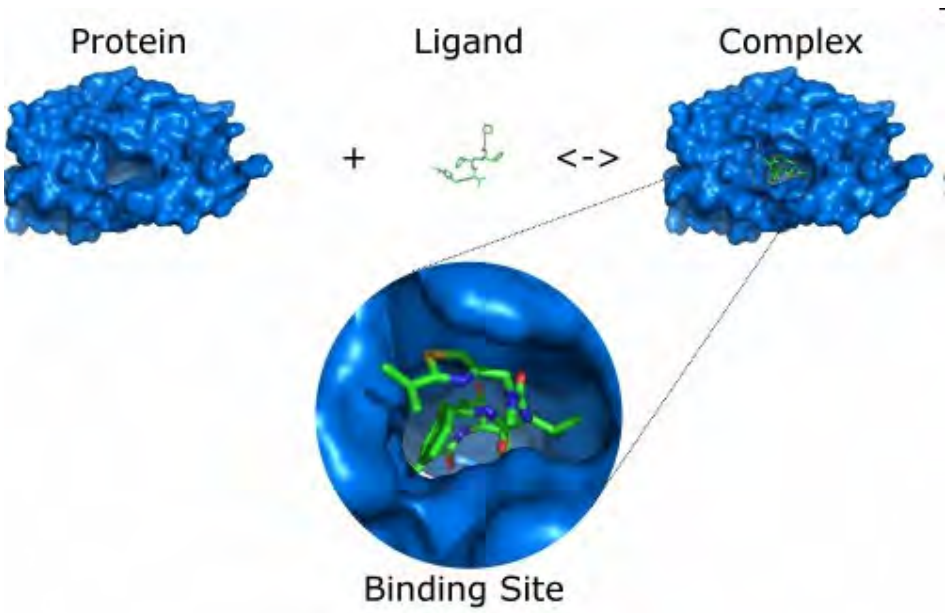
Protein - ligand (L) interakcije

(Aktivnost proteina: šta protein radi?)

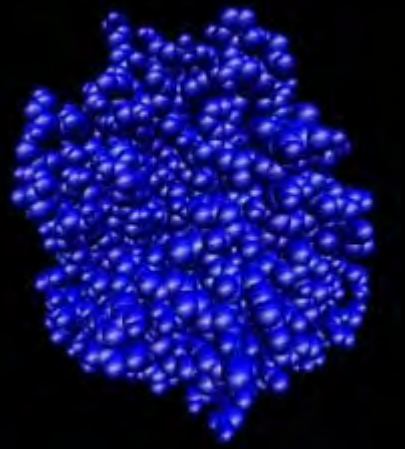


Zajednička osobina biološki aktivnih proteina je da specifično interagujusa malim brojem od prisutnih molekula u svom okruženju - ligandima (L) dajući protein-ligand (PL) kompleks.

Sadržaj predavanja

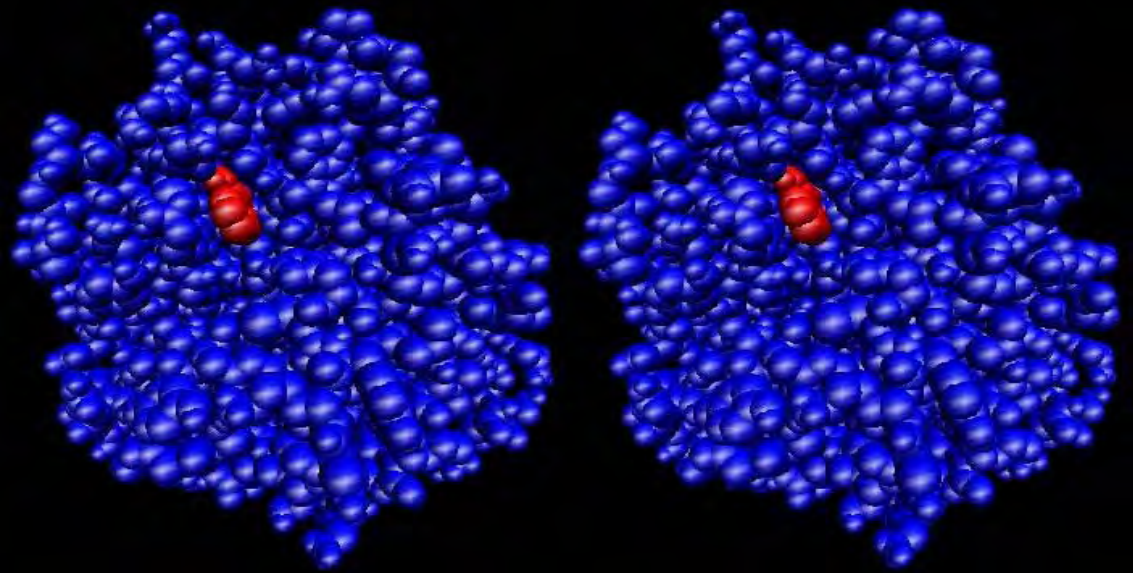
- Aktivnost proteina se zasniva na protein-ligand interakcijama:
  - Primeri za protein-ligand interakcije
    - Primeri za male molekule
    - Primeri za koenzime/kofaktore/prostetične grupe
    - Primeri za proteine
- Strukturne osnove specifičnog prepoznavanja i vezivanja liganda:
  - komplementarnost površina vezivnog mesta na molekulu proteina i molekula liganda
  - konstanta vezivanja i vezivna energija

Primer: aktivno mesto na molekulu enzima

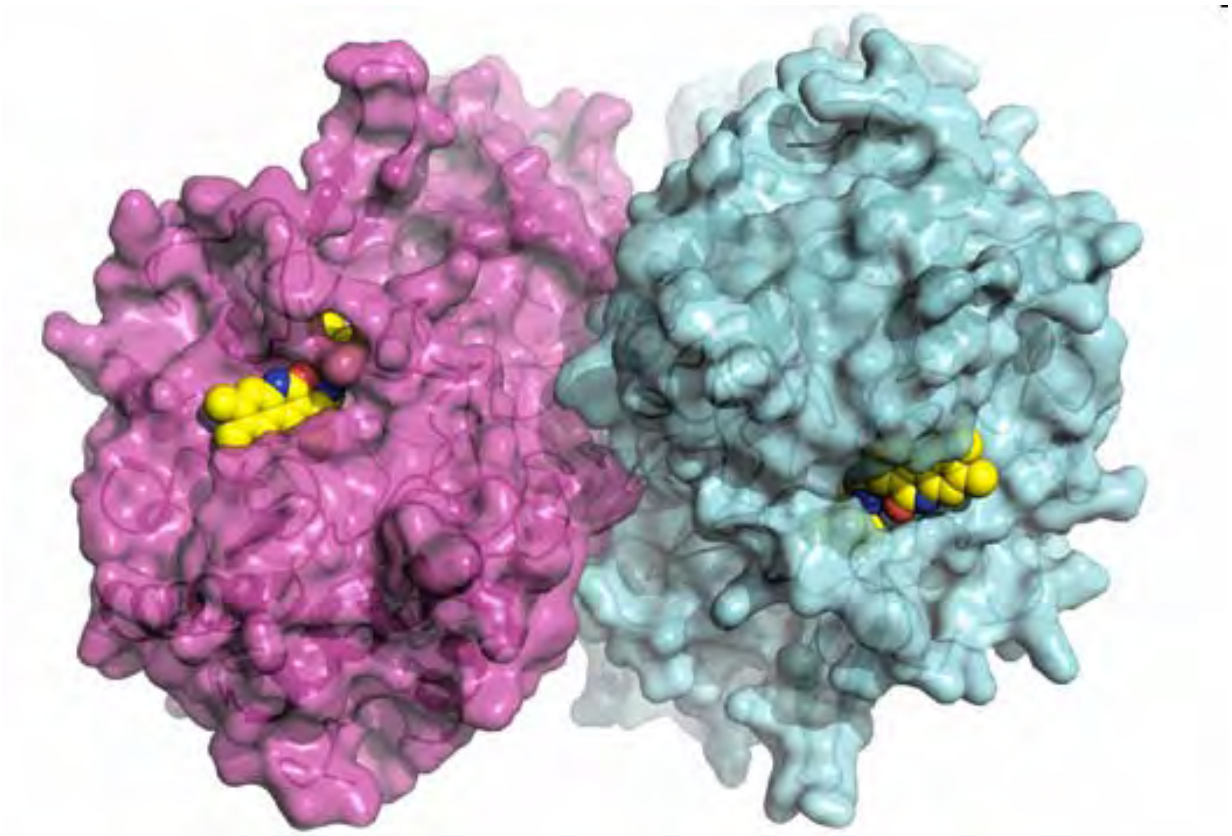


Tripsin

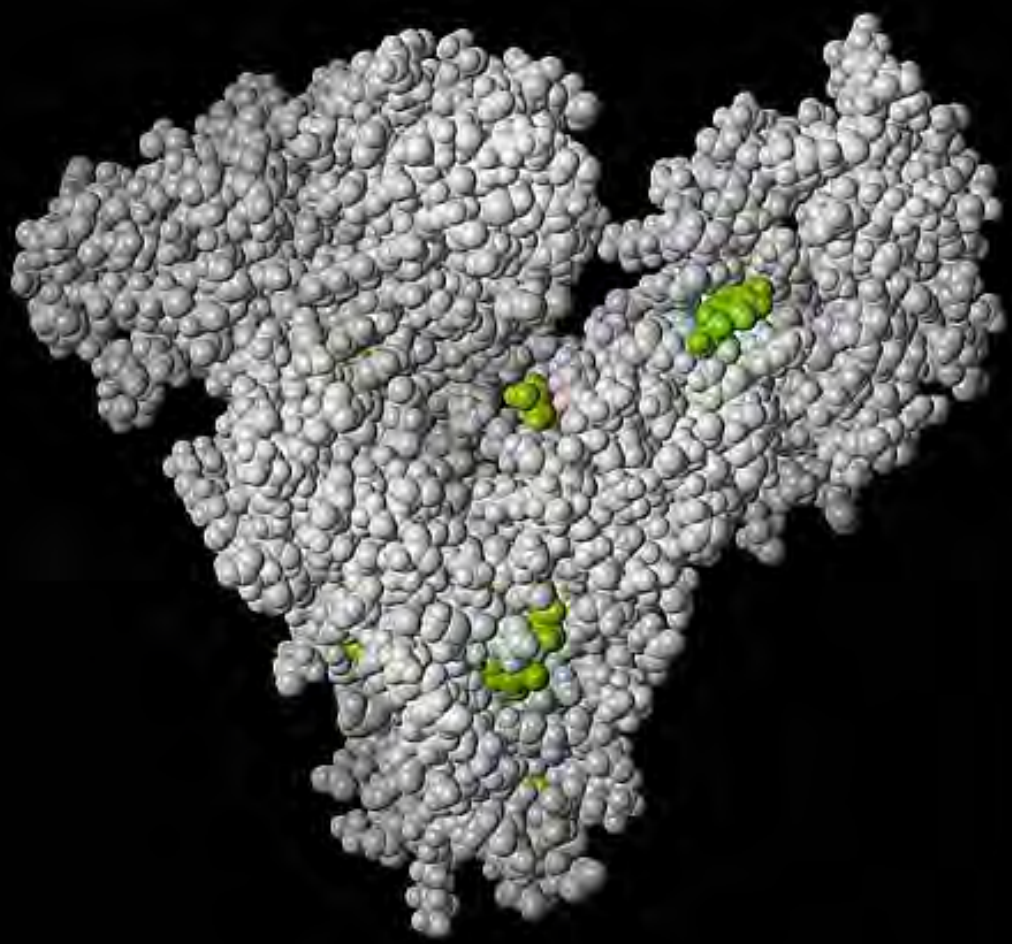
Benzidin: inhibitor tripsina



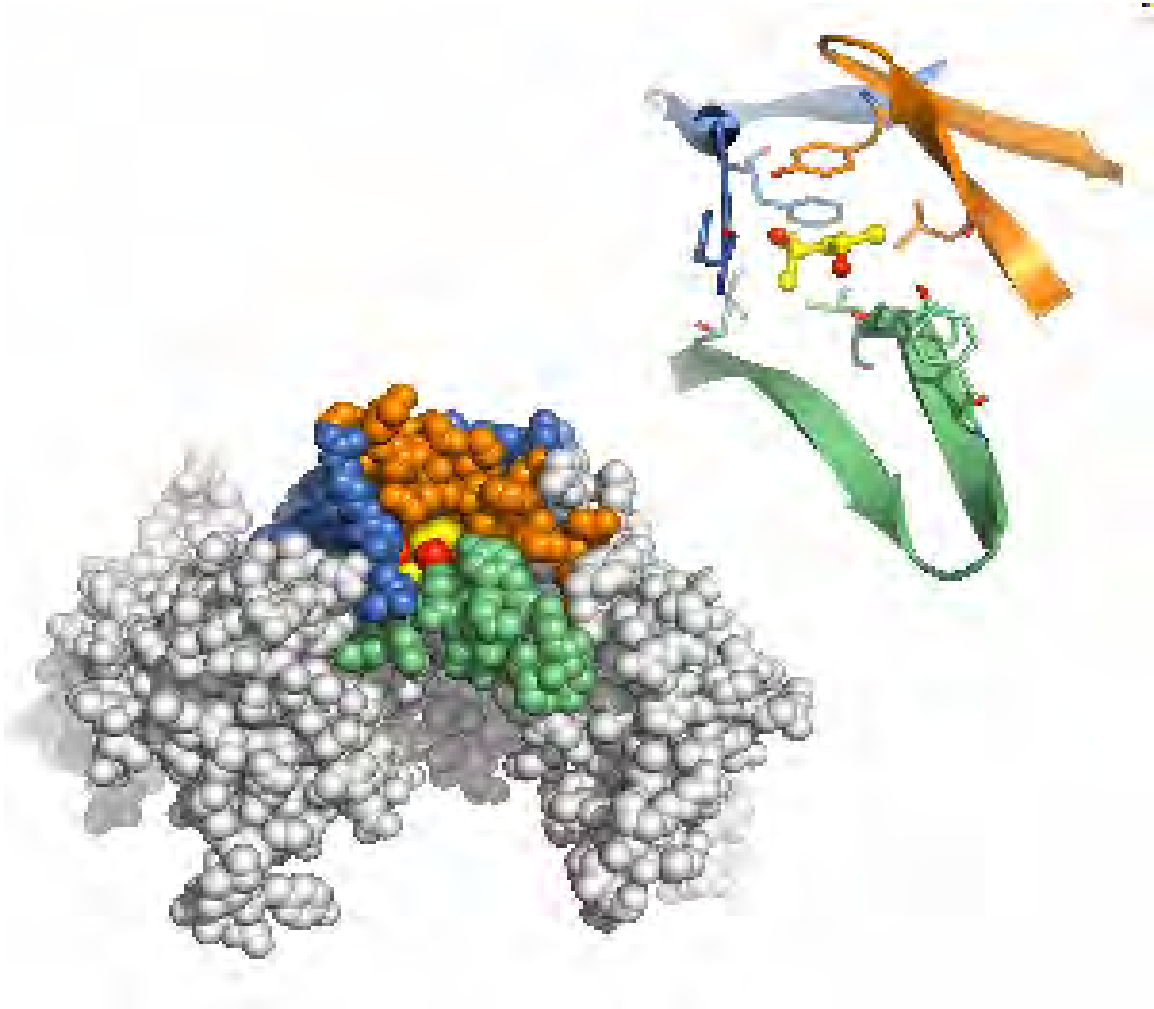
Stereo slika tripsin – benzidin kompleksa



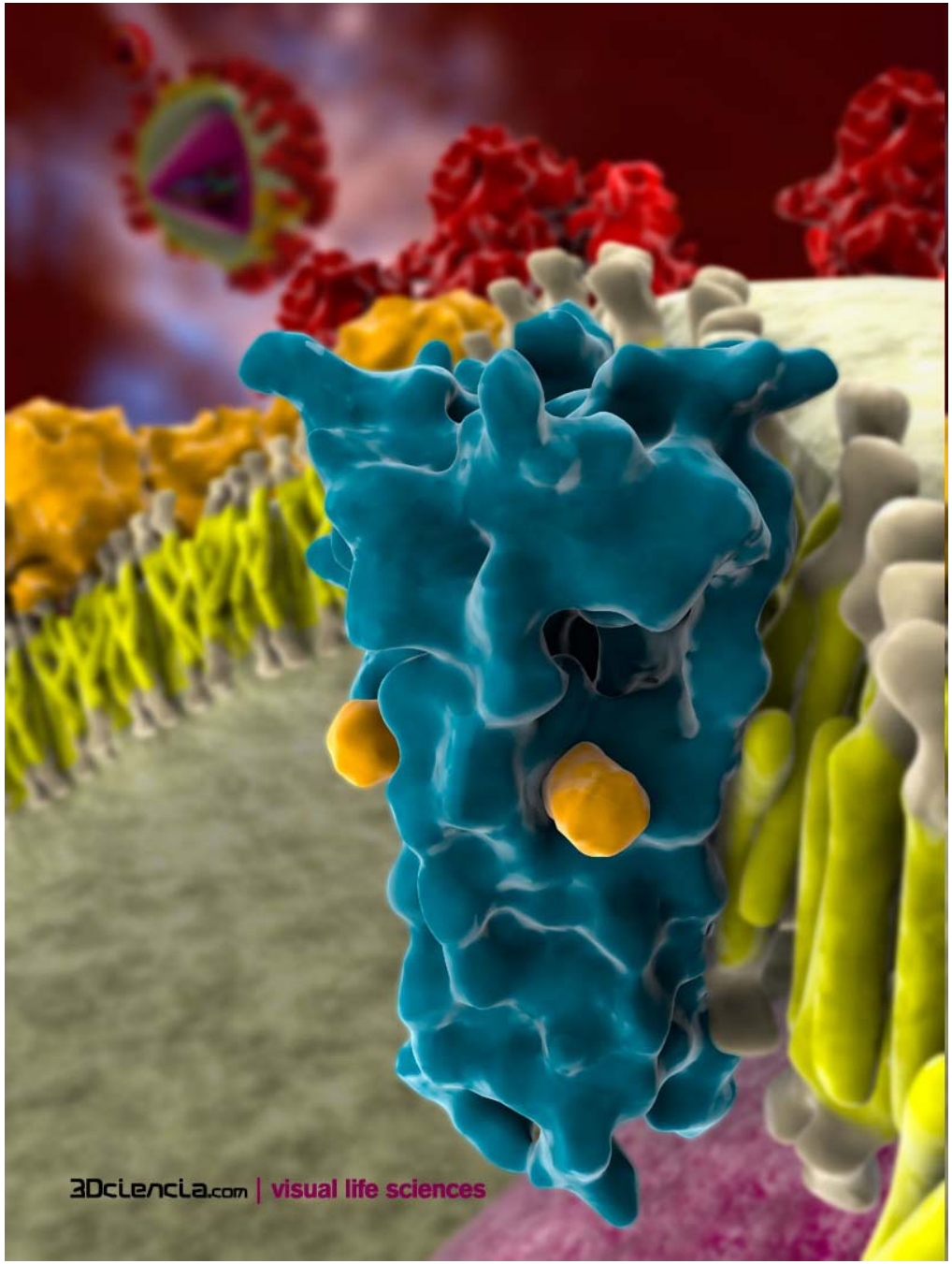
Toxicity of the anticancer drug CPT-11 is alleviated by inhibiting a specific enzyme (pictured here in purple and blue) in human symbiotic gastrointestinal bacteria: The trouble with CPT-11 begins presumably after it has already killed tumor cells and been rendered inert.When it reaches the intestines, beta glucuronidase enzymes in the gut bacteria snip a sugar off the inactivated cancer drug, essentially reactivating it, where it causes tissue damage.Compound (yellow) target and bind to the enzyme, blocking the reaction that leads to the drug's debilitating side effects.



Serum albumin (grey) binding ligands (green) nonspecifically.



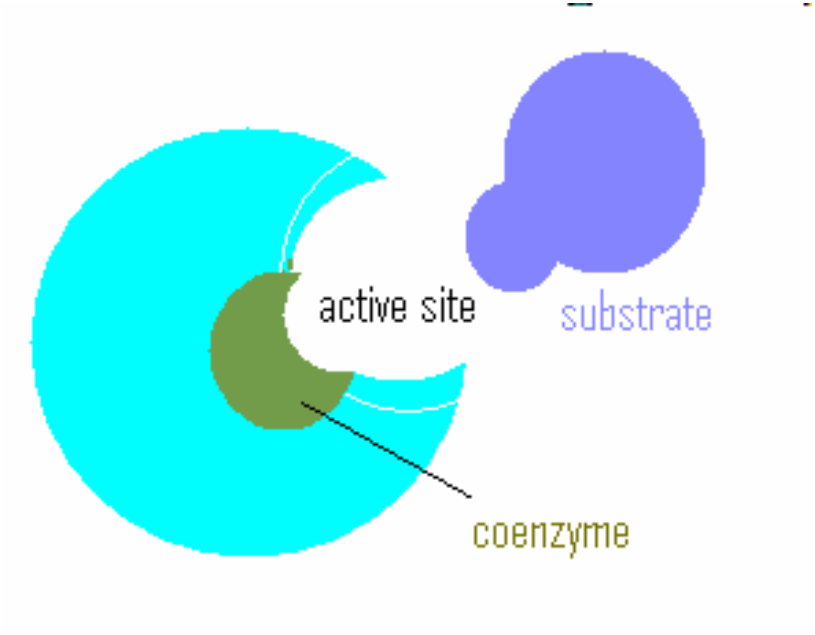
Bottom: X-ray crystallography revealed a binding site for alcohol in an ion channel that plays a key role in several brain functions associated with drugs of abuse and seizures.
Top: Detailed view of the alcohol binding pocket with a an alcohol moleculeshown in yellow and red.

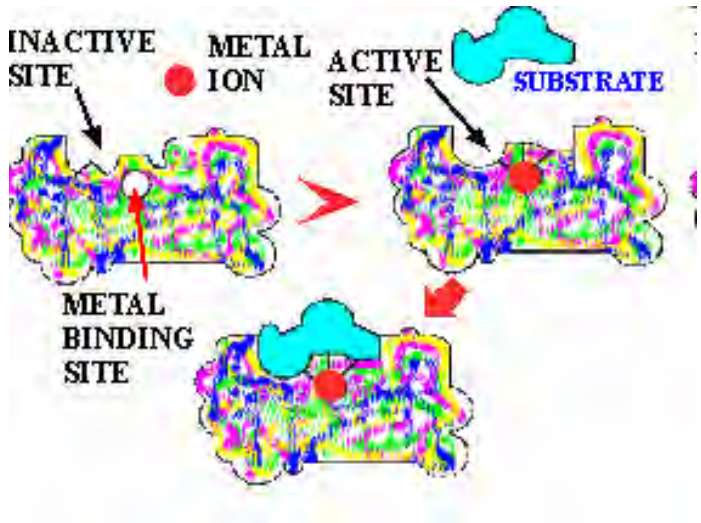


Flu virus surface proteins, M2 in blue, target of adamantane viral drugs in orange.

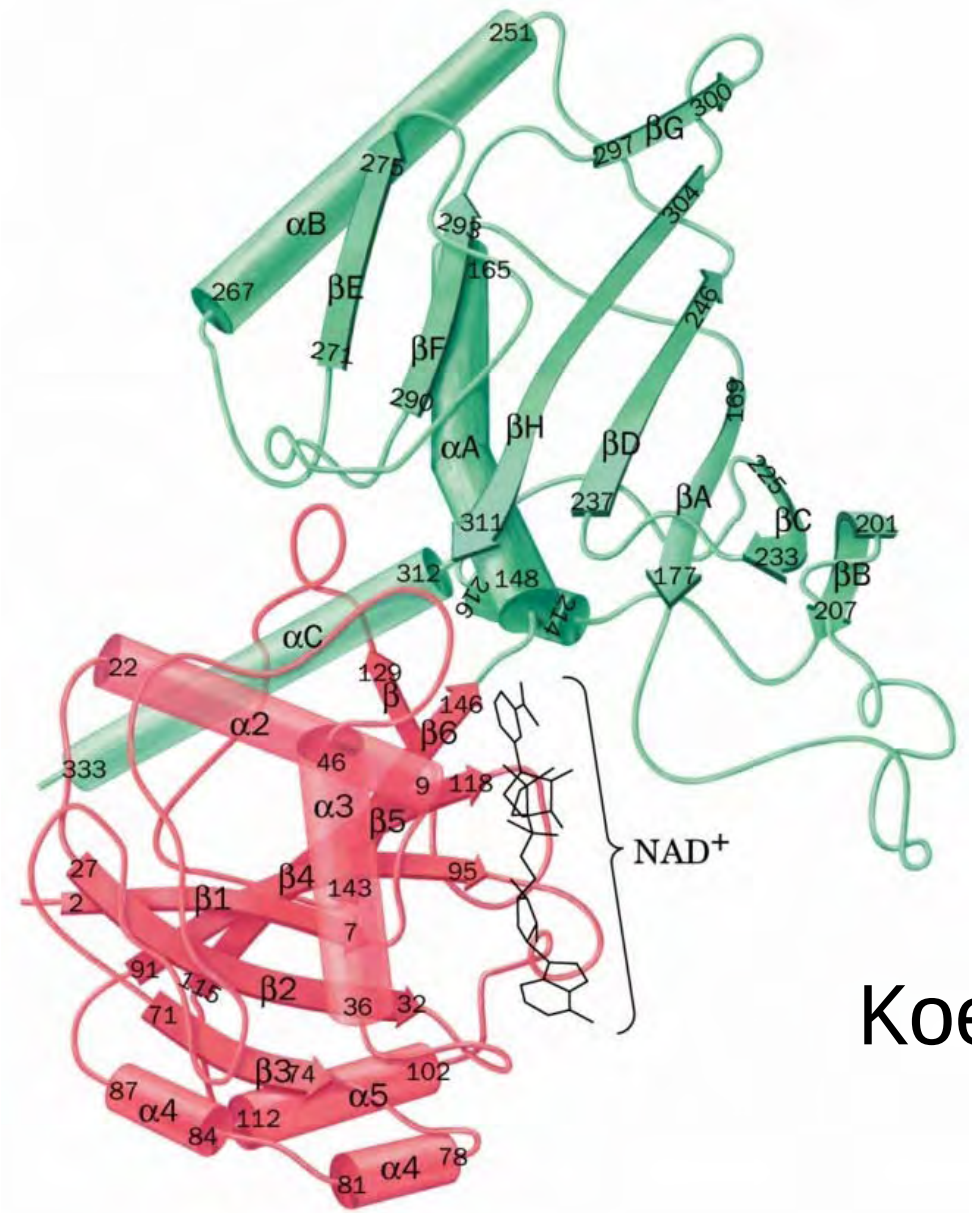
Adamantane antivirals, such as amantadine, inactivate M2 and thus stop the virus proliferation.

Koenzimi/kofaktori/prostetične grupe



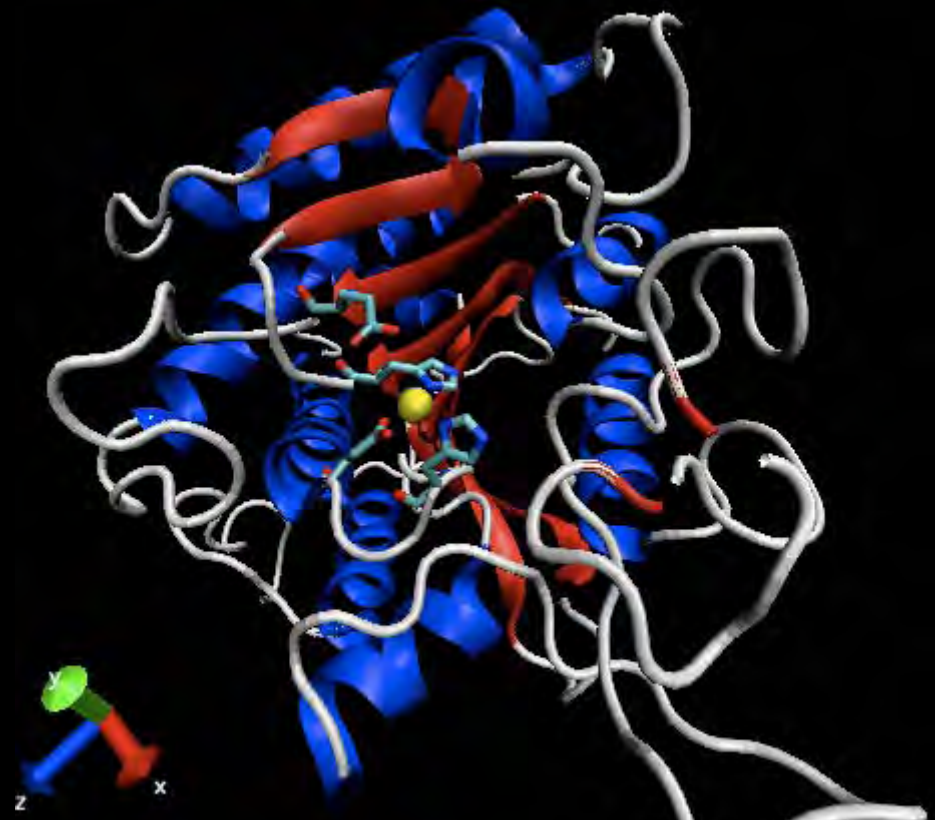


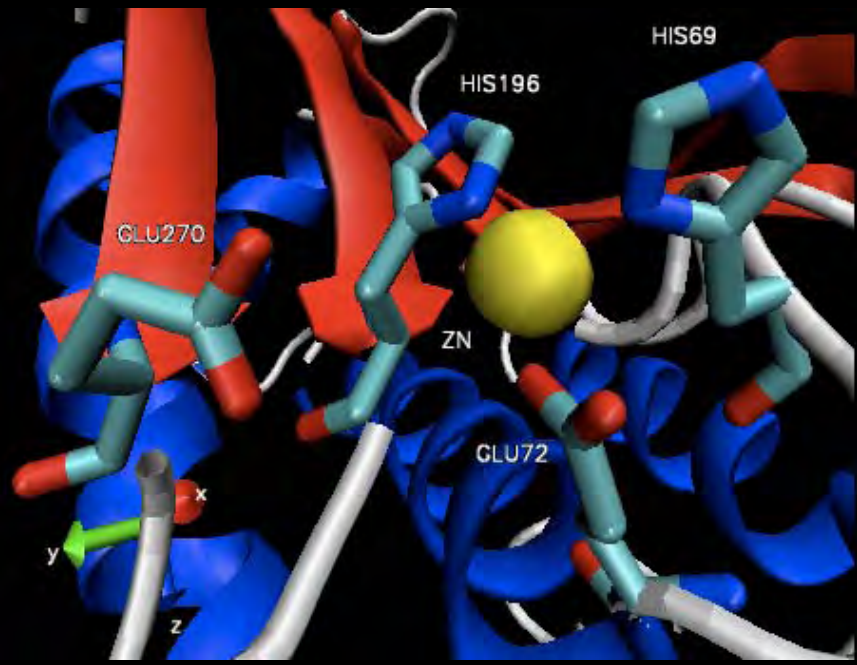
Gliceraldehid-3-fosfat dehidrogenaza



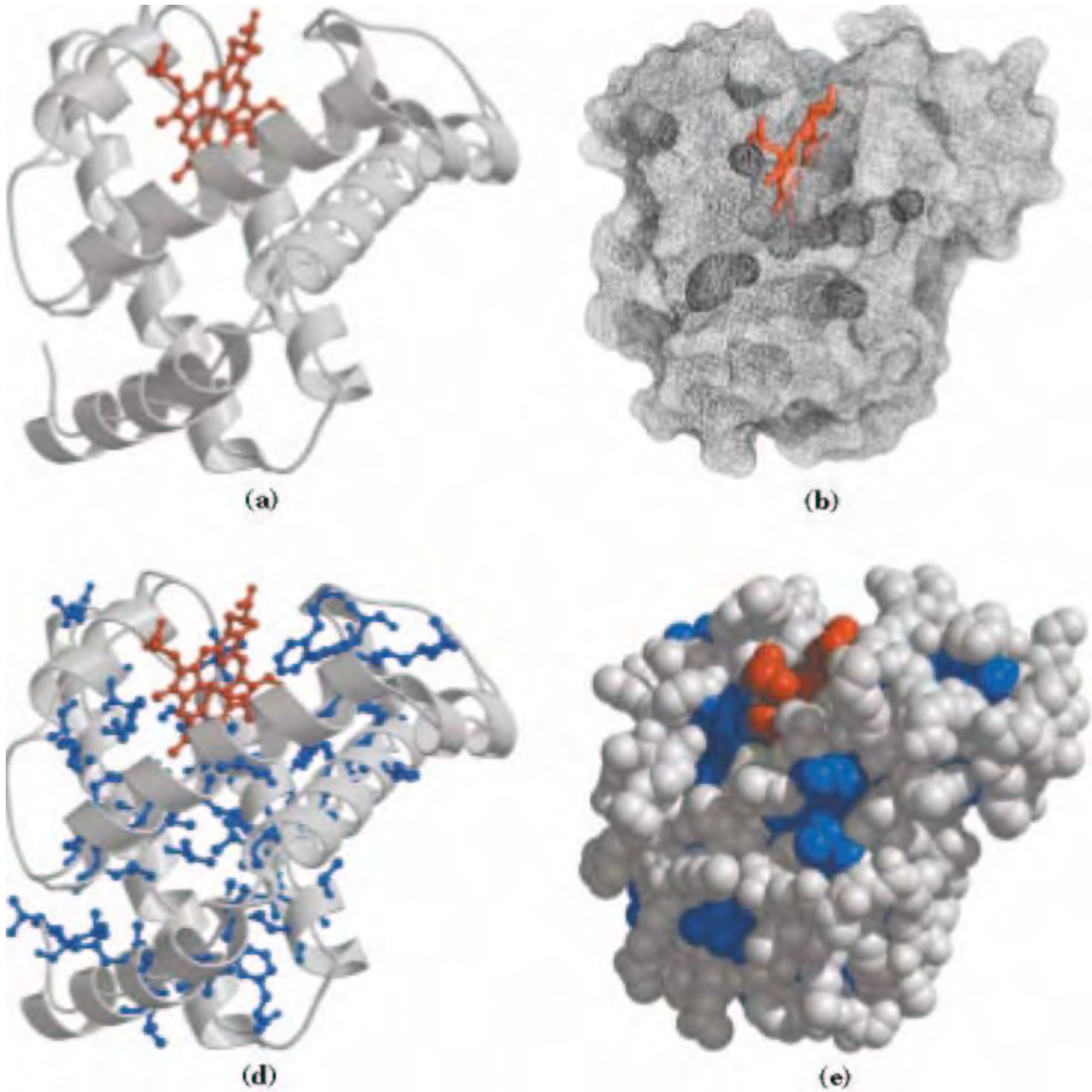
Koenzim: NAD^+

Karboksipeptidaza: kofaktor Zn^{2+}

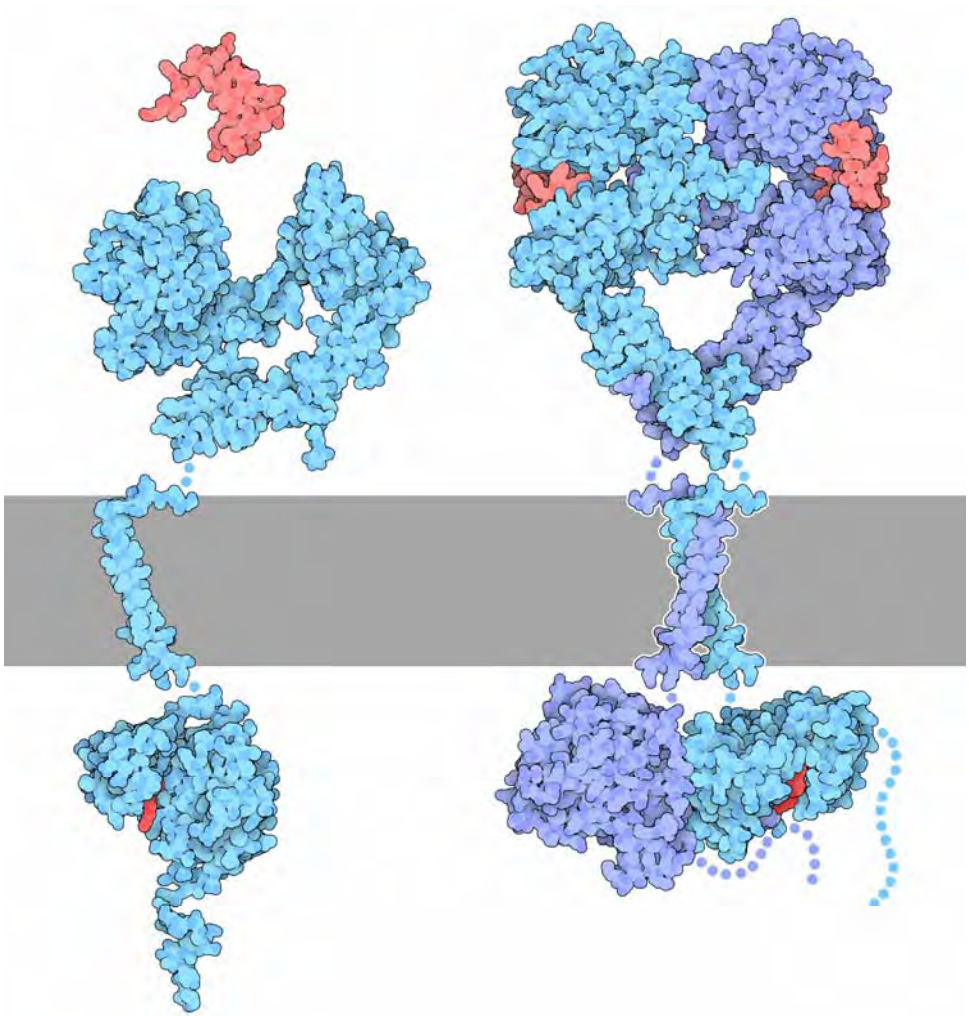


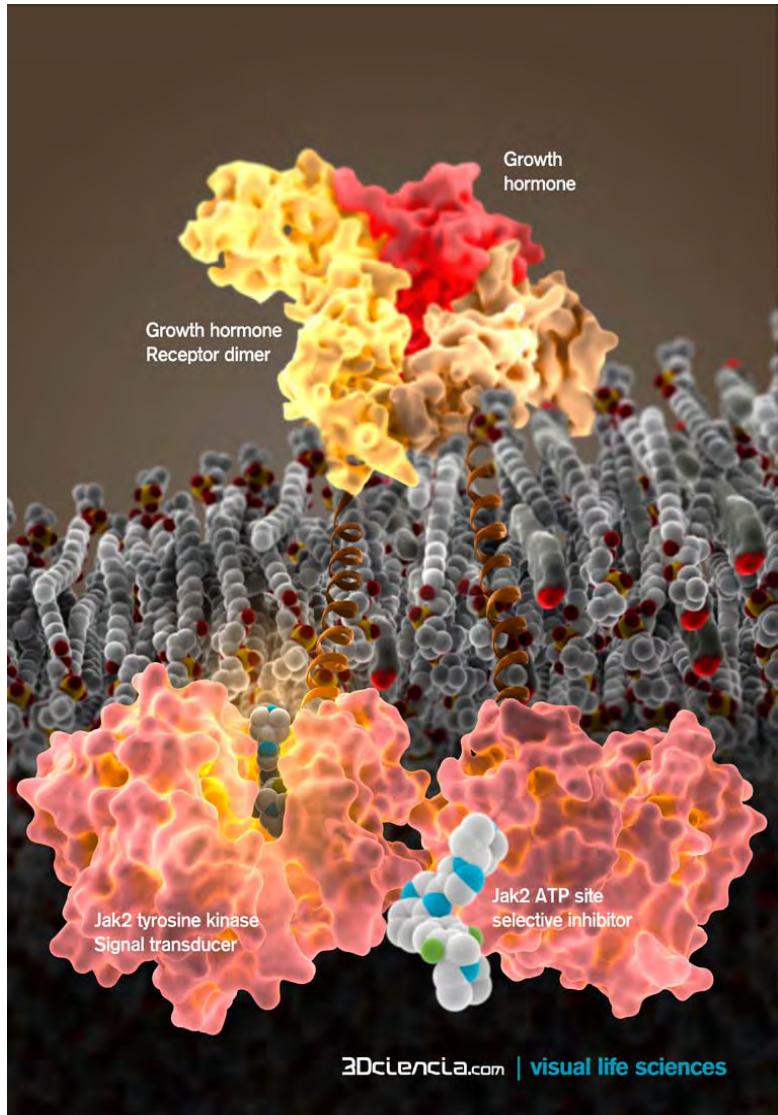


Hem u mioglobinu: prostetična grupa

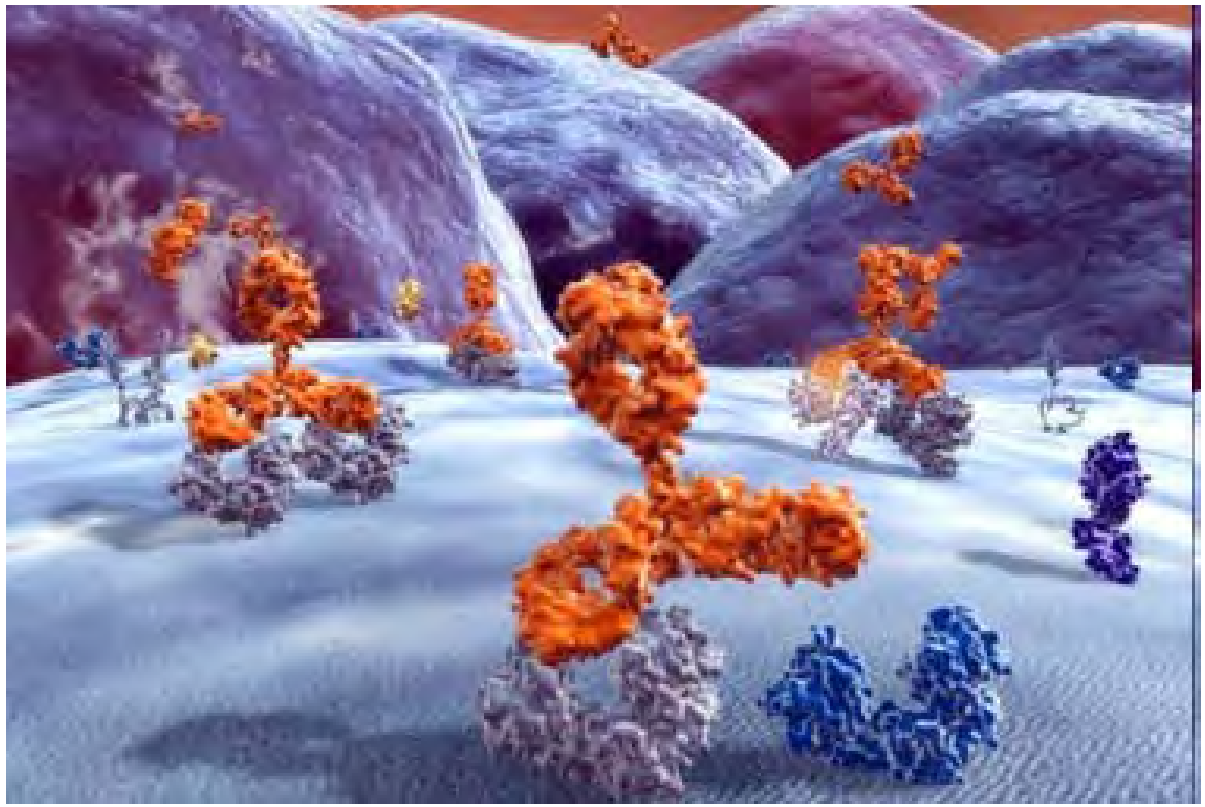


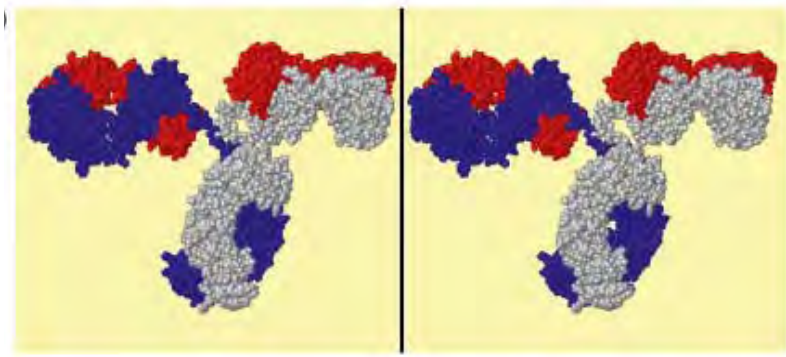
Protein – protein interakcije:
epidermalni faktor rasta (EGF) i EGF receptor



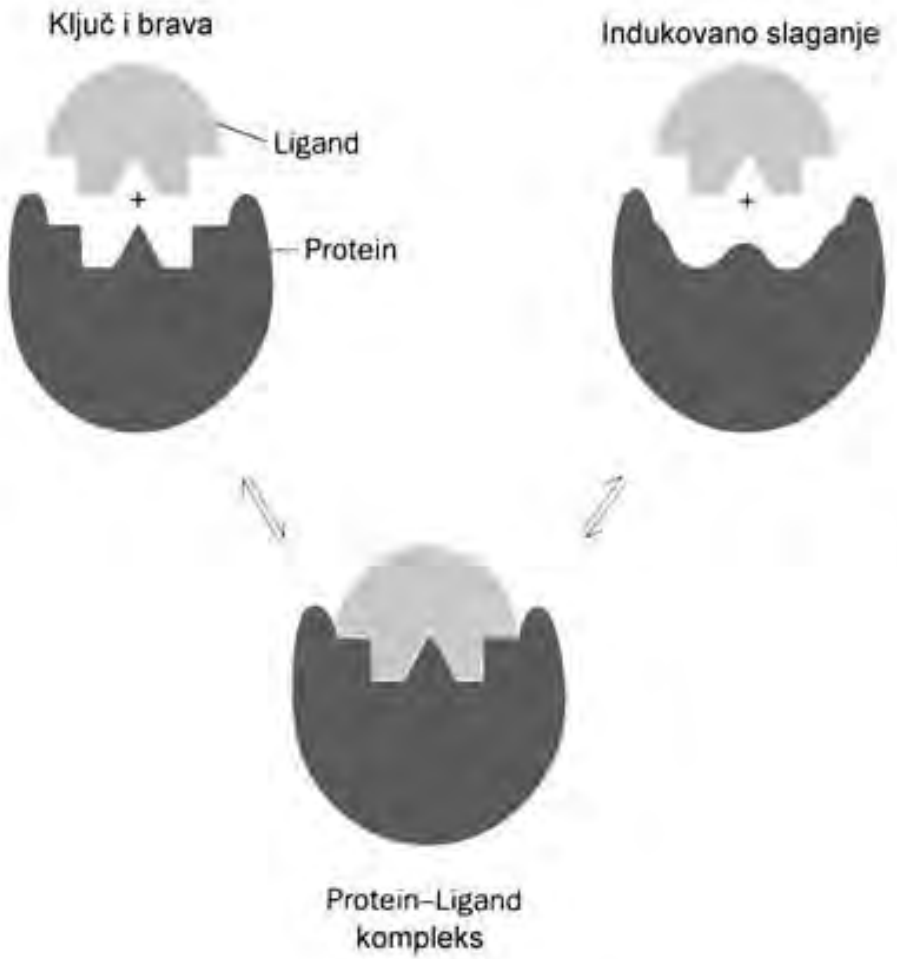


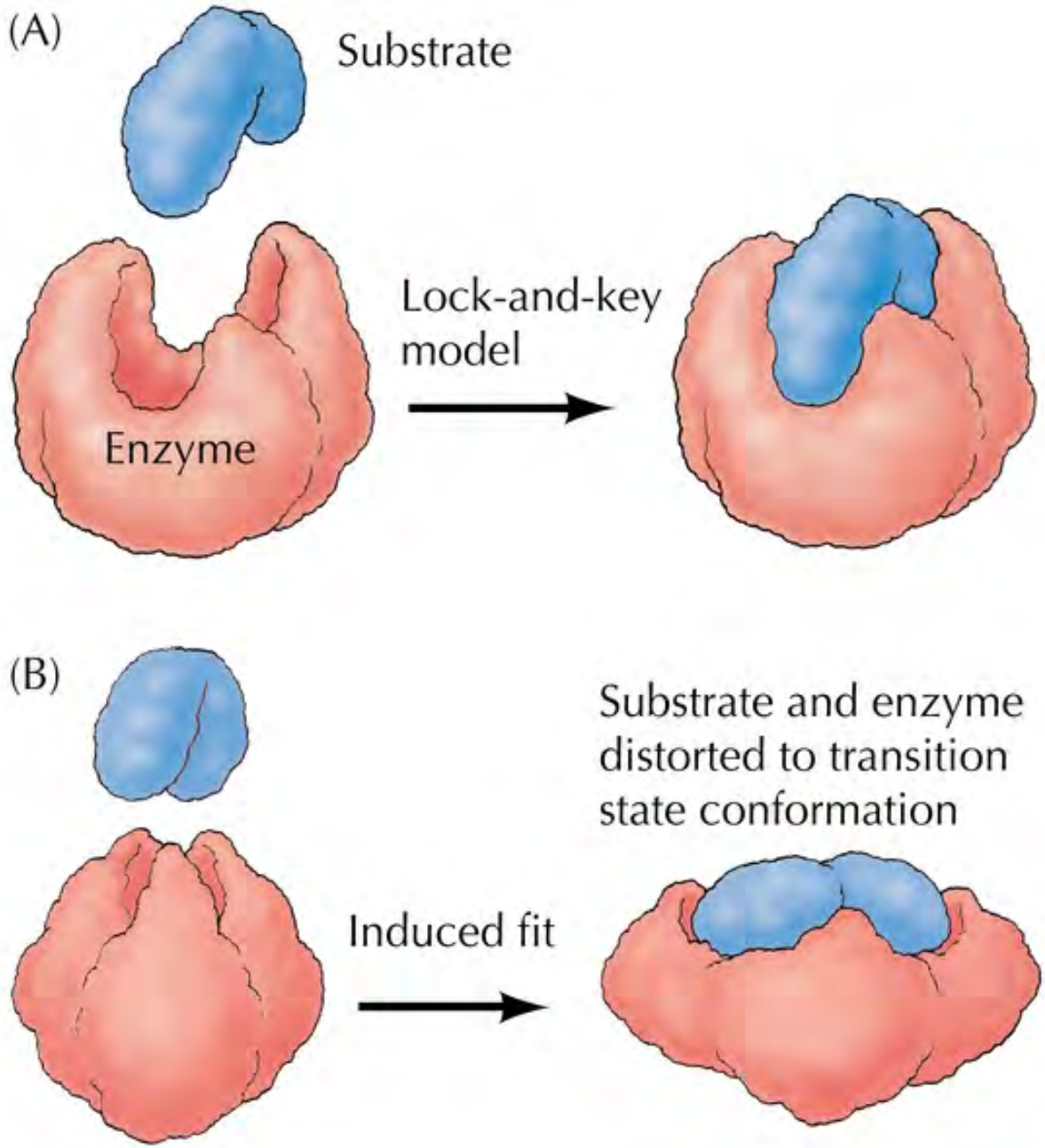
Antitela za vezivanje EGF receptora u terapiji kancera



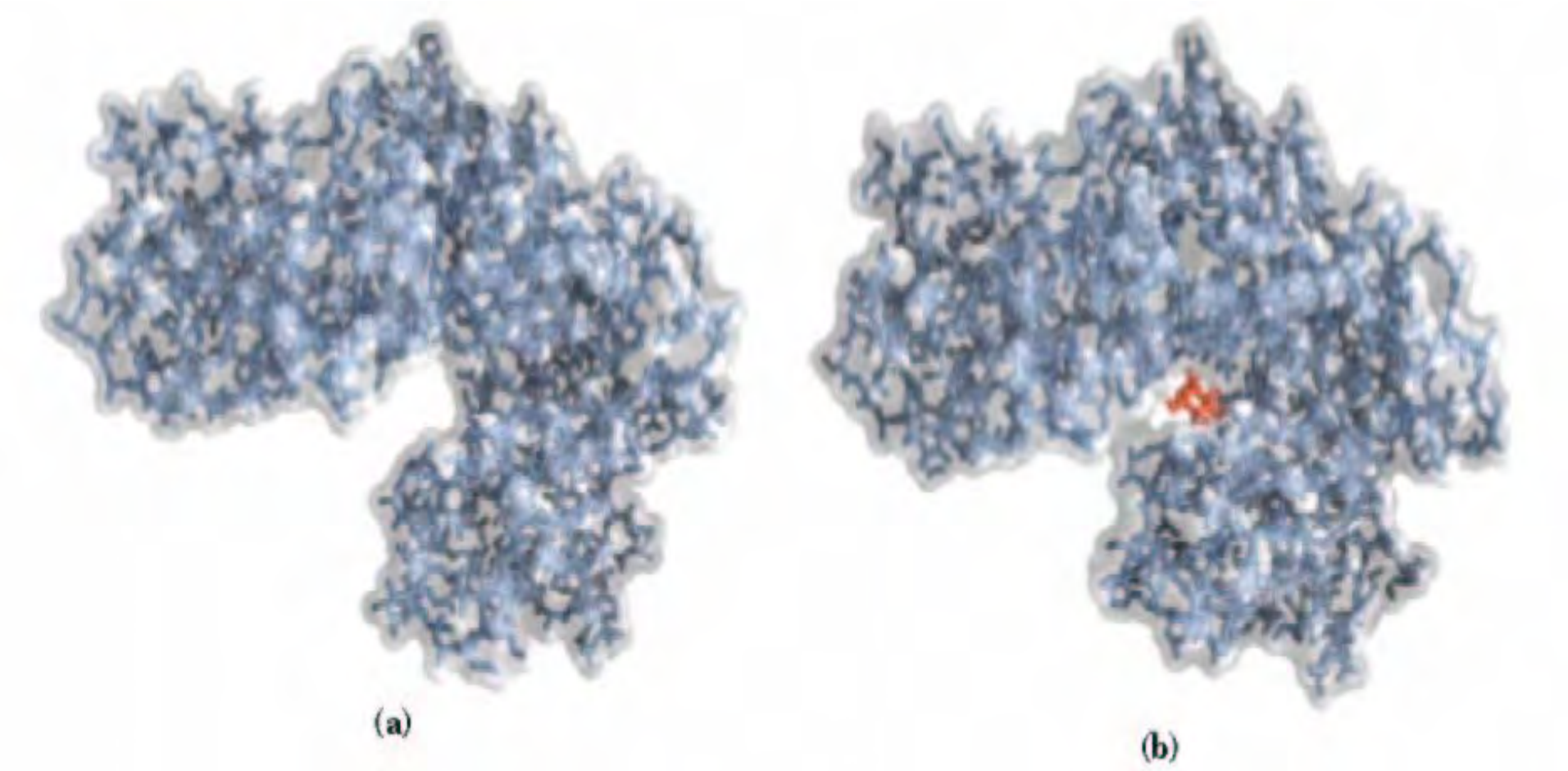


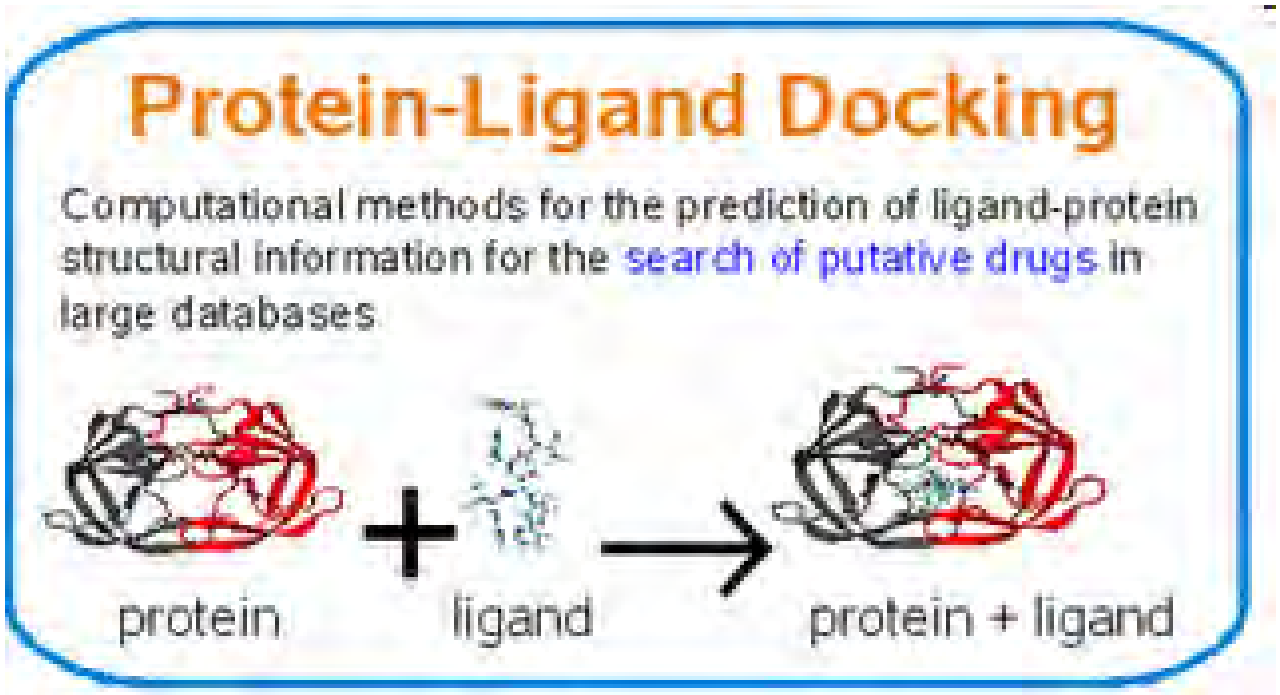
Specifično prepoznavanje proteina i liganda: komplementarnost površina molekula liganda i vezivnih mesta na proteinu



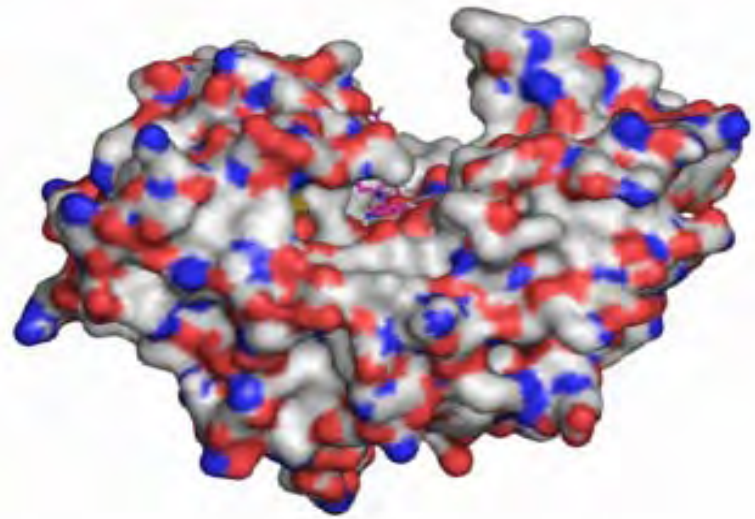


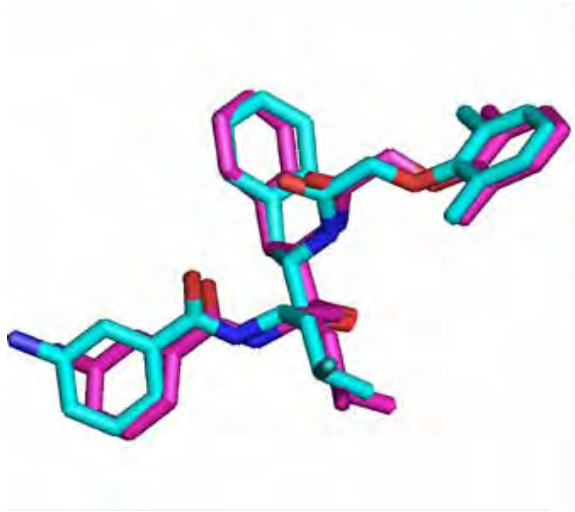
Vezivanje heksoza za heksokinazu



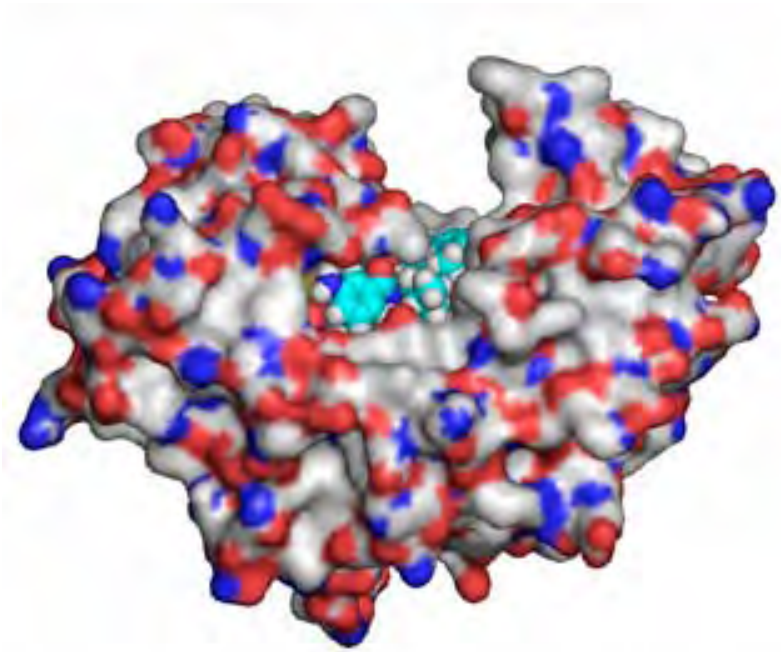


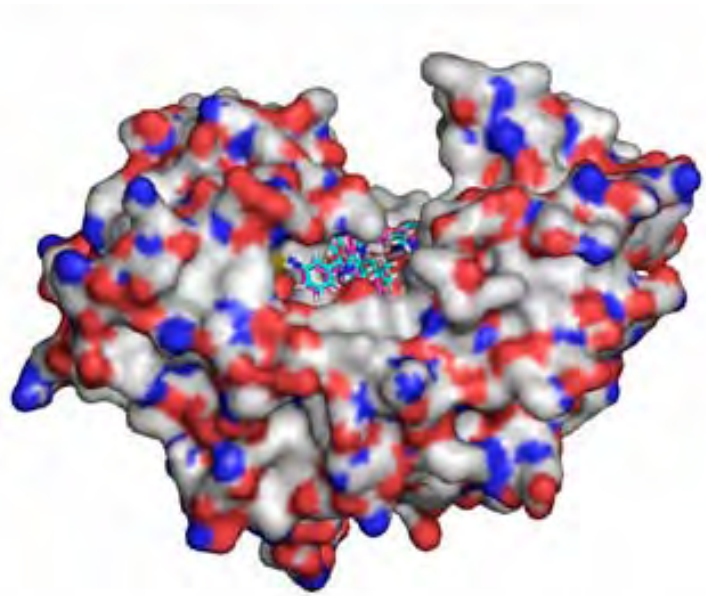
Protein – ligand usidravanje



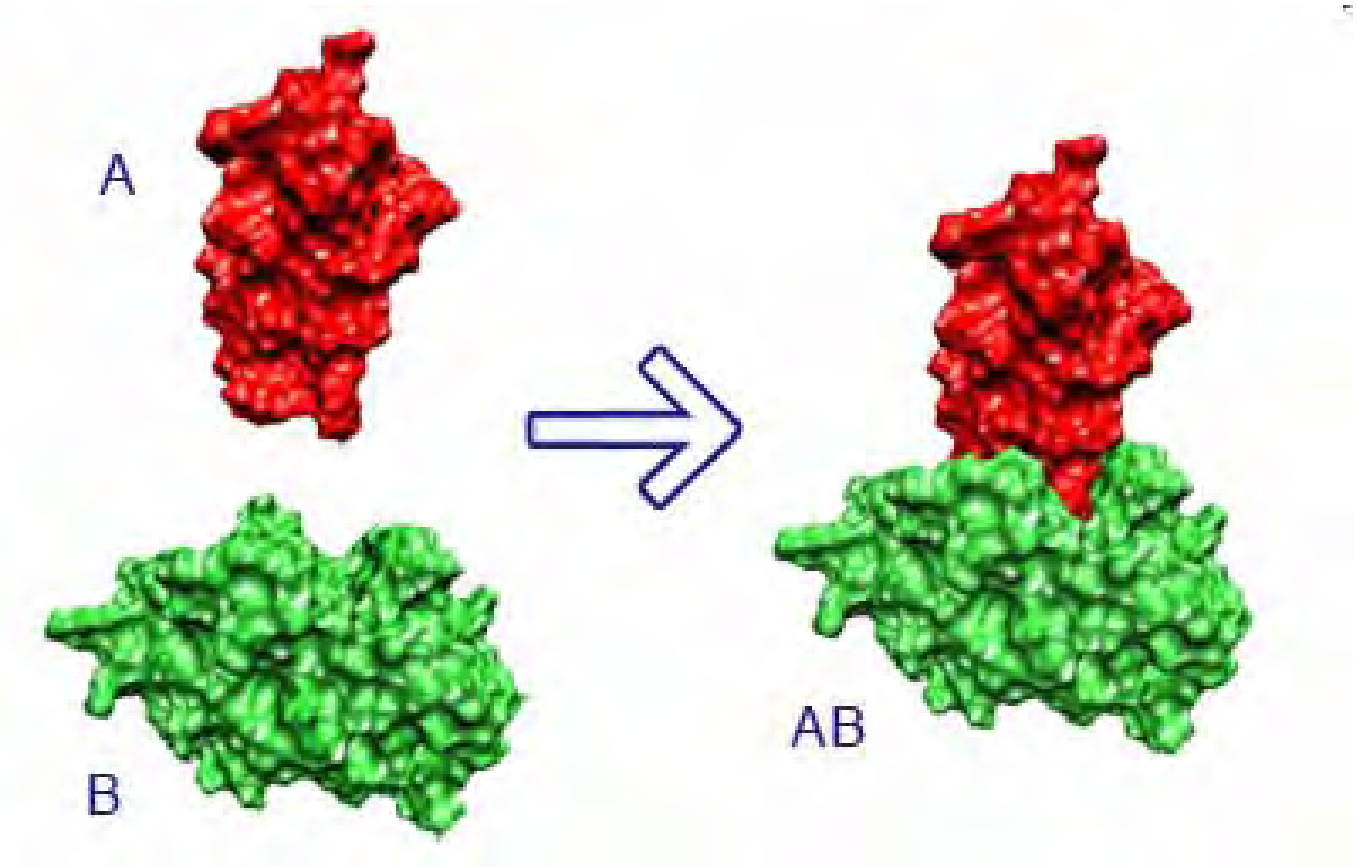


Molekulsko usidravanje



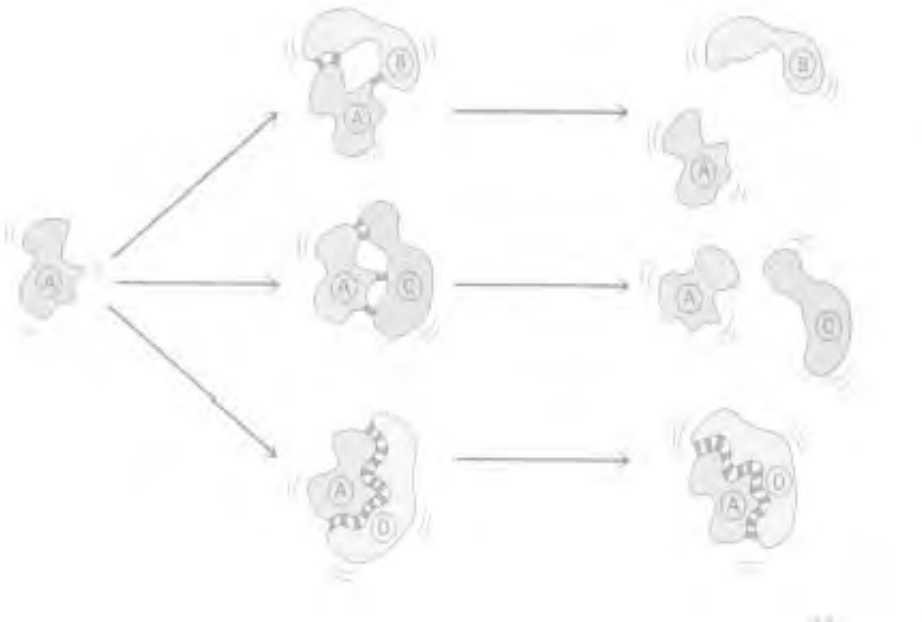


Usidravanje proteina sa drugim proteinom



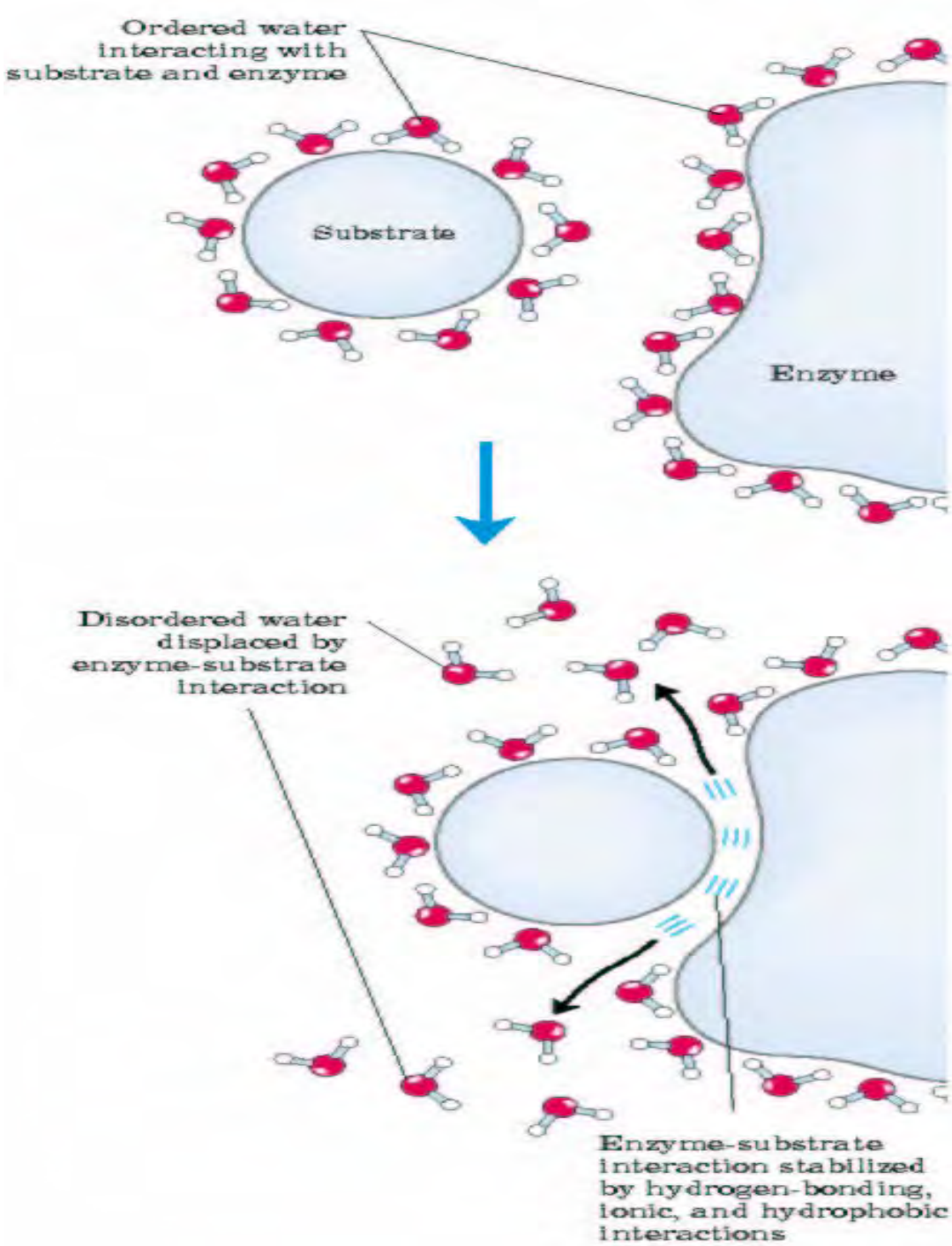
Kako dolazi do vezivanja proteina i liganda?

Difuzija molekula u rastvoru.



Prepoznavanje i vezivanje: komplementarnost površina i međumolekulske interakcije.

Mumolekulske sile
i hidrofobni efekat
stabilizuju
PL kompleks



Konstanta vezivanja P i L

Vezivanje proteina i liganda je ravnotežni proces

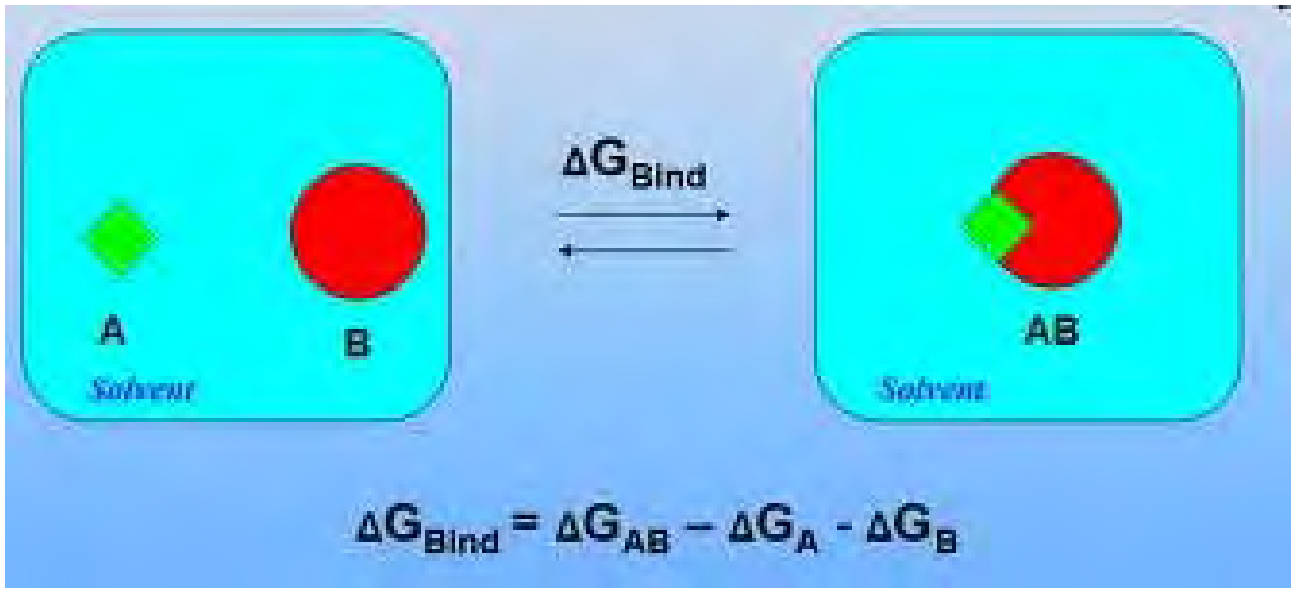
$$\mathrm{P} + \mathrm{L} \rightleftharpoons \mathrm{PL}$$

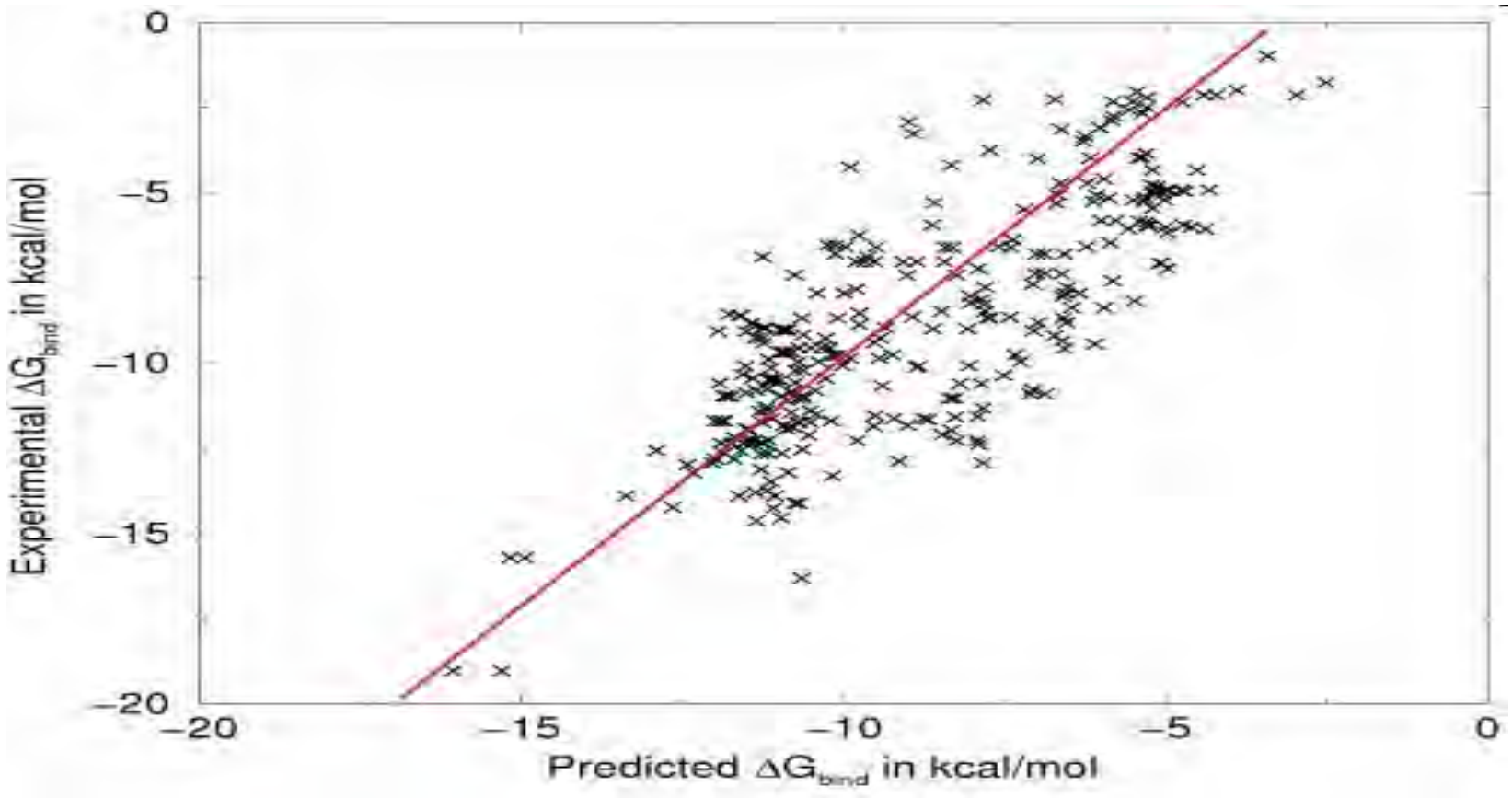
$$K = \frac{[\mathrm{PL}]}{[\mathrm{P}][\mathrm{L}]} \qquad K_d = \frac{[\mathrm{P}][\mathrm{L}]}{[\mathrm{PL}]}$$

K = K_a Konstanta asocijacije

Konstanta vezivanja i energija vezivanja

$$\Delta G = -RT\ln K_a = \Delta H - T\Delta S$$



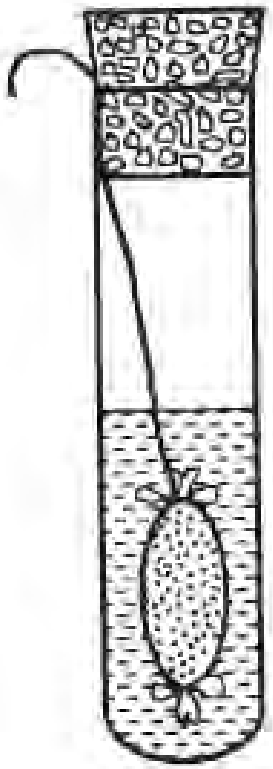


Korelacija između predviđenih i eksperimentalno određenih vezivnih energija za protein–ligand komplekse (na osnovu Ligand Protein Data Base).

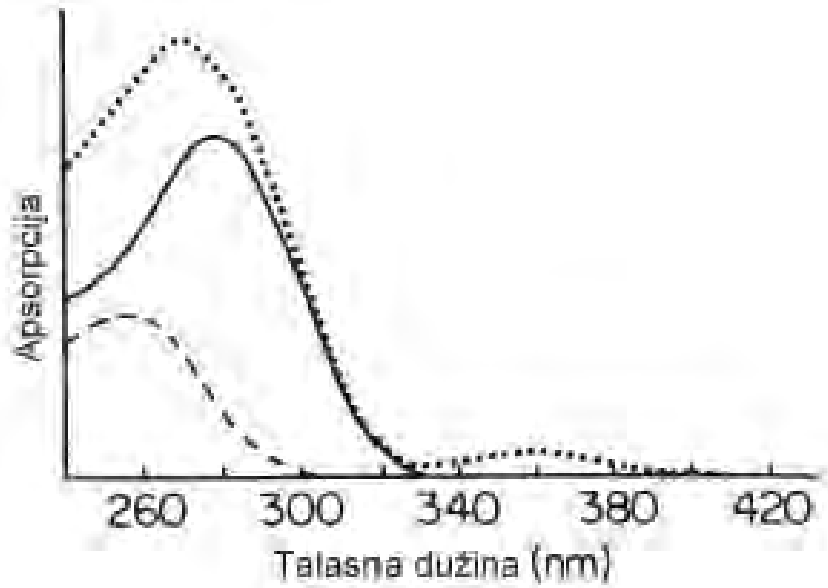
Tabela 11.1: Metode za ispitivanje vezivanja malih liganada za proteine (makromolekule uopšte)

Odredjivanje koncentracije slobodnog liganda: Biološka aktivnost; Odvajanje liganda: dijaliza, ultrafiltracija, gel filtracija, ultracentrifuga; Elektrohemija: specifične elektrode, konduktometrija, polarografija.
Perturbacija osobina vezanog liganda: Optička spektroskopija, NMR, fluorescencija, ESR.
Perturbacija osobina PL kompleksa (u odnosu na protein bez liganda): Biološka aktivnost; Termodinamičke: osmotski pritisak, ΔpI, sedimentacija, rasejavanje svetlosti; Elektromagnetne: spektroskopske (UV/VIS, ORD, NMR, rendgenska strukturna analiza), elektroforetske.

Primeri metoda



Ekvilibraciona dijaliza



NAD ---- Enzim _____

NAD vezan za enzim .....

Jednačina vezivanja liganda i proteina sa jednim centrom vezivanja

$$\nu = \frac{\text{Ukupna koncentracija L vezanog za P}}{\text{Ukupna koncentracija proteina}}$$

ν = stepen zasićenja

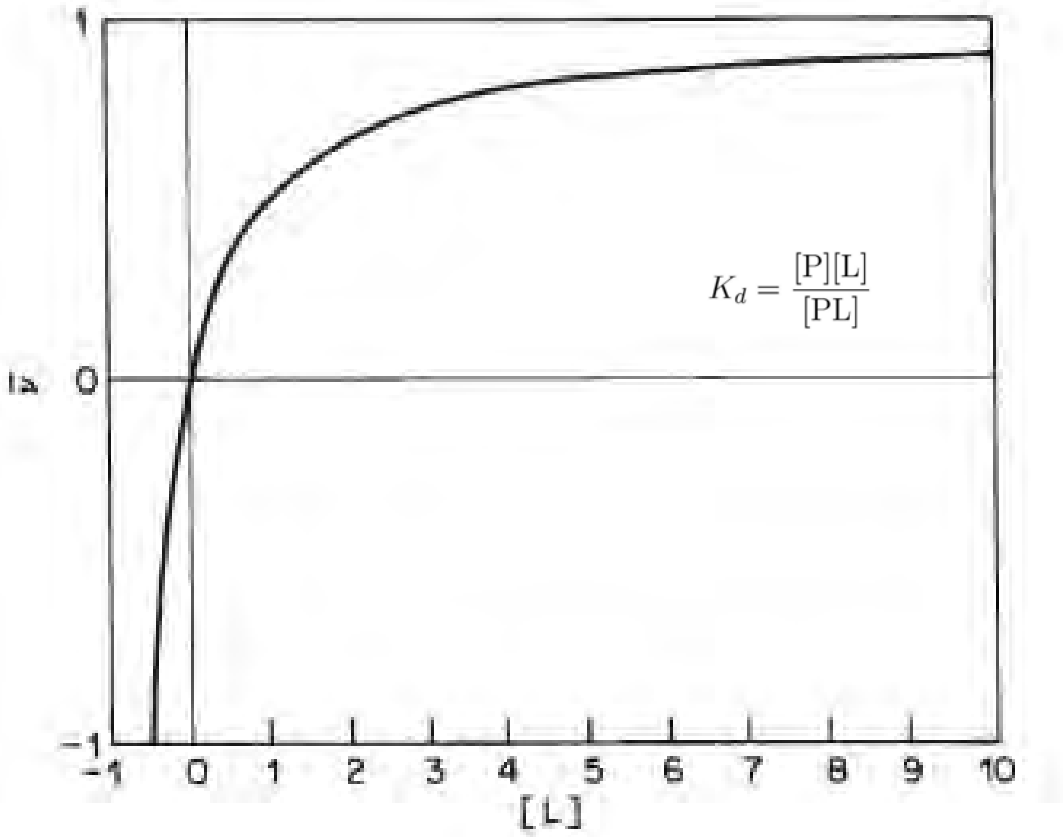
$$\nu = \frac{[L]}{K_d + [L]}$$

$\nu = ½$

$K_d = [L]$

K_d - makroskopska konstanta disocijacije PL kompleksa

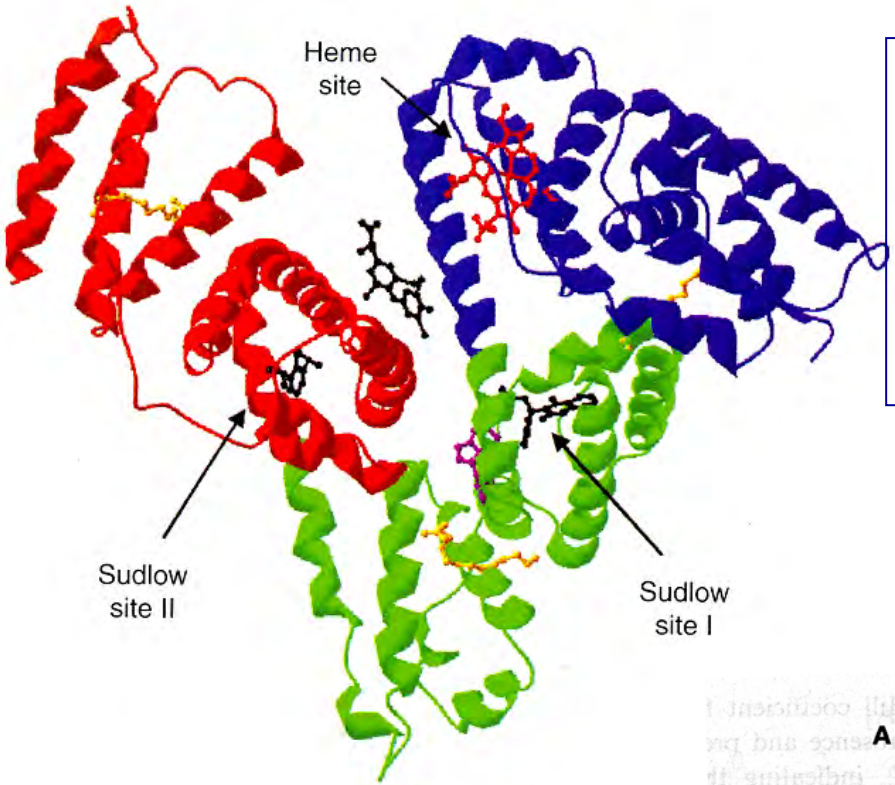
Kriva vezivanja: hiperbola



$\nu = ½$

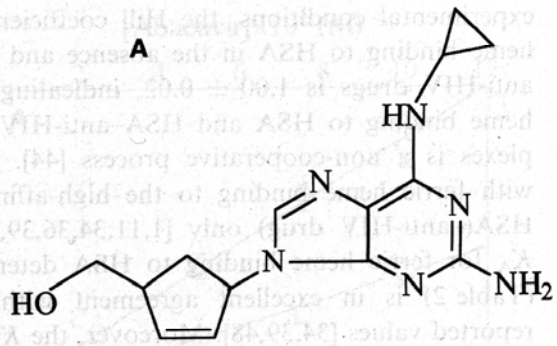
$K_d = [L]$

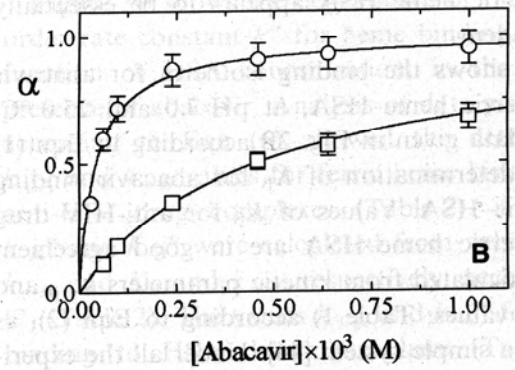
Primer: interakcije anti-HIV leka sa humanim serum-albuminom (HSA)



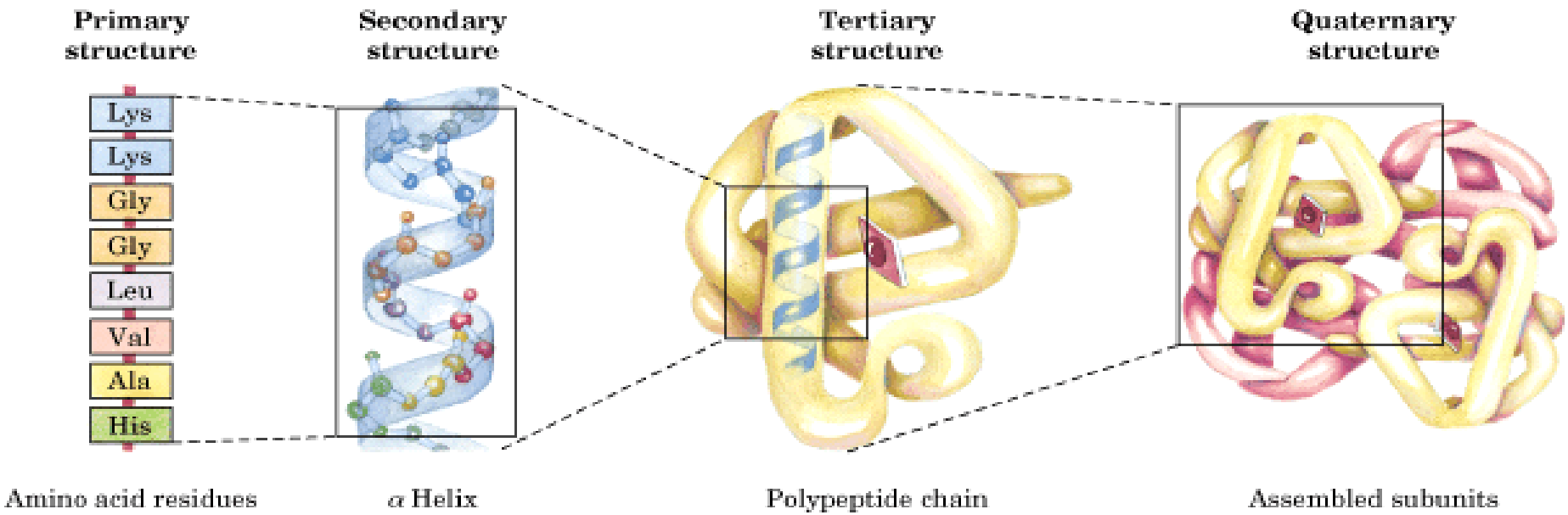
HSA ima izuzetan kapacitet da vezuje razne ligande: npr hem i lekove. Sastoji se iz 3 domena: I, II i III (svaki od po 2 subdomena). Razni ligandi se vezuju za različitedomene!)

Vezivanje leka (abacevir) za HSA (o) i hem-HSA (□) određeno ekvilibracionom dijalizom! ▶





Kvaternarna struktura proteina



Šta je kvaternarna struktura?

Veliki proteini (npr globularni proteini mase veće od 100 kD) često se javljaju u obliku agregata u kojima su subjedinice asosovane na geometrijski određen način.

Asocijacija subjedinica u oligomer je ravnotežni proces koji se karakteriše konstantom asocijacije (ili disocijacije). Disocijacija može da se vrši po mehanizmu *"sve ili ništa"* ili postepeno:

$$P_n \rightleftharpoons P_{n-1} + P \rightleftharpoons P_{n-2} + 2P \rightleftharpoons \cdots \rightleftharpoons nP$$

Zašto kvaternarna struktura?

- Kompleksna funkcija - kompleksna struktura!
- Nastajanje strukture određene geometrije!
- Osmotski pritisak!
- Stabilnost tercijarne strukture proteina!
- Regulacija aktivnosti!!!
- Komunikacija proteina sa okolinom!!!!

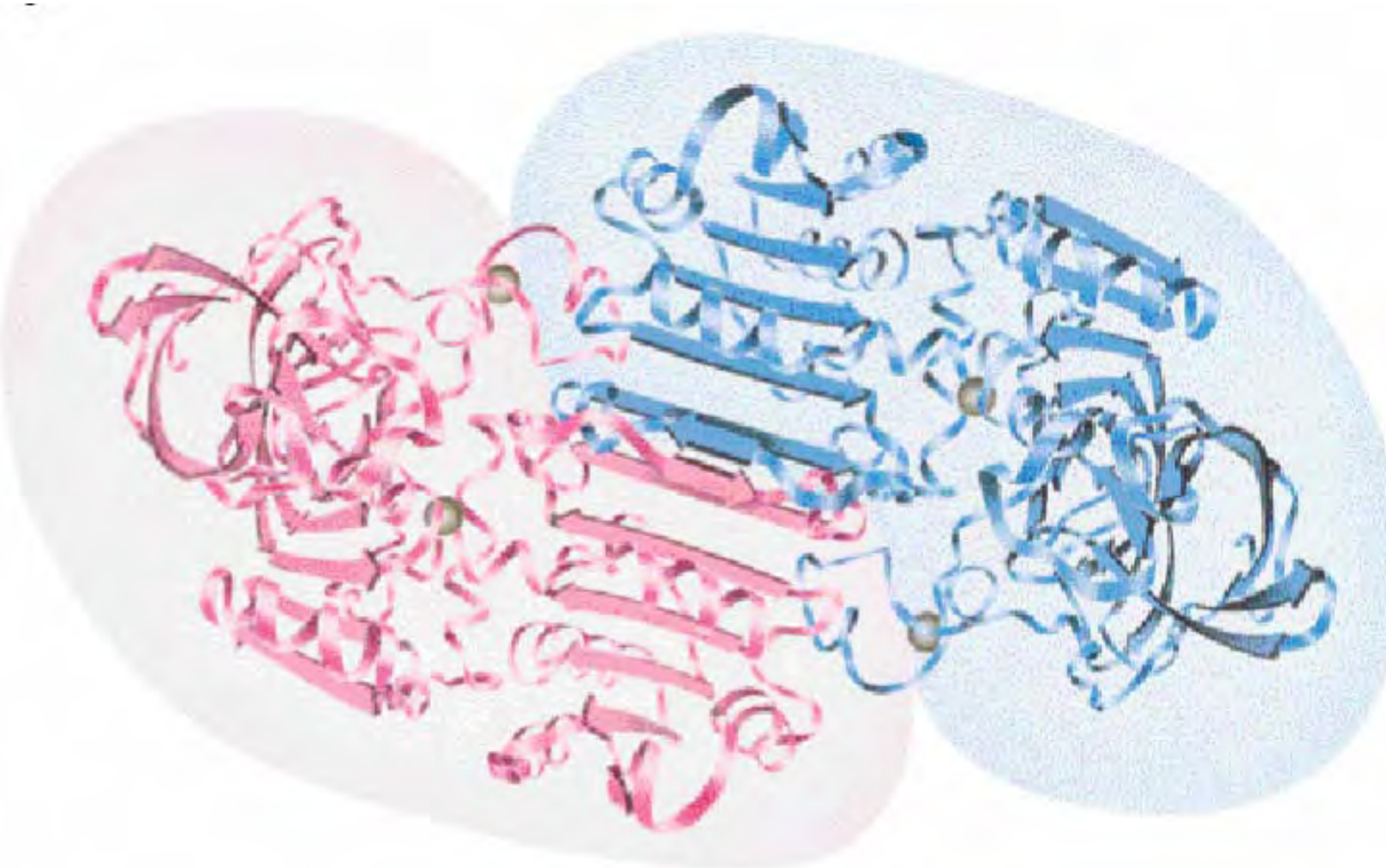
Opisivanje kvaternarne strukture:

Stehiometrija: tip i broj subjedinica

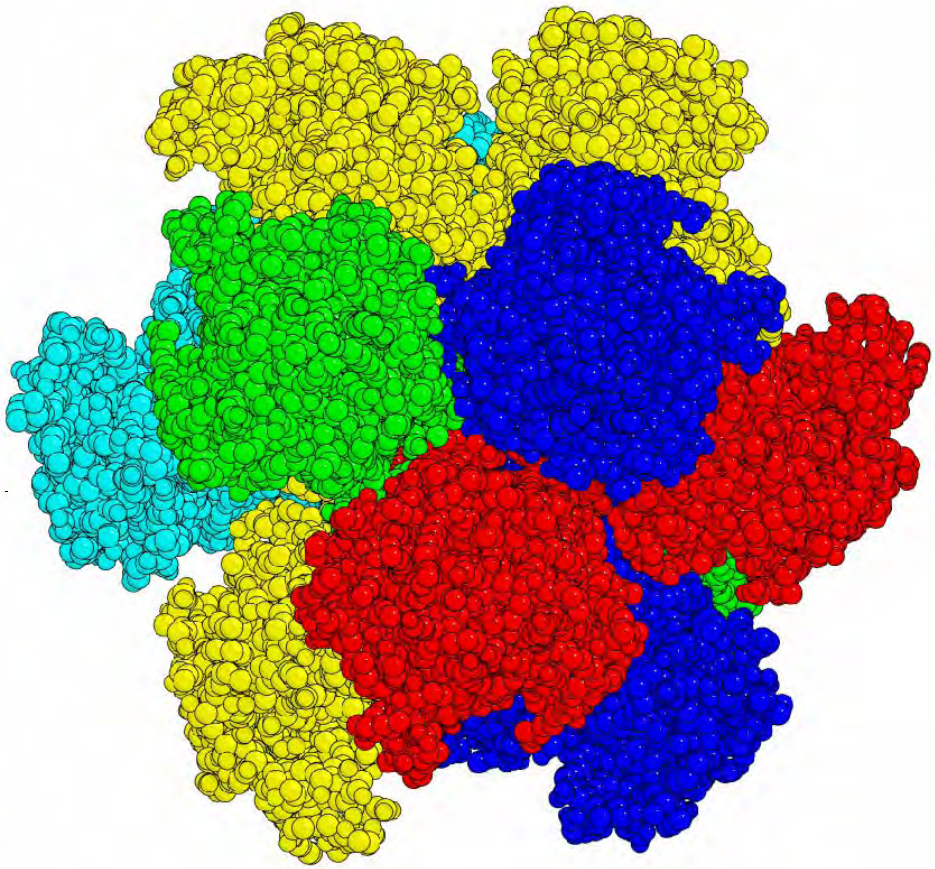
Geometrija: geometrijski raspored (tip simetrije)

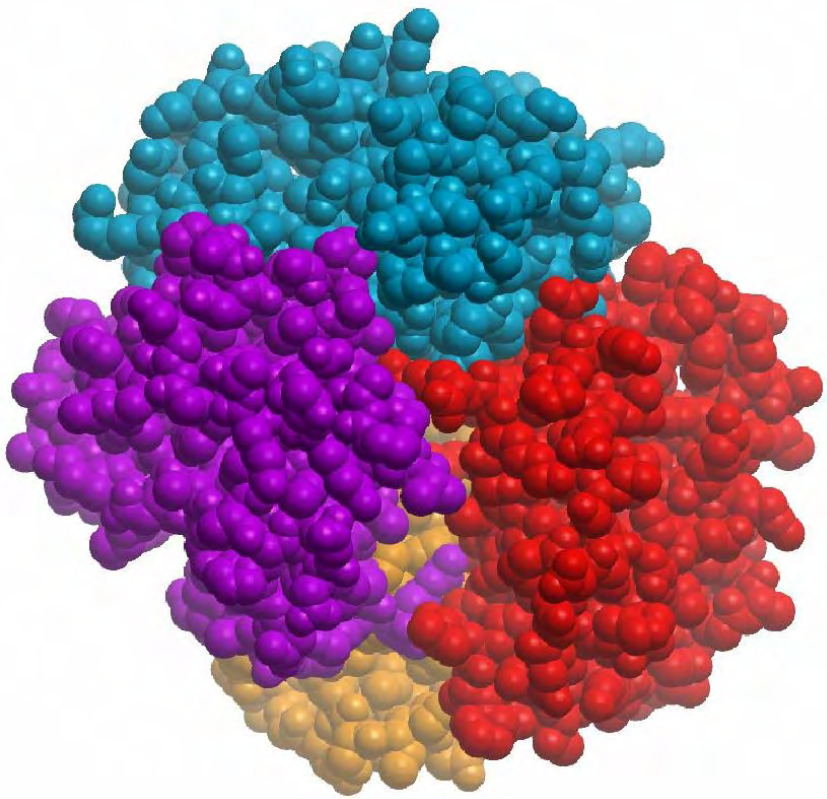
Stabilnost kvaternarne strukture

2 identične subjedinice

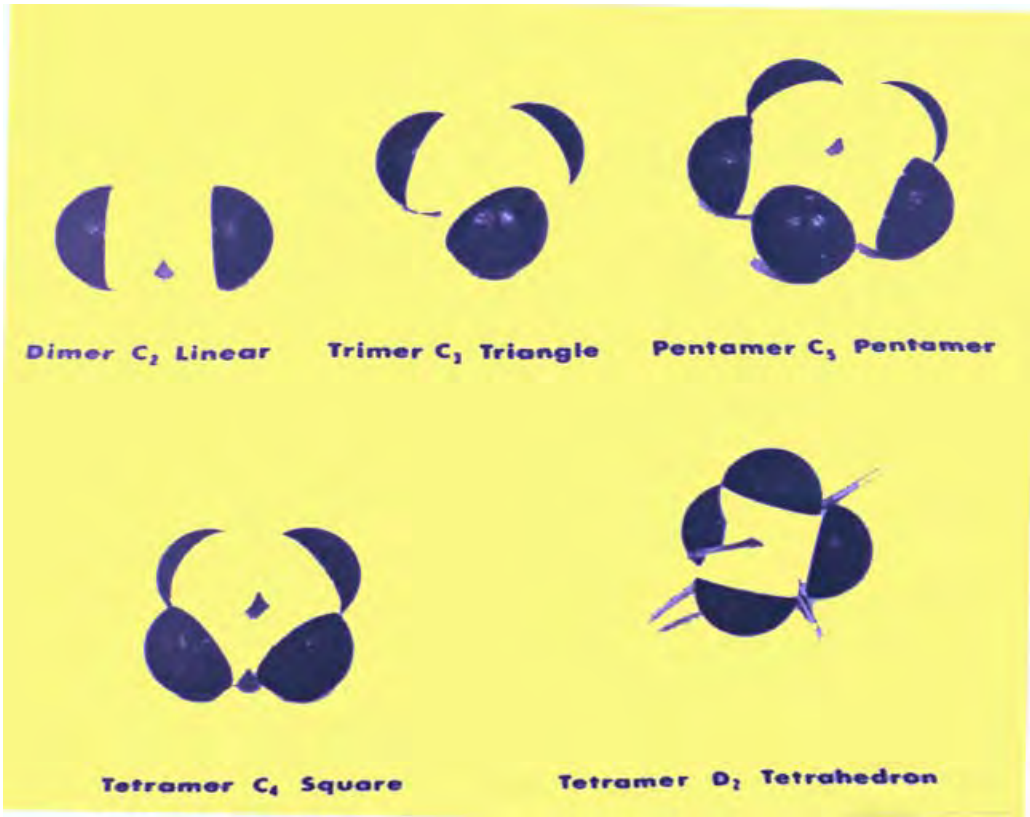


Obratiti pažnju na komplementarnost dodirnih površina među subjedinicama!





Geometrijski raspored subjedinica



Sve subjedinice se nalaze u ekvivalentnoj ili pseudo-ekvivalentnoj okolini: linearni raspored subjedinica se isključuje!

Stabilnost kvaternarne strukture

- Dodirne površine subjedinica su komplementarne!!!
- Aminokiselinski sastav na dodirnim površinama subjedinica je sličan sastavu u unutrašnjosti molekula globularnih proteina: dominiraju nepolarni ostaci (Ile, Leu, Phe, Val, Cys i Met)
- Arg se češće javlja na dodirnim površinama nego što bi se očekivalo na osnovu prosečnog sadržaja u Arg u proteinima!

Zaključak o doprinosu stabilnosti 4^0 strukture:

(1) Hidrofobni efekat: najveći doprinos!

(2) Vodonične veze/soni mostovi na dodirnim površinama subjedinica!!!

Interakcije sa ligandima: nezavisni centri

Makroskopske i mikroskopske konstante

Jednačina i kriva vezivanja

Scatchardov grafik

PROTEIN-LIGAND INTERAKCIJE : KVANTITATIVNI ASPEKTI

JEDAN CENTAR VEZIVANJA :

$P + L \rightleftarrows PL \qquad K = \frac{[PL]}{[P][L]}$ = konstanta protein-ligand asocijacije

$K_{DIS} = \frac{1}{K_{AS}}$ = konstanta disocijacije (ENZIMI)

$$\nu = \frac{\text{BROJ MOLOVA VEZANOG LIGANDA}}{\text{UKUPAN BROJ MOLOVA PROTEINA}} = \frac{[PL]}{[P] + [PL]}$$

$$\nu = \frac{K[L]}{1 + K[L]} = \frac{1}{1 + \frac{1}{K[L]}} \qquad \nu = \frac{[L]}{K_d + [L]}$$

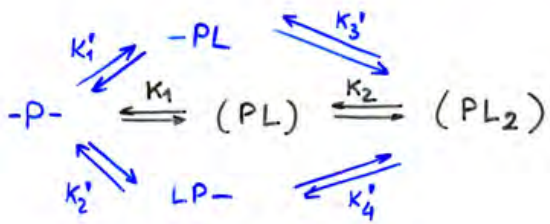
$\nu = \frac{1}{2} \quad \therefore$

$K_d = [L]$

$K = ?$

DVA (i VIŠE) ISTIH NEZAVISNIH (NEINTERAGUJUĆIH) CENTARA VEZIVANJA :

PROTEIN SE SASTOJI IZ DVE SUBJEDINICE (PROTOMERA)



$K_1' = K_2' = K_3' = K_4'$

Mikroskopske konstante (afinitet svakog centra za L)

$K_1 = \frac{[PL]}{[P][L]} \qquad K_2 = \frac{[PL_2]}{[PL][L]}$

K_1, K_2 = Makroskopske konstante (koje se određuju eksperimentalno).

$P + L \rightleftarrows PL$

$$K_1 = \frac{[PL]}{[P][L]} = \frac{[-PL] + [LP-]}{[P][L]} = \frac{(K_1' + K_2')[P][L]}{[P][L]} = K_1' + K_2'$$

$$\boxed{K_1 = 2K'} \qquad k' = \frac{K_1}{2}$$

$$K_2 = \frac{[PL_2]}{[PL][L]} = \frac{[PL_2]}{([-PL] + [LP-])[L]} = \ldots = \frac{1}{\frac{1}{K_3'} + \frac{1}{K_4'}} \left(= \frac{1}{\frac{1}{K_1'} + \frac{1}{K_1'}} \right)$$

$$\boxed{K_2 = \frac{K'}{2}} \qquad k' = 2K_2$$

$\frac{K_1}{K_2} = \frac{2K'}{K'/2} = 4$ STATISTIČKI FAKTOR ZA DVA CENTRA

$K_1 = 4K_2$: NA OSNOVU VEROVATNOĆE K_1 = 4 x VEĆE OD K_2.

$$\nu = \frac{[PL] + 2[PL_2]}{[P] + [PL] + [PL_2]} = \frac{K_1[P][L] + 2K_1K_2[P][L]^2}{[P] + K_1[P][L] + K_1K_2[P][L]^2} =$$

$$= \frac{K_1[L] + 2K_1K_2[L]^2}{1 + K_1[L] + K_1K_2[L]^2} \quad \text{ALI} \quad K_1 = 2K' \quad i \quad K_2 = K'/2$$

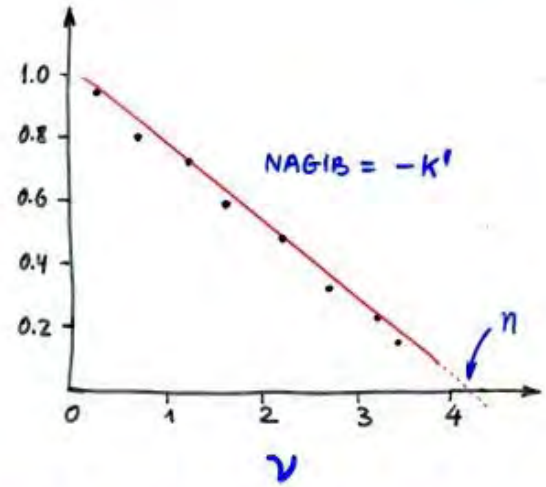
$$\nu = \frac{2K'[L] + 2K'^2[L]^2}{1 + 2K'[L] + 2K'^2[L]^2} = \frac{2K'[L](1 + K'[L])}{(1 + K'[L])^2} =$$

$$= \frac{2K'[L]}{1 + K'[L]} = \frac{2}{\frac{1}{K'[L]} + 1}$$

$$\nu = \frac{n \cdot K'[L]}{1 + K'[L]}$$

U OPŠTEM SLUČAJU ZA n ISTIH NEINTERAGUJUĆIH CENTARA

$$\nu = \frac{n \cdot K'[L]}{1 + K'[L]} \quad / : [L] \quad \Rightarrow \quad \frac{\nu}{[L]} = nK' - \nu K'$$



... A $\frac{1}{L}$ i $\frac{1}{\nu}$?
DIJAGRAM ("PLOT")

SCATCHARD-OV DIJAGRAM ZA LAKTAT-DEHIDROGENAZU / NADH