Hemoglobin (Hb): model za alosterne proteine (zavisne centre)

Krive vezivanja Hb/Mb i O_2:

- kooperativna priroda vezivanja Hb i O_2
- fiziološki značaj

Kvaternarna struktura Hb

Molekulski (Perutzov) mehanizam kooperativnog vezivanje Hb i O_2:

- Hb ima dve konformacije
- Simetrični vs sekvencionalni model

Komunikacija Hb sa okolinom

Kriva vezivanja O_2 za hemoglobin

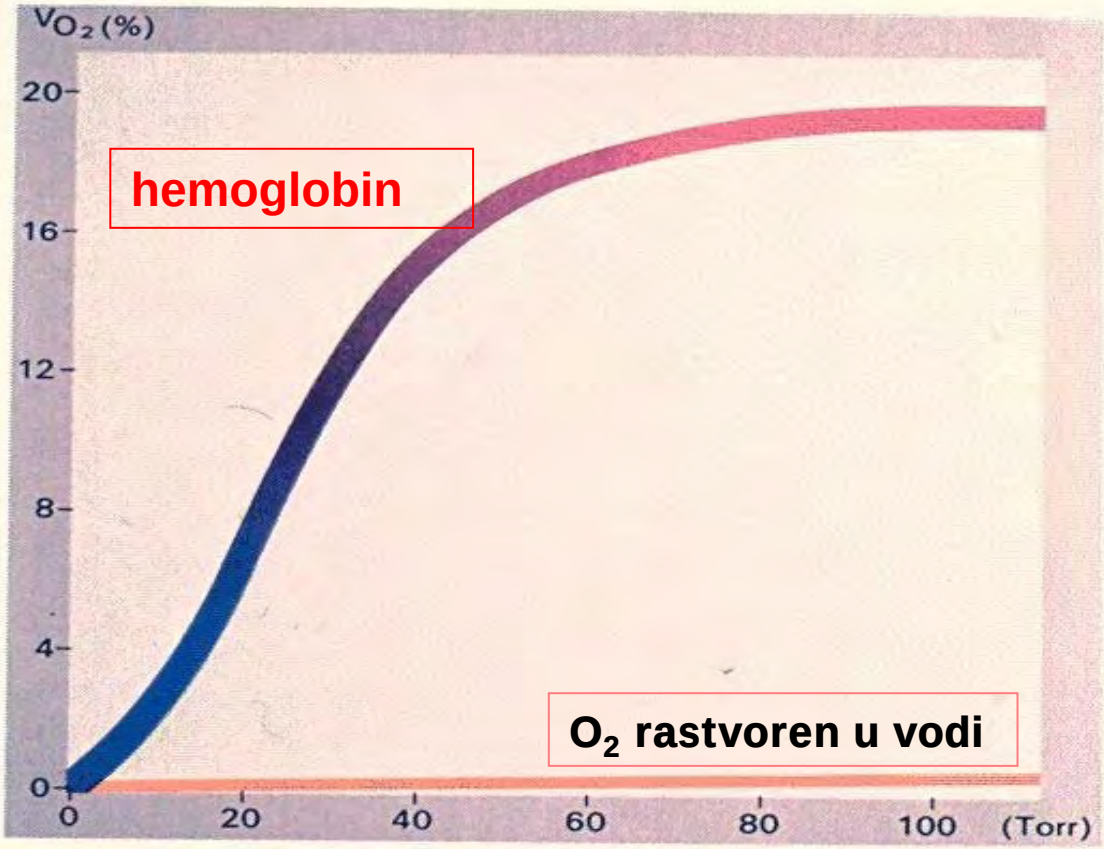
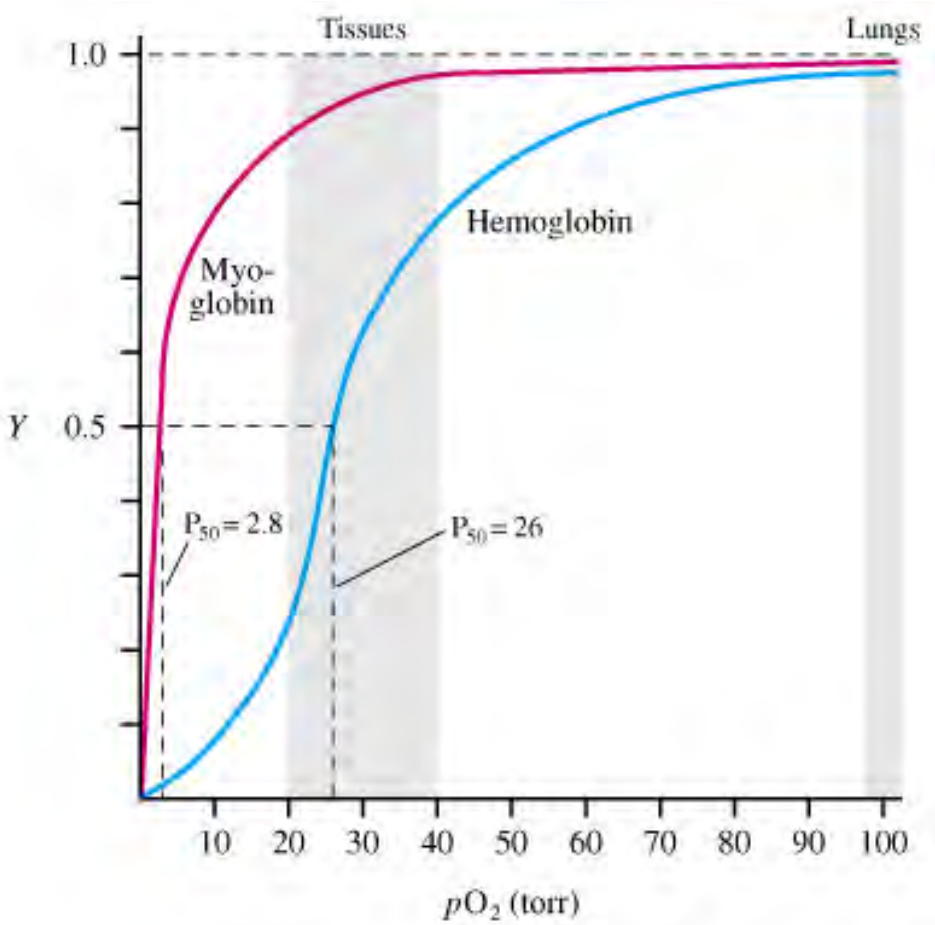


Abb. 10: Gelöster (orange) und gebundener Anteil (blaurot) des im Blut transportierten O_2
Koordinaten wie in Abb. 9

Kriva vezivanja O_2 za hemoglobin/mioglobin

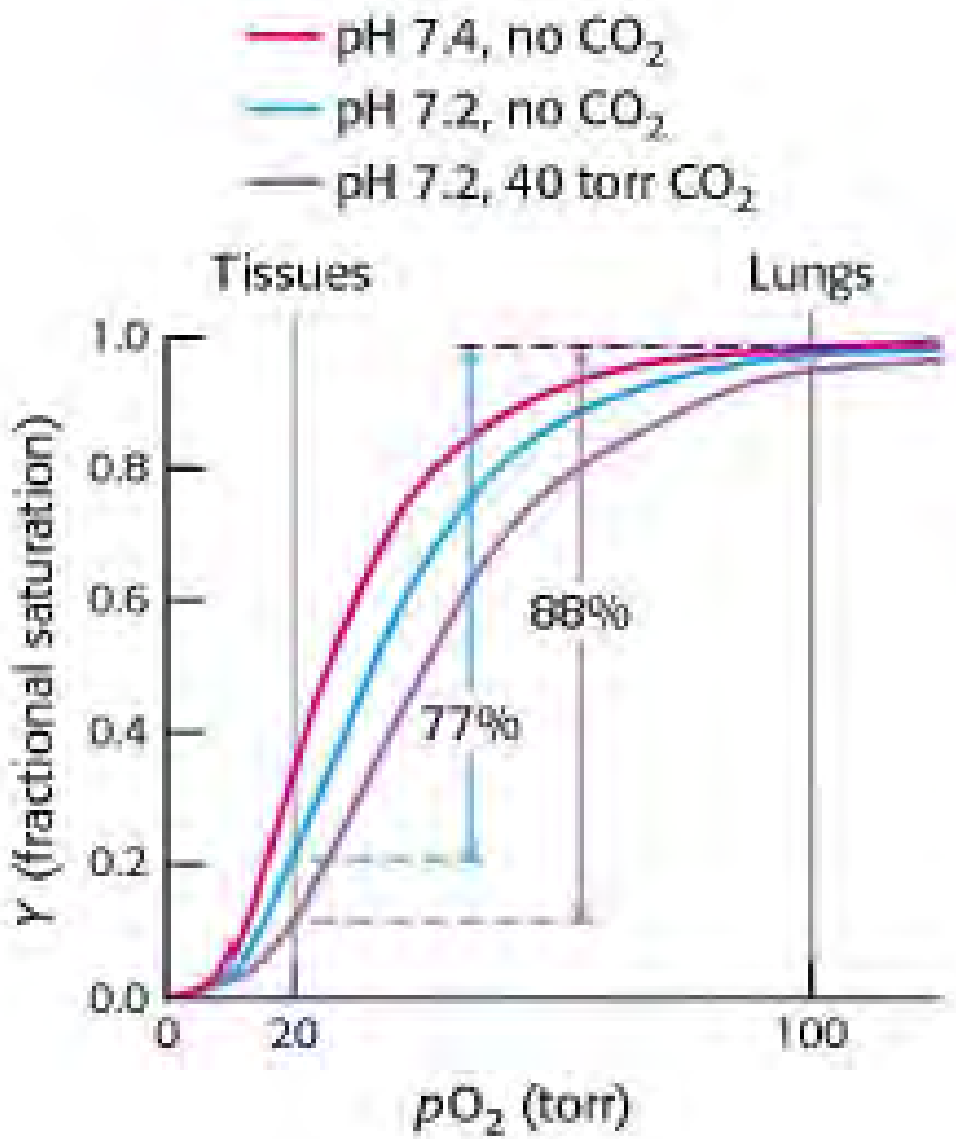


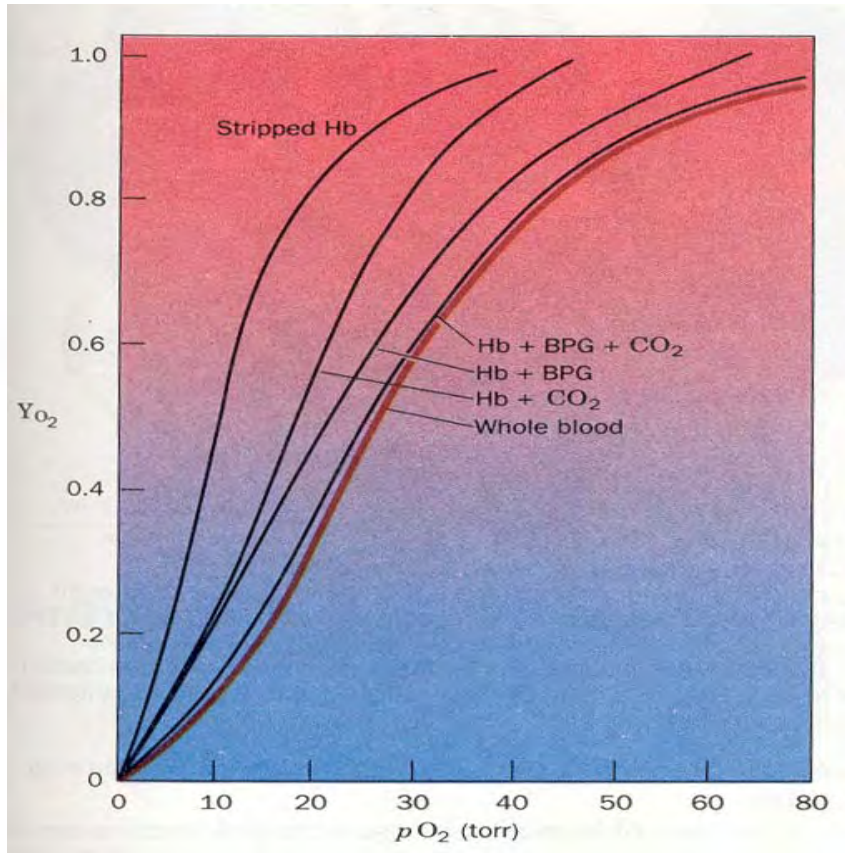
- Mioglobin:
  - Veći afinitet za O_2
  - Kriva vezivanja je hiperbola
    - O_2:Mb 1:1
  - Mb vezuje O_2 u tkivima

- Hemoglobin:
  - Manji afinitet za O_2
  - Sigmoidna kriva
  - O_2:Hb 4:1
  - Hb vezuje O_2 u plućima,
    - a oslobađa ga u tkivima
  - Hb je idealno podešen
    - za funkciju koju obavlja!!!!

Regulacija vezivanja O_2 za Mb i Hb

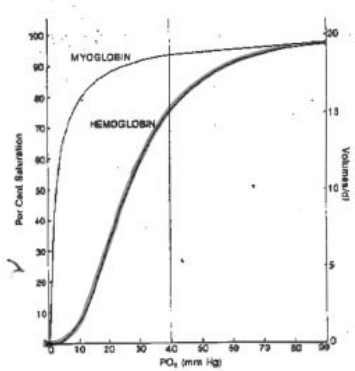
Efekat pH, CO_2 i BPG na vezivanje O_2 za Hb



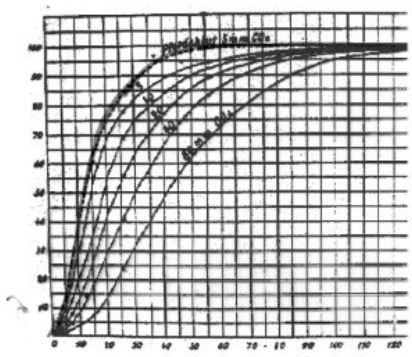


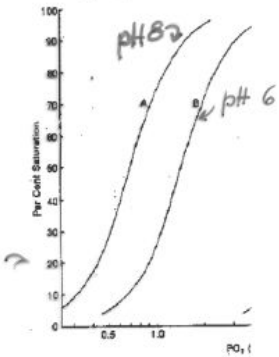
Vezivanje O_2 za hemoglobin: prelomna istraživanja

1904



Efekat CO_2 na vezivanje O_2 za Hb





Efekat pH na vezivanje O_2 za Hb (Bohrov efekat)

1910

$$Hb + n\,O_2 \rightleftharpoons Hb(O_2)_n$$

$$K = \frac{[Hb][O_2]^n}{[Hb(O_2)_n]}$$

$$\nu = \frac{[O_2]^n}{K + [O_2]^n}$$

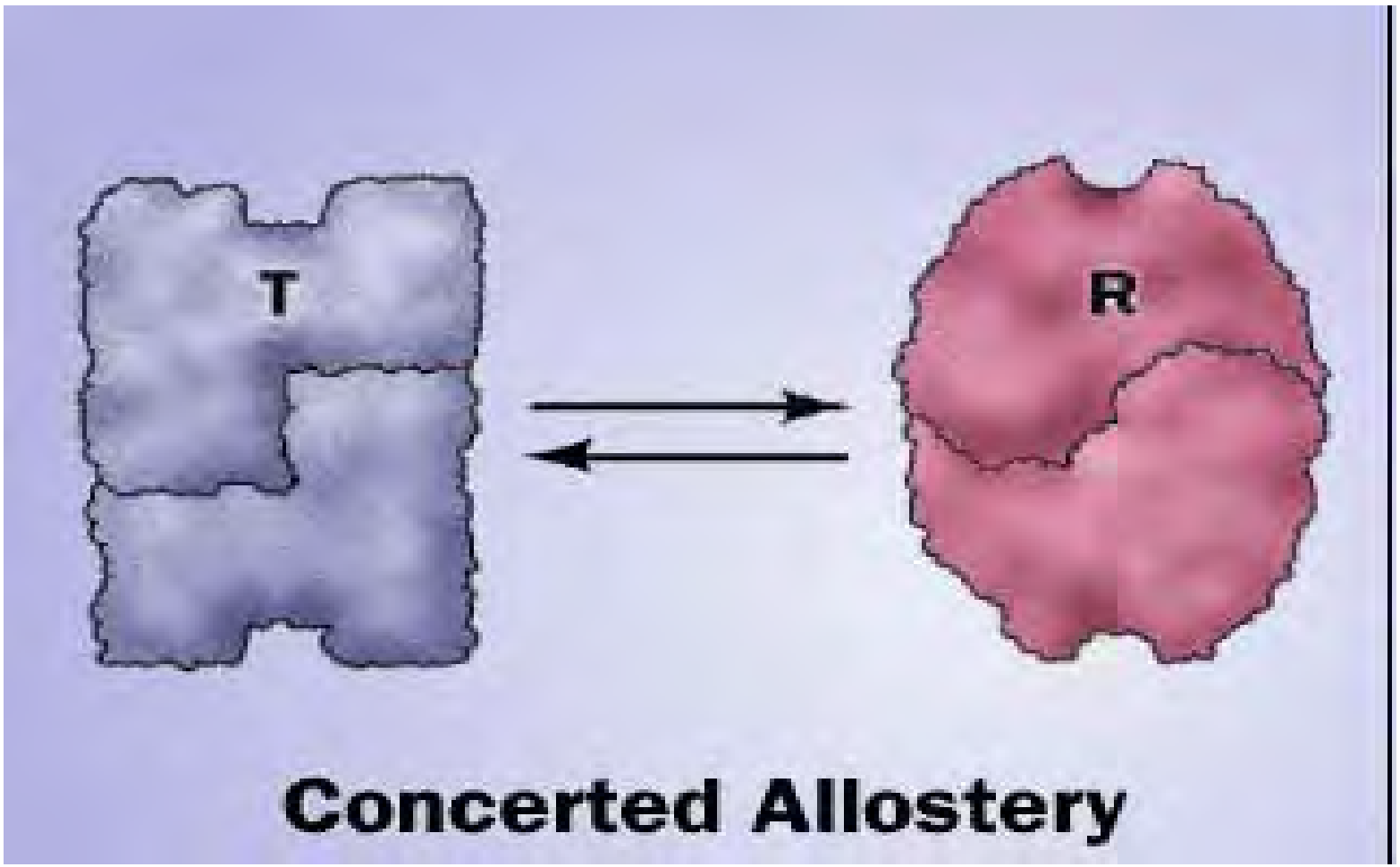
↳ % ZASIĆENJA Hb sa O_2

A.Hill: kooperativno vezivanje O_2 za Hb!!!

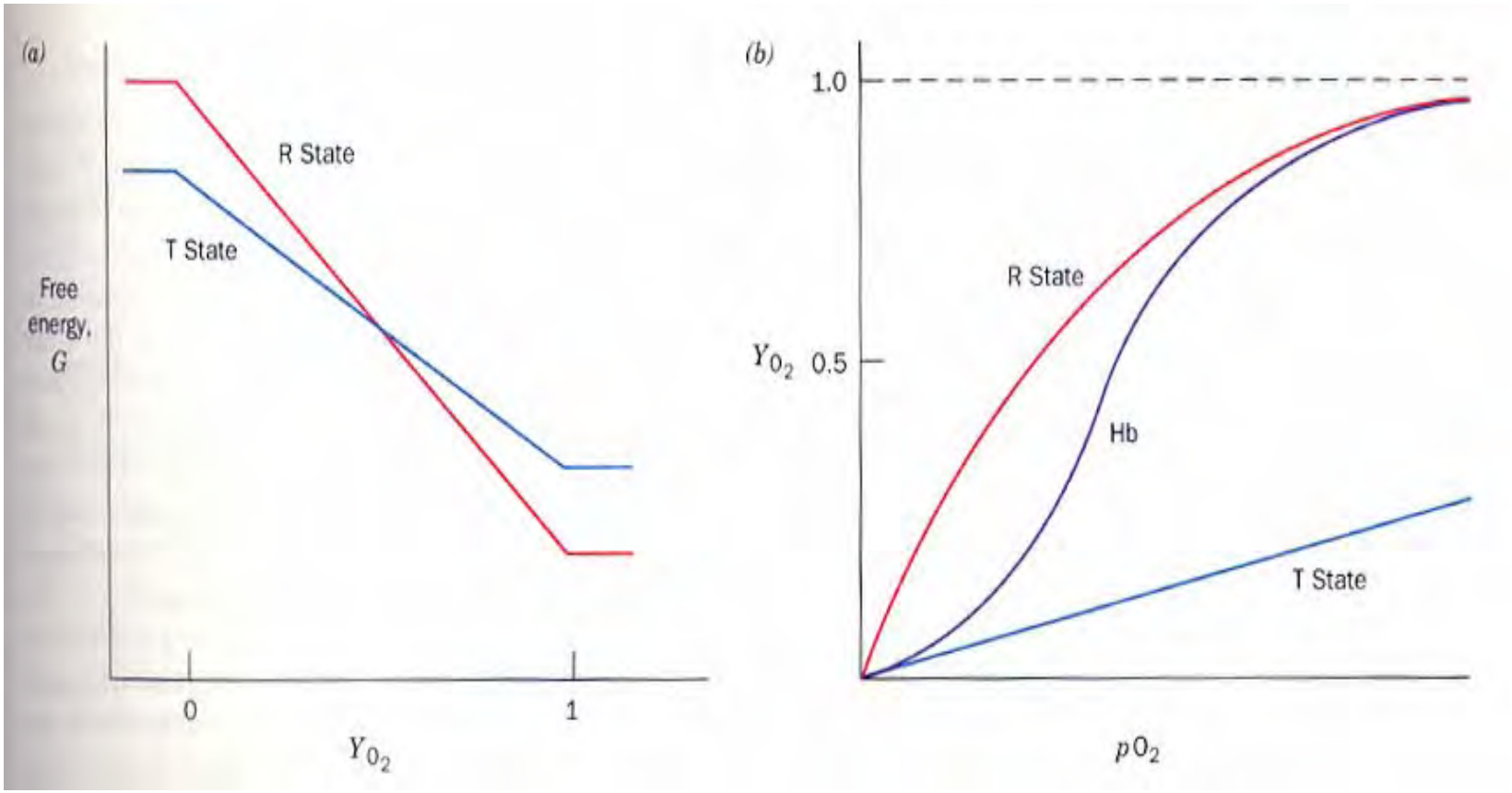
Kako objasniti kooperativnost?

Simetrični MWC model

(Monod, Wyman i Changeux)



R i T stanje Hb u funkciji koncentracije O_2



K_1 = 8,8 tora K_2 = 6.1 tora K_3 = 0.85 tora K_4 = 0.25 tora

Eksperimentalno određene konstante disocijacije za $Hb(O_2)_4$

MAX PERUTZ

1936	ZAPOČEO DOKTORAT .....KRISTALNA STRUKTURA HEMOGLOBINA
1959	KRISTALNA STRUKTURA MET-HEMOGLOBINA
1962	NOBELOVA NAGRADA
1962	KRISTALNA STRUKTURA DEZOKSI-HEMOGLOBINA

"Making a discovery is such a wonderful thing. It s like falling in love and getting to the top of the mountain all in one. When you get to the top after a hard climb, a view of a new landscape open before you."

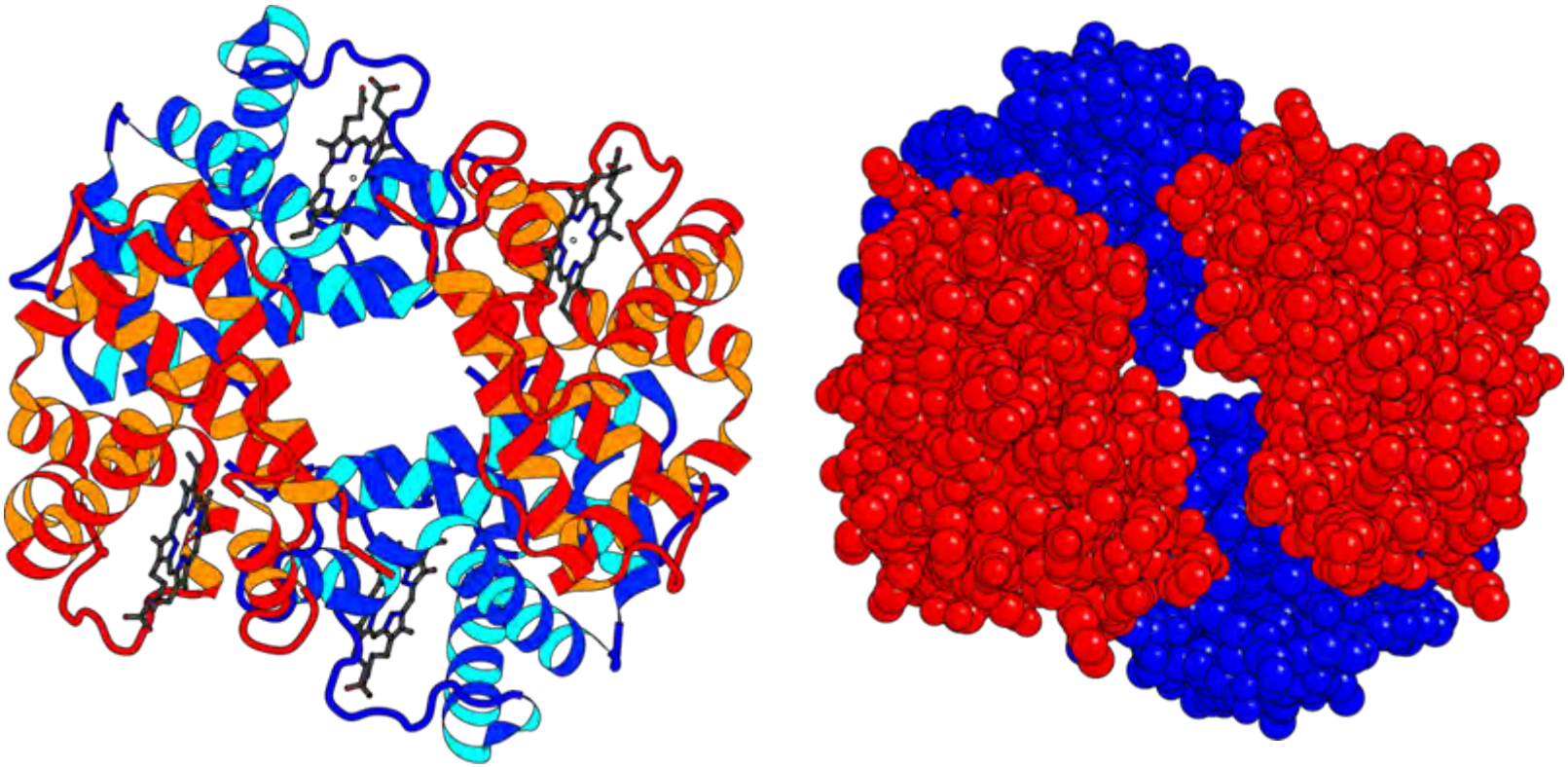
M. Perutz:

(1) 3-D struktura Hb

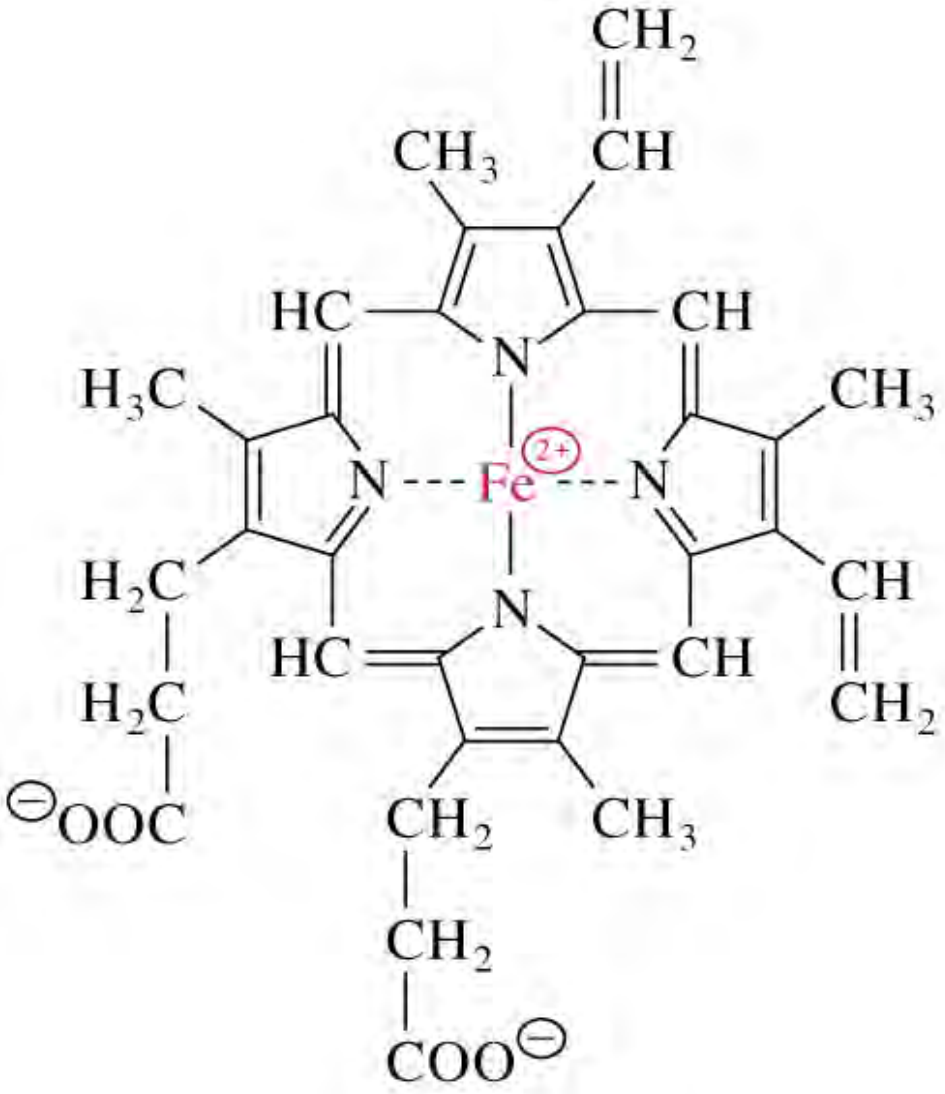
(2) Molekulski mehanizam kooperativnog vezivanja O_2

Alan Fersht (2004):
"Max sought the truth in science and what was right and worthwhile in life".

Struktura molekula hemoglobina

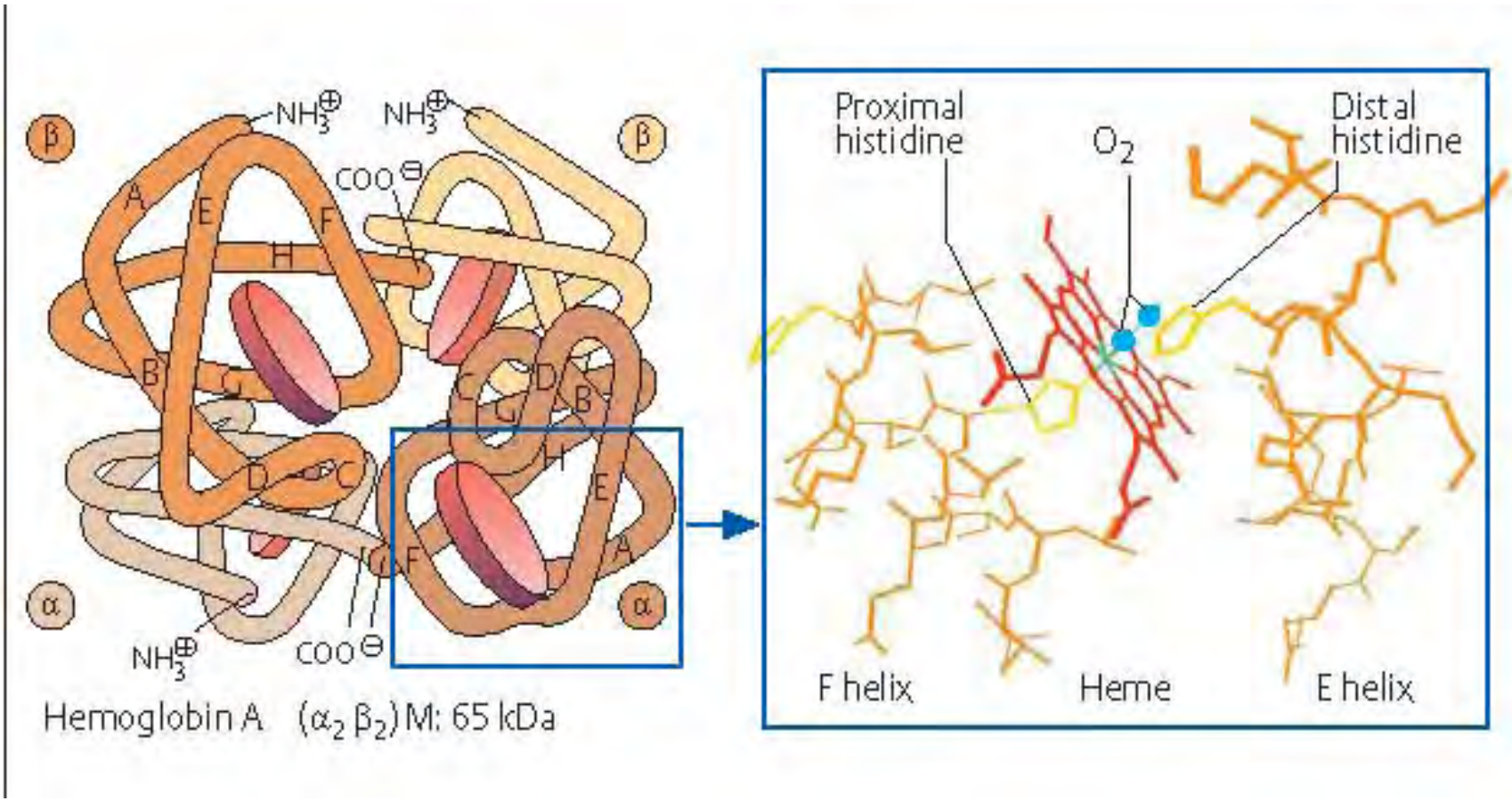


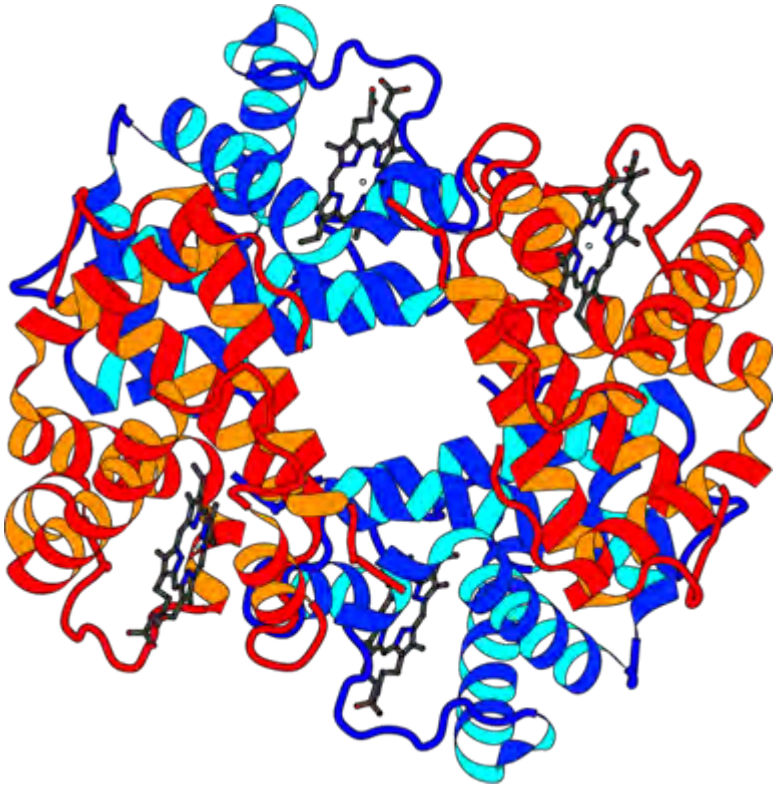
Hb i Mb su crveni zbog prisustva hema



Struktura hemoglobina

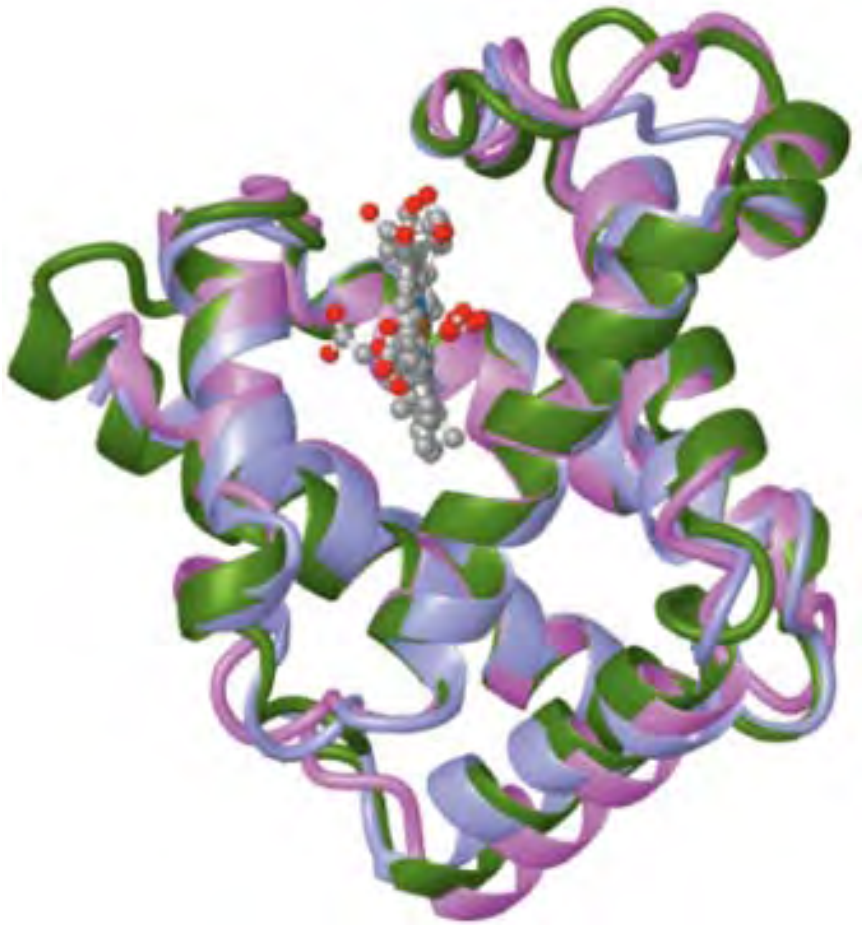
Hem se nalazi u hidrofobnom džepu





3-D strukture mioglobina i subjedinica Hb su vrlo slične!!!

Zaključak (odakle potiče kooperativnost Hb)?

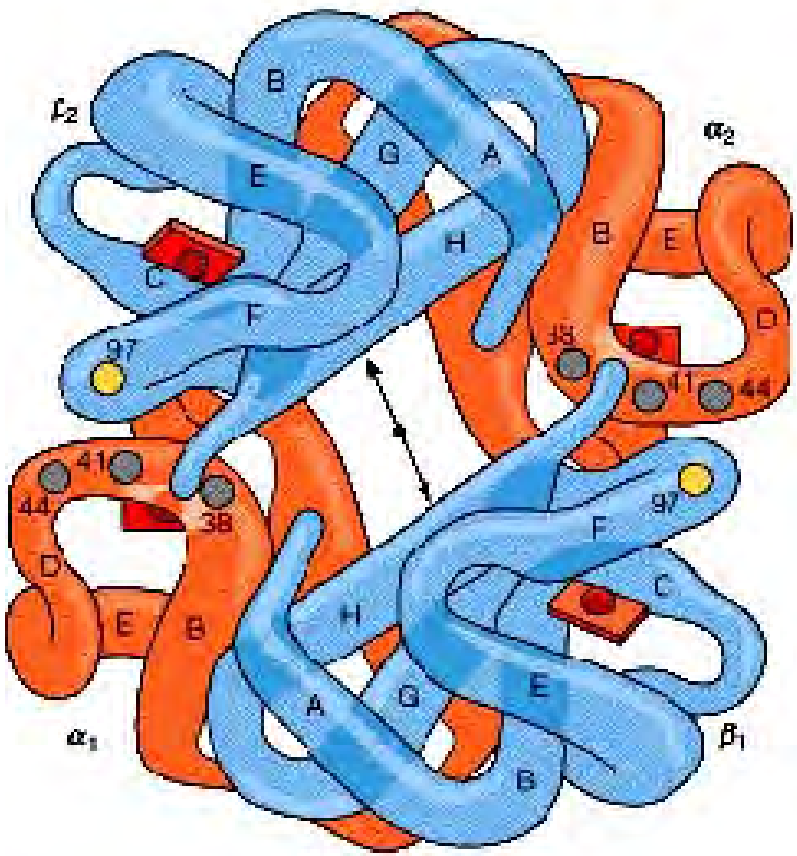


α- niz Hb (plav)

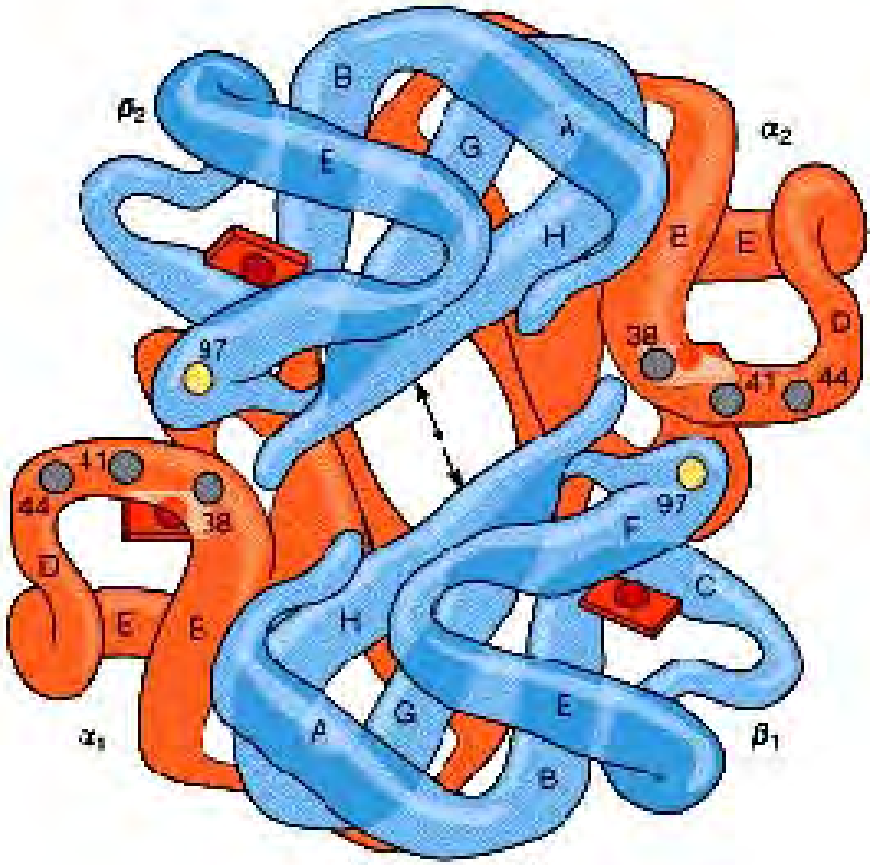
β-niz Hb (roza)

Mioglobin (zelen)

DezoksiHb (T) i oksiHb (R) su u ravnoteži

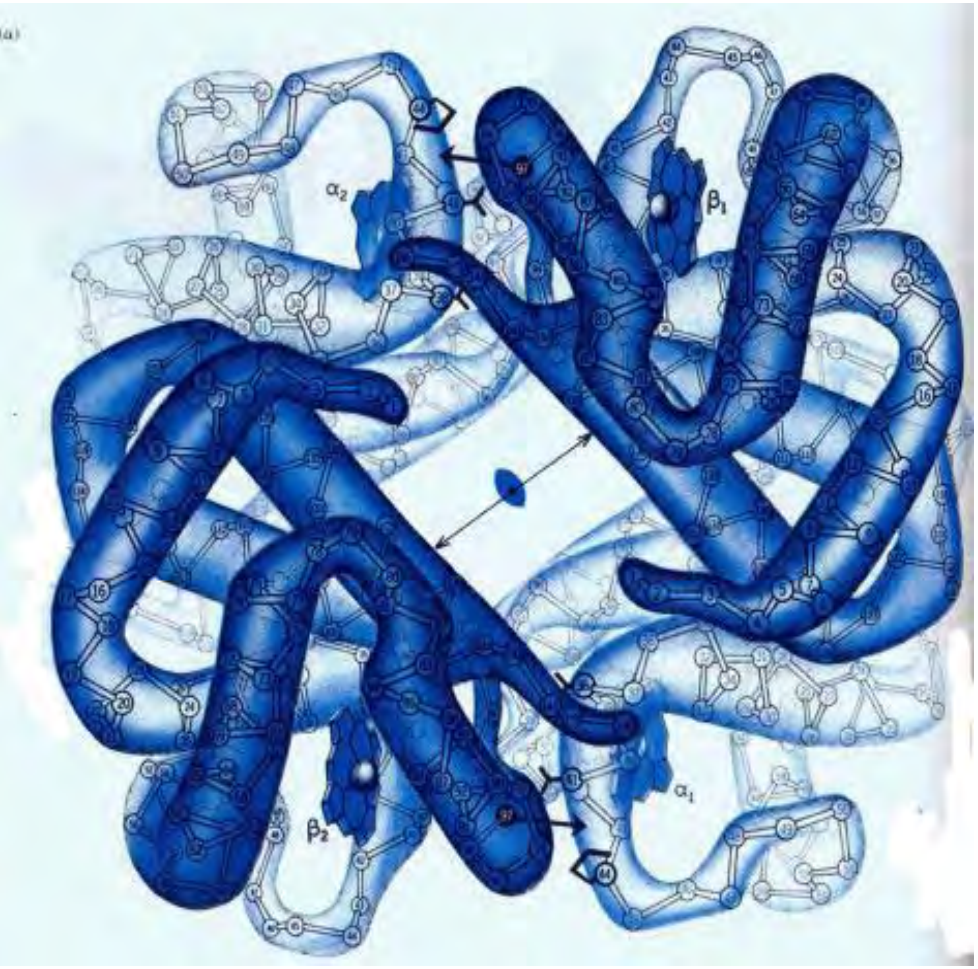


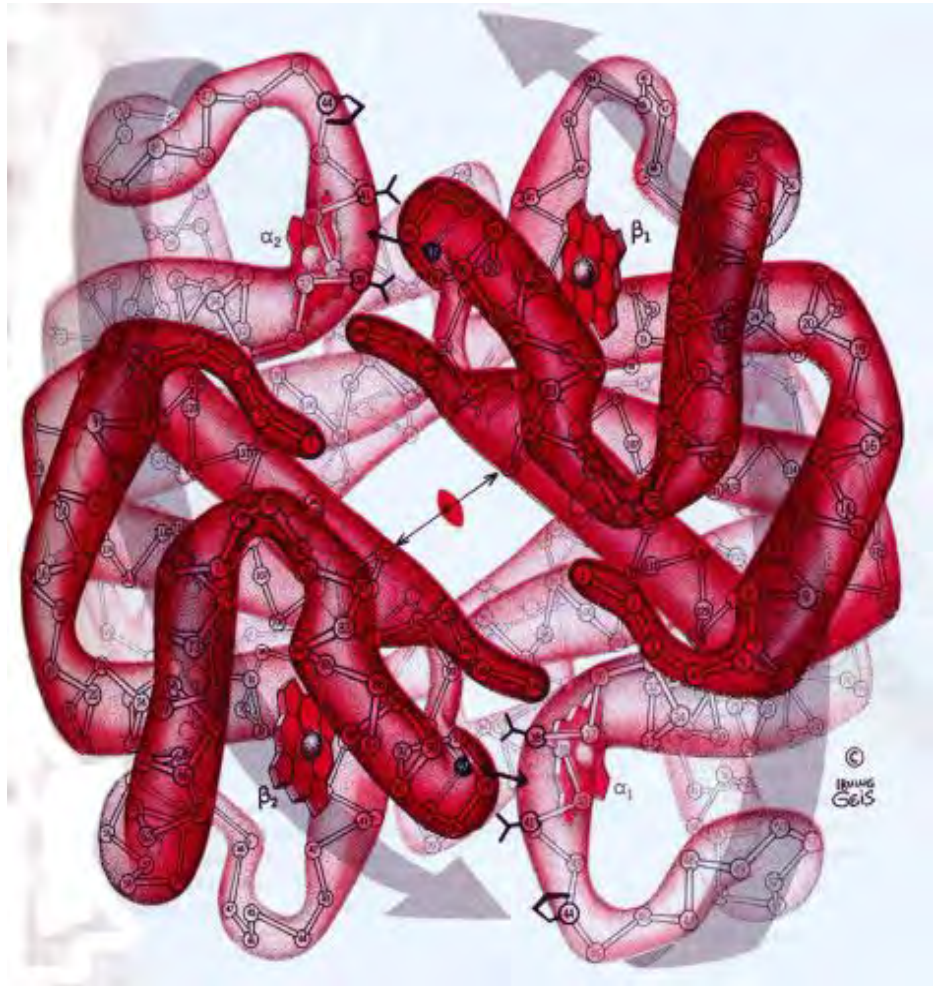
Deoxyhemoglobin

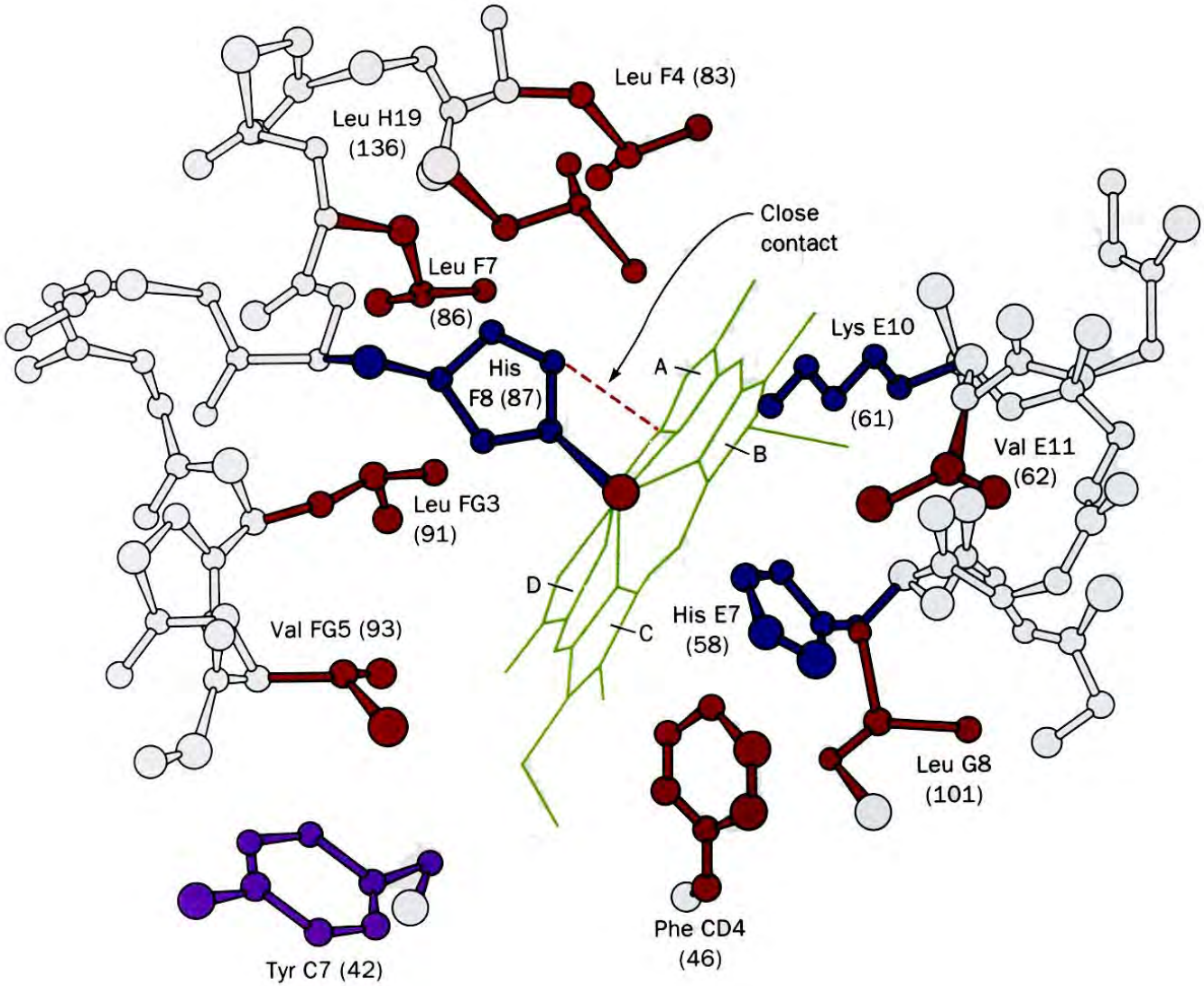


Oxyhemoglobin

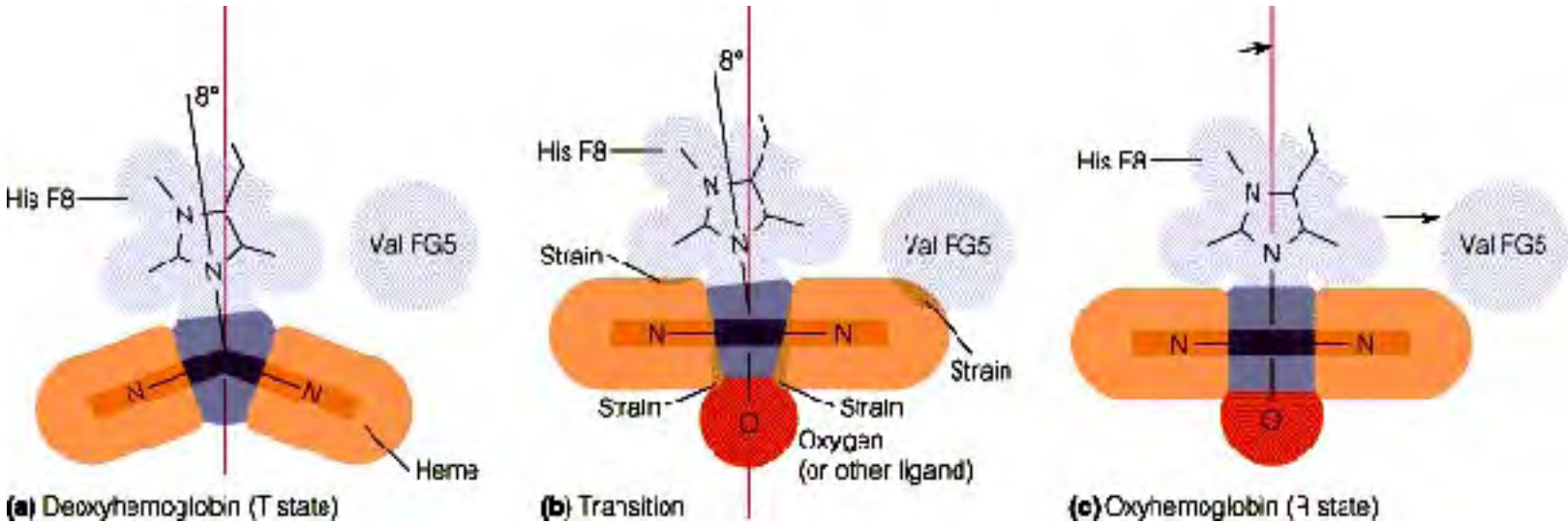
Struktura dezoksi (T) i oksi (R)-hemoglobina

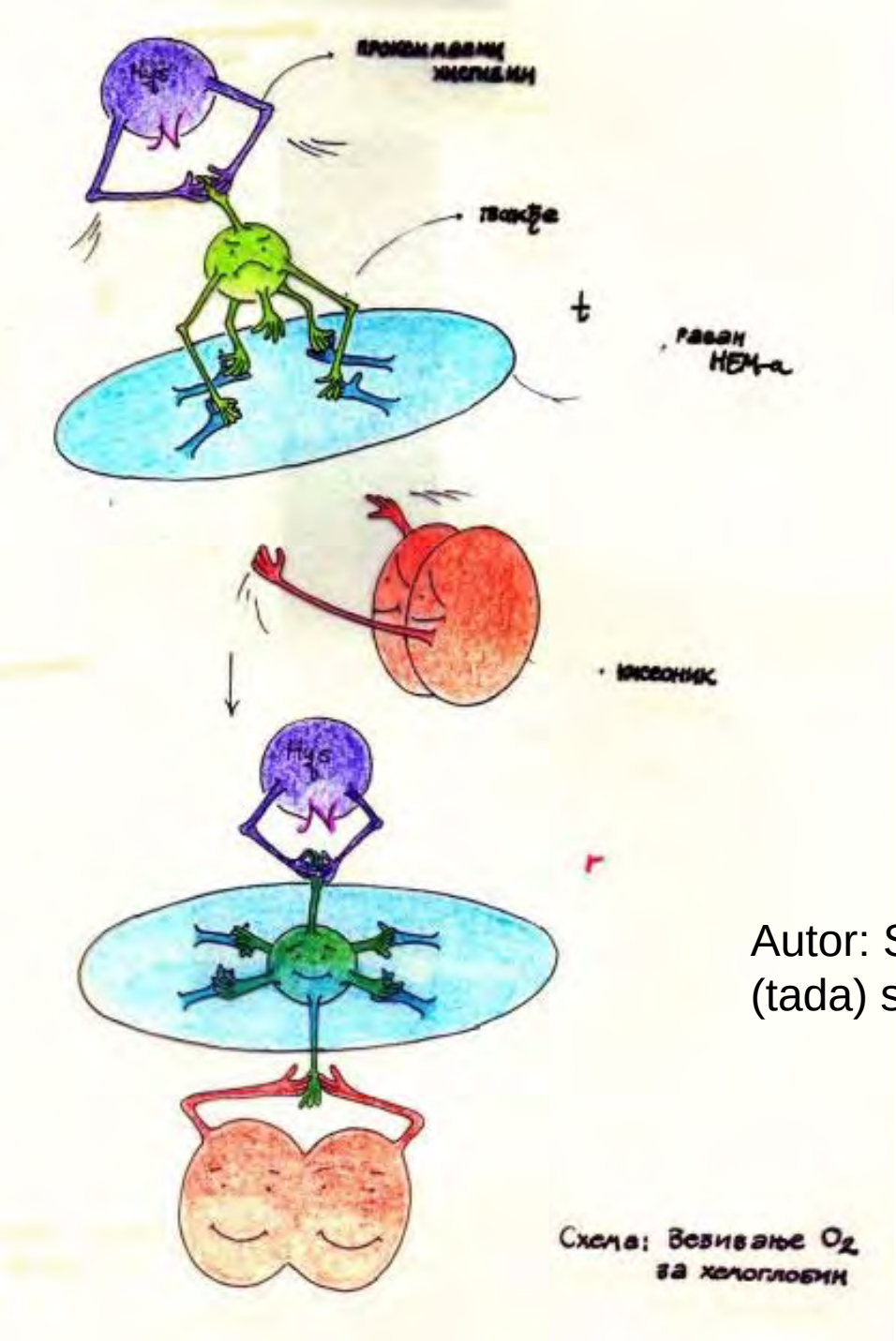




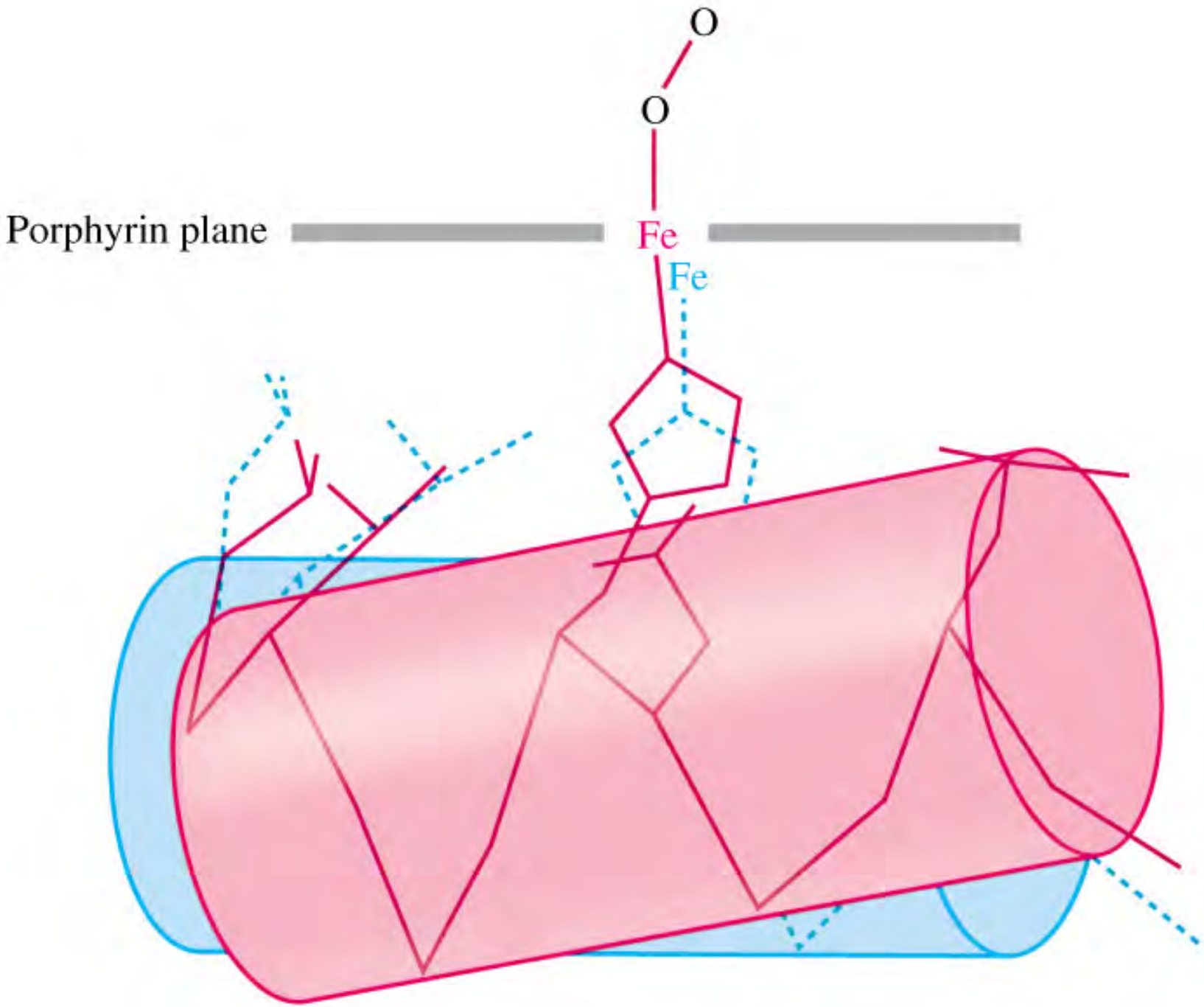

Leu F4 (83)
Leu H19
(136)
Close
contact
Leu F7
(86)
Lys E10
(61)
His
F8 (87)
A
B
Val E11
(62)
Leu FG3
(91)
D
C
His E7
(58)
Val FG5 (93)
Leu G8
(101)
Phe CD4
(46)
Tyr C7 (42)

Mehanizam okidača za prelaz T u R: vezivanje O_2 za hem

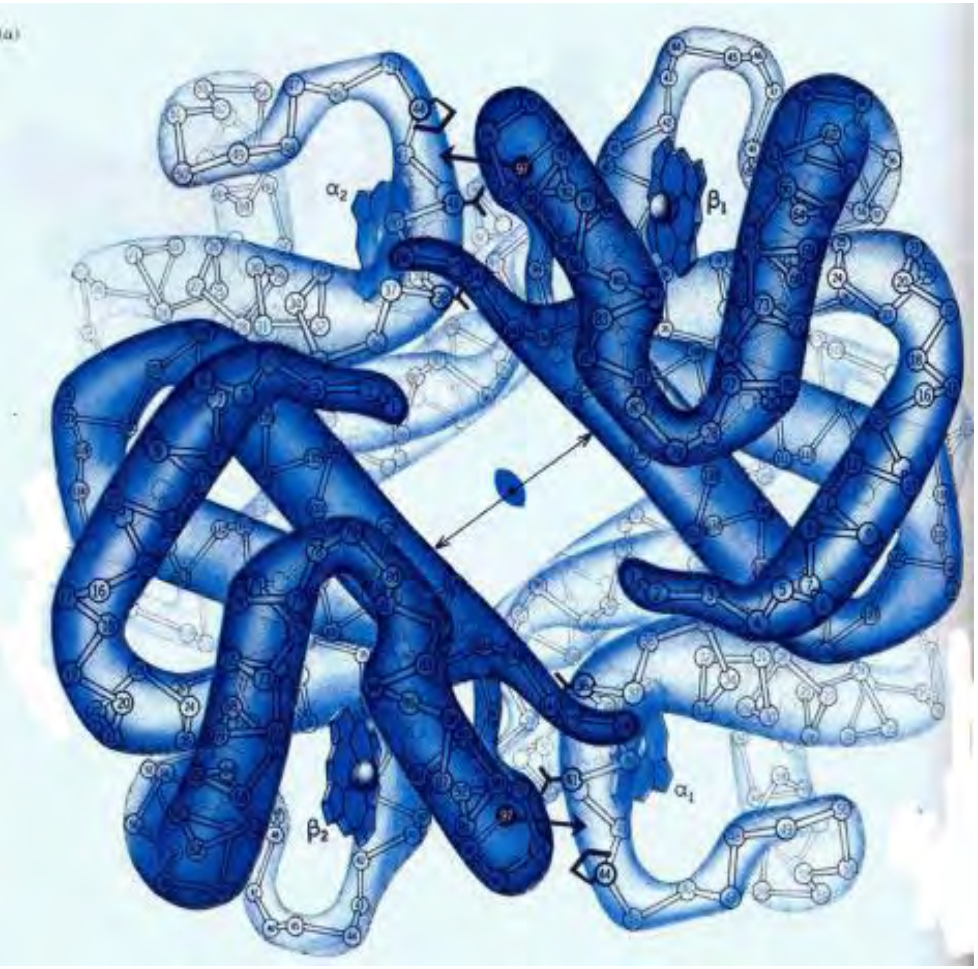


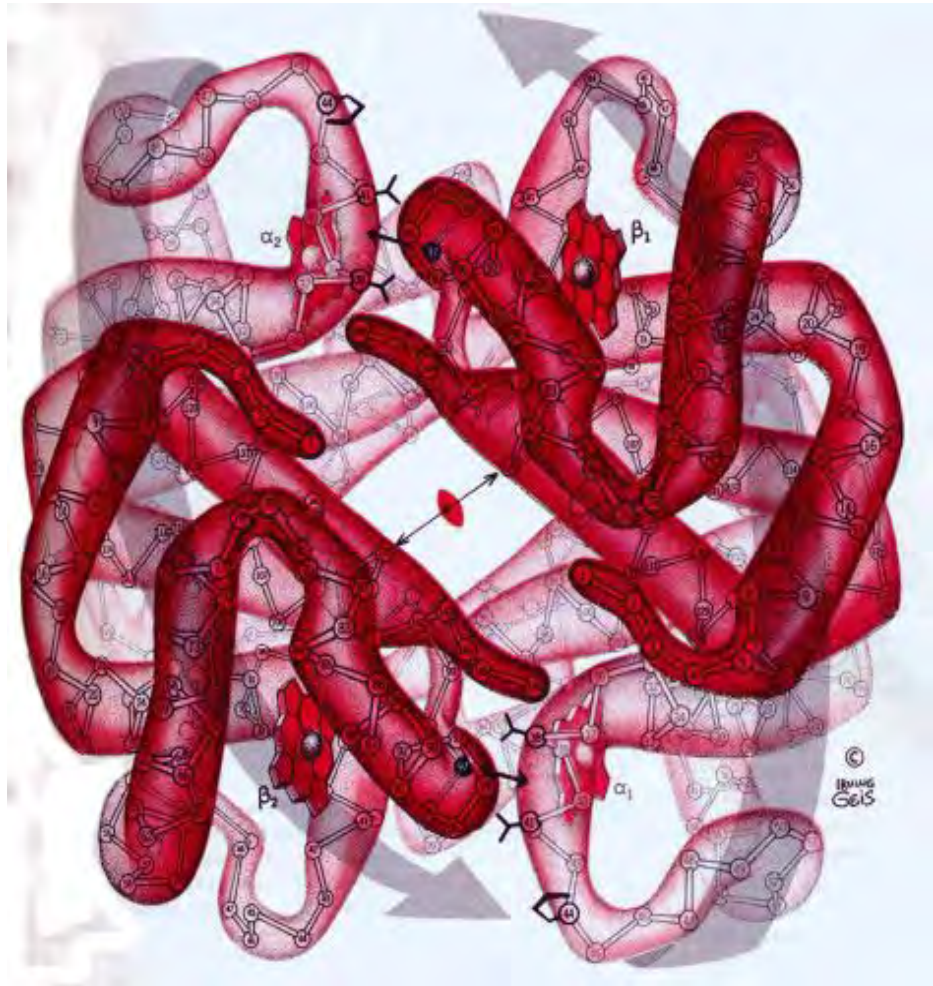


Autor: Selena Milićević
(tada) student III godine hemije


O
O
Porphyrin plane
Fe
Fe

Struktura dezoksi (T) i oksi (R)-hemoglobina



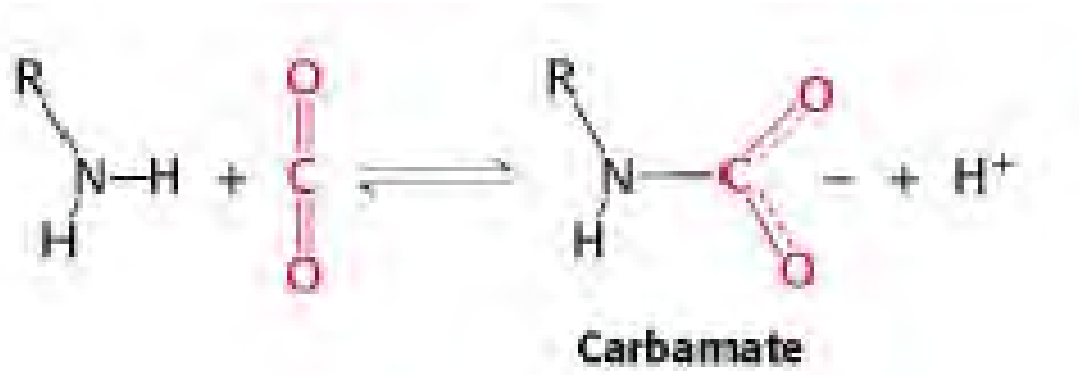


Komunikacija Hb sa okolinom:

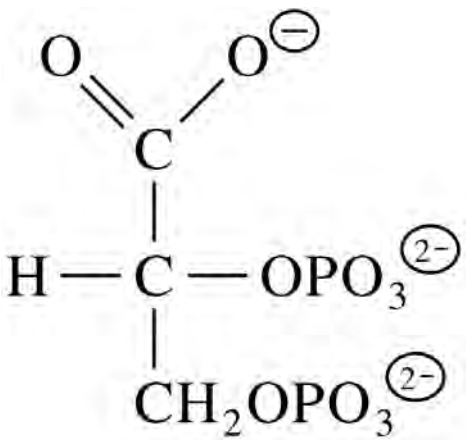
vezivanje CO_2

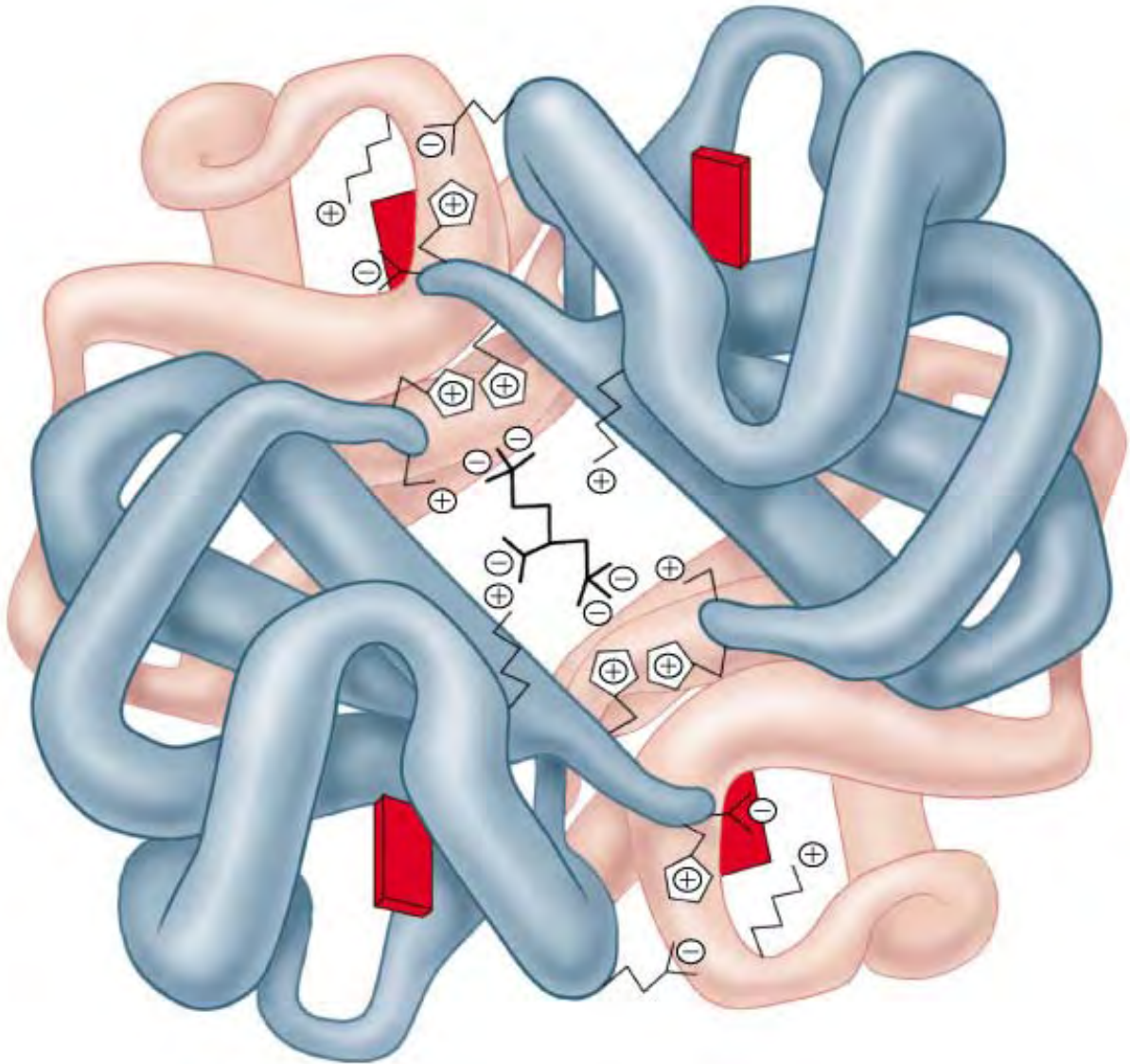
vezivanje BPG

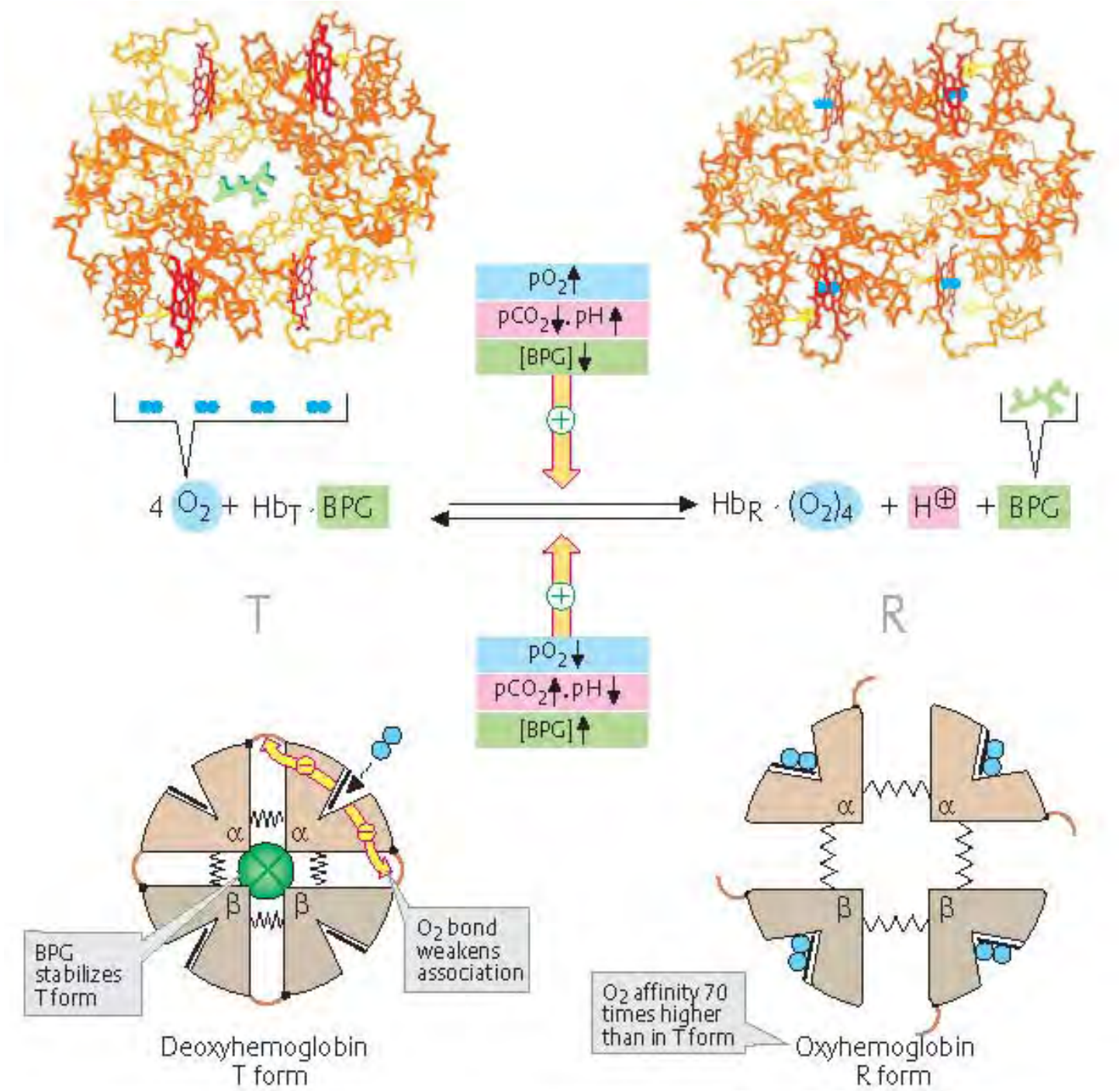
Vezivanje CO_2 za amino terminal α niza

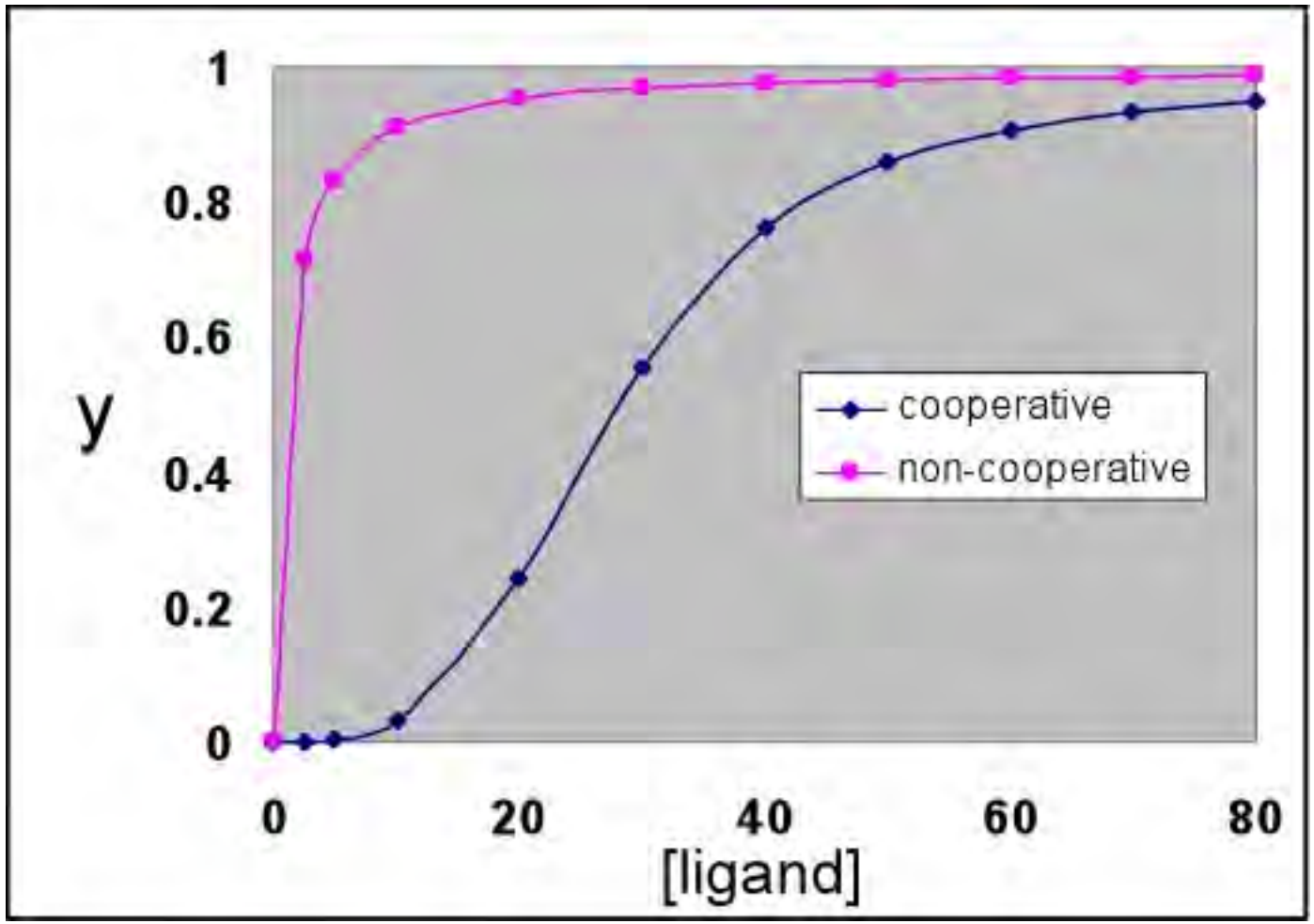


2,3 bis fosfoglicerat (BPG) je alosterni inhibitor Hb

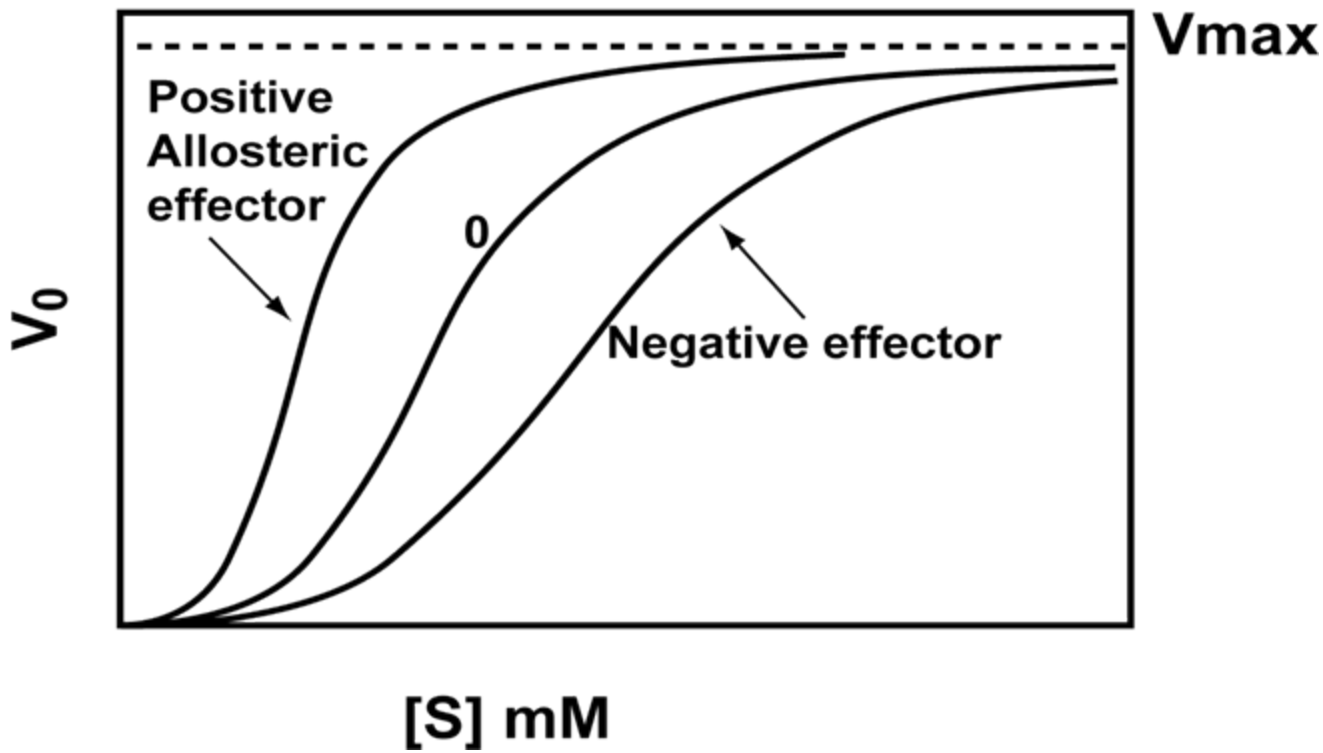





pO2↑
pCO2↓. pH↑
[BPG]↓
4 O2 + HbT · BPG
HbR · (O2)4 + H⊕ + BPG
T
R
pO2↓
pCO2↑. pH↓
[BPG]↑
α
α
β
β
BPG stabilizes T form
O2 bond weakens association
Deoxyhemoglobin T form
O2 affinity 70 times higher than in T form
Oxyhemoglobin R form


1
0.8
0.6
0.4
0.2
0
y
0
20
40
60
80
[ligand]
cooperative
non-cooperative

Interakcije sa ligandima: zavisni centri

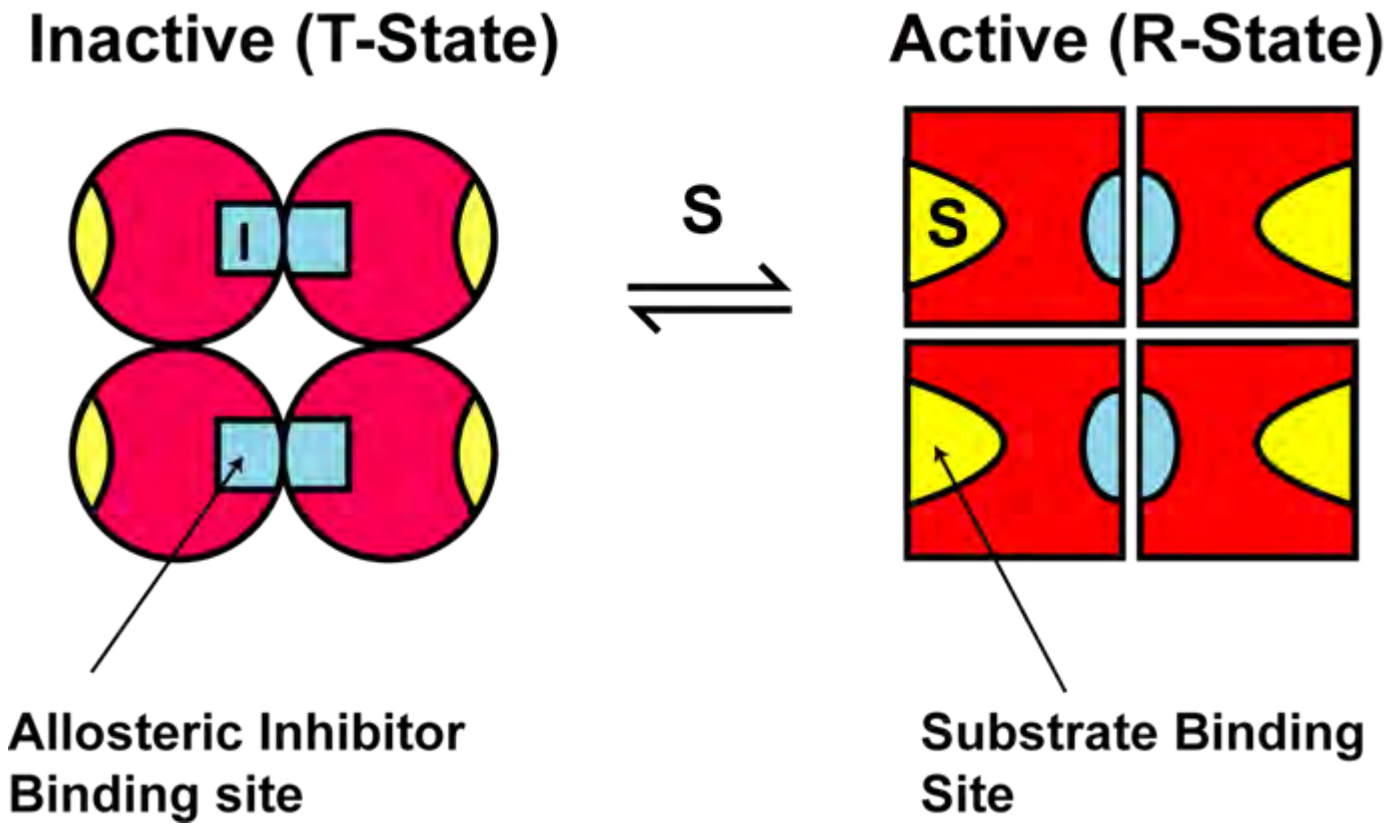


Regulacija aktivnosti!!!

Komunikacija sa okolinom!!!!

Mehanizam?

Simetrični MWC model



- **uprošćen prikaz!**

Alosterizam

- Alosterni efektori se vezuju za centar različit od aktivnog centra i menjaju konformaciju i aktivnost proteina
  - Aktivatori stabilizuju R stanje
  - Inhibitori stabilizuju T stanje

- Hemoglobin je model za alosterne proteine/enzime