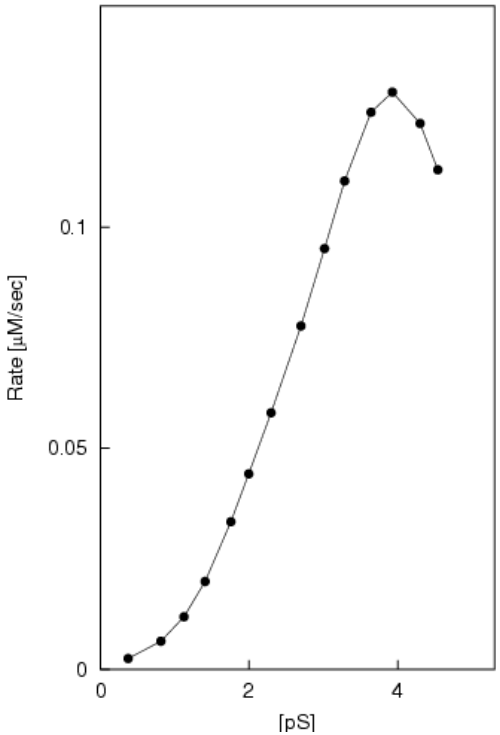
Proučevanje začetnih hitrosti:

1. ravnotežna in stacionarna predpostavka, (E) <<(S)
2. ne-ravnotežna predpostavka, (E) <<(S)
3. brez predpostavk

Proučevanje časovnega poteka reakcij (progress curves):

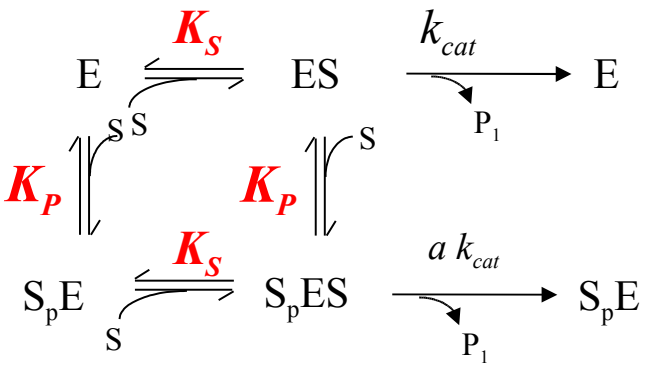
1. ravnotežna ali/in stacionarna predpostavka, (E) <<(S); analitične rešitve
2. brez predpostavk, ne-togi sistemi
3. brez predpostavk, togi sistemi





Hitre kinetične metode in analiza podatkov

Homotropična parcialna nekompetitivna inhibicija - substratna inhibicija



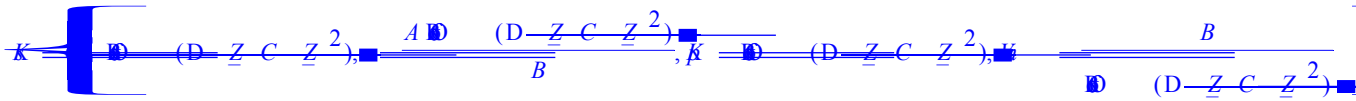
Webb's model

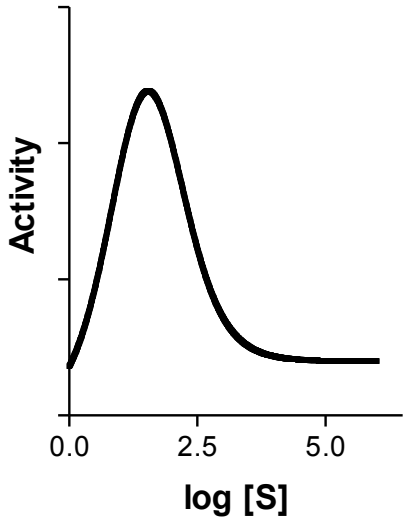
$$v = \frac{V_{MAX}[S]\left(K_P + a[S]\right)}{\left([S]+K_S\right)\left([S]+K_P\right)}$$

$$v = \frac{A[S]^2 + B[S]}{[S]^2 + C[S] + D}$$

$$A = aV_{MAX}$$
$$B = K_P V_{MAX}$$
$$C = K_S + K_P$$
$$D = K_S K_P$$

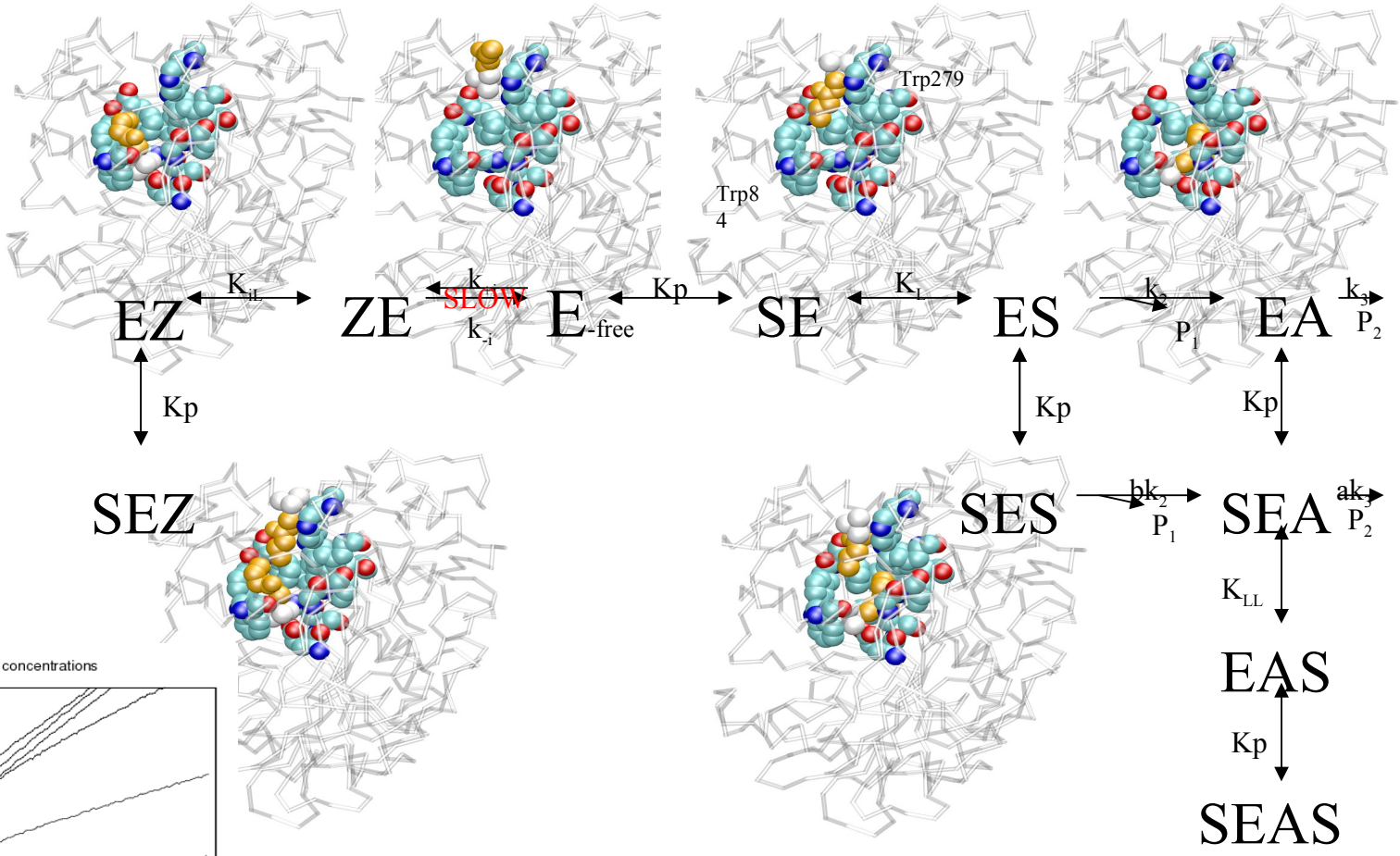
```
> solve( {A=a*Vmax, B=Kp*Vmax, C=Ks+Kp,
D=Kp*Ks}, {Ks,Kp,a,Vmax} );
```

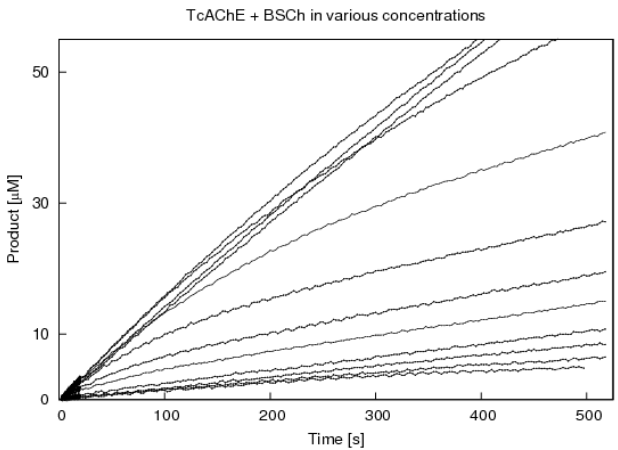




- grafični prikazi, klasična kinetična analiza, konstrukcija mehanističnih shem
- izpeljava enačb in določitev kinetičnih parametrov
- ločitev med konkurenčnimi modeli

Hitre kinetične metode in analiza podatkov





Neproduktivno vezanje - nonproductive binding

Matjaž Zorko
5. predavanje

Ponovitev predavane snovi

Kolokvij 1