

STRUTTURA-FUNZIONE DELLE PROTEINE

UN BINOMIO
INSCINDIBILE

Funzioni delle proteine

<u>Strutturale</u>	extracellulari(proteine della matrice) intracellulari (proteine citoscheletriche)
<u>Trasporto</u>	extracellulari(proteine trasportatrici del sangue) intracellulari transmembrana
<u>Catalisi</u>	Enzimi
<u>Riconoscimento</u>	Recettori Immunoglobuline
<u>Modulatori di attività biologiche:</u>	ormoni, fattori di crescita
<u>Motilità:</u>	Proteine contrattili del muscolo.

Funzioni di trasporto

Proteine trasportatrici
del sangue

ligandi

Albumina

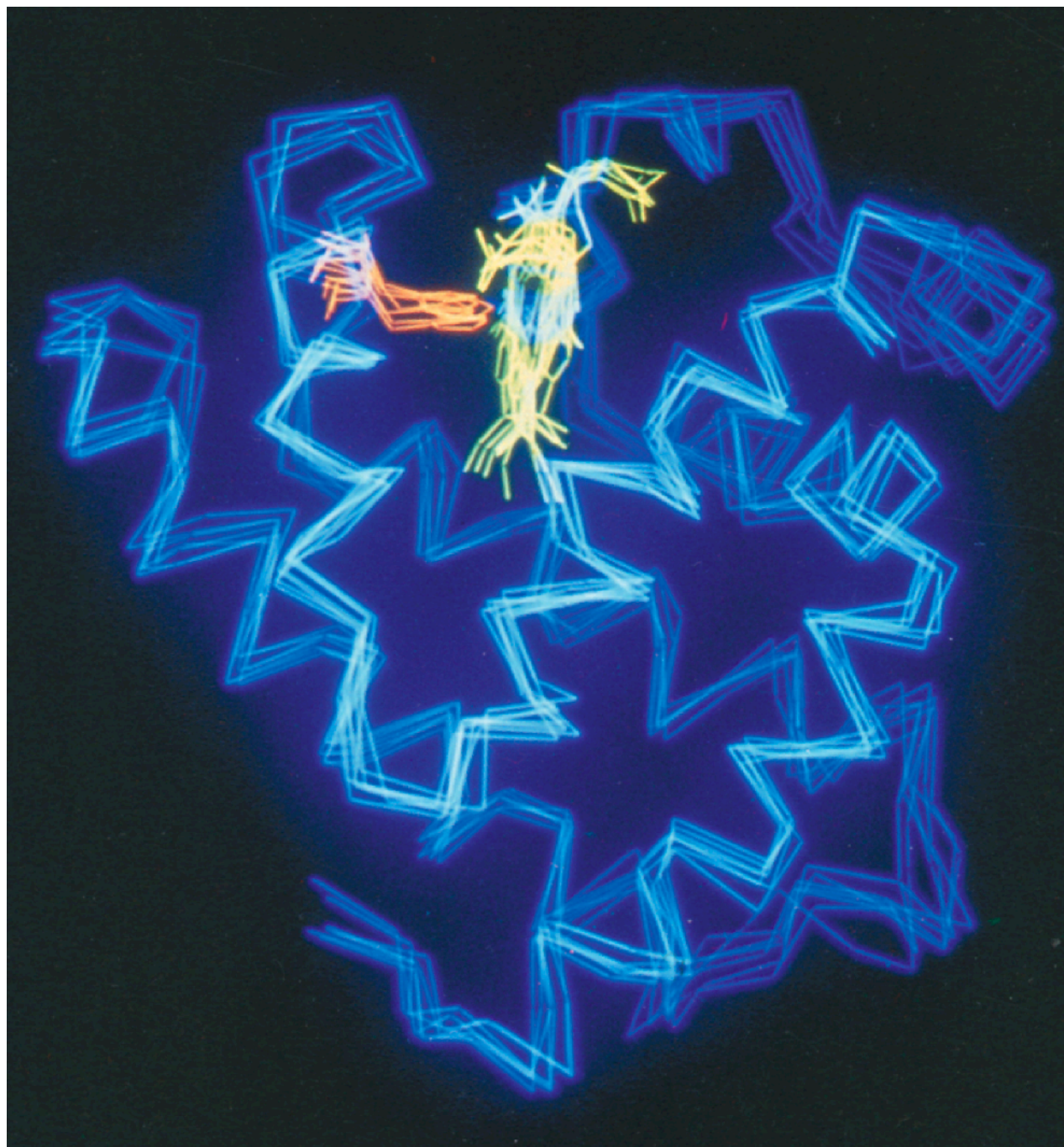
Cationi bivalenti, bilirubina, acidi grassi

Ceruloplasmina
Transferrina

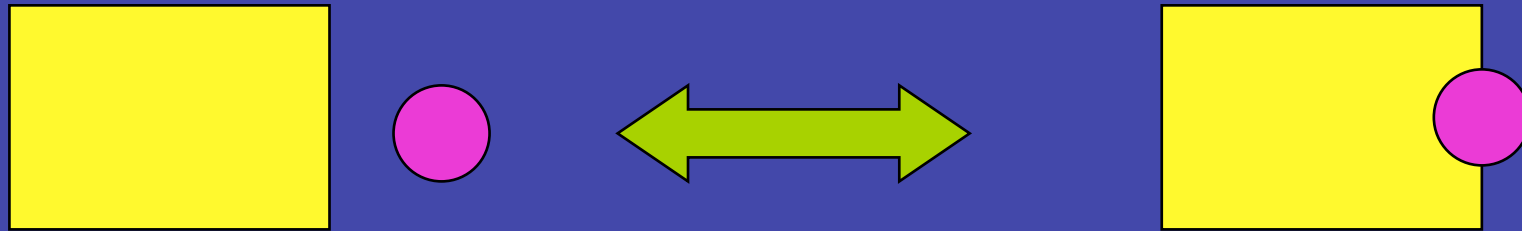
Cu^{2+}
 Fe^{2+}

TBG
SHBG
CBG

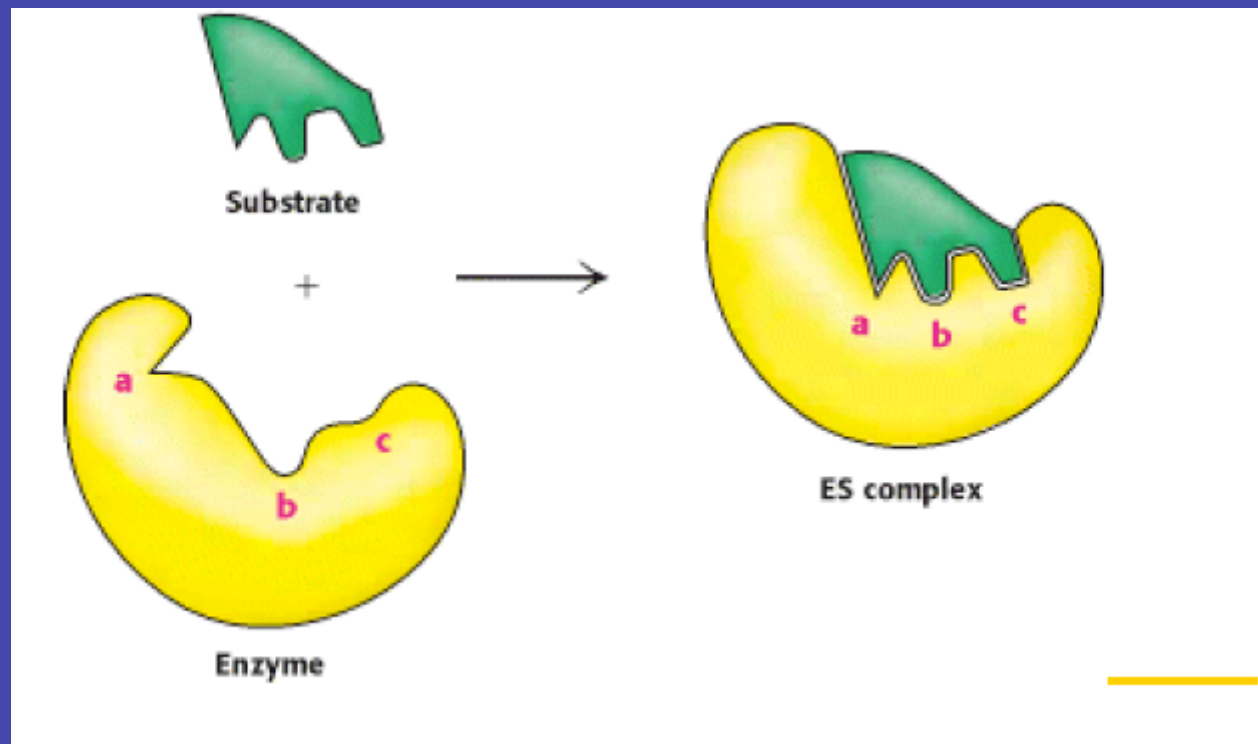
Ormoni tiroidei
Ormoni sessuali
Cortisolo



Processo di legame

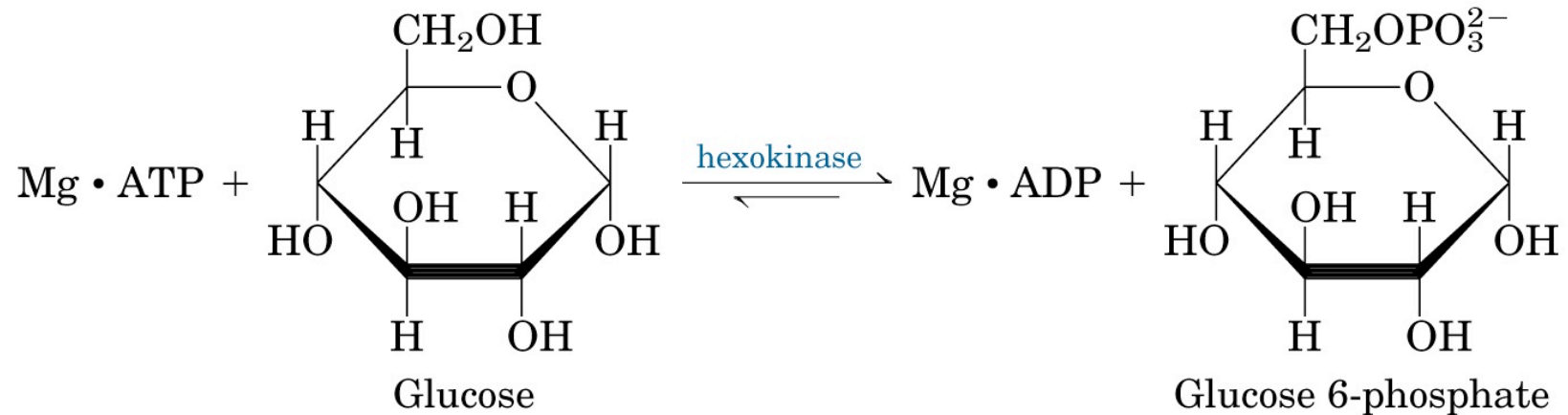
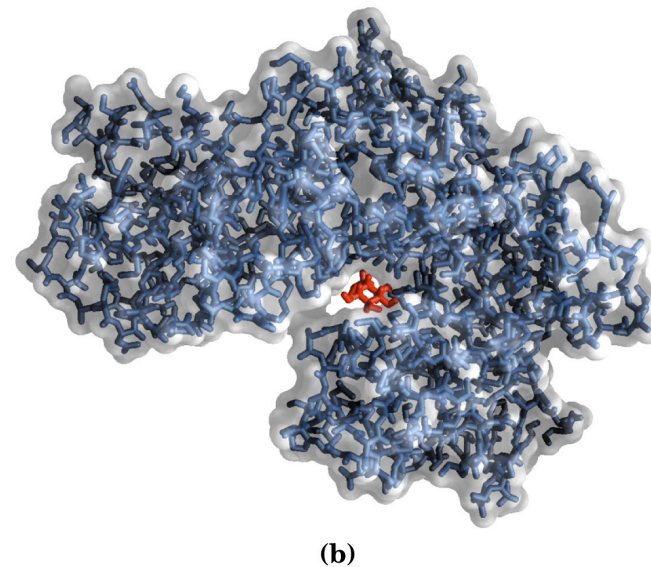
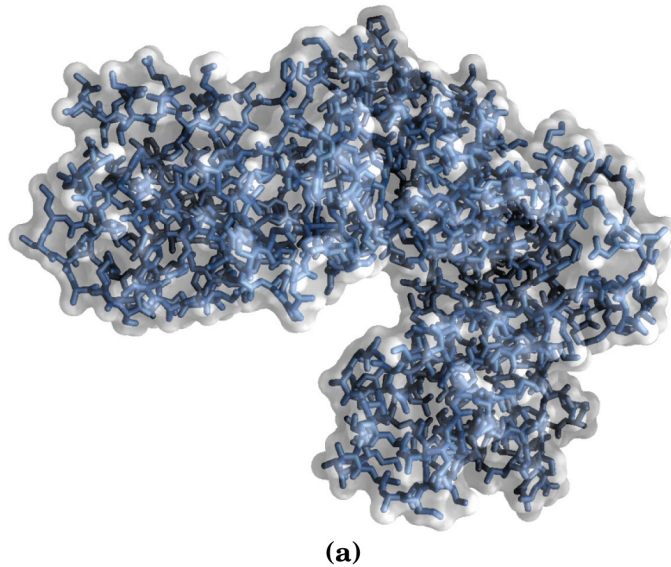


Adattamento indotto

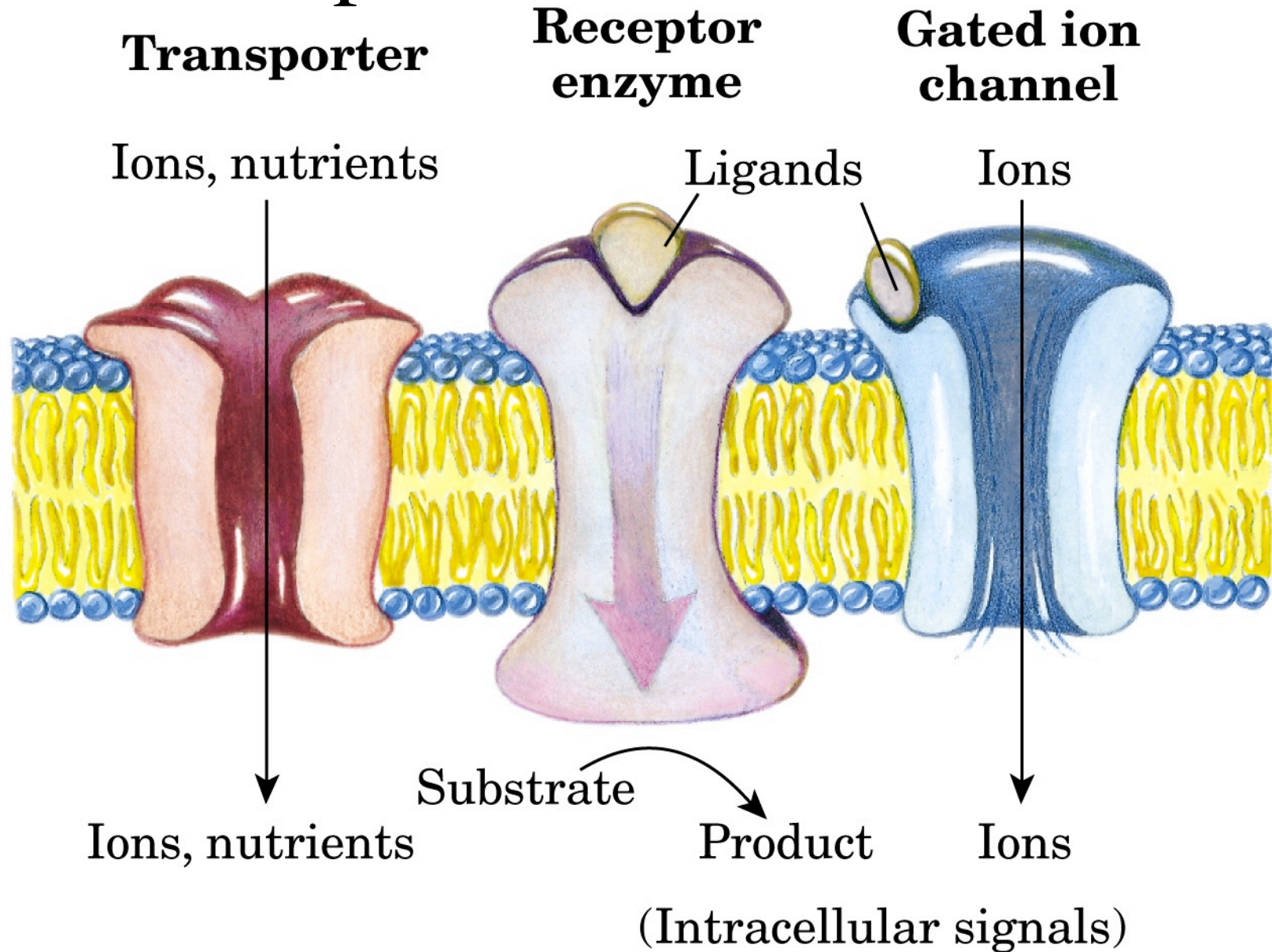


Esempio di induced fitting: esochinasi

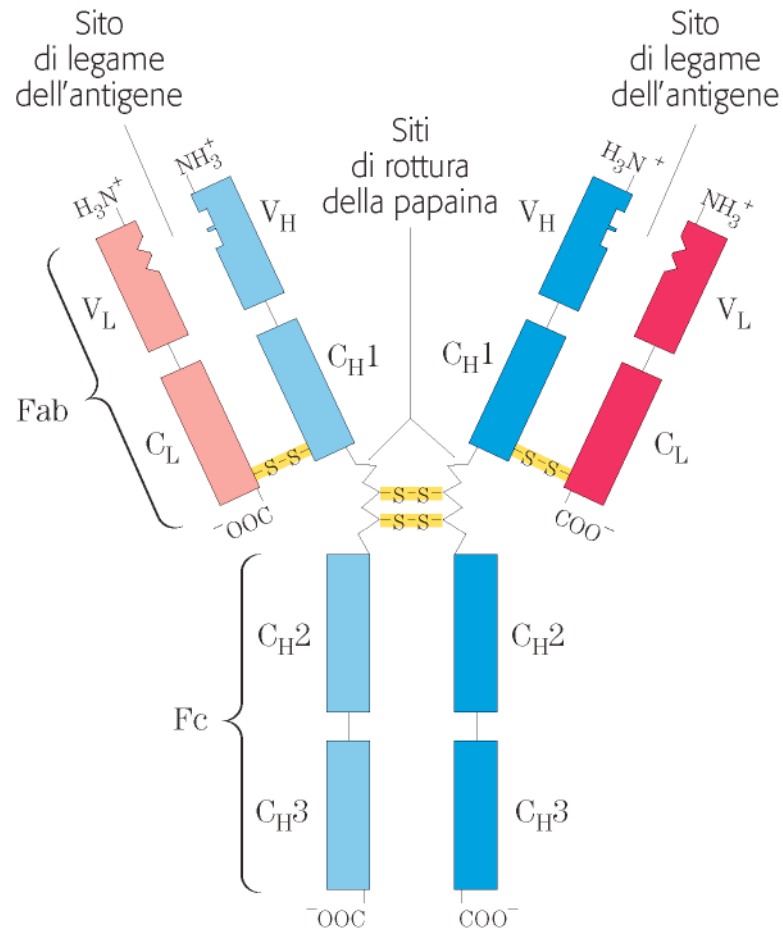
Il legame del substrato (glucosio) all'enzima promuove un cambiamento configurazionale che stabilizza l'interazione substrato-enzima



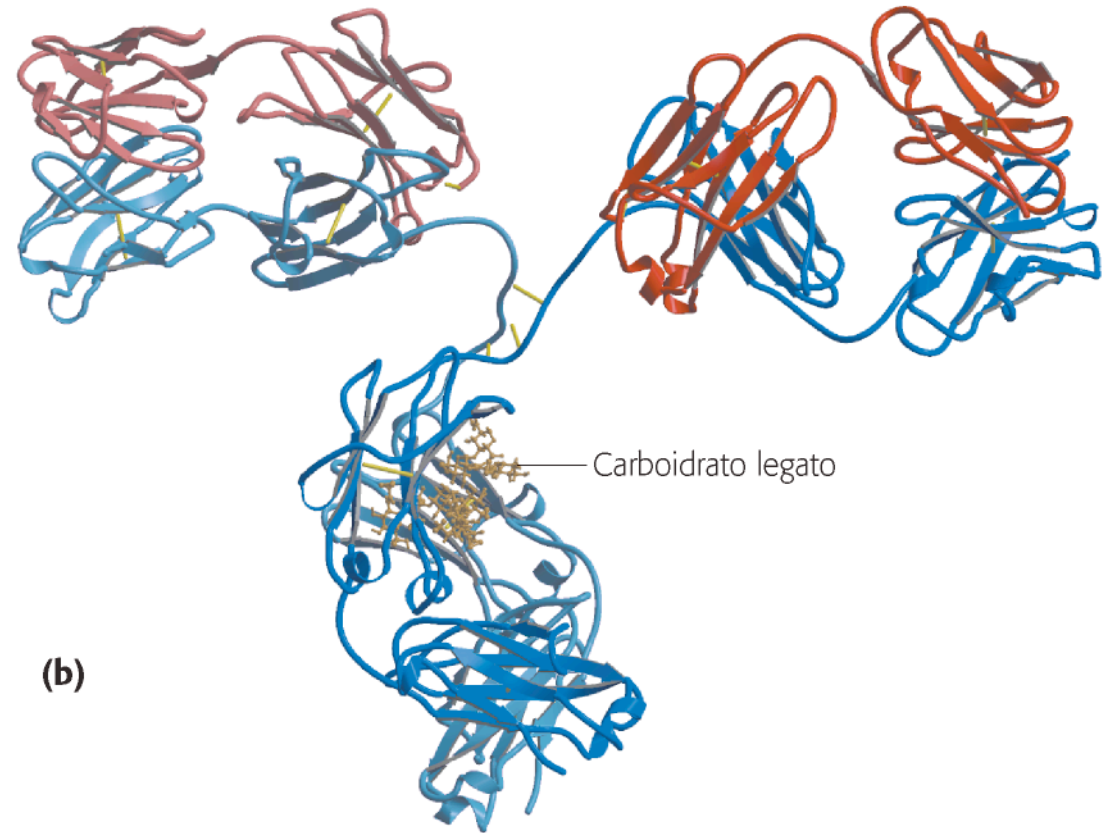
Trasportatori di membrana



Proteine con funzione di “riconoscimento”



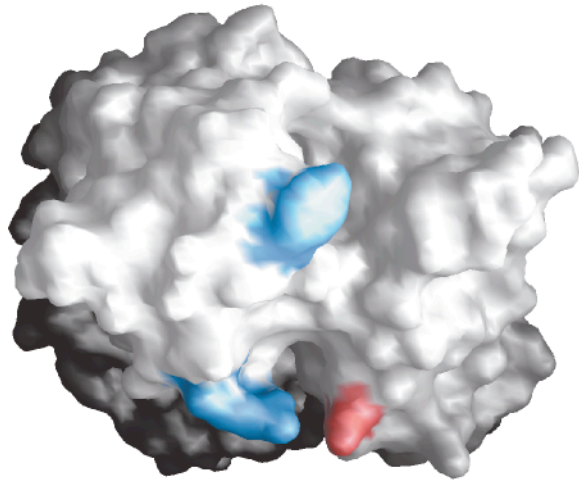
C = dominio costante
V = dominio variabile
H, L = catena pesante; catena leggera



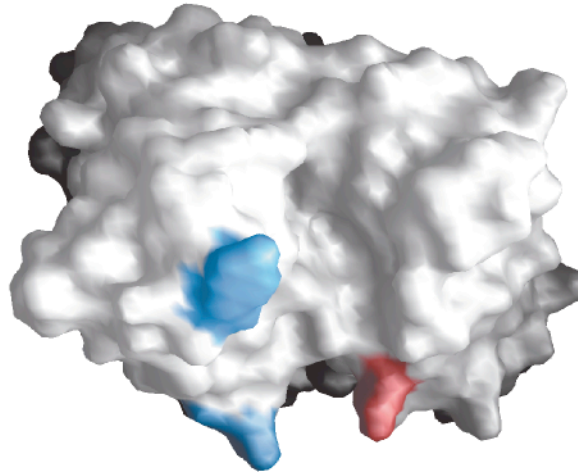
(a)

(b)

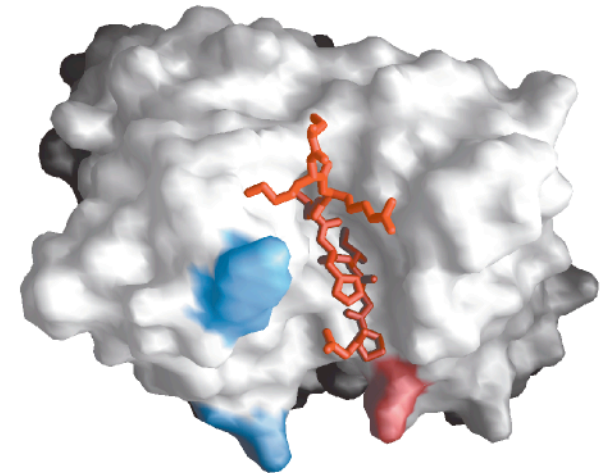
Adattamento Indotto



(a) Conformazione senza antigene



(b) Conformazione con l'antigene (non mostrato)



(c) Conformazione con l'antigene (mostrato)

Legame proteina-ligando



$$K_a = \frac{[PL]}{[P][L]} = \text{costante di associazione}$$

$$K_d = \frac{[P][L]}{[PL]} = \text{costante di dissociazione}$$

$$K_a = \frac{[PL]}{[P][L]} = \text{costante di associazione} \quad K_a[L][P] = [PL]$$

$$\Theta = \frac{\text{siti di legame occupati}}{\text{Siti di legame totali}} = \frac{[PL]}{[PL] + [P]}$$

$$\Theta = \frac{K_a[L][P]}{K_a[L][P] + [P]} = \frac{K_a[L]}{K_a[L] + 1} = \frac{[L]}{[L] + 1/K_a}$$

Costante di dissociazione (molto più usata)

$$K_d = \frac{[P][L]}{[PL]} = \text{costante di dissociazione}$$

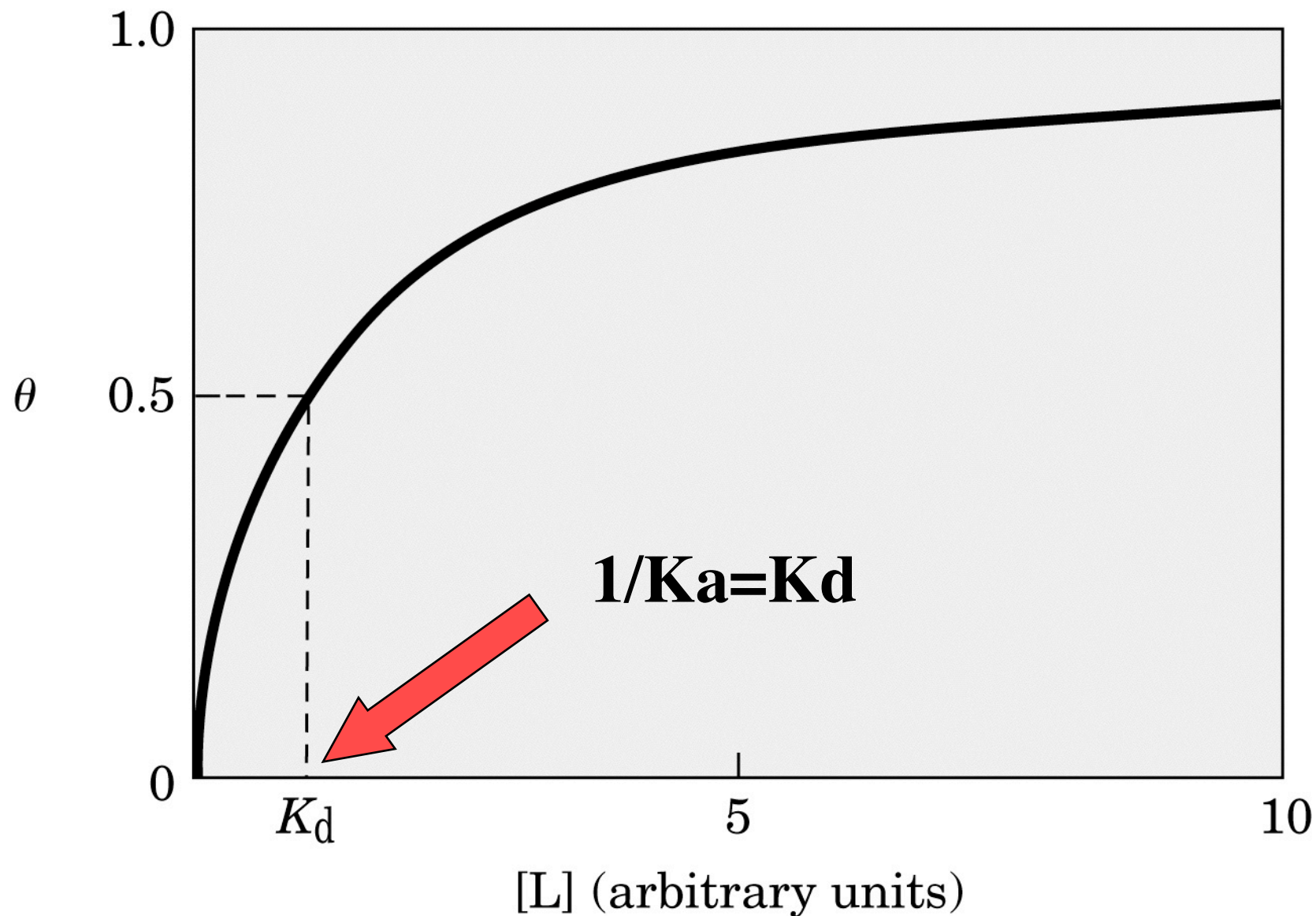
$$[PL] = \frac{[P][L]}{K_d}$$

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

$$X = \frac{Y}{Y+Z}$$

Cosa succede quando $L = K_d$?

Legame proteina-ligando



(a)

Tabella affinità

TABLE 5-1 Some Protein Dissociation Constants

<i>Protein</i>	<i>Ligand</i>	K_d (M)*
Avidin (egg white) [†]	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human) [‡]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni ²⁺	1×10^{-7}
Calmodulin (rat) [§]	Ca ²⁺	3×10^{-6}
		2×10^{-5}

*A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

[†]Interaction of avidin with biotin, an enzyme cofactor, is among the strongest noncovalent biochemical interactions known.

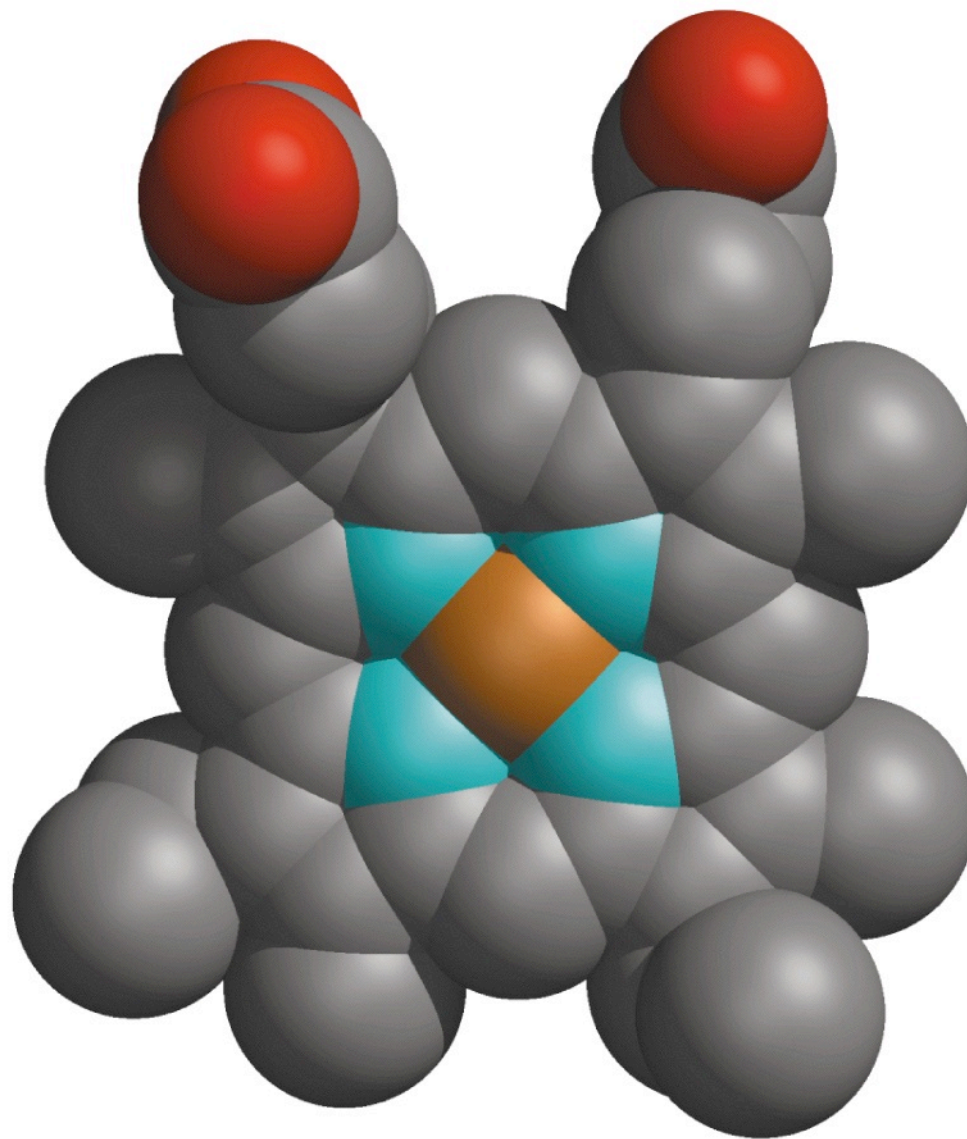
[‡]This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

[§]Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Proteine trasportatrici per l'ossigeno

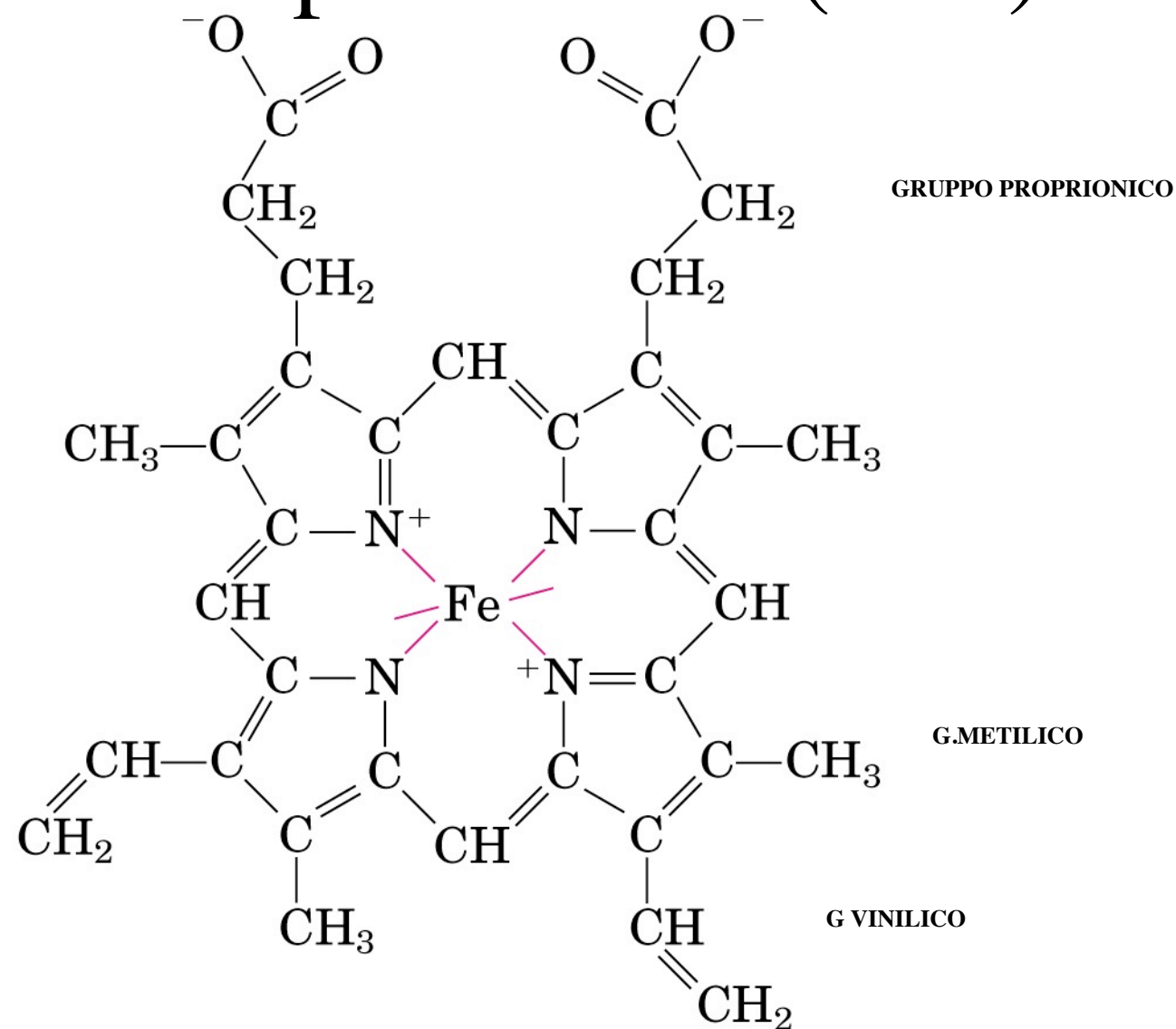
RUOLO DELL'EME

Fe-Protoporfirina IX (eme)



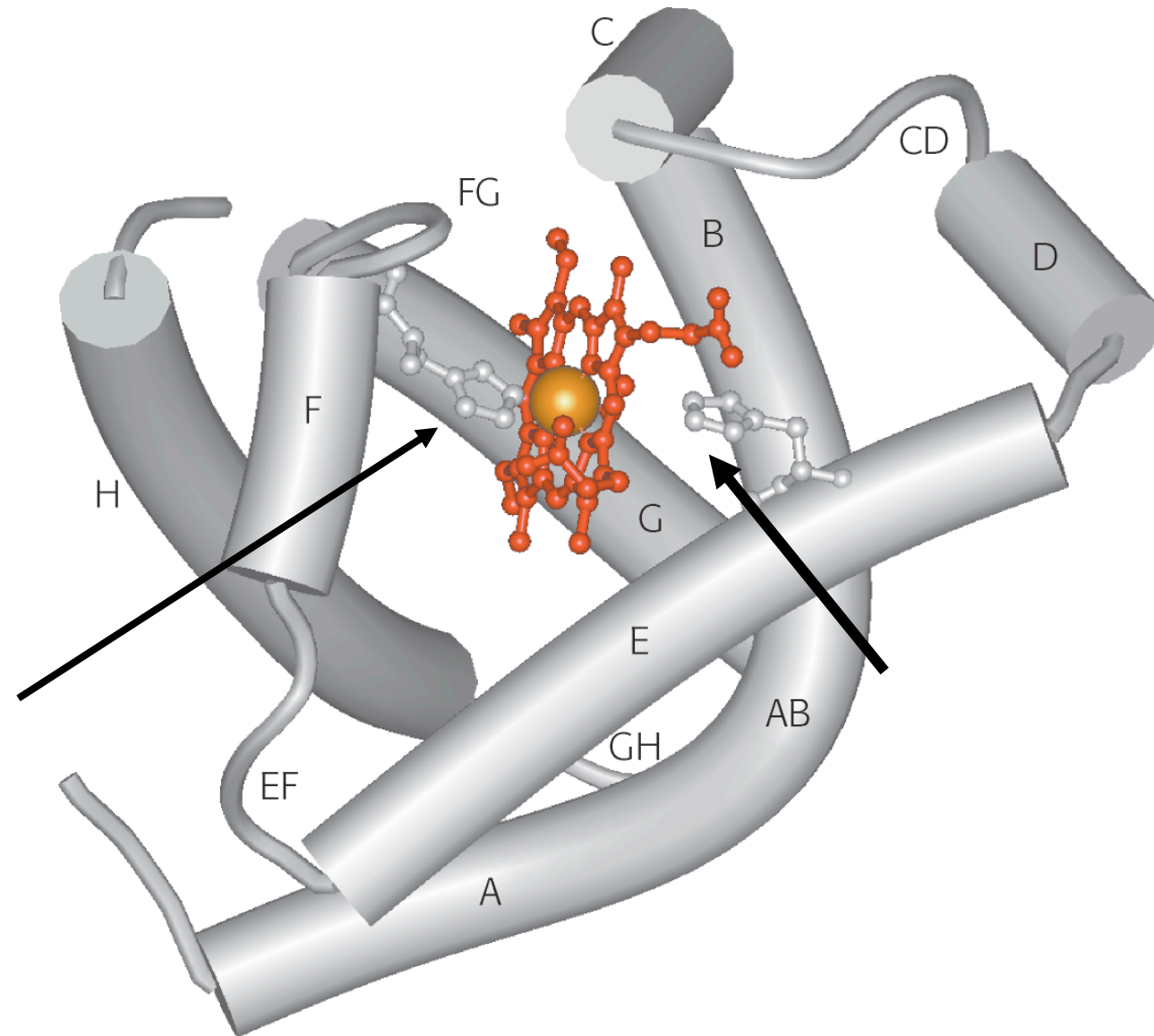
(c)

Fe-Protoporfirina IX (eme)

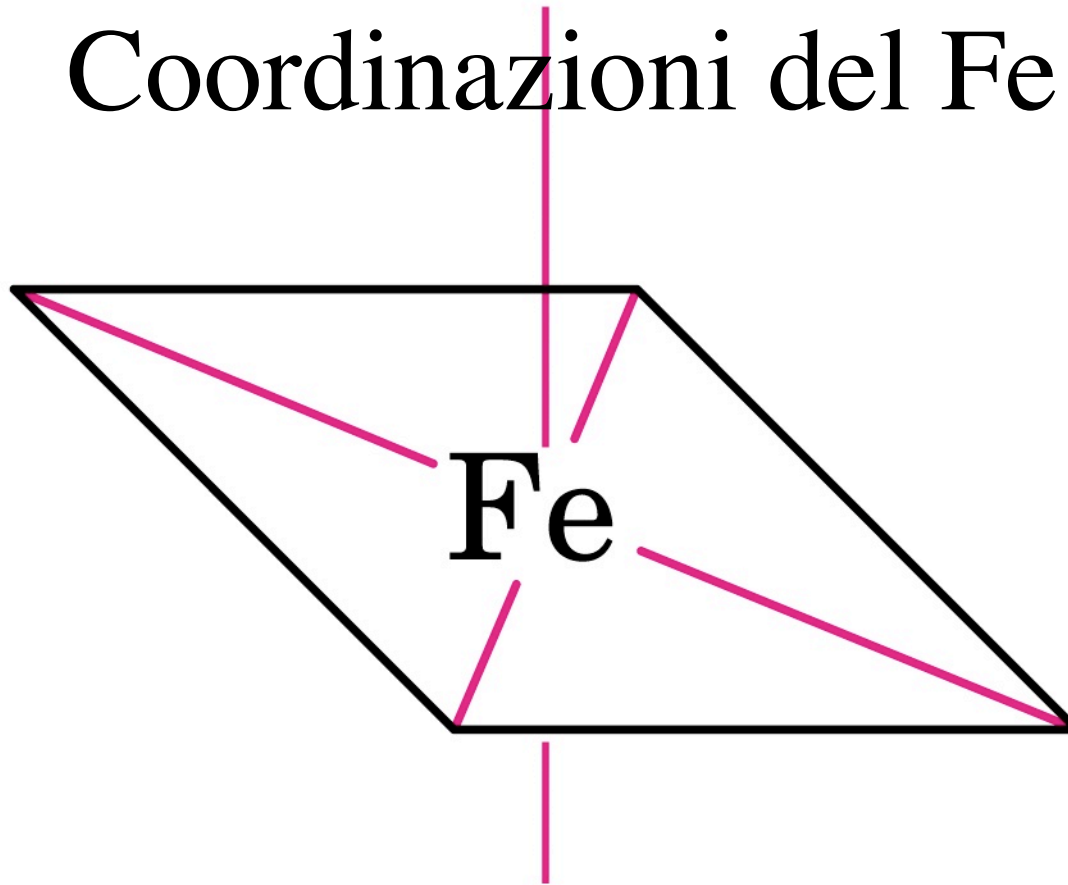


(b)

LA MIOGLOBINA: la prima proteina di cui conosciamo la struttura



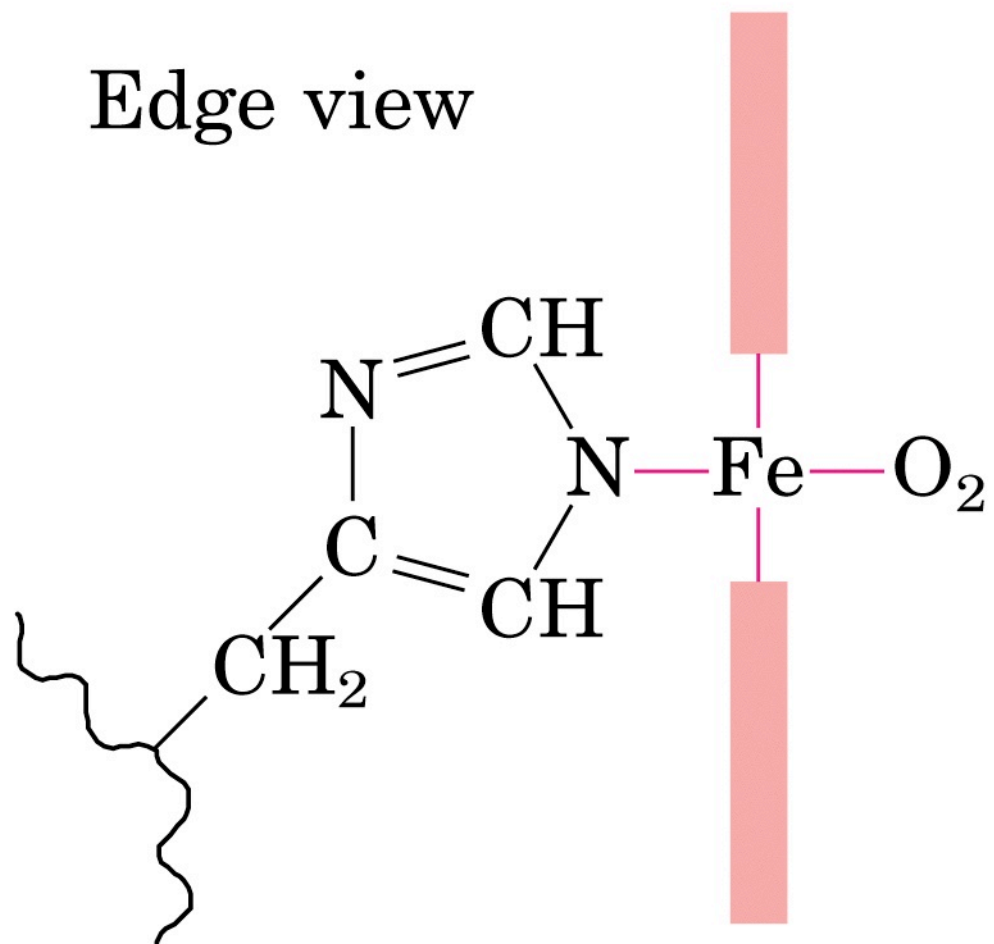
Coordinazioni del Fe



(d)

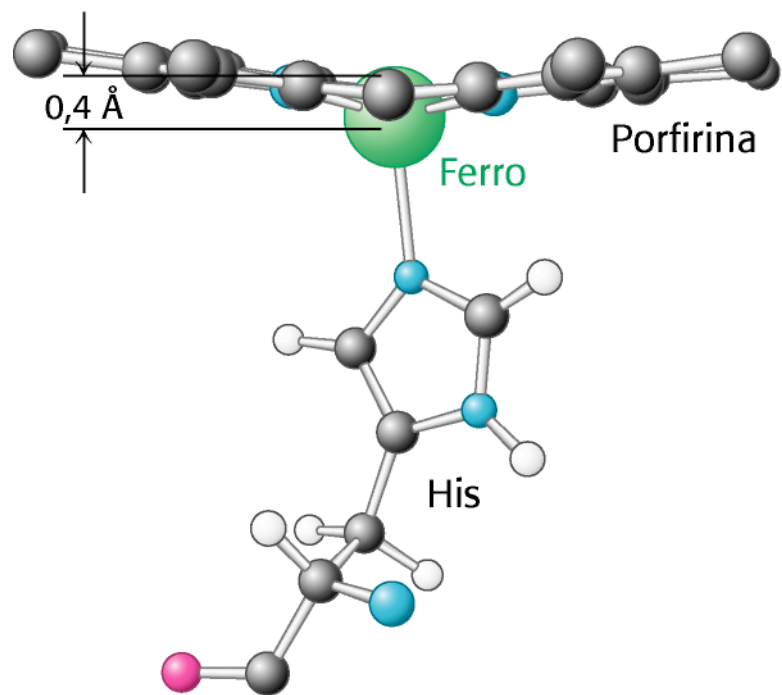
Coordinationi del Fe

Edge view

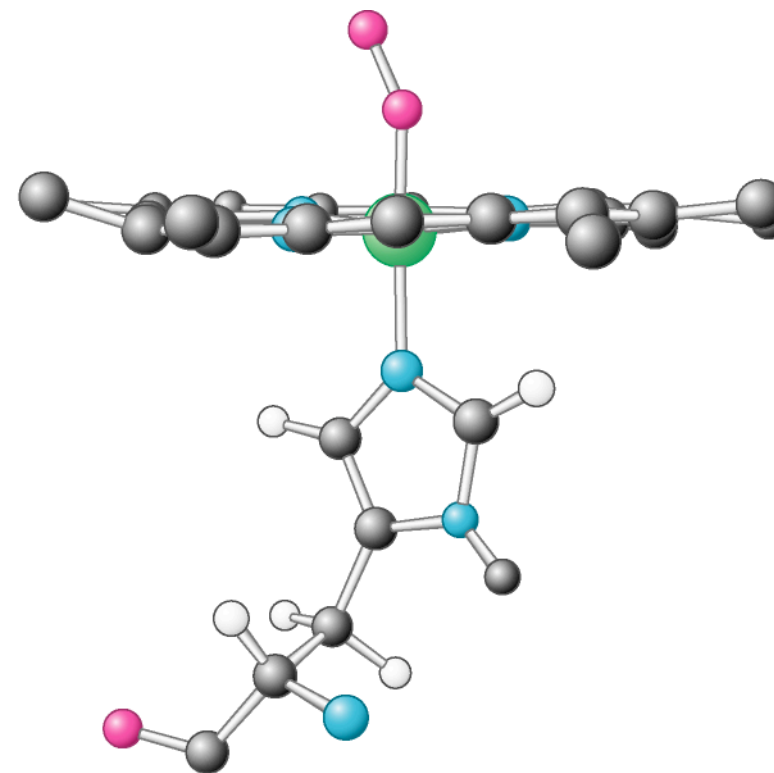


Histidine
residue

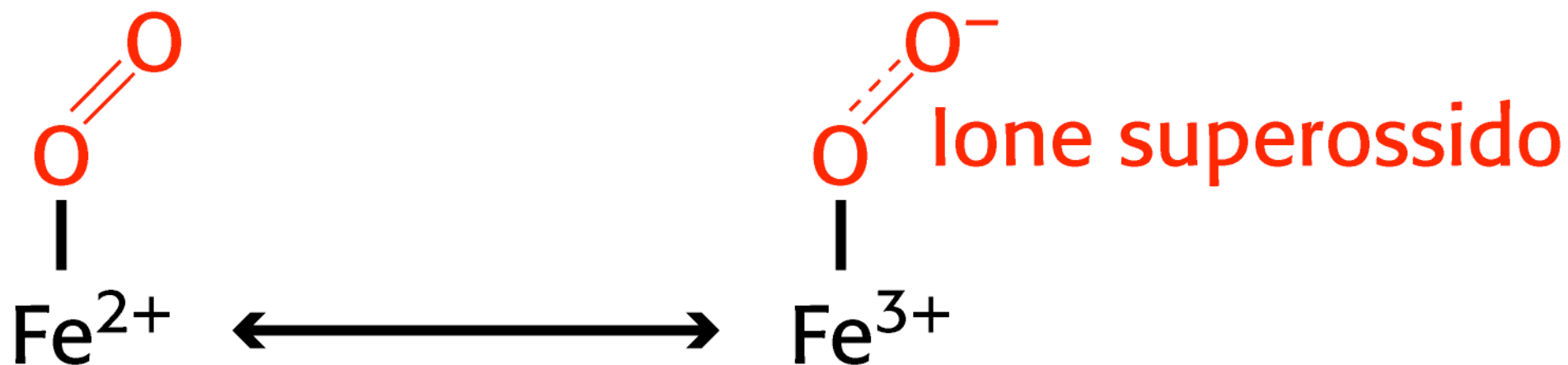
Plane of
porphyrin
ring system



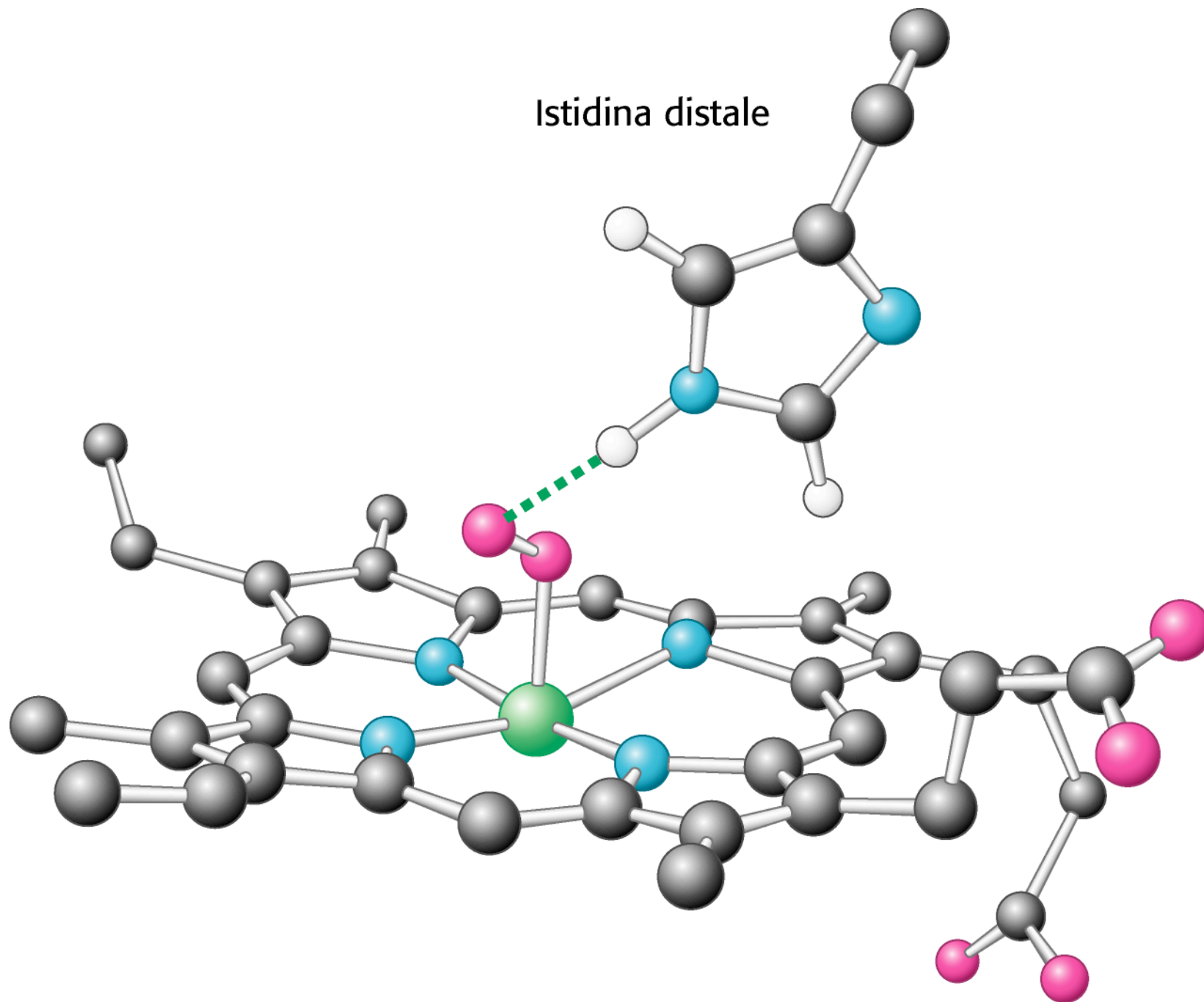
Nella deossioglobina



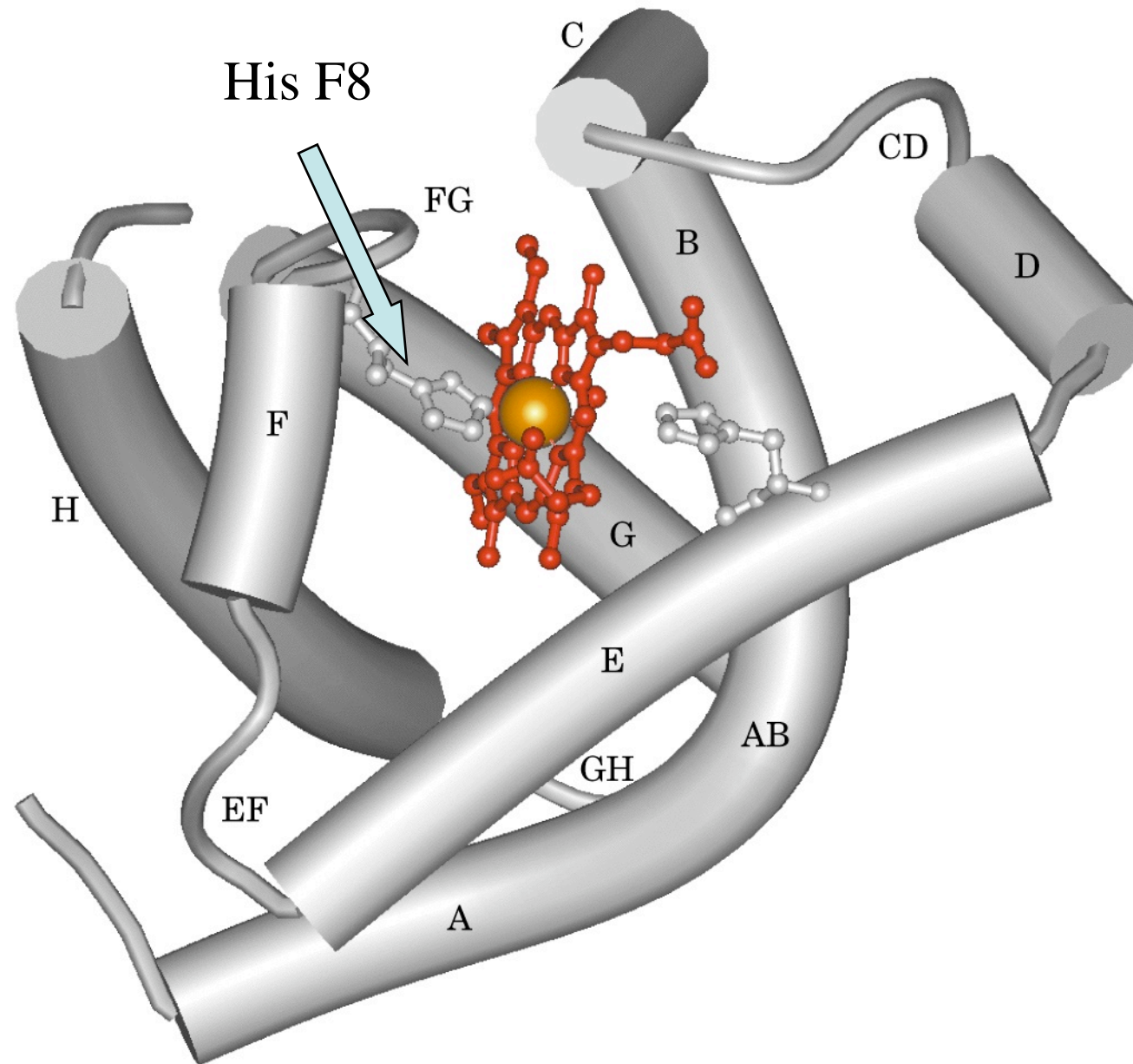
Nell'ossiemoglobina



Istidina distale



Mb-struttura terziaria



$$K_d = \frac{[P][L]}{[PL]} = \text{costante di dissociazione}$$

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

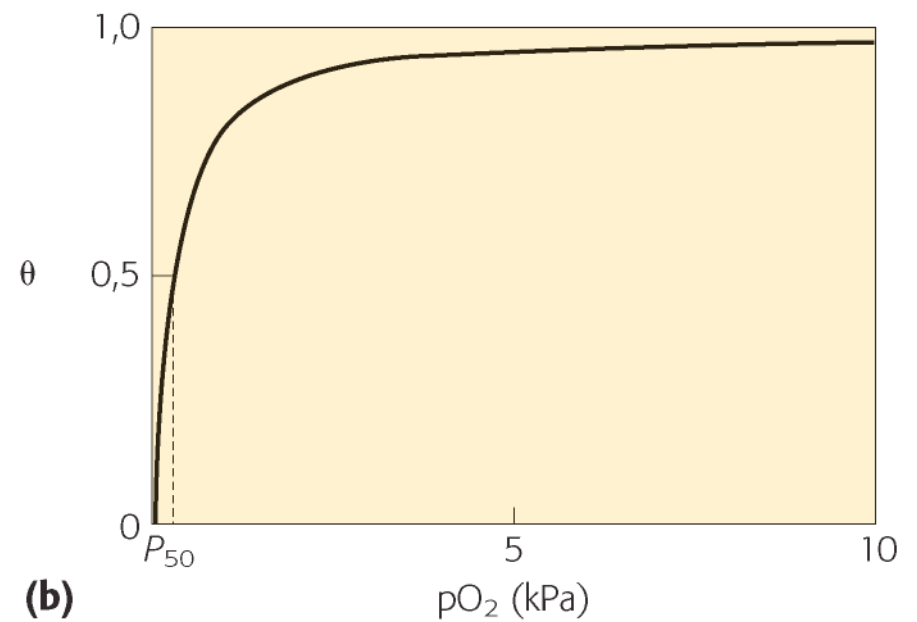
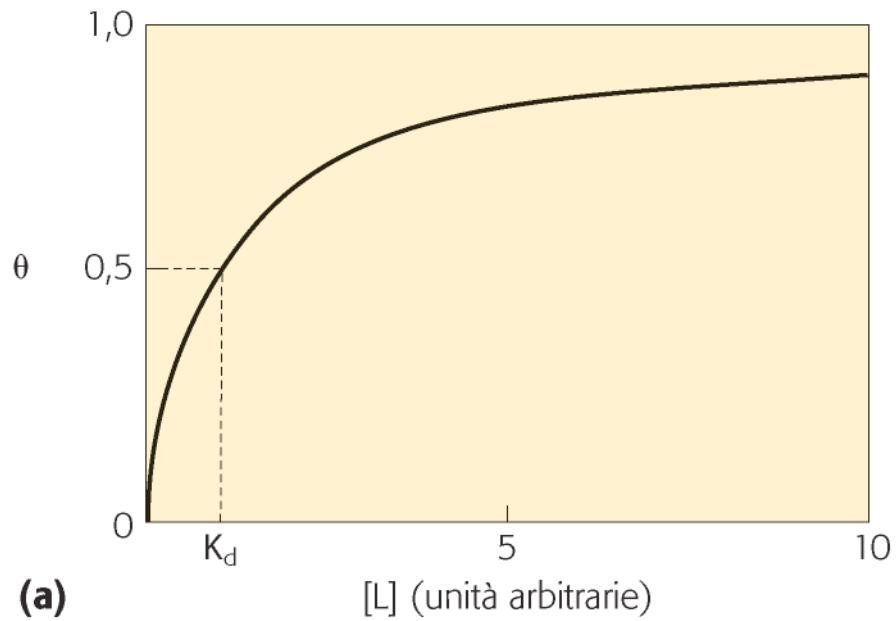
$$\Theta = \frac{[L]}{[L] + K_d}$$

PER SEMPLICITA' PARLIAMO DI PRESSIONE PARZIALE

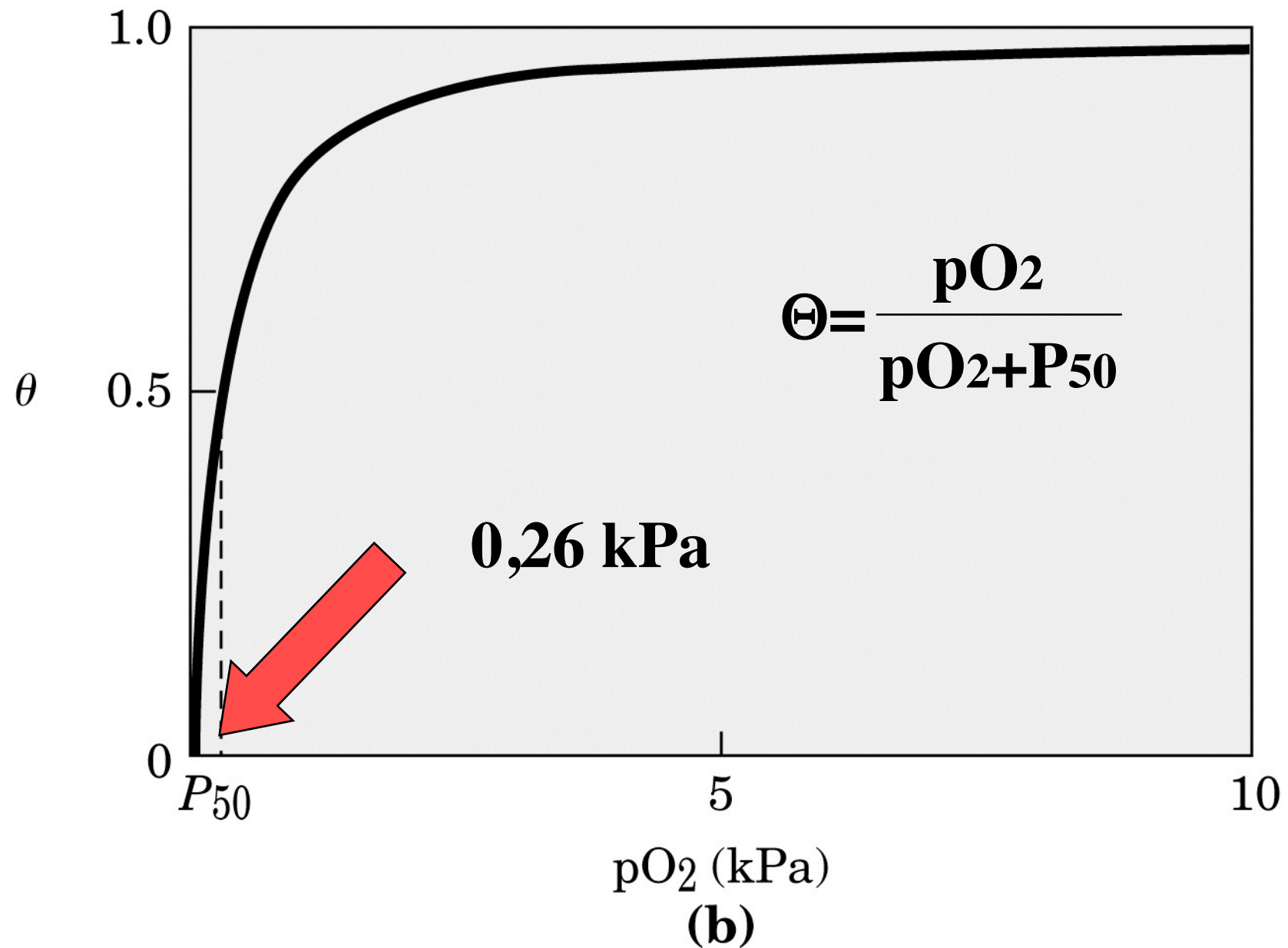
$$\Theta = \frac{[L]}{[L] + K_d}$$

$$\Theta = \frac{[O_2]}{[O_2] + P_{50}}$$

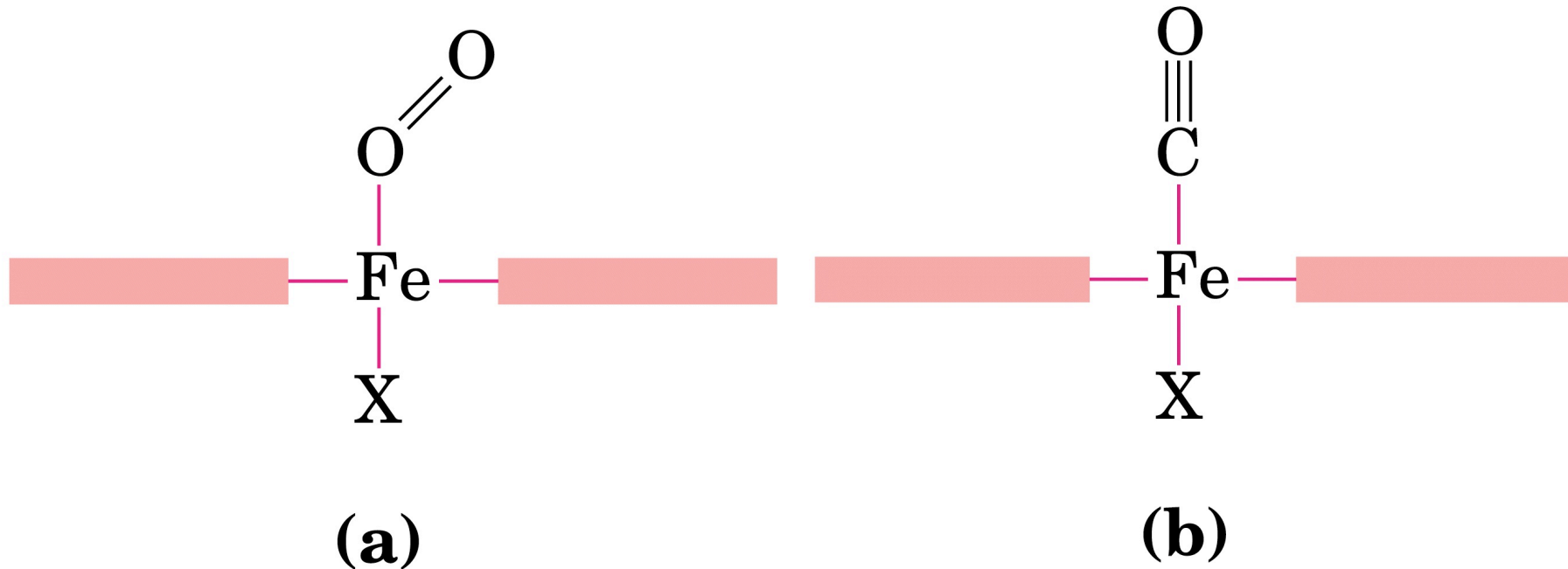
CURVE DI LEGAME



Legame mioglobina ossigeno

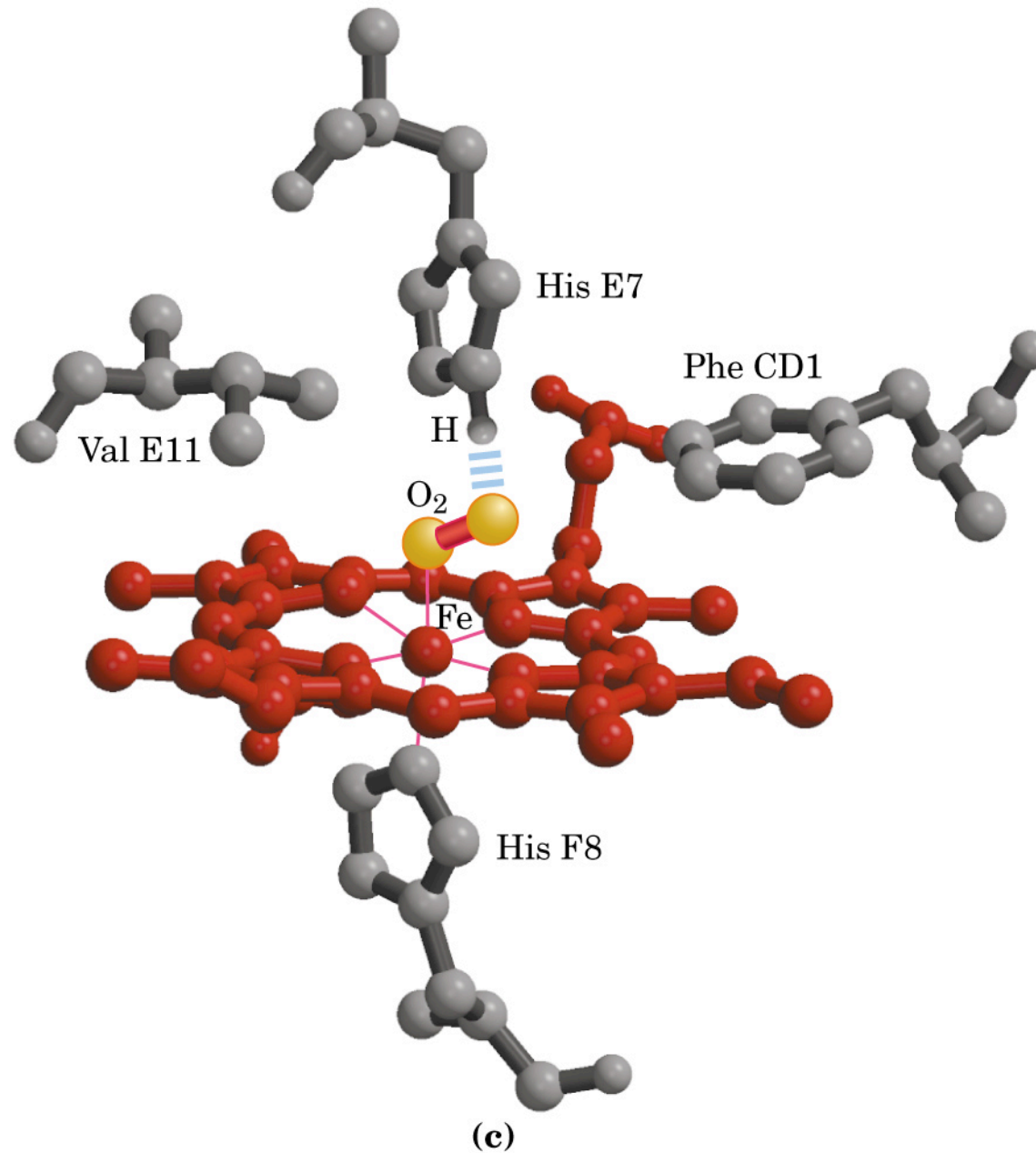


L' AFFINITÀ PER I LIGANDI È MEDIATA DALLA STRUTTURA PROTEICA

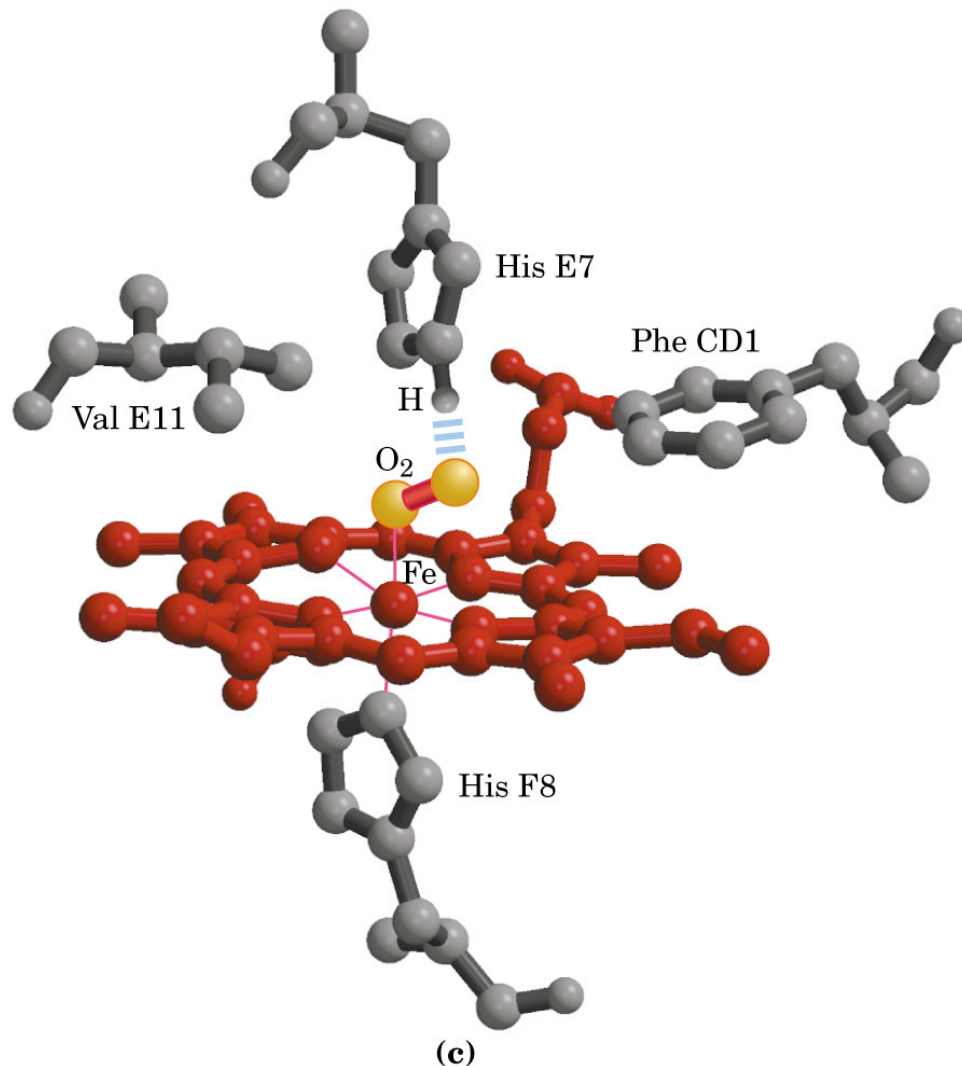


Eme	x	20.000 x
Hb	y	200 y

Interazione con His E7

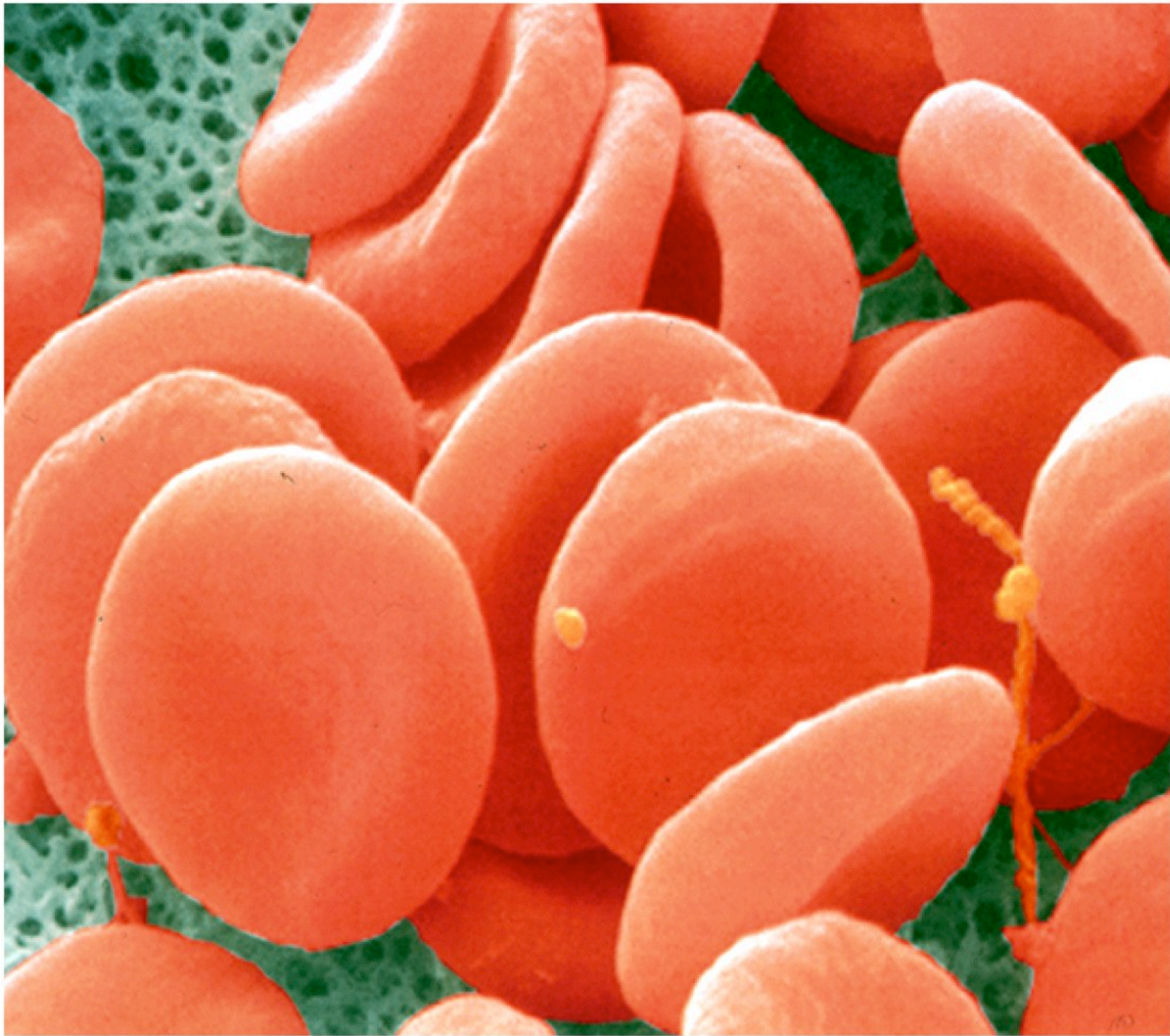


IL GRUPPO EME è “PROTETTO”



**Struttura è talmente compatta che anche l'O₂ non riuscirebbe ad entrare,
A meno che.....**

**Anche le proteine
“respirino”
(E7 ogni ns si muove)**



Emoglobina

Protomeri	4 ($\alpha_2\beta_2$)
pM	64 kDa
Localizzazione	G.R.

Mioglobina

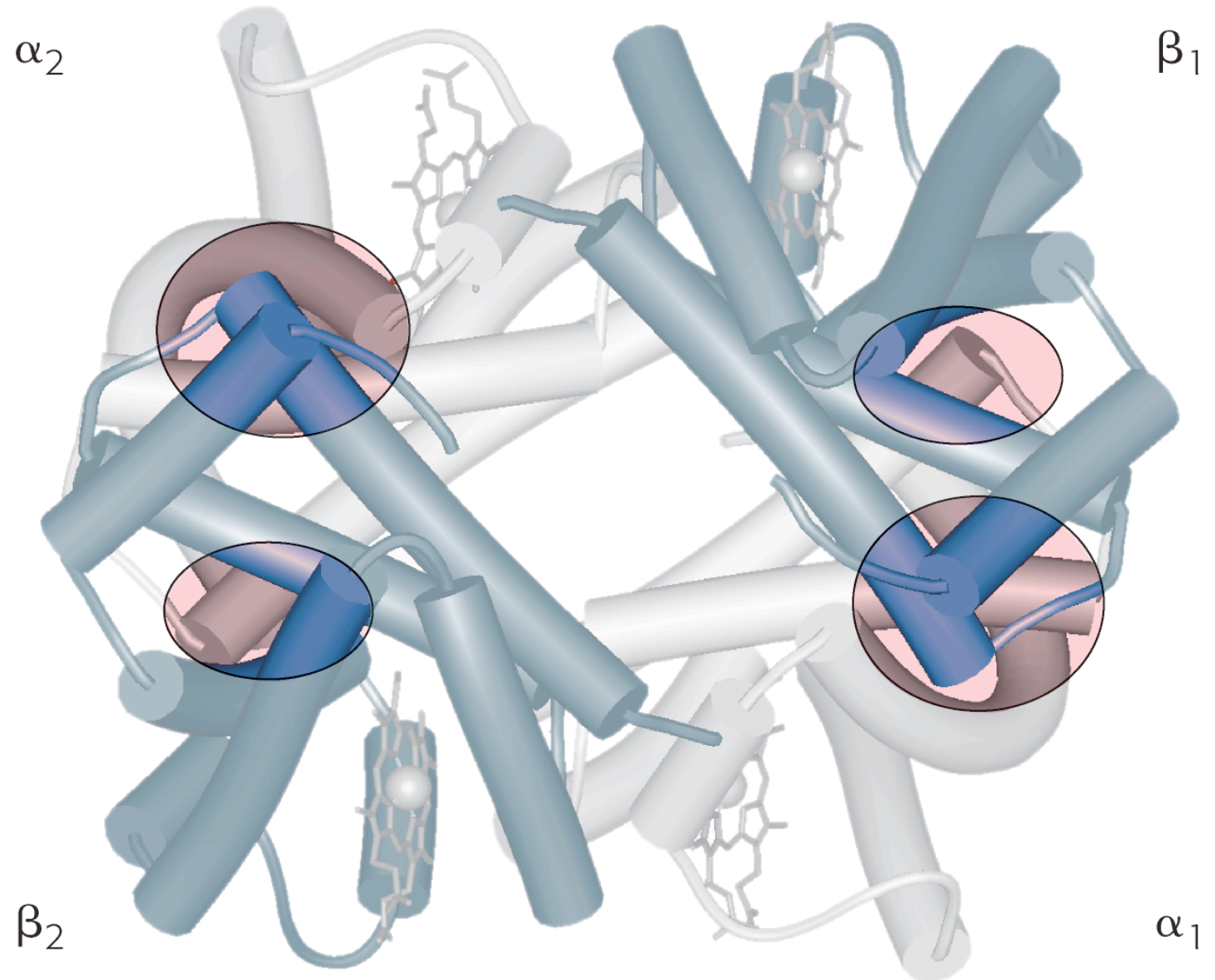
1
16 kDa
Muscolo



Hb è un tetramero

2 protomeri $\alpha\beta$

Siti d'interazione



Grigio: residui uguali
Rosso: Residui nella
stessa posizione

	Mb	Hb α	Hb β
NA1	1 V	1 V	1 V
	—	—	H
	L	L	L
A1	---S	S	T---
	E	P	P
	G	A	E
	E	D	E
	W	K	K
	Q	T	S
	L	N	A
	V	V	V
	L	K	T
	H	A	A
	V	A	L
	W	W	W
	A	G	G
	K	K	K
	V	V	V
A16	---E	G	----
	A	A	—
B1	--20D	20H	N---
	V	A	20V
	A	G	D
	G	E	E
	H	Y	V
	G	G	G
	Q	A	G
	D	E	E
	I	A	A
	L	L	L
	I	E	G
	R	R	R
	L	M	L
	F	F	L
	K	L	V
B16	---S	S	V---
C1	---H	F	Y---
	P	P	P
	E	T	W
	T	T	T
	40L	40K	Q
	E	T	40R
C7	---K	Y	F---
	F	F	F
	D	P	E
	R	H	S
	F	F	F
	K	—	G
	H	D	D
	L	L	L
	K	S	S
D1	---T	H	T---

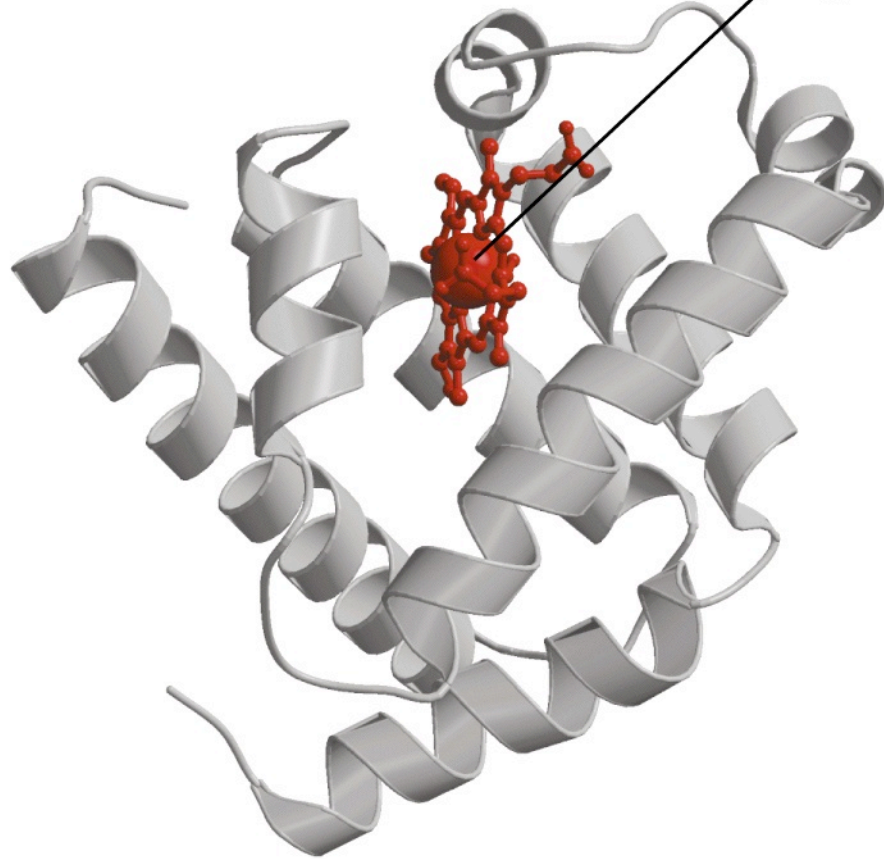
	Mb	Hb α	Hb β
	E	—	P
	A	—	D
	E	—	A
	M	—	V
	K	—	M
D7	---A	G	G---
E1	---S	S	N---
	E	A	P
	60D	Q	K
	L	V	60V
	K	K	K
	K	G	A
Distal His	E7	H	H
	G	G	G
	V	60K	K
	T	K	K
	V	V	V
	L	A	L
	T	D	G
	A	A	A
	L	L	F
	G	T	S
	A	N	D
	I	A	G
E19	---L	V	L---
	K	A	A
	K	H	H
	K	V	L
	80G	D	D
	H	D	80N
	H	M	L
	E	P	K
	A	N	G
	E	A	T
F1	---L	80L	F---
	K	S	A
	P	A	T
	L	L	L
	A	S	S
	Q	D	E
	S	L	L
Proximal His	F8	H	H
	F9	A	C
	T	H	D
	K	K	K
	H	L	L
	K	R	H
	I	V	V
G1	--100P	D	D---
	I	P	100P
	K	V	E
	Y	N	N

	Mb	Hb α	Hb β
	L	F	F
	E	K	R
	F	100L	L
	I	L	L
	S	S	G
	E	H	N
	A	C	V
	I	L	L
	I	L	V
	H	V	C
	V	T	V
	L	L	L
	H	A	A
	S	A	H
G19	---R	H	H---
	H	L	F
	120P	P	G
	G	A	120K
	D	E	E
	F	F	F
H1	---G	T	T---
	A	P	P
	D	120A	P
	A	V	V
	Q	H	Q
	G	A	A
	A	S	A
	M	L	Y
	N	D	Q
	K	K	K
	A	F	V
	L	L	V
	E	A	A
	L	S	G
	F	V	V
	R	S	A
	140K	T	N
	D	V	140A
	I	L	L
	A	T	A
H21	---A	S	H---
	K	K	K
	Y	140Y	Y
	K	141R	146H
	E		
H26	---L		
	G		
	Y		
	Q		
	153G		

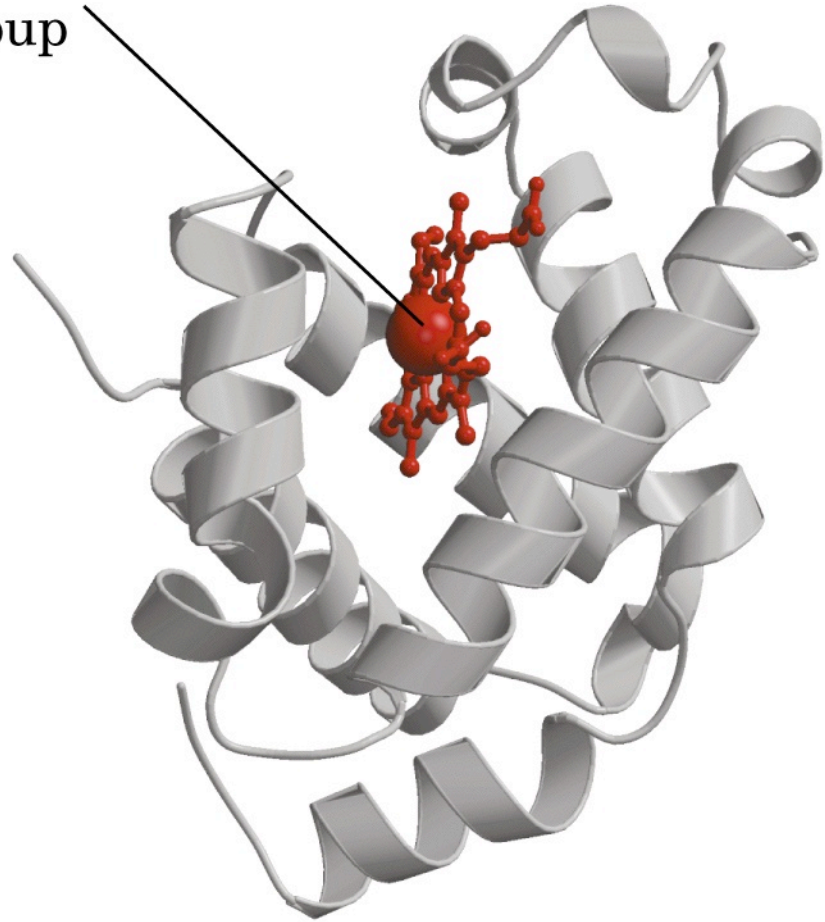
Hb α and
Hb β only

Mb e catena β di Hb

Heme
group

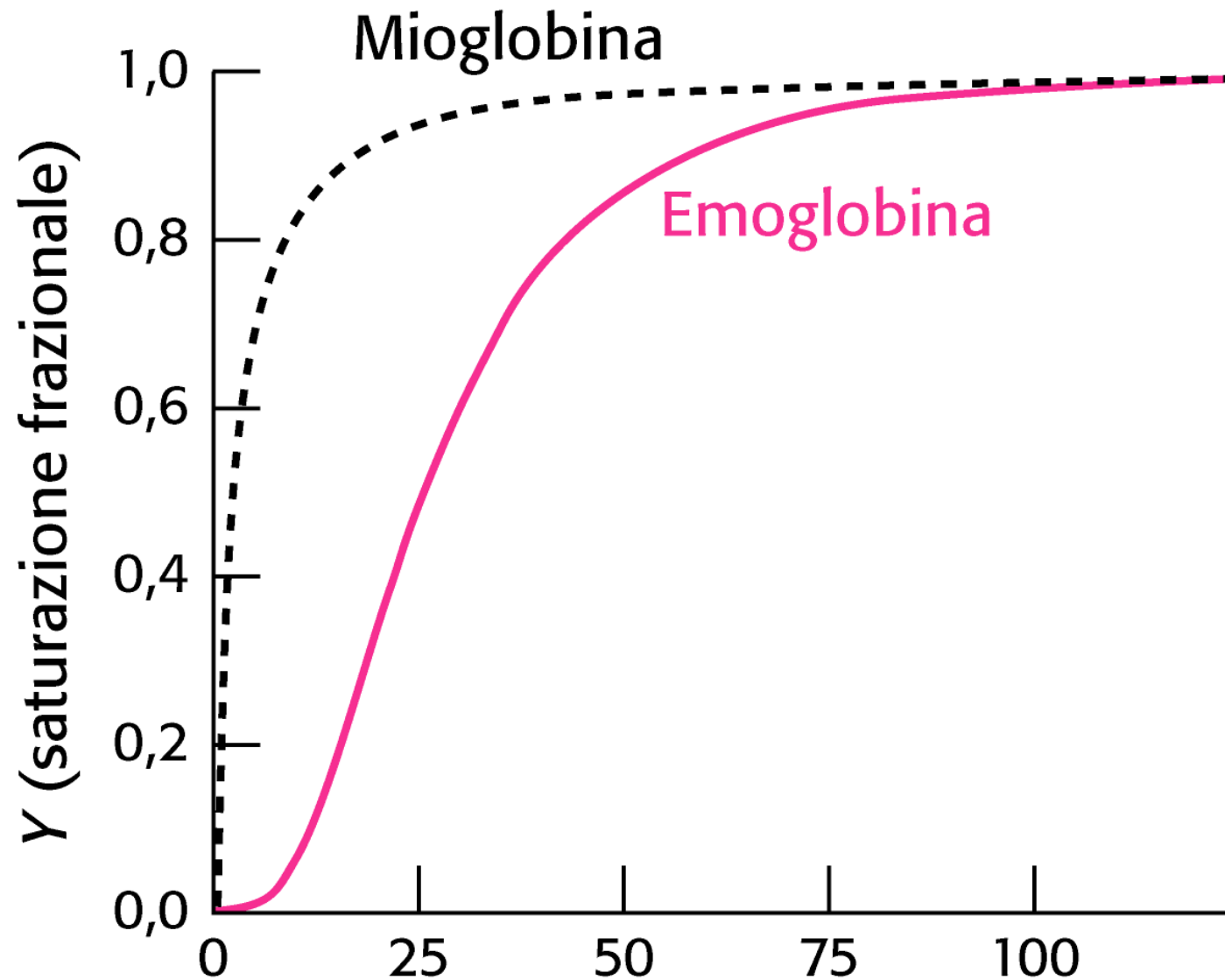


Myoglobin



β subunit of
hemoglobin

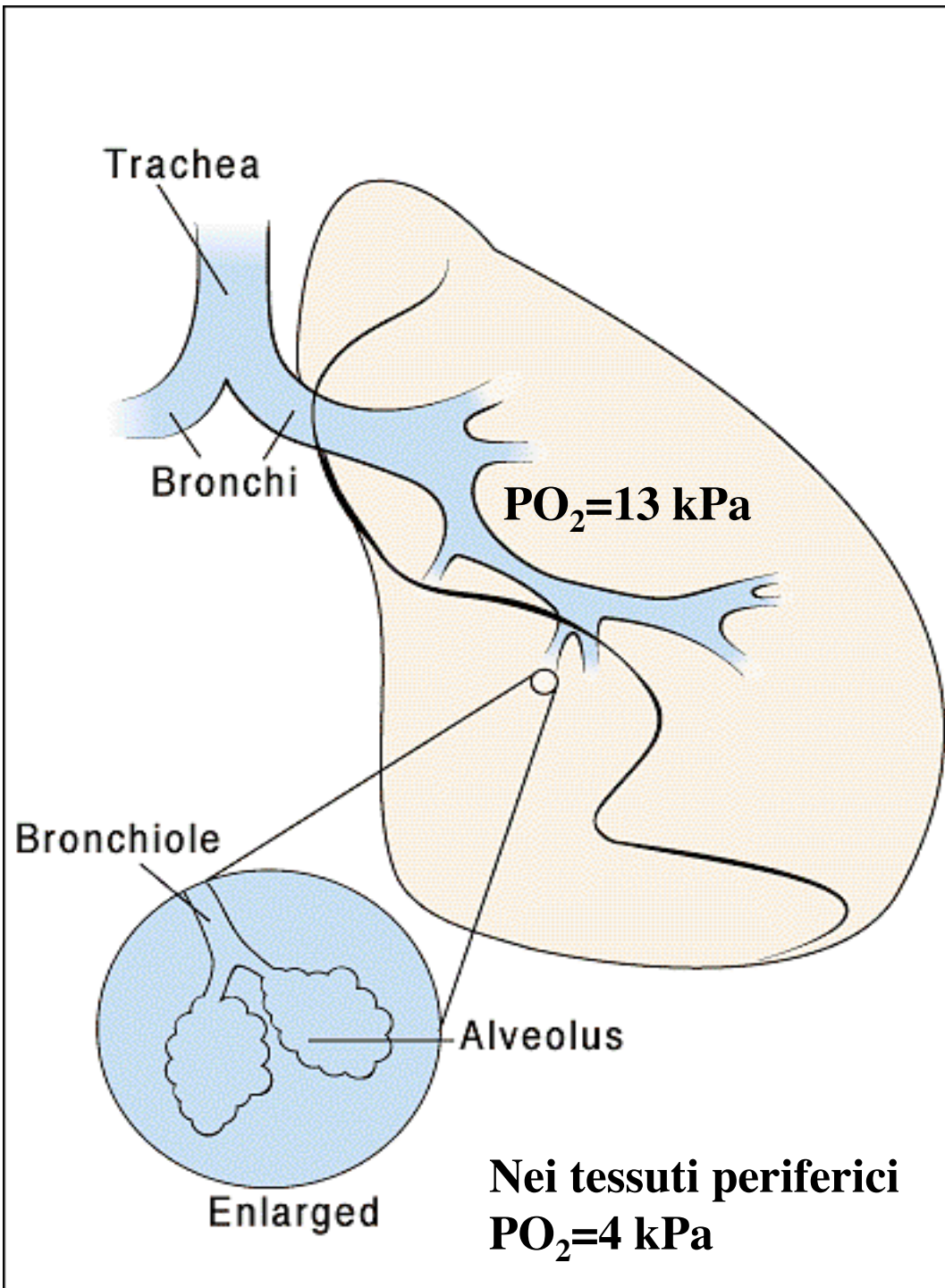
Una differenza essenziale: la curva di legame

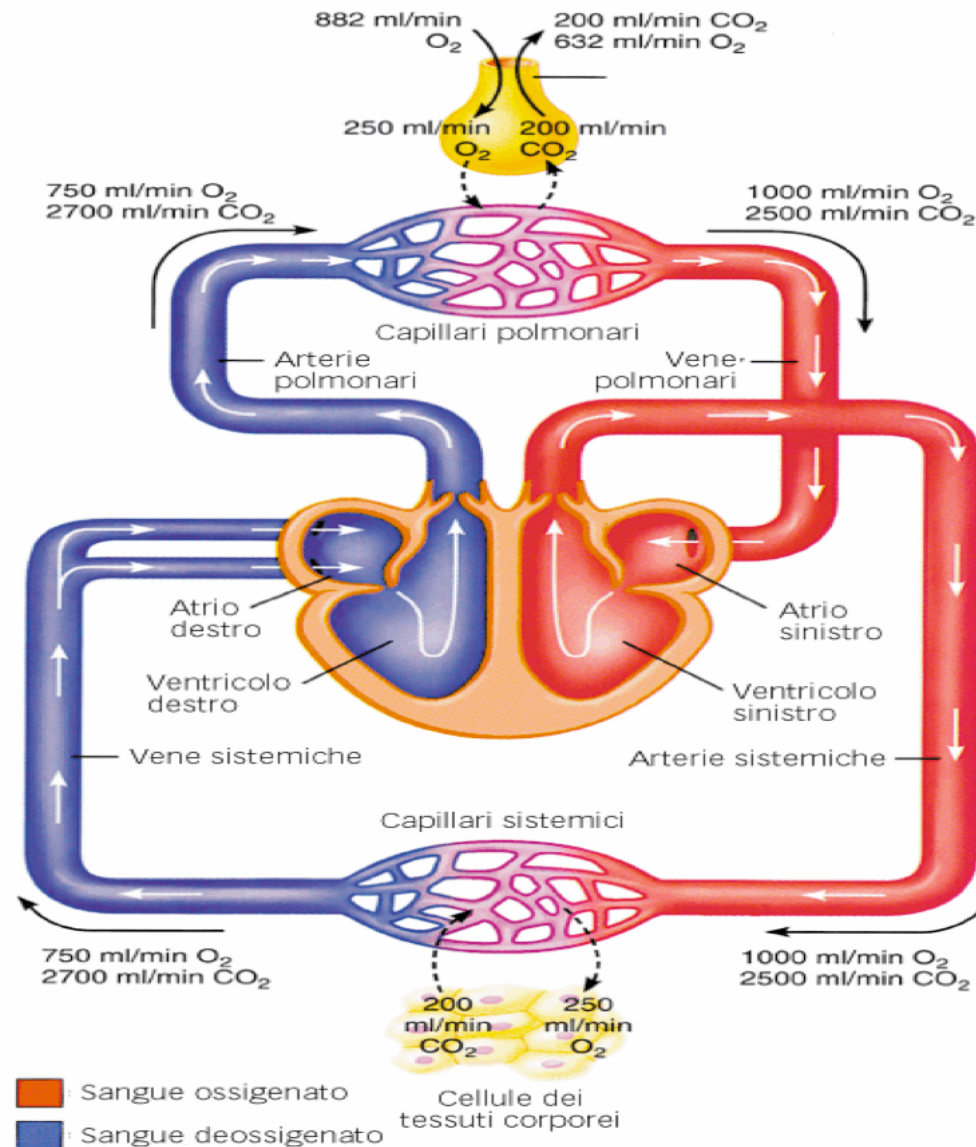


1torr=133Pa

pO_2 (Torr)

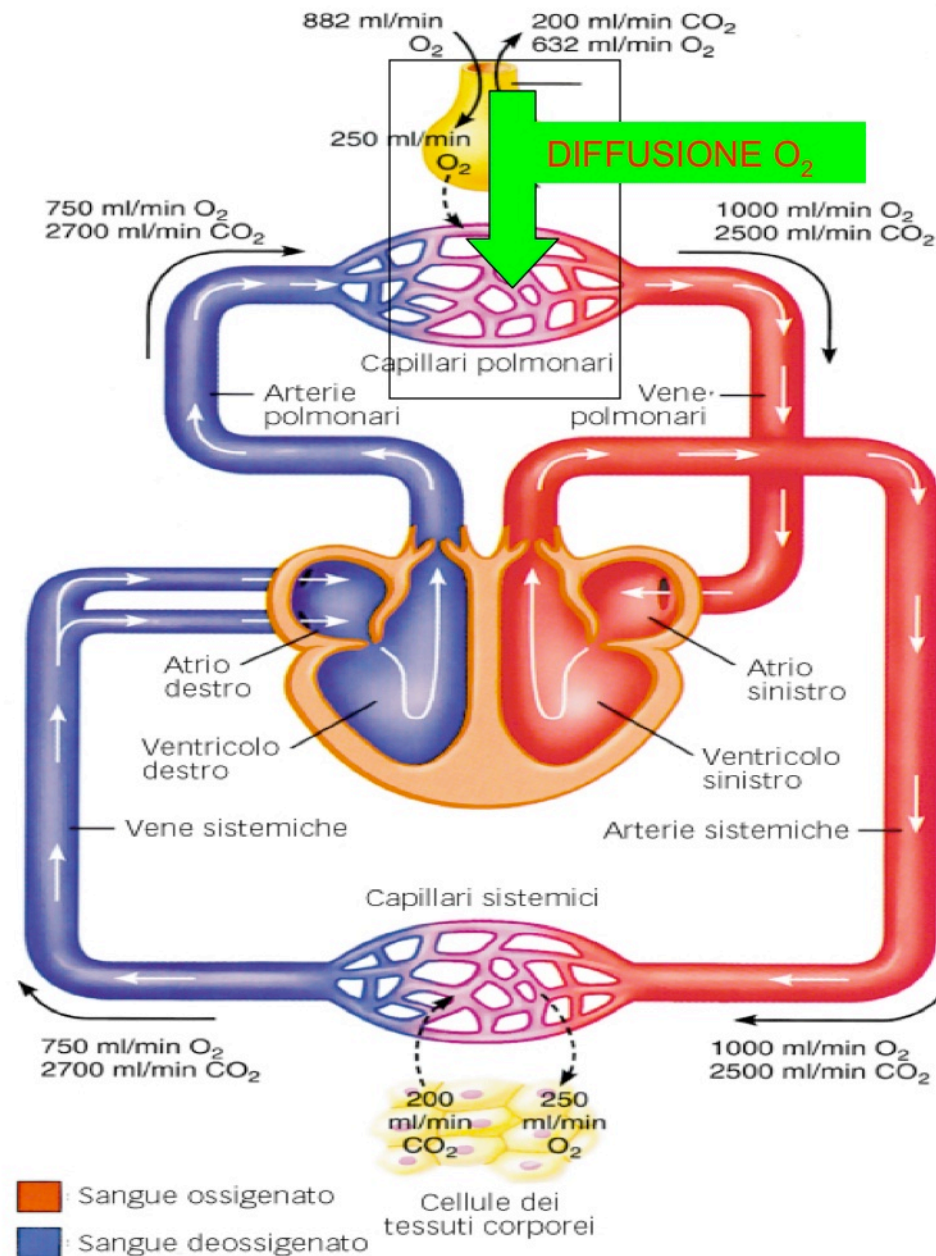
**Hb deve saturarsi
facilmente e liberare
facilmente O₂ a
seconda dei casi**





GER. **FIGURA 16.1** Movimenti dell'ossigeno e dell'anidride carbonica nei tessuti polmonari e sistemici in condizioni di riposo. A livello dei capillari nei polmoni, l'ossigeno diffonde dagli alveoli al sangue e l'anidride carbonica diffonde dal sangue agli alveoli. A livello dei capillari sistemici, l'ossigeno diffonde dal sangue alle cellule, mentre l'anidride carbonica diffonde dalle cellule al sangue.

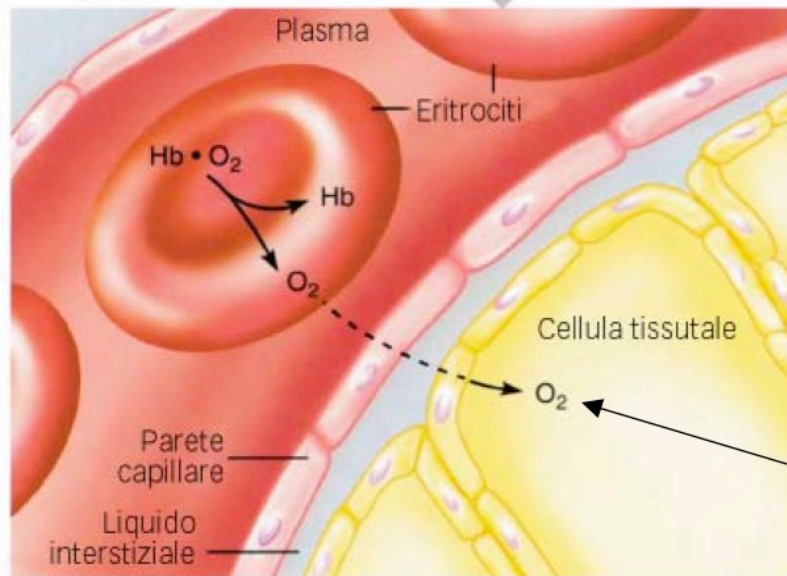
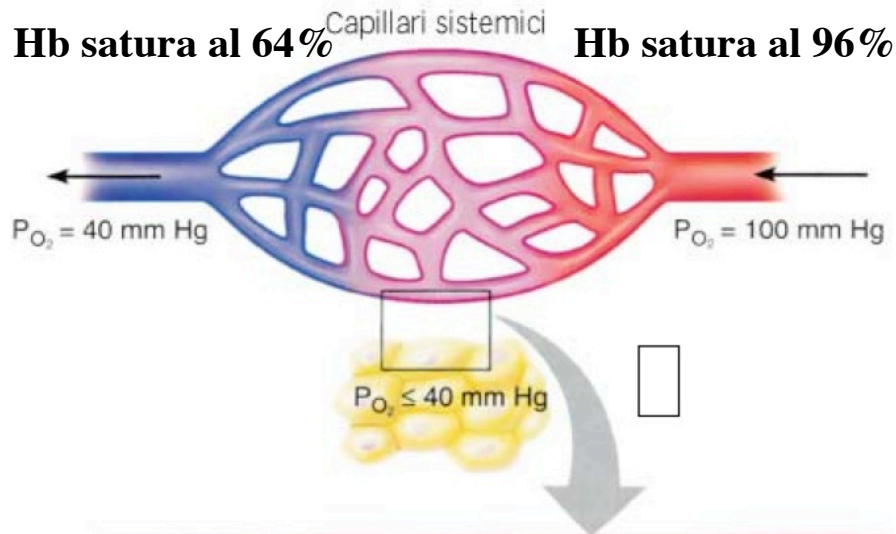
DIFFUSIONE O_2 A LIVELLO POLMONARE E TRASPORTO NEL SANGUE



Hb satura al 96%
Hb 34% in peso
in Eritrociti

TRASPORTO
 O_2

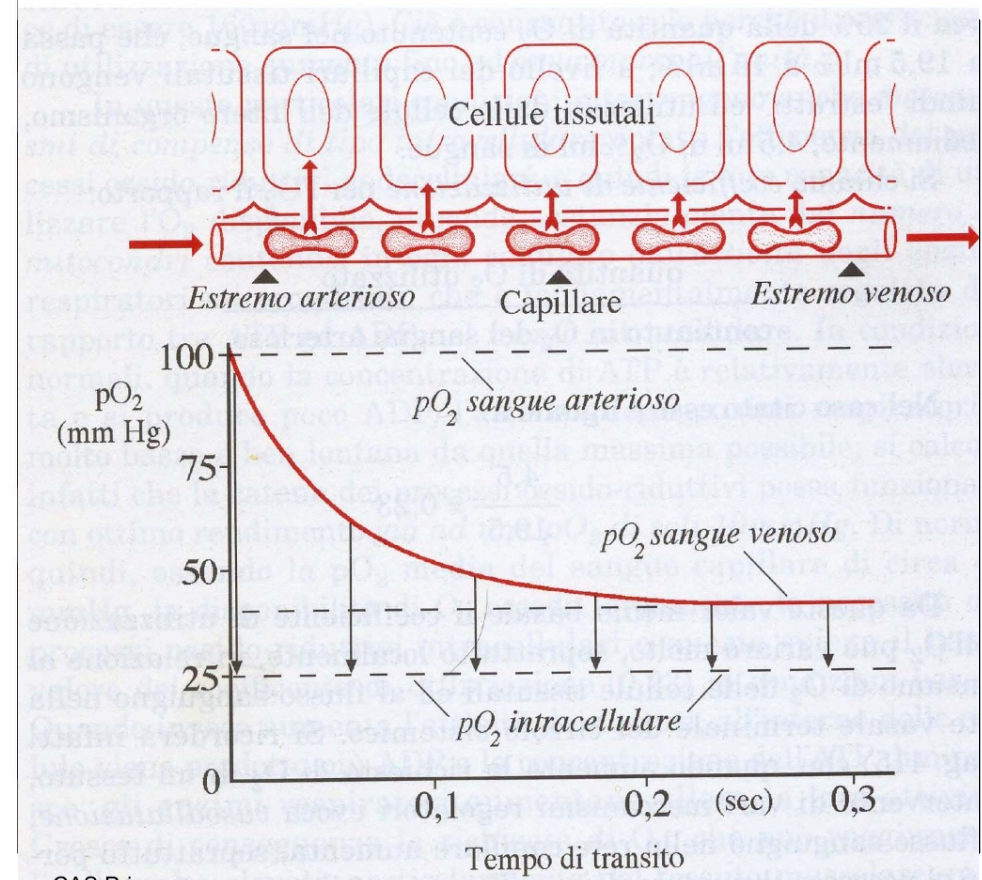
DIFFUSIONE DI O₂ A LIVELLO TISSUTALE

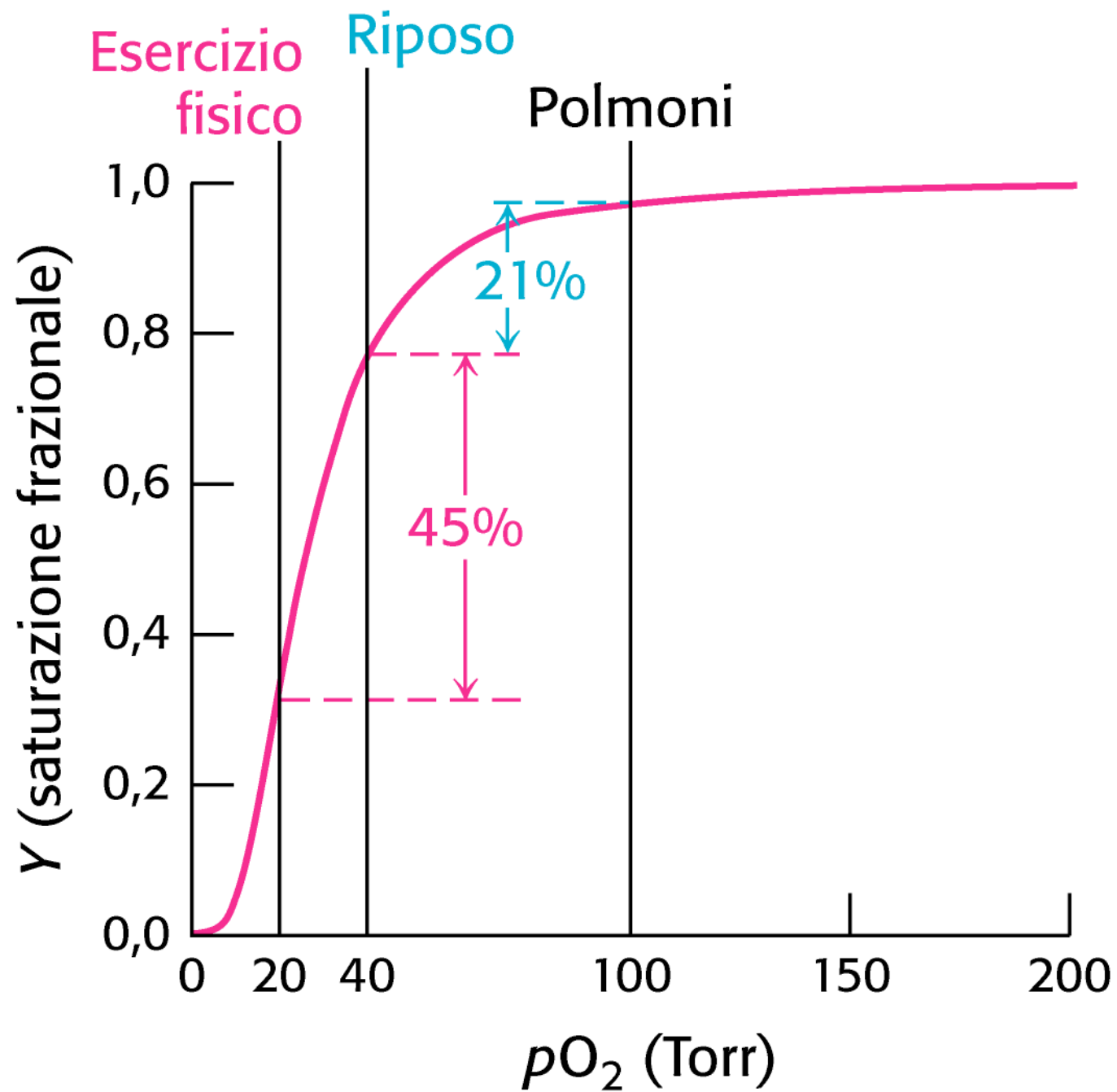


(b) **100 ml di sangue cedono 6,5 ml O₂**
(1/3 dell'O₂ trasportato)

MIOGLOBINA (Riserva di O₂) solo
 nei Muscoli Cardiaco e Scheletrico

Negli altri tessuti l'APPORTO DI O₂
 = O₂ Sang.Art. X **FLUSSO S.Cap***





FISIOLOGIA RESPIRATORIA

SCAMBIO DI O_2 E CO_2 A LIVELLO POLMONARE

TRASPORTO DI O_2 NEL SANGUE

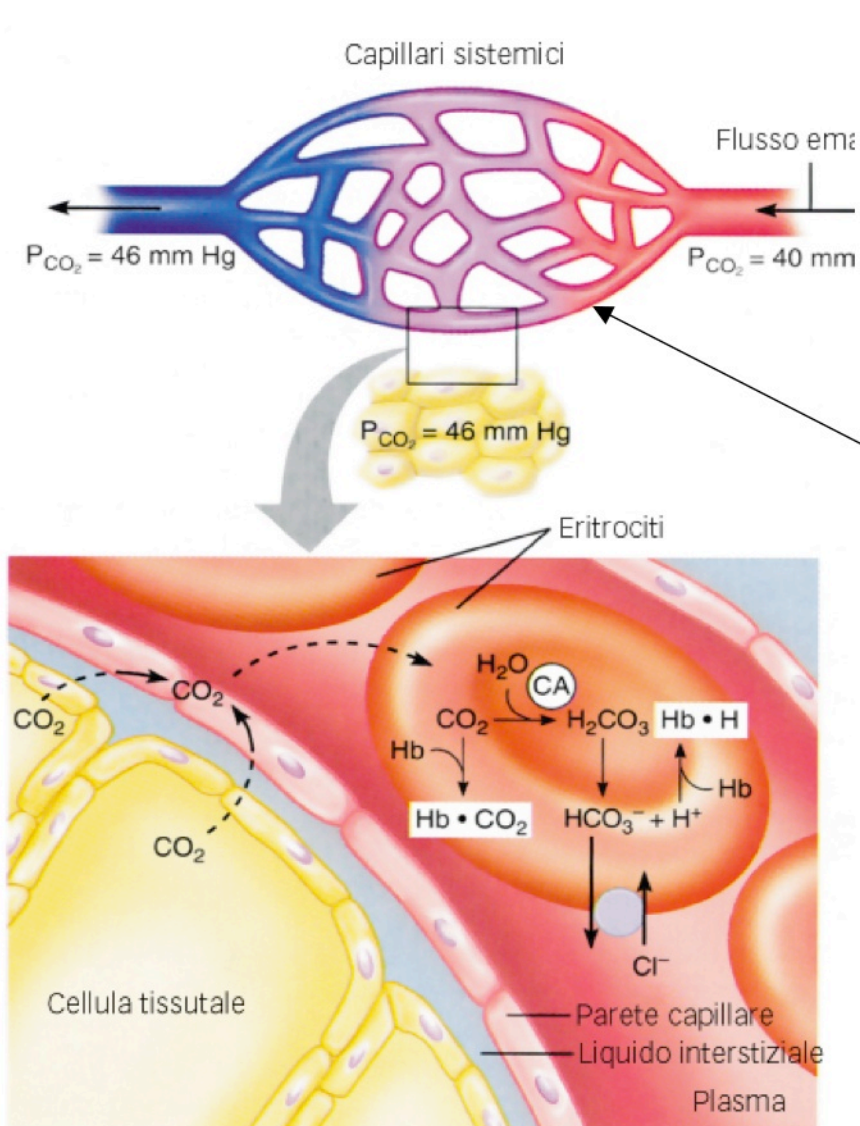
DIFFUSIONE DI O_2 A LIVELLO DEI TESSUTI

DIFFUSIONE DI CO_2 A LIVELLO DEI TESSUTI

TRASPORTO DI CO_2 NEL SANGUE

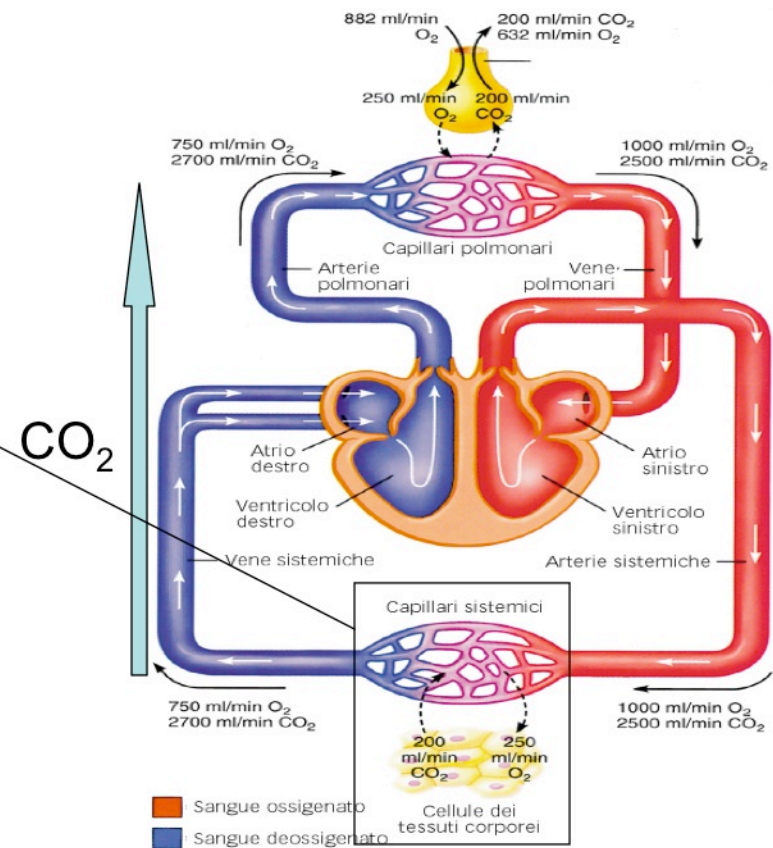
DIFFUSIONE DI CO_2 A LIVELLO POLMONARE

DIFFUSIONE DI CO₂ A LIVELLO DEI TESSUTI

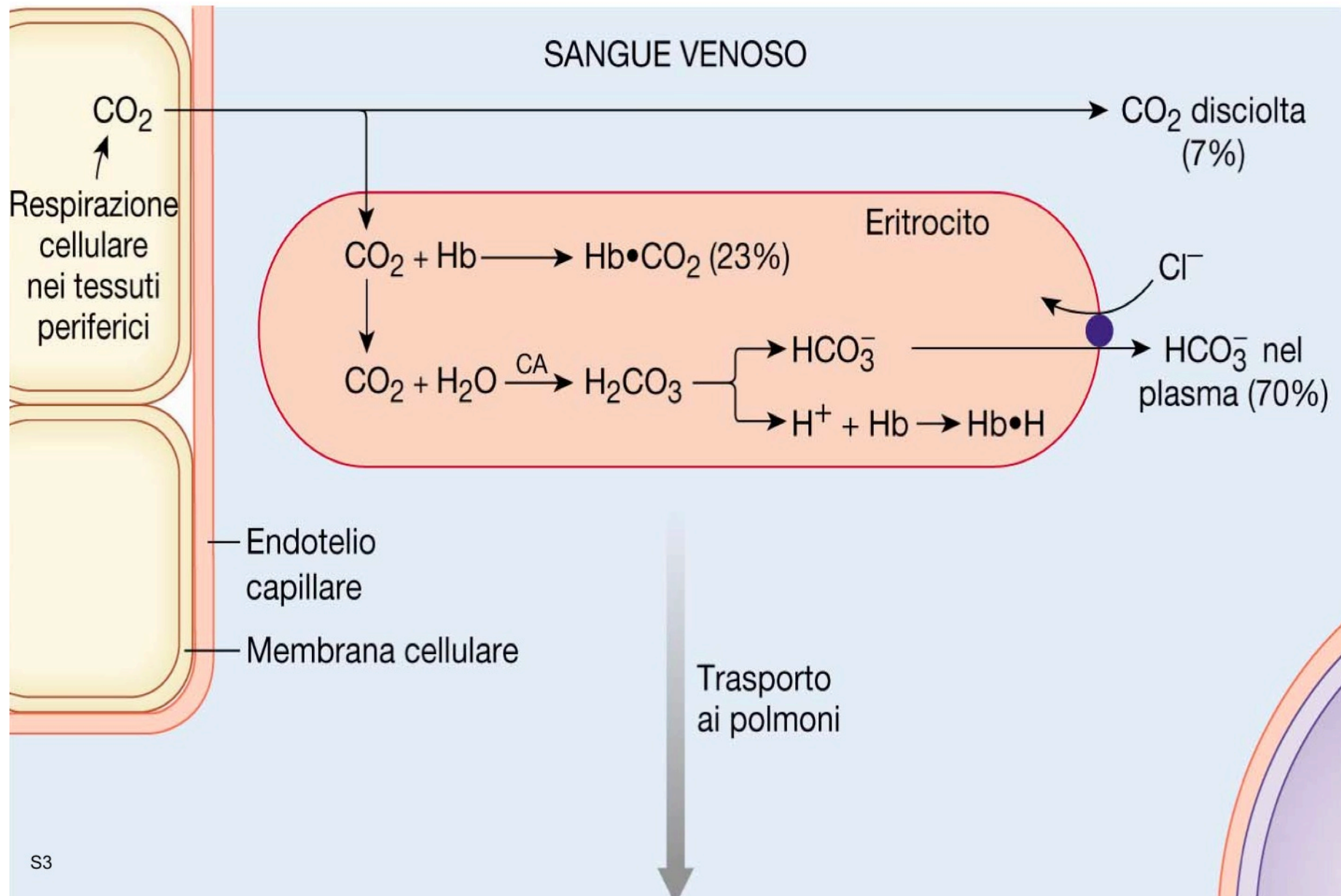


(a)

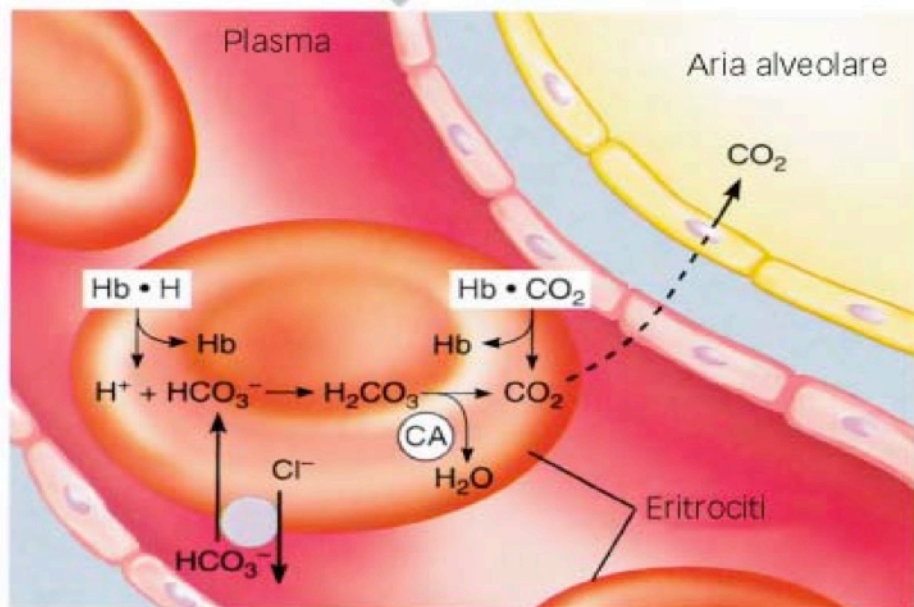
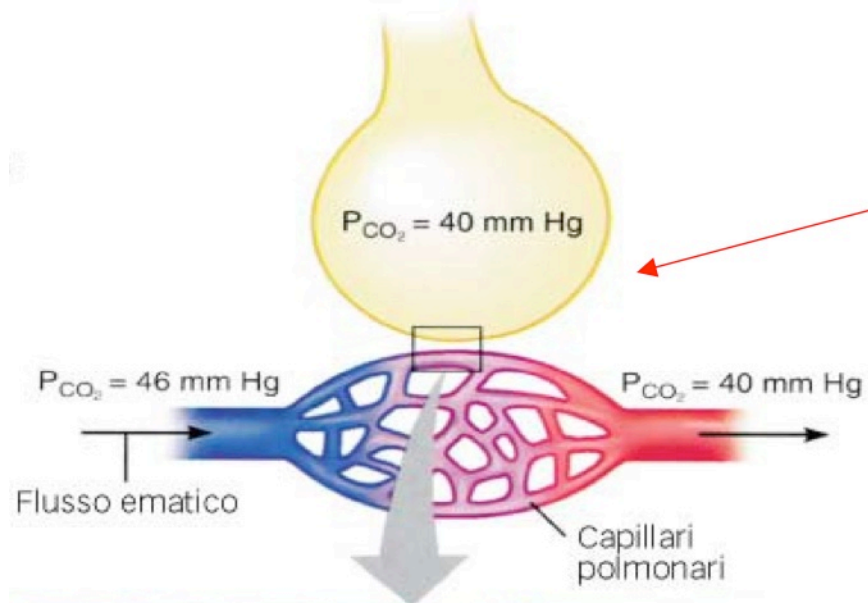
GER



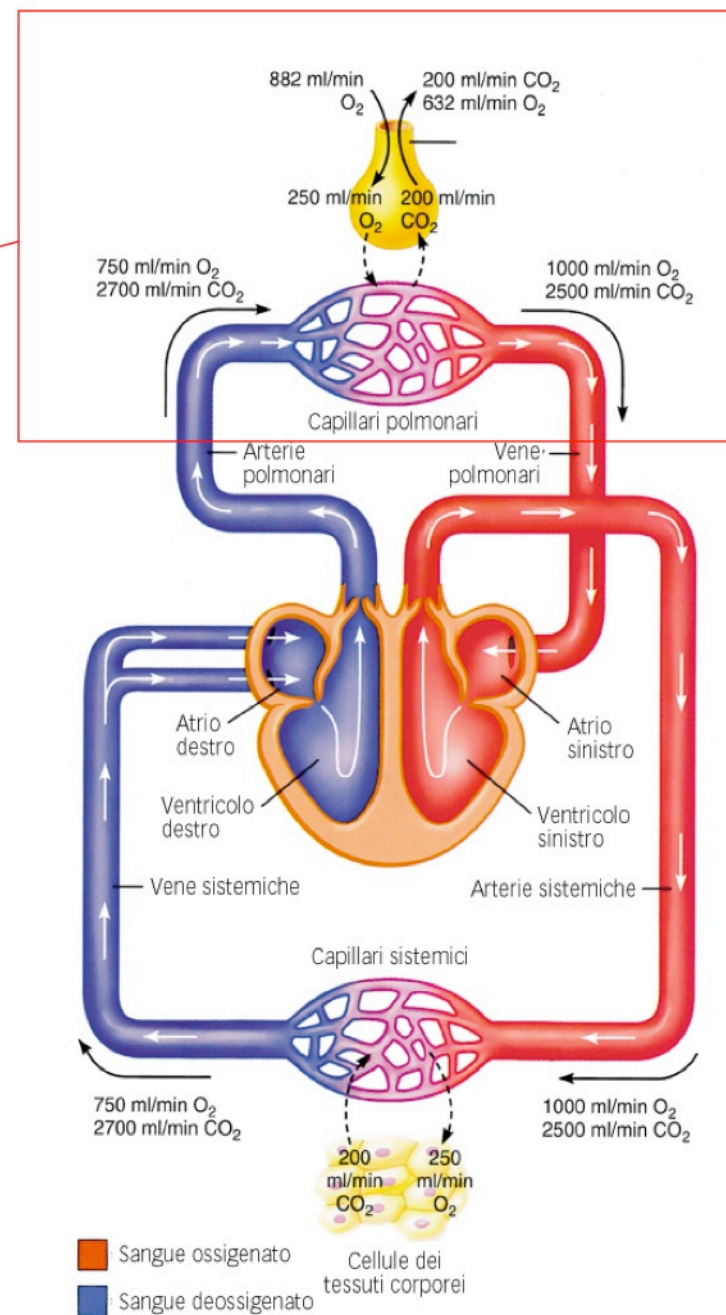
TRASPORTO DI CO₂ NEL SANGUE : DIFFERENTI FORME



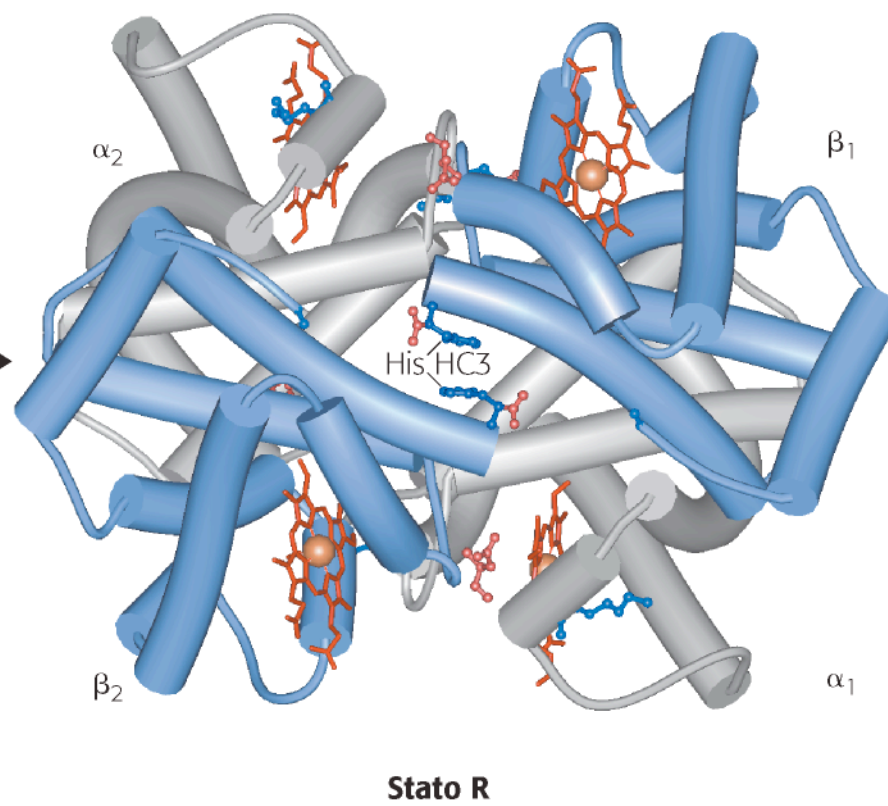
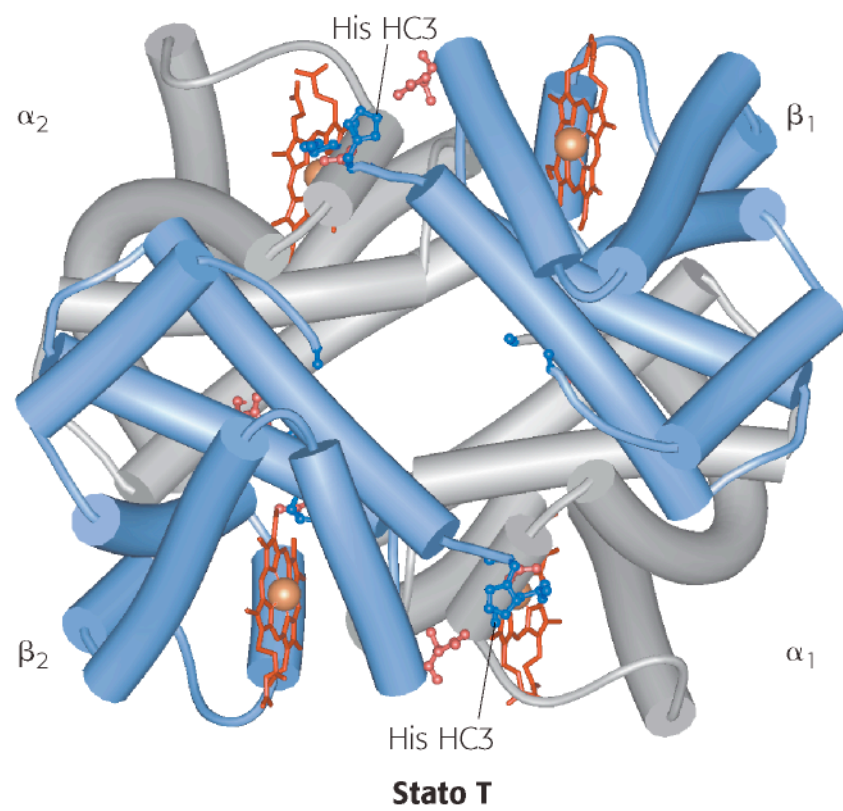
DIFFUSIONE DI CO₂ A LIVELLO POLMONARE

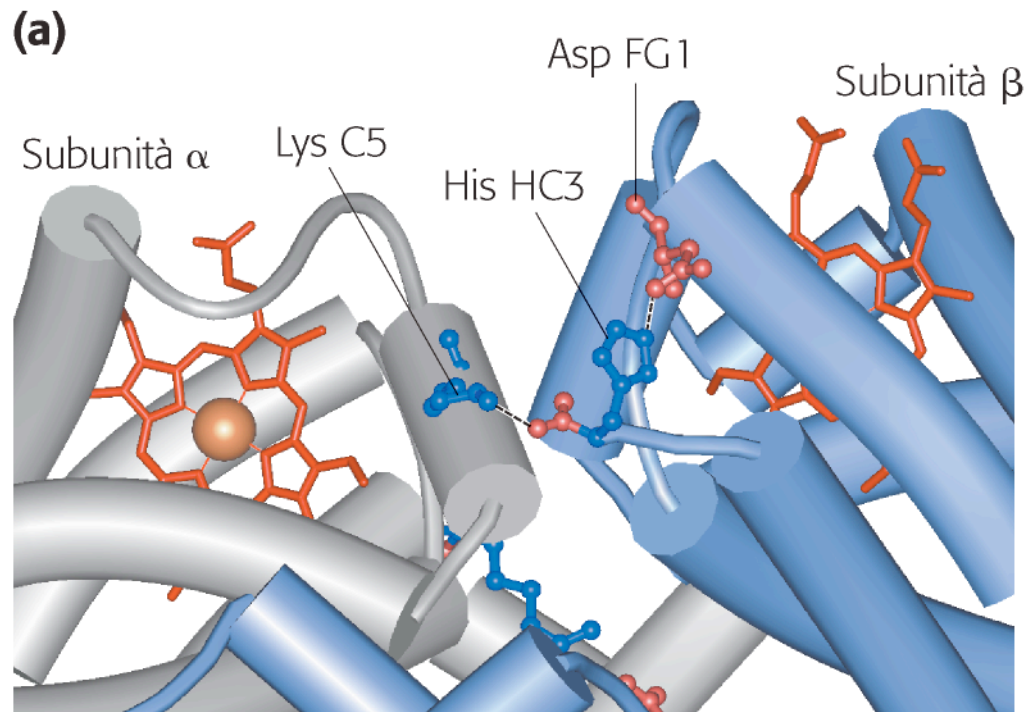


(b)

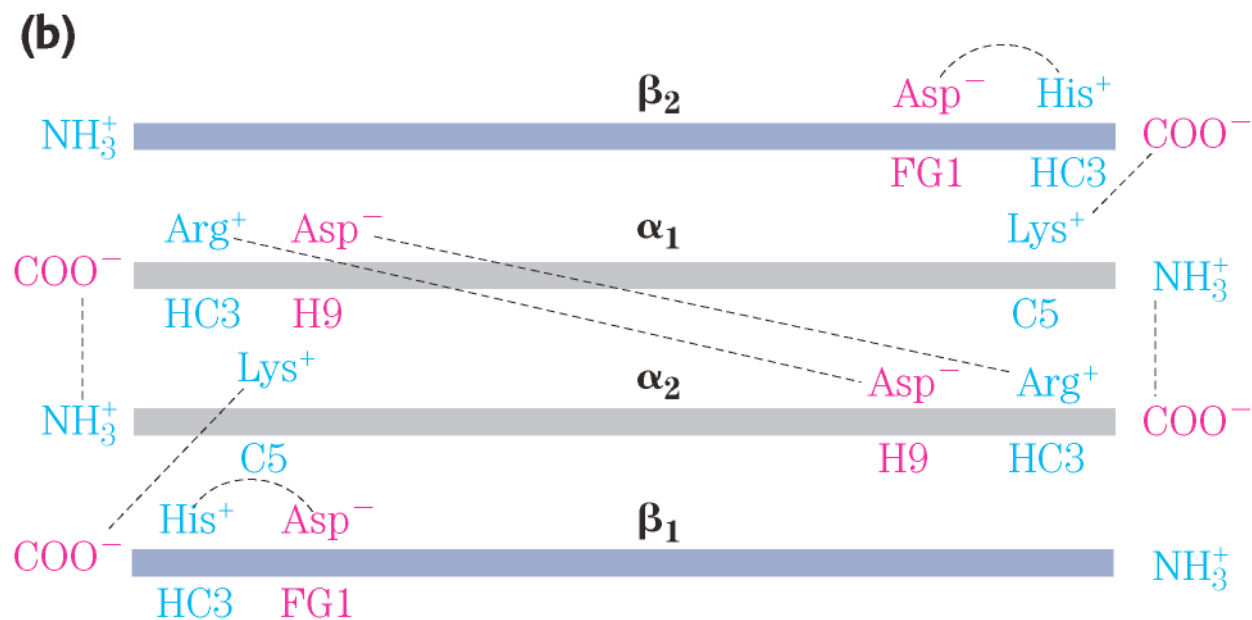


**CRISTALLI DI DEOSSIH_b ESPOSTI
ALL'OSSIGENO SI FRANTUMANO**

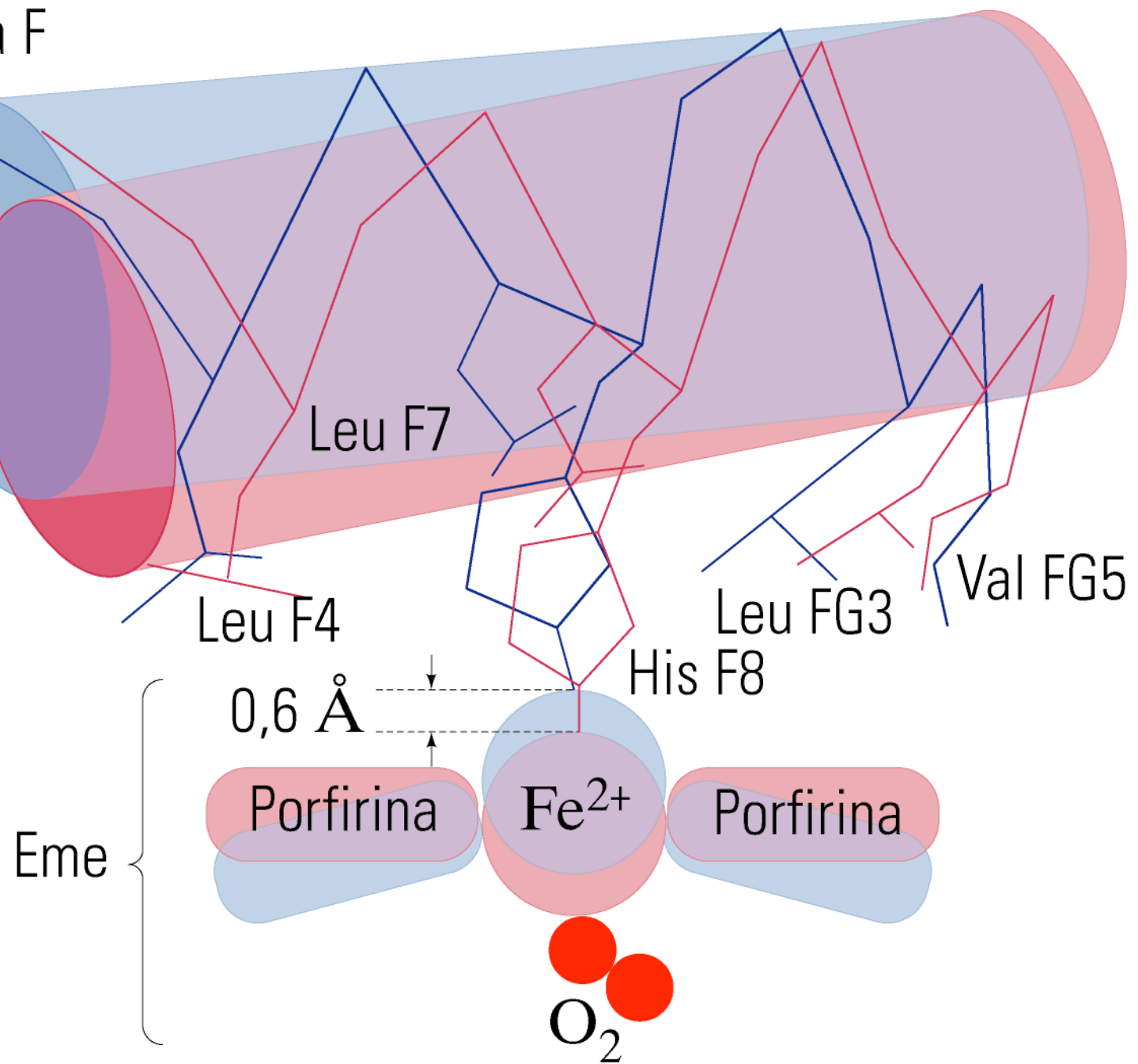




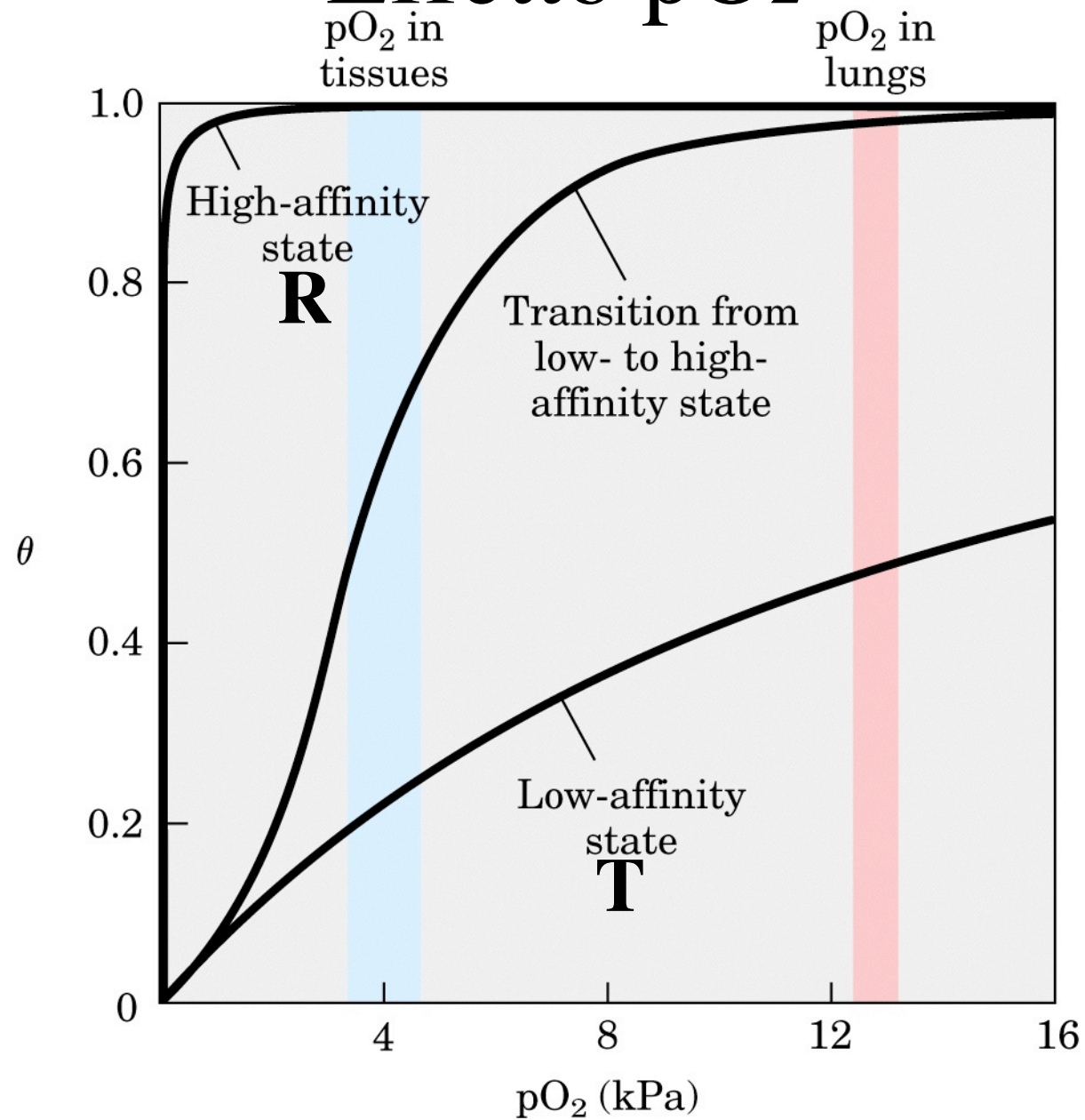
LO STATO **T è
STABILIZZATO DA
NUMEROSE
COPPIE IONICHE**

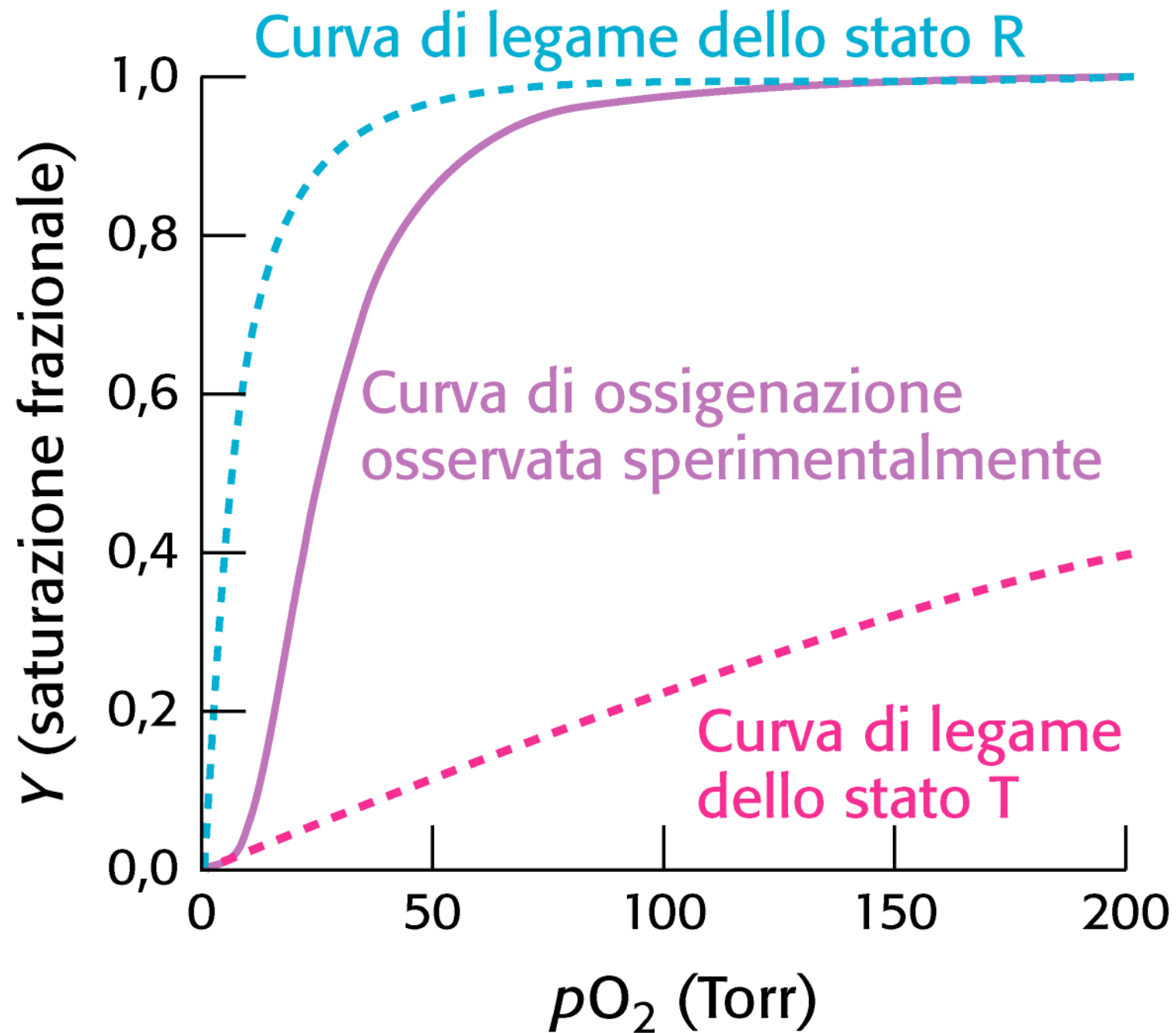


Elica F



Effetto pO_2





L'EMOGLOBINA è UNA PROTEINA ALLOSTERICA

LIGANDO-MODULATORE

EFFETTO OMOTROPICO

EFFETTO ETEROTROPICO

QUANTIFICAZIONE DELLA COOPERATIVITA'

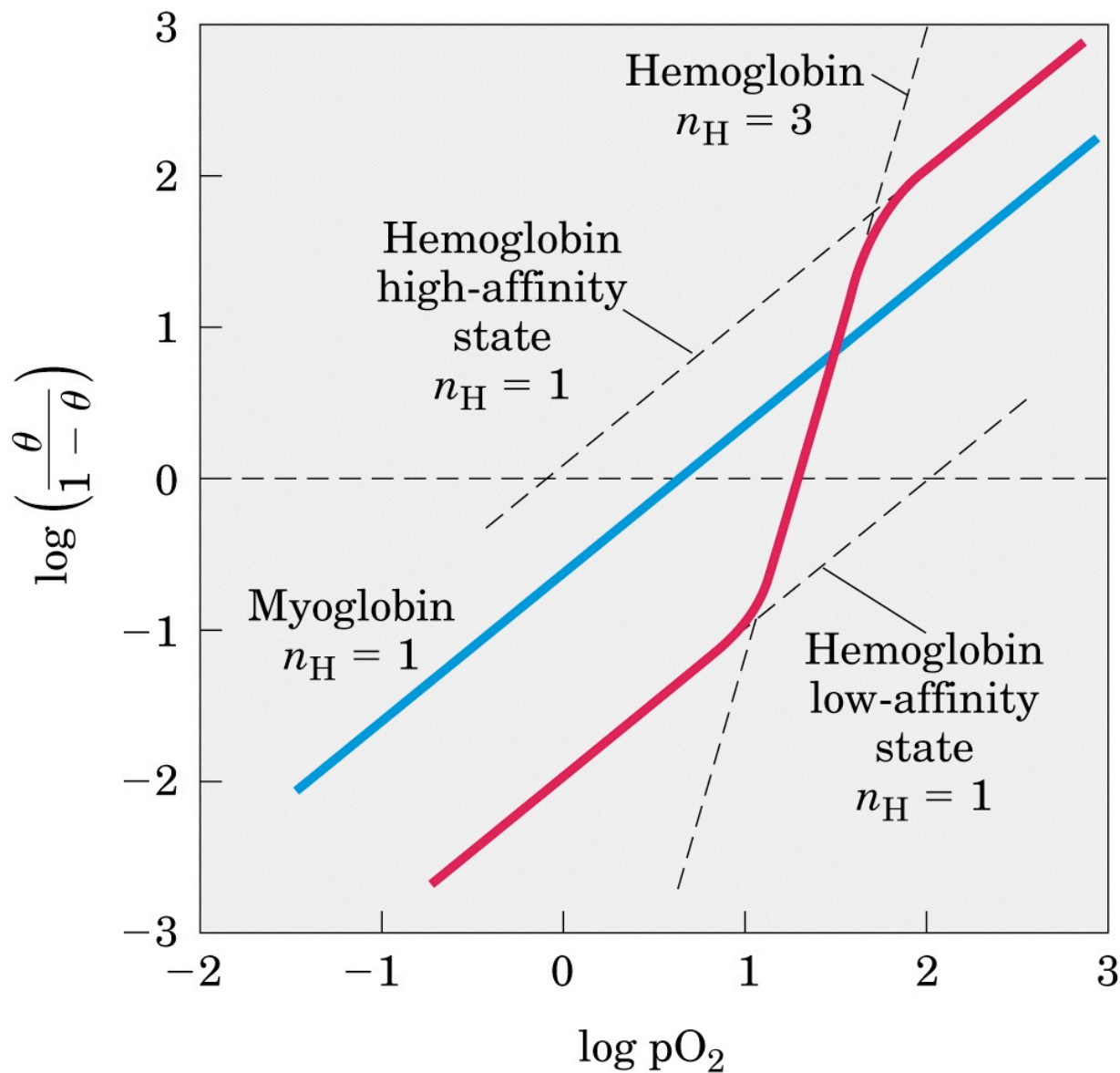


$$\Theta = \frac{[L]^n}{[L]^n + K_d}$$

$$\frac{\Theta}{1 - \Theta} = \frac{[L]^n}{K_d}$$

$$\log \frac{\Theta}{1 - \Theta} = n \log [L] - \log K_d \quad (K_d = [L]_{0,5}^n)$$

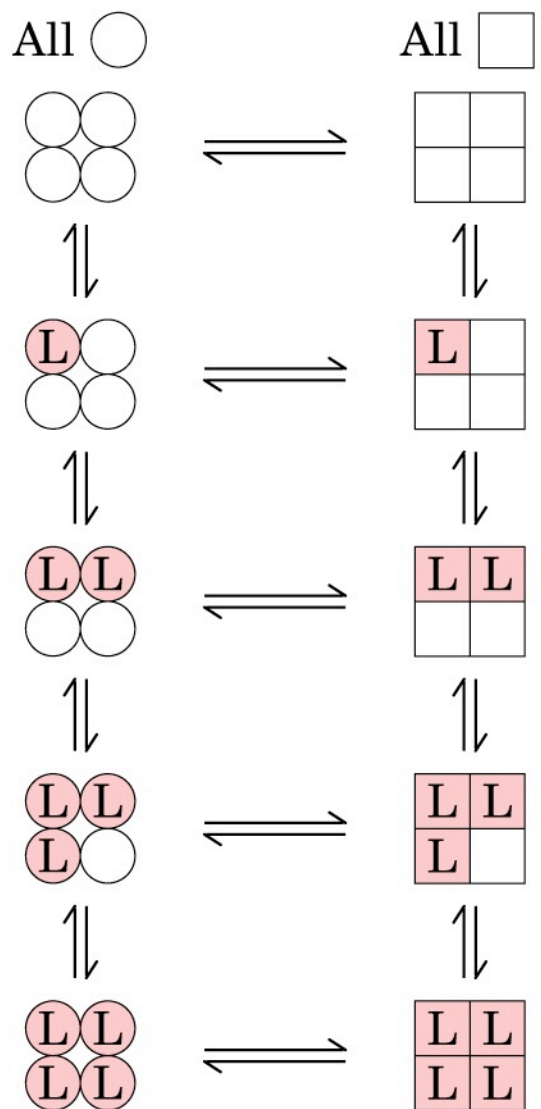
Plot di Hill



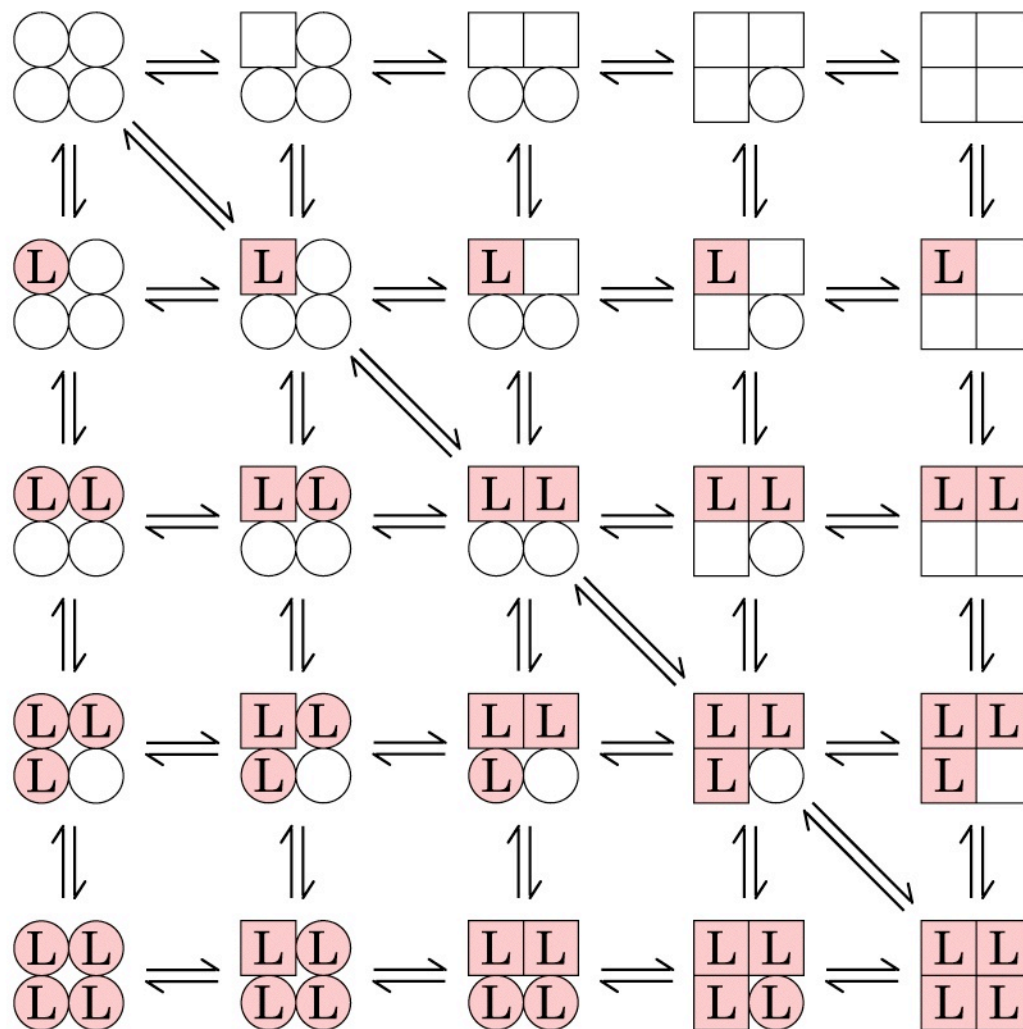
$$N = N_H$$

IMPOSSIBILE, RICHIEDEREBBE LA
CONTEMPORANEITÀ

Modelli di cooperatività di legame



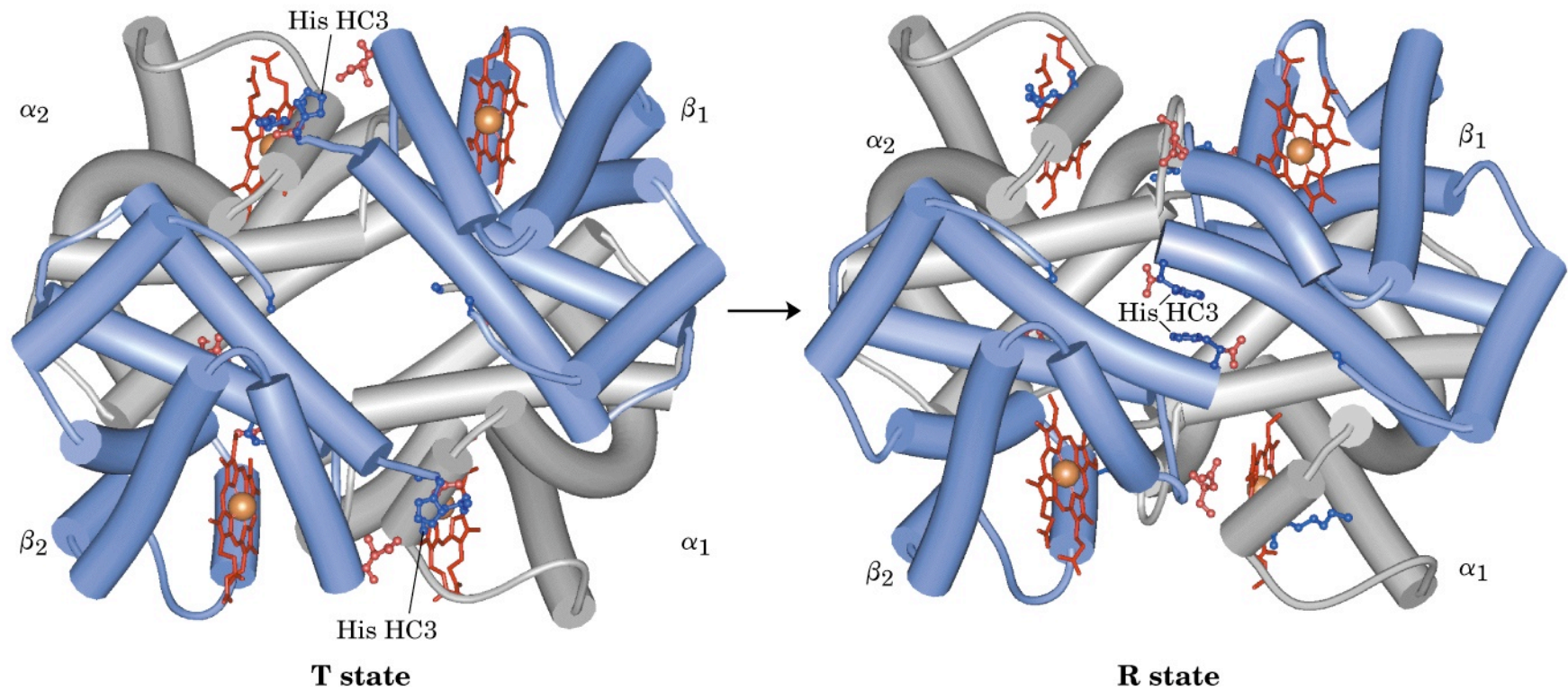
(a)



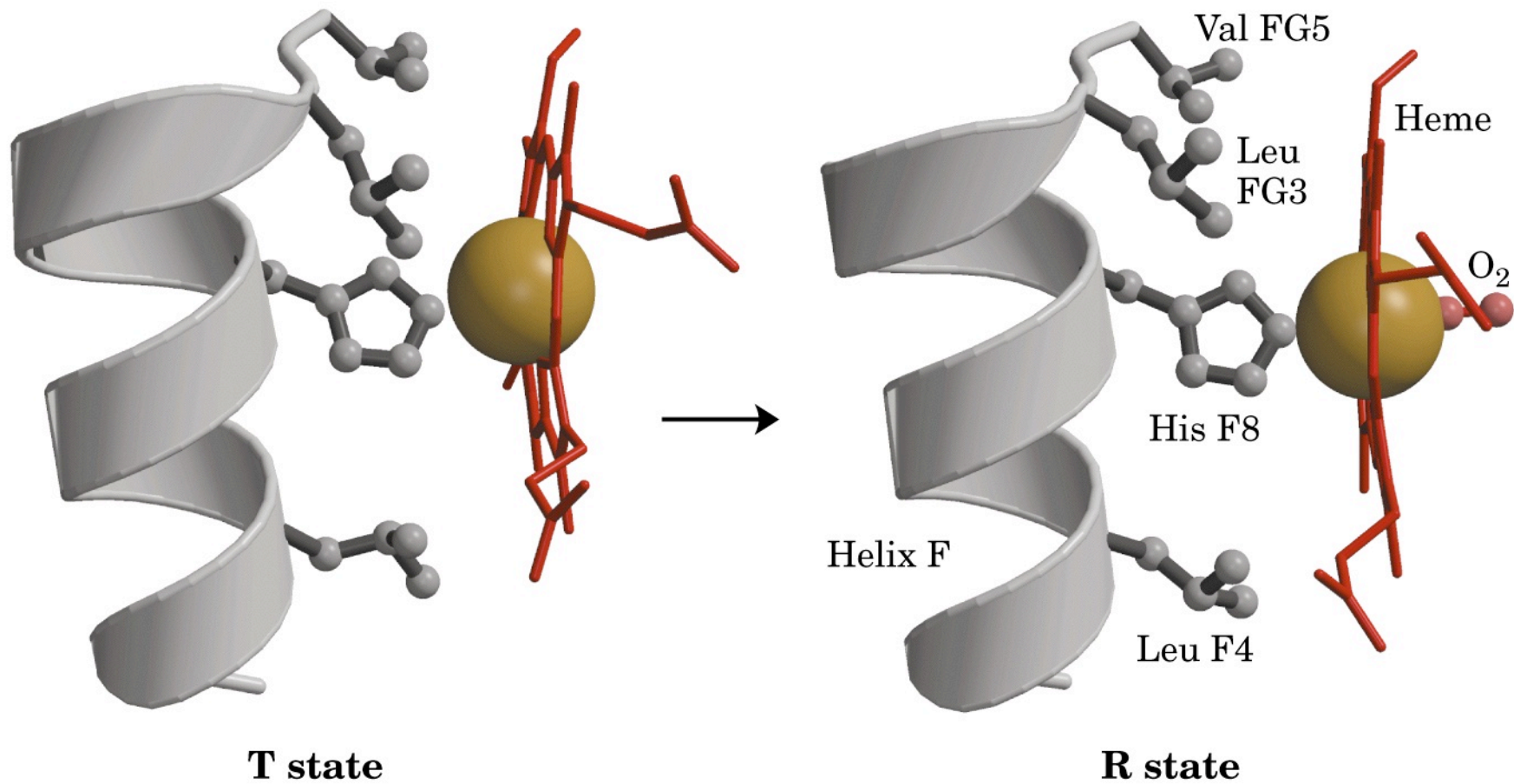
(b)

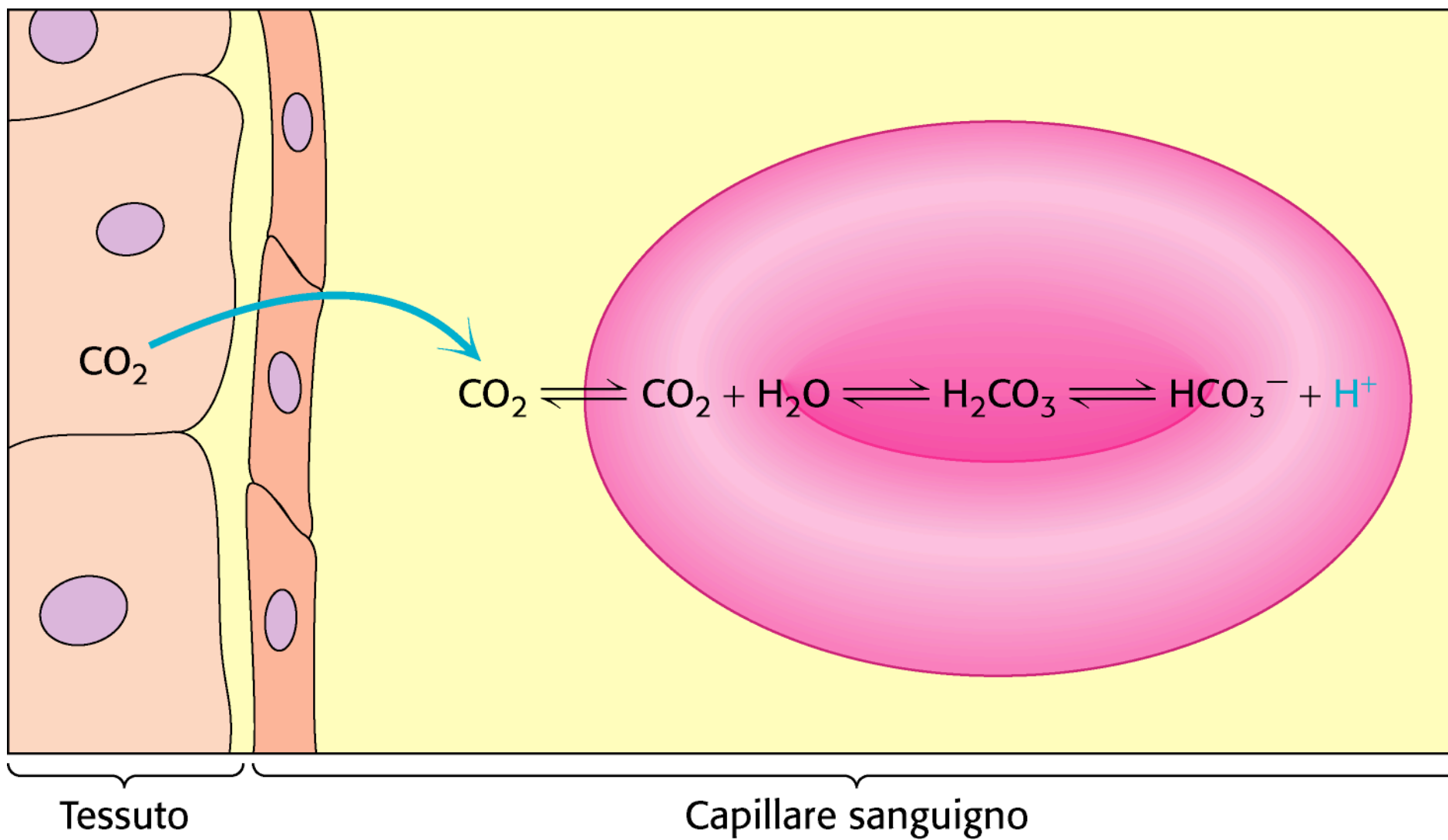
Conformazioni T ed R

Ligando: O₂ kD HbT > kD HbR

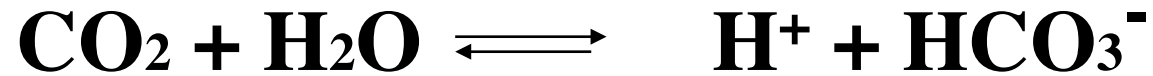


Legame con ossigeno e conformazioni Hb

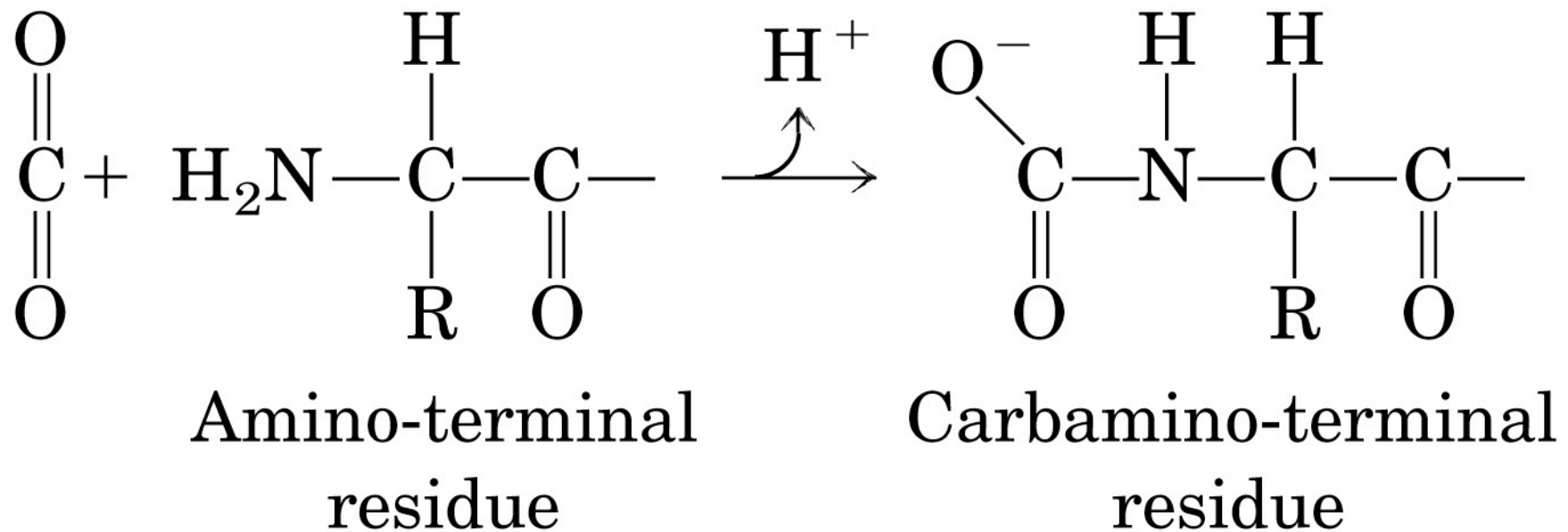




ANIDRASI CARBONICA

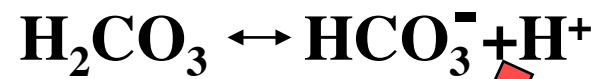
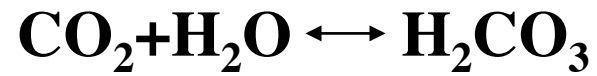


Trasporto CO_2



EFFETTO DEL pH

Effetto Bohr

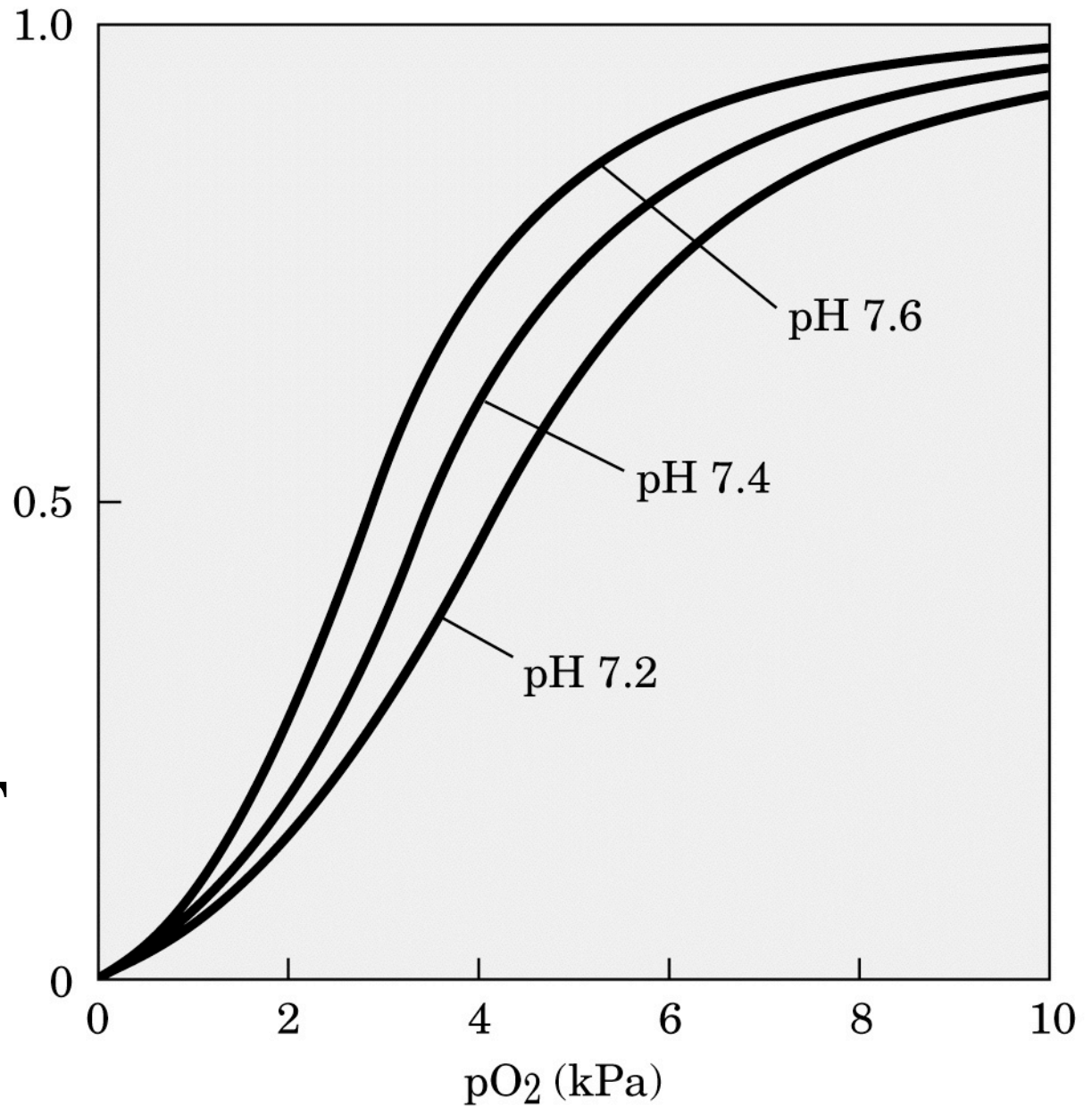


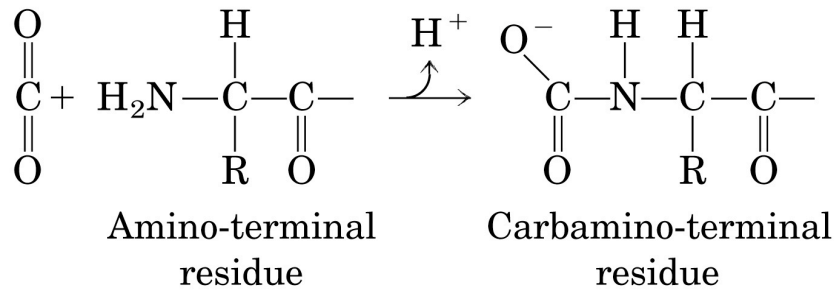
θ

$\beta(\text{Hys143})$

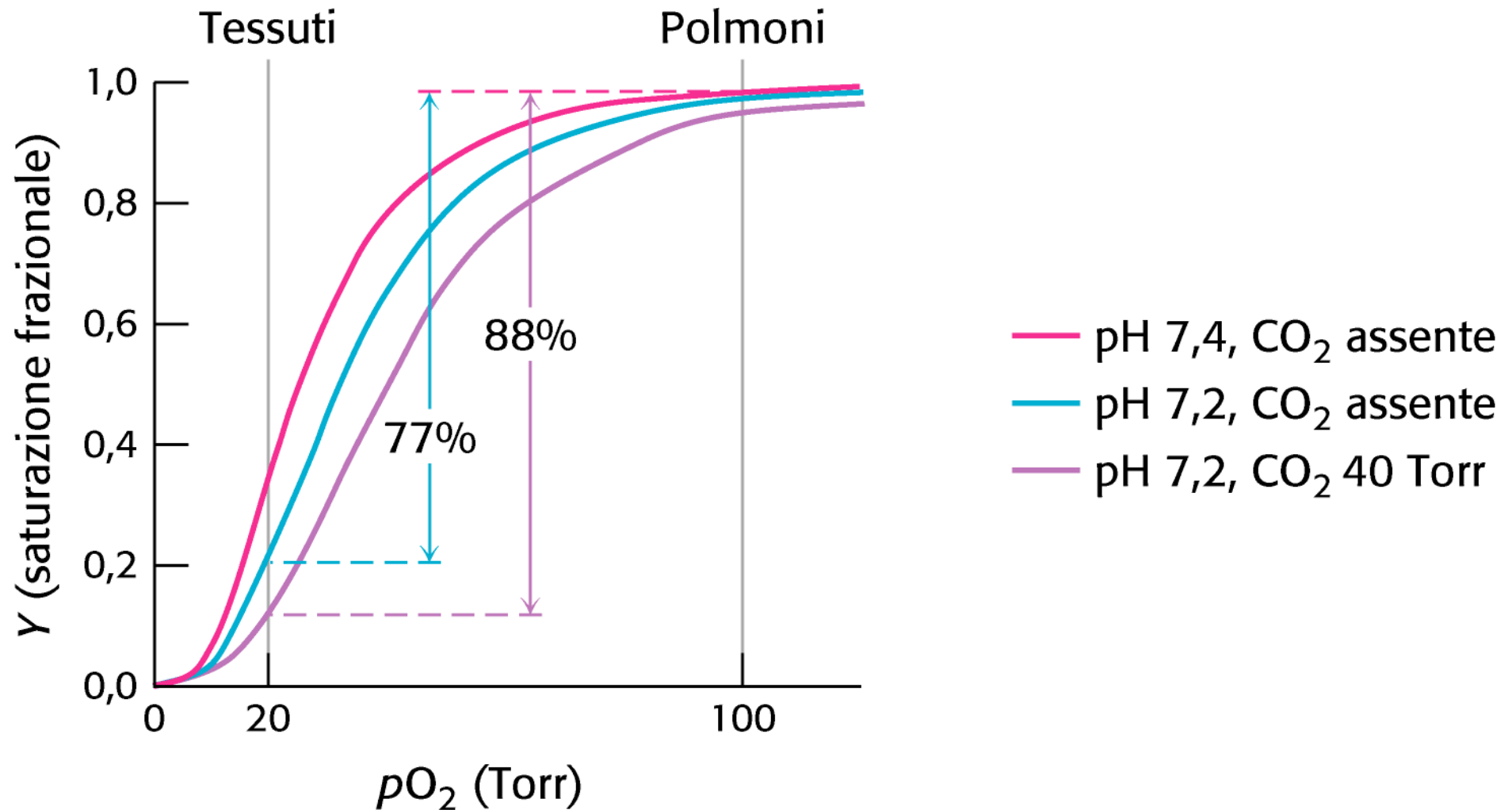


ASP94 FORM **T**

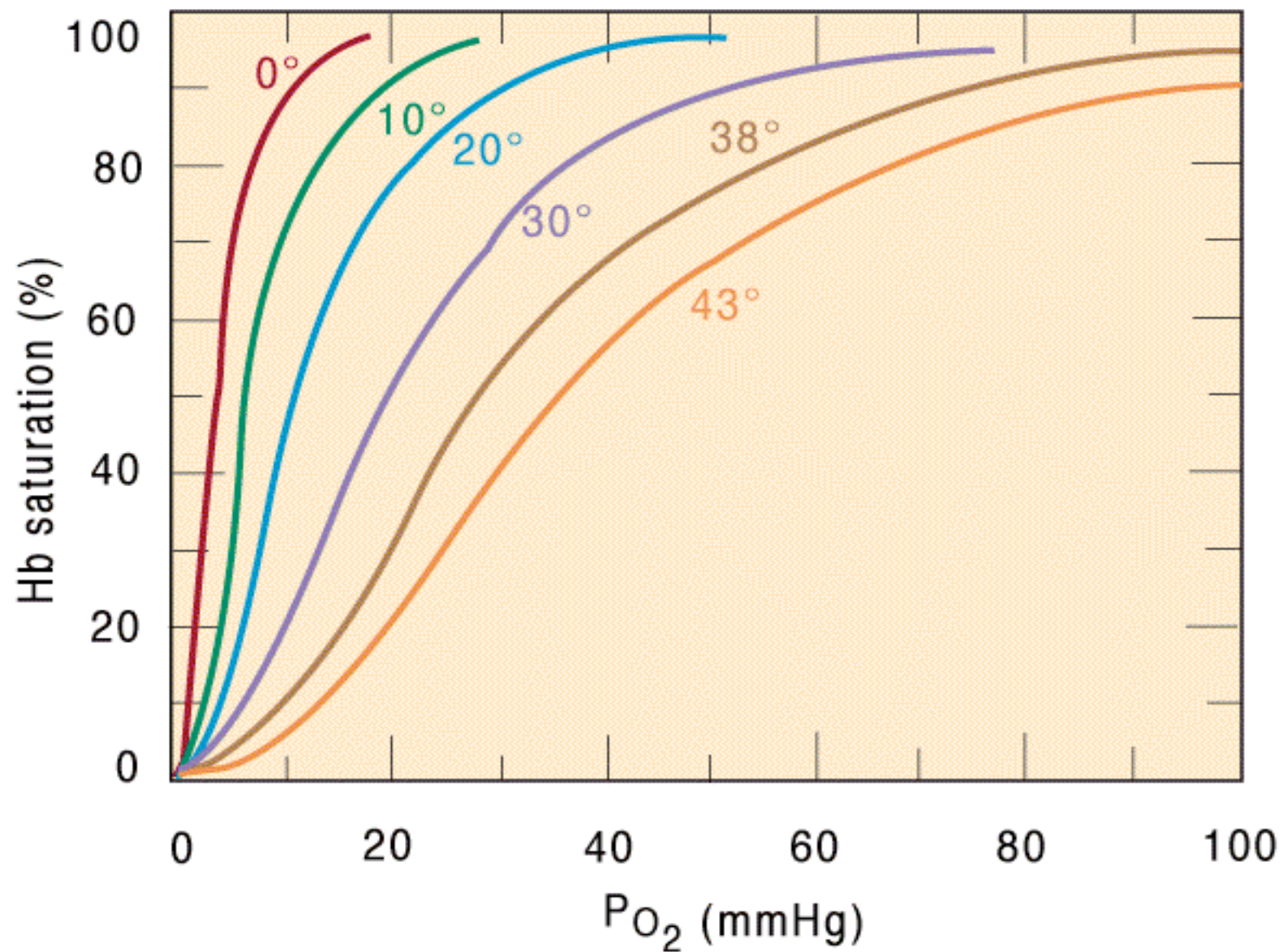


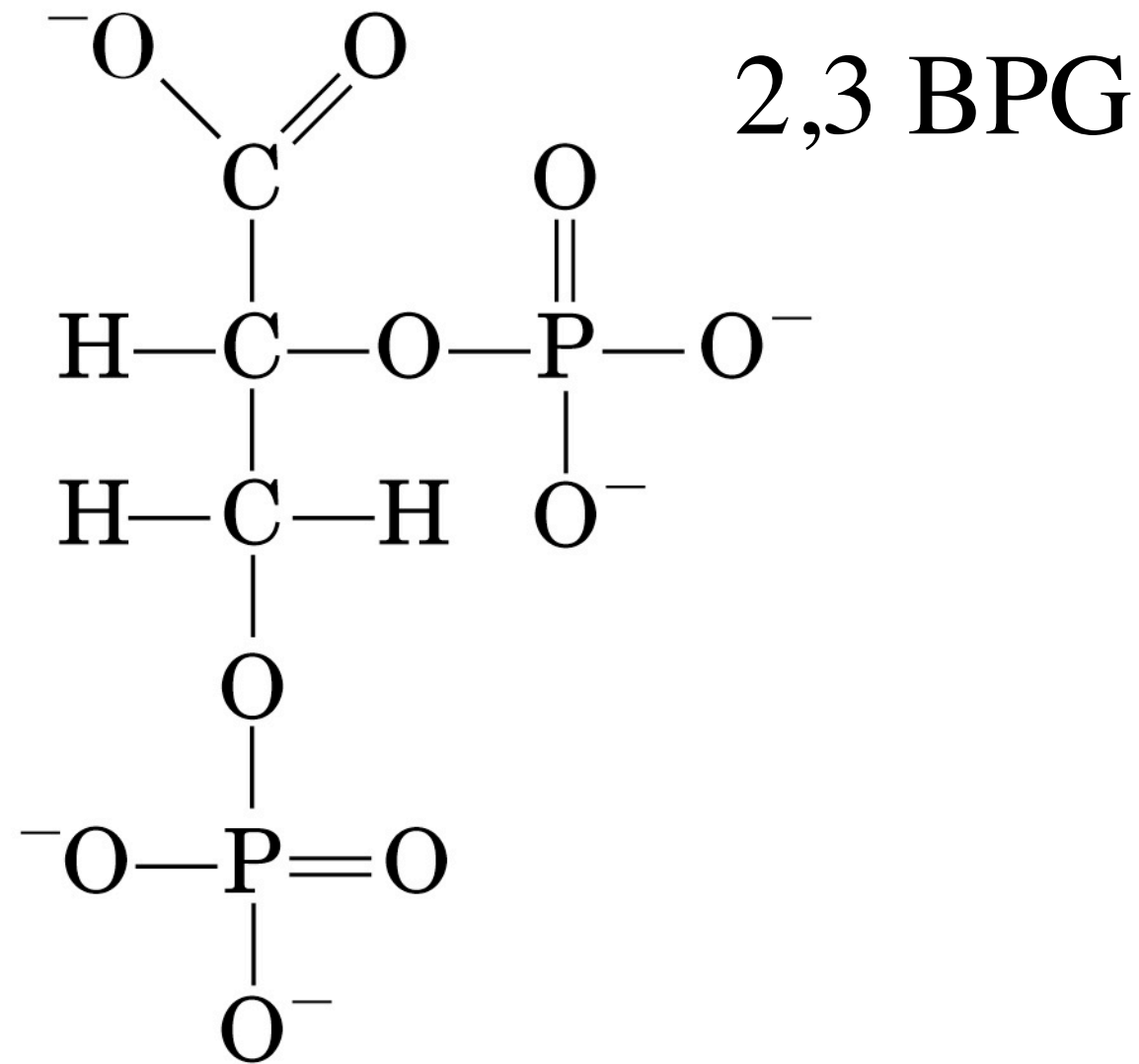


H⁺ E PONTI SALINI



EFFETTO DELLA TEMPERATURA

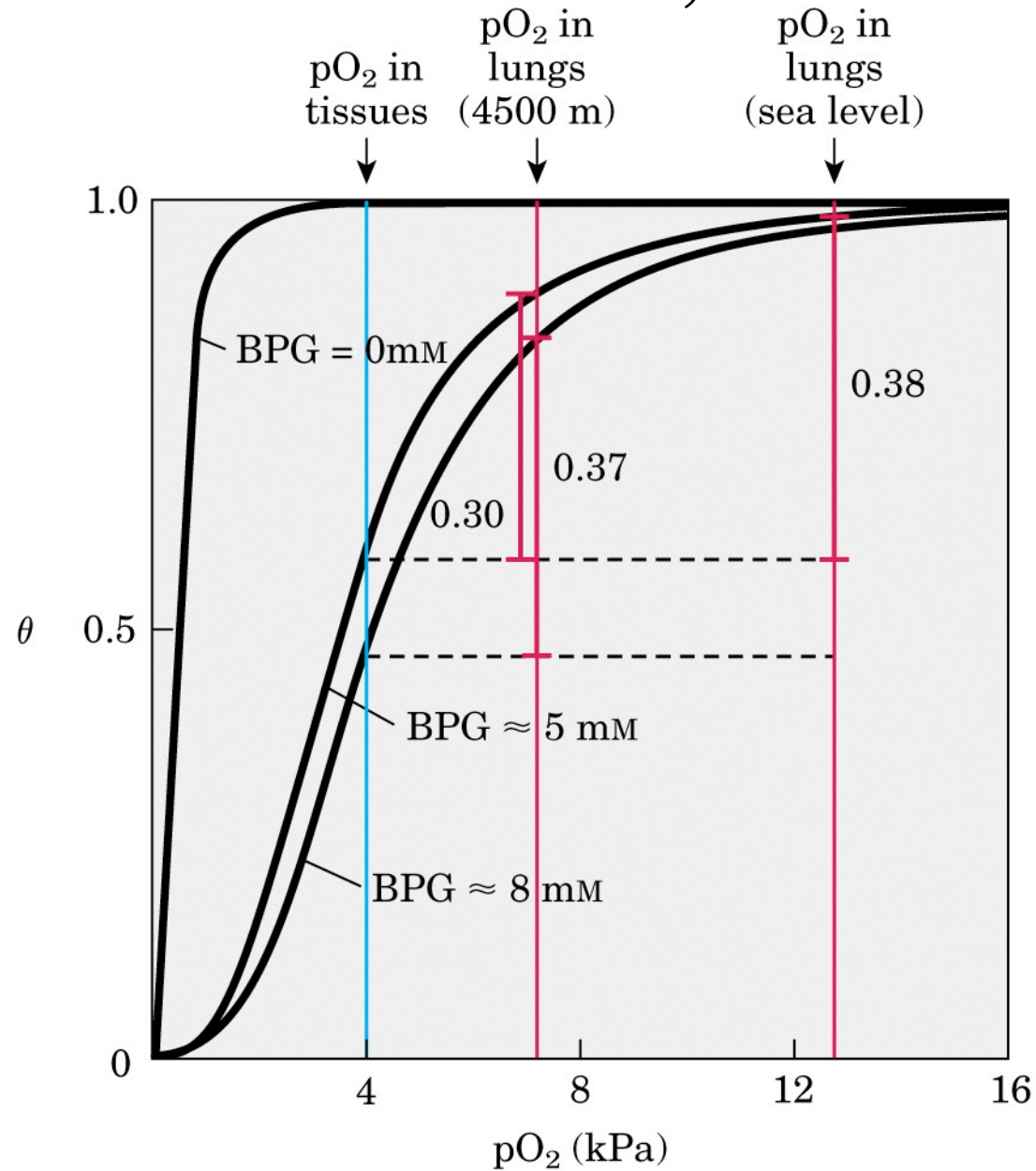


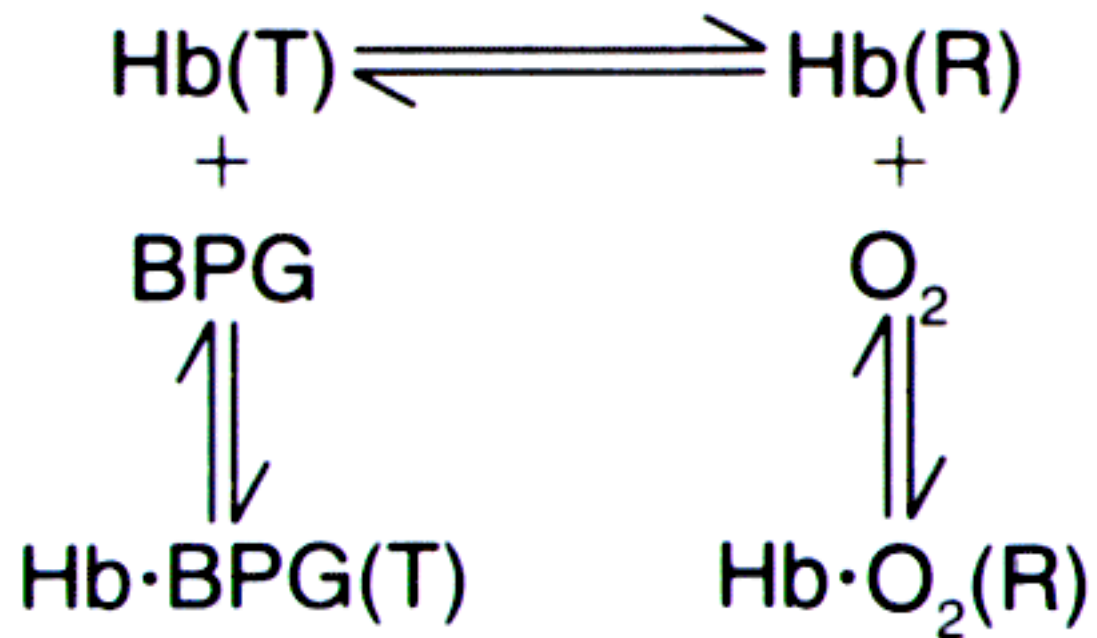


2,3-Bisphosphoglycerate

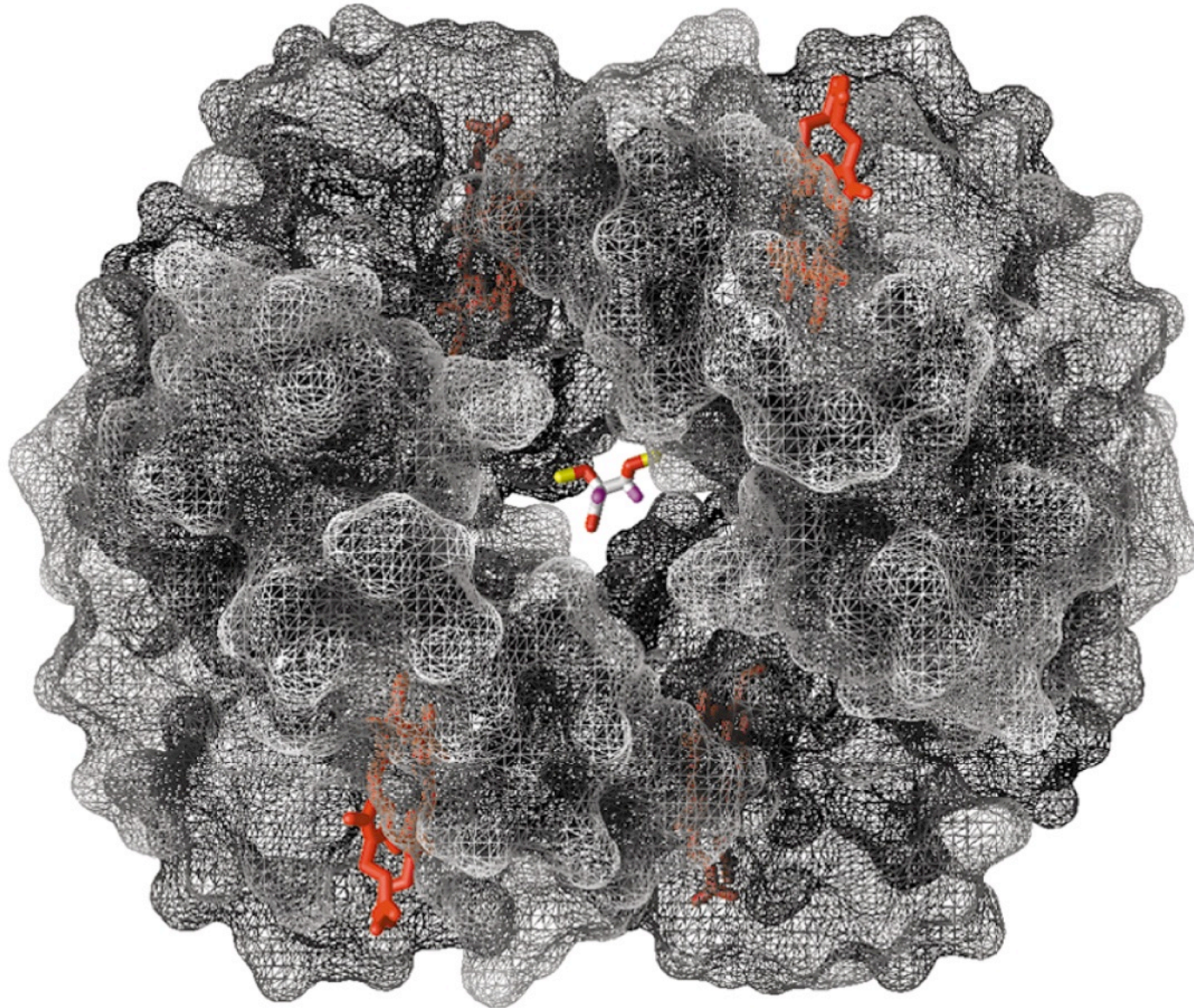
REGOLAZIONE ETEROTROPICA

Effetto di 2,3BPG

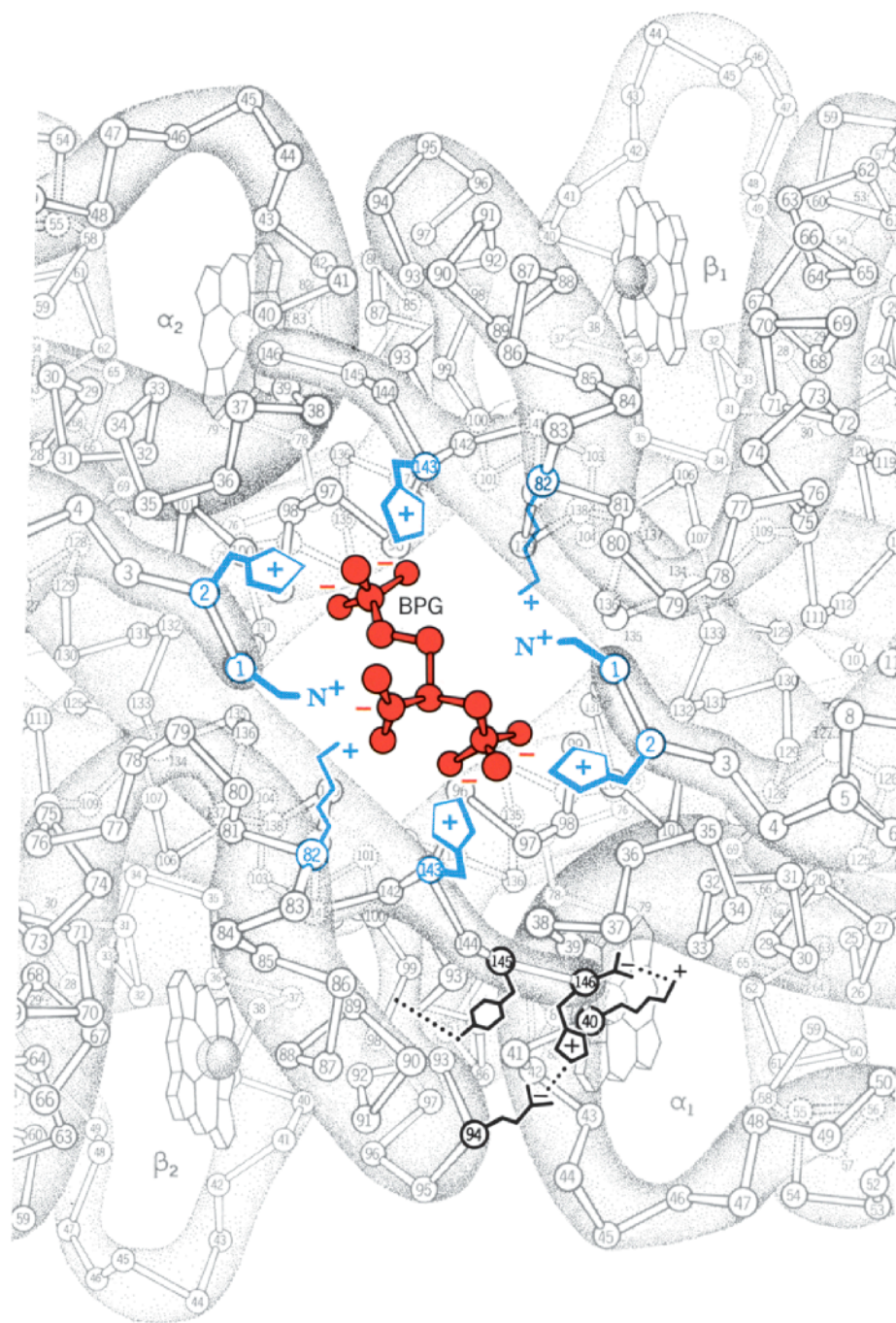




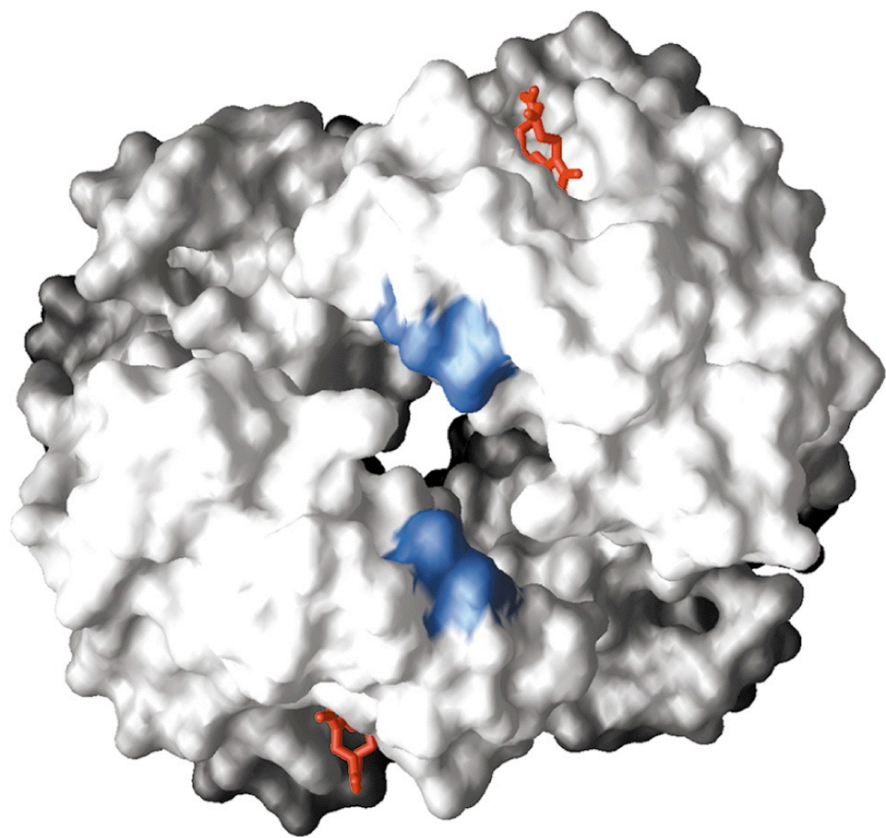
2,3 BPG-Hb



(a)

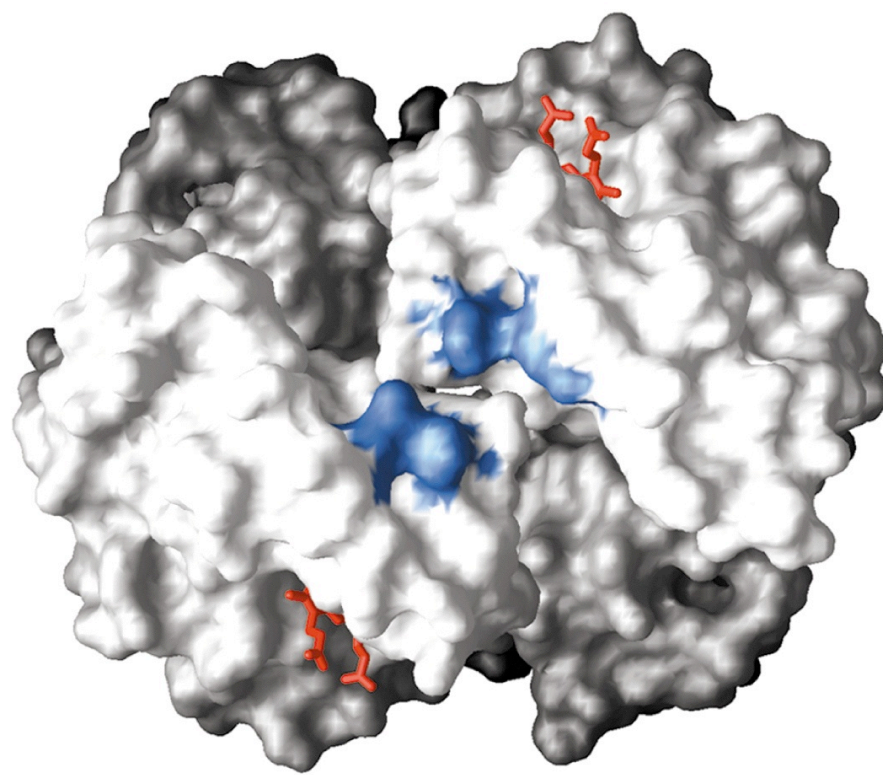


Deossi Hb



(b)

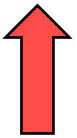
Ossi Hb



(c)

Fattori che influenzano l'affinità di Hb per O₂

pO₂



H⁺



CO₂

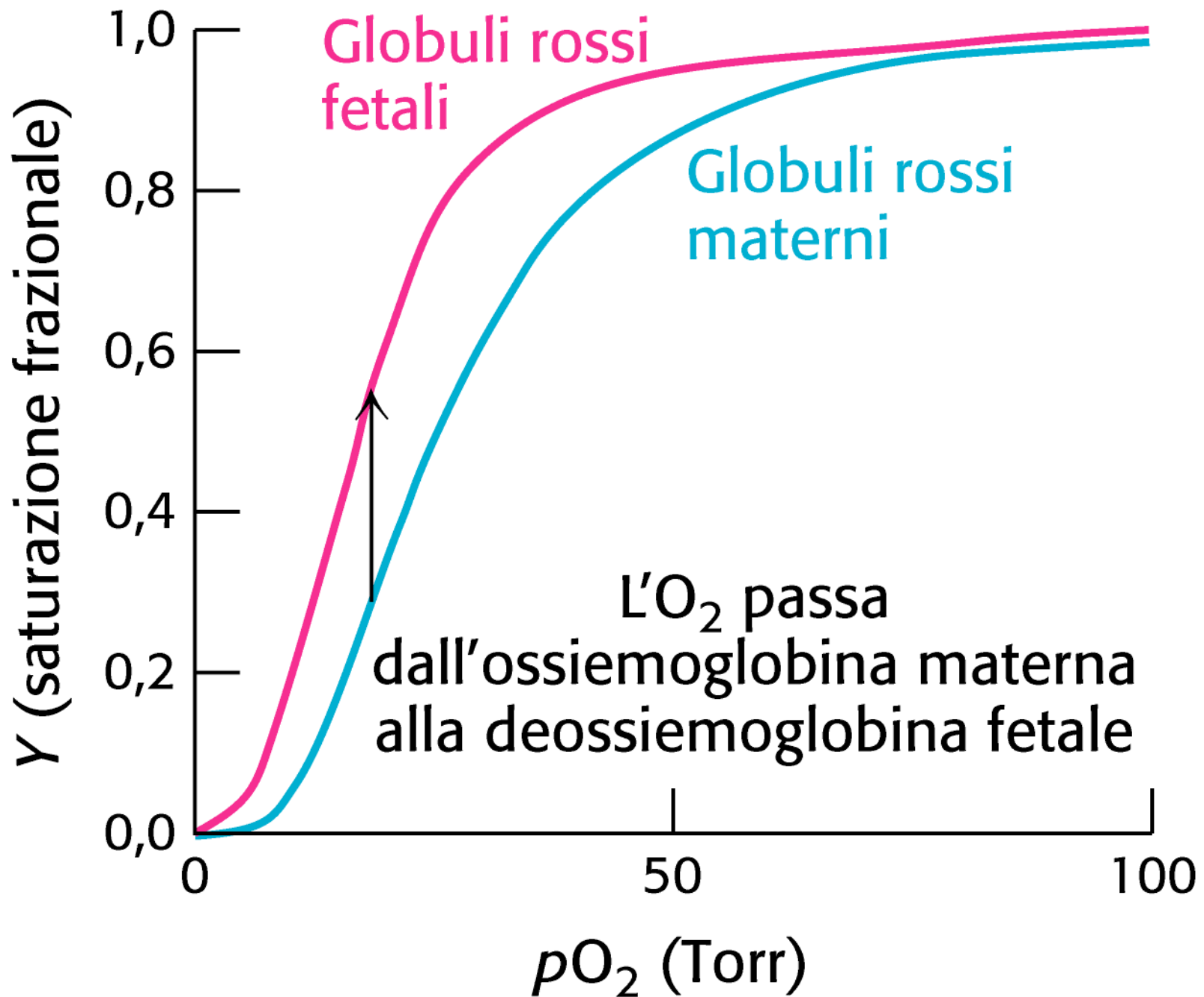


2,3 DPG

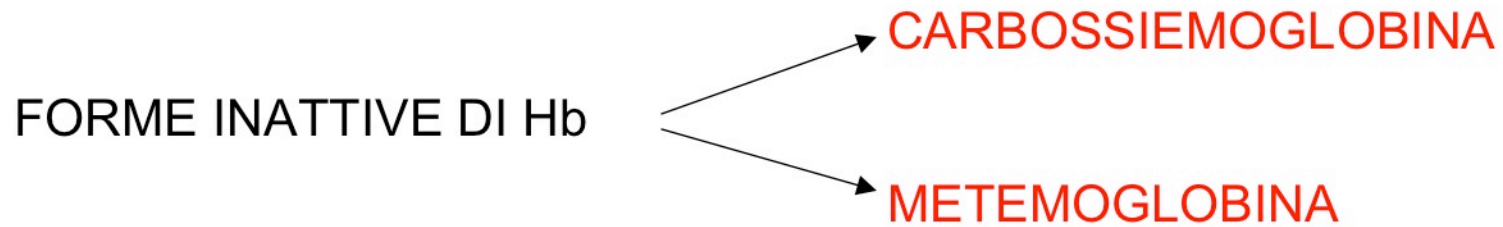


T





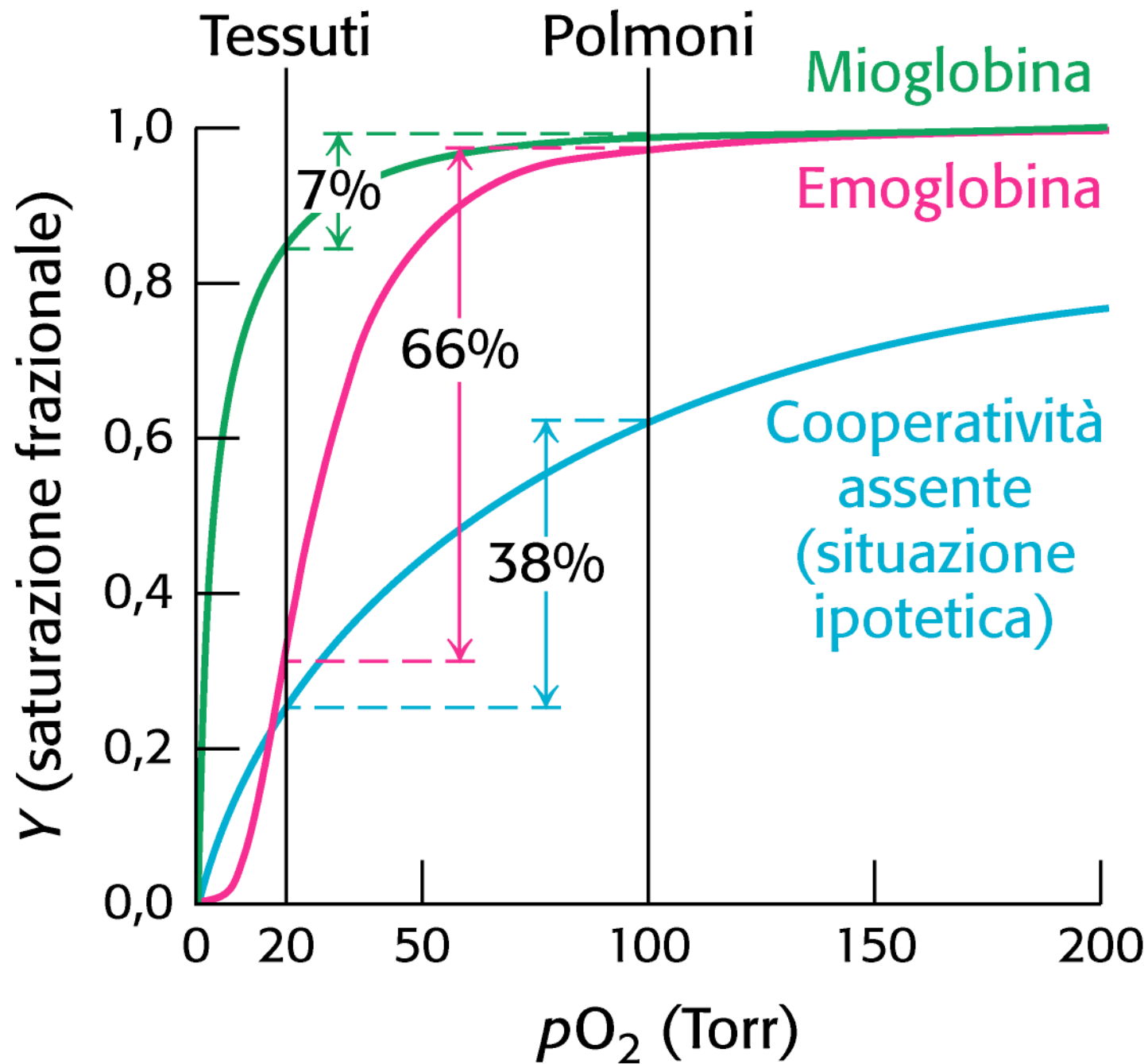
EMOGLOBINE PATOLOGICHE UMANE



MODIFICAZIONI QUALITATIVE → ANEMIA FALCIFORME

MODIFICAZIONI QUANTITATIVE (Es. SINDROMI TALASSEMICHE)





METEGLOBINEMIE : Fe+++

SITUAZIONE NORMALE

TENDENZA DEL Hb AD OSSIDARSI $\longleftrightarrow$ SISTEMA ENZIMATICO MANTIENE Fe⁺⁺

0.5 % ALLO STATO DI METEMOGLOBINA (Fe+++)

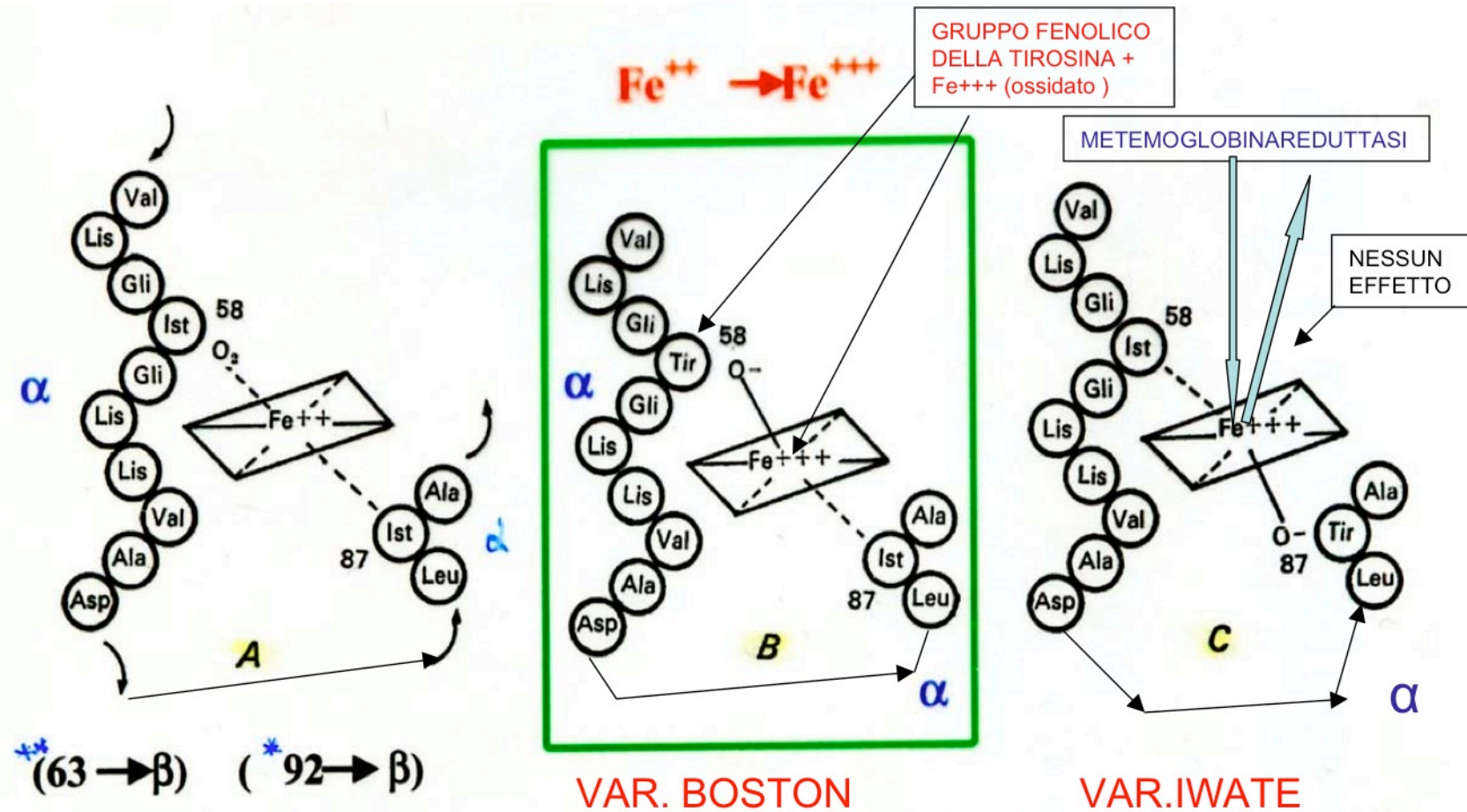
ANOMALIE:

MANCANZA CONGENITA DI METEMOGLOBINA REDUTTASI

SOSTITUZIONE DI UN AMINOACIDO

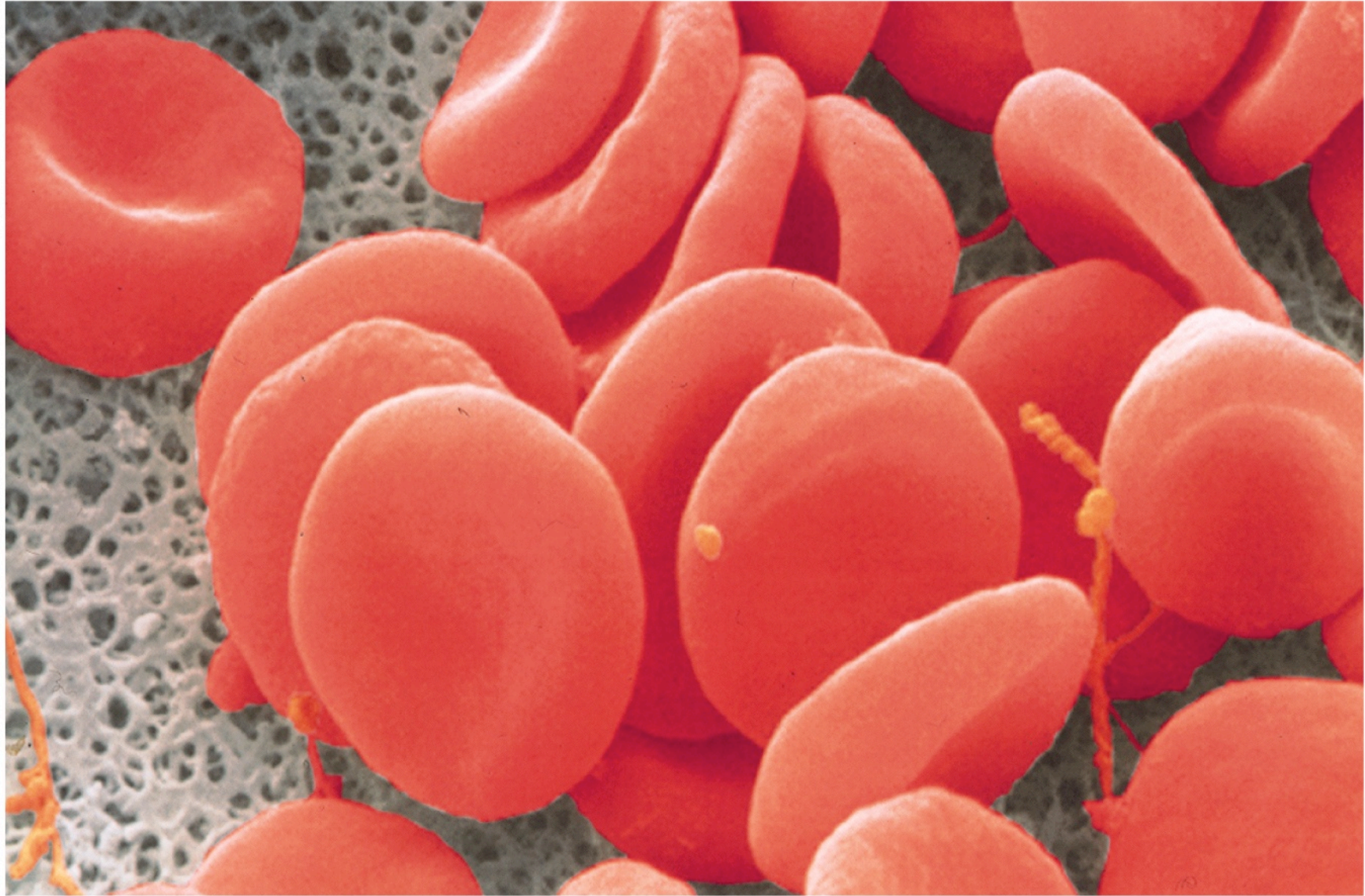
GRAN PARTE DEL FERRO CIRCOLANTE E' ALLO STATO FERRICO (Fe+++)

METEMOGLOBINE



GH. Fig. 3-36. Effetto della sostituzione di una delle istidine che legano l'eme con la tirosina. A, ossiemoglobina normale; B, metemoglobina M Boston; C, metemoglobina M Iwate. Come si vede, si può formare un complesso fra il ferro ossidato e il residuo di tirosina (da Harris).

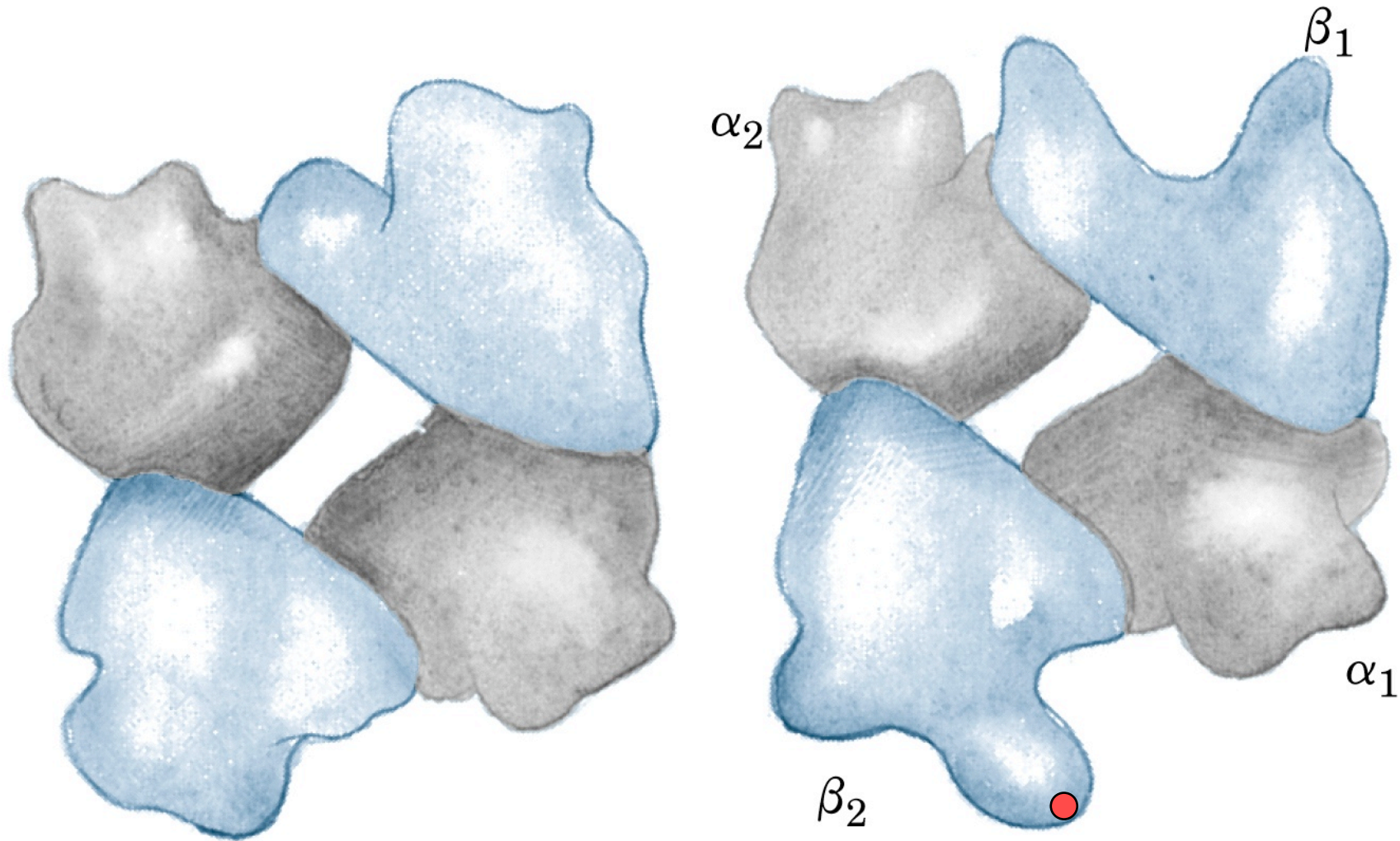
Globuli rossi normali



(a)

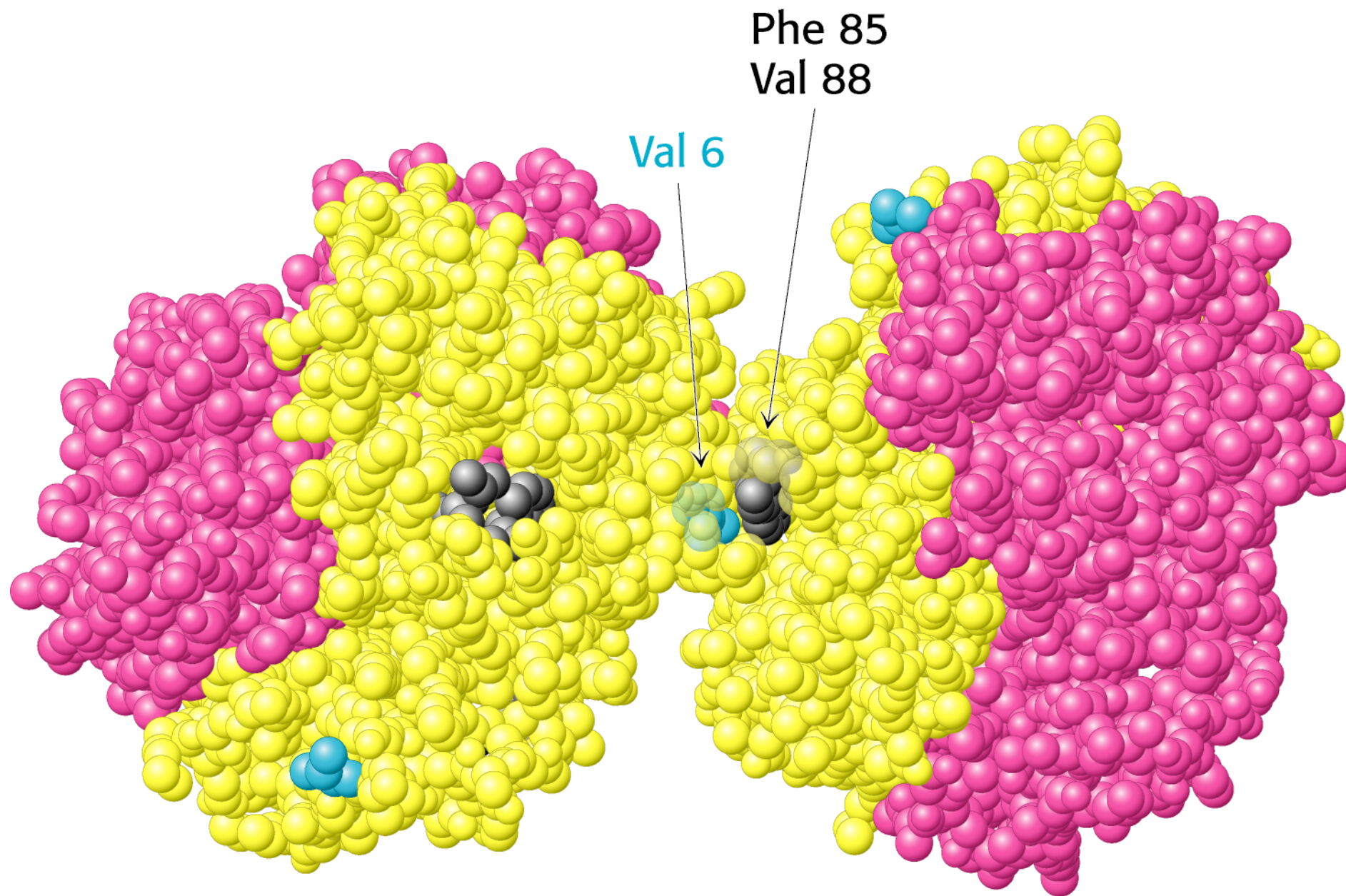
2 μm

Hemoglobin A HbA HbS Hemoglobin S

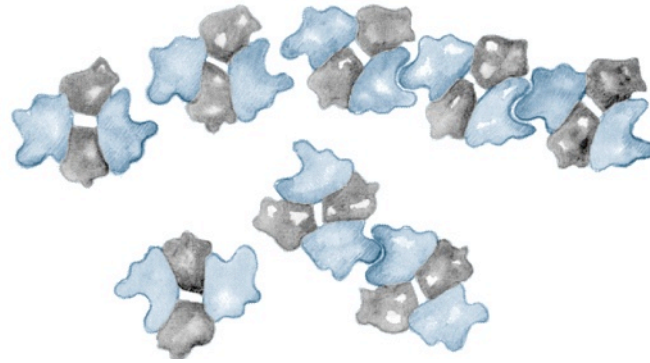


(a)

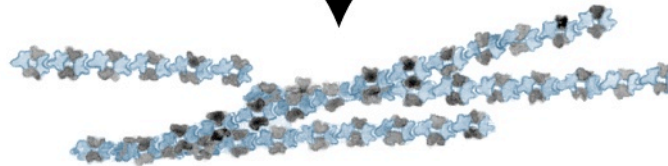
β_6 glu $\Rightarrow$ val



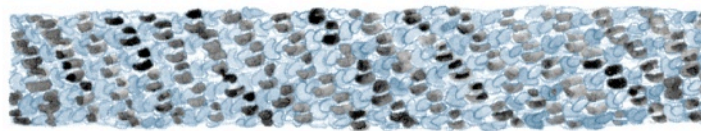
HbS



Interazione tra molecole deossigenate



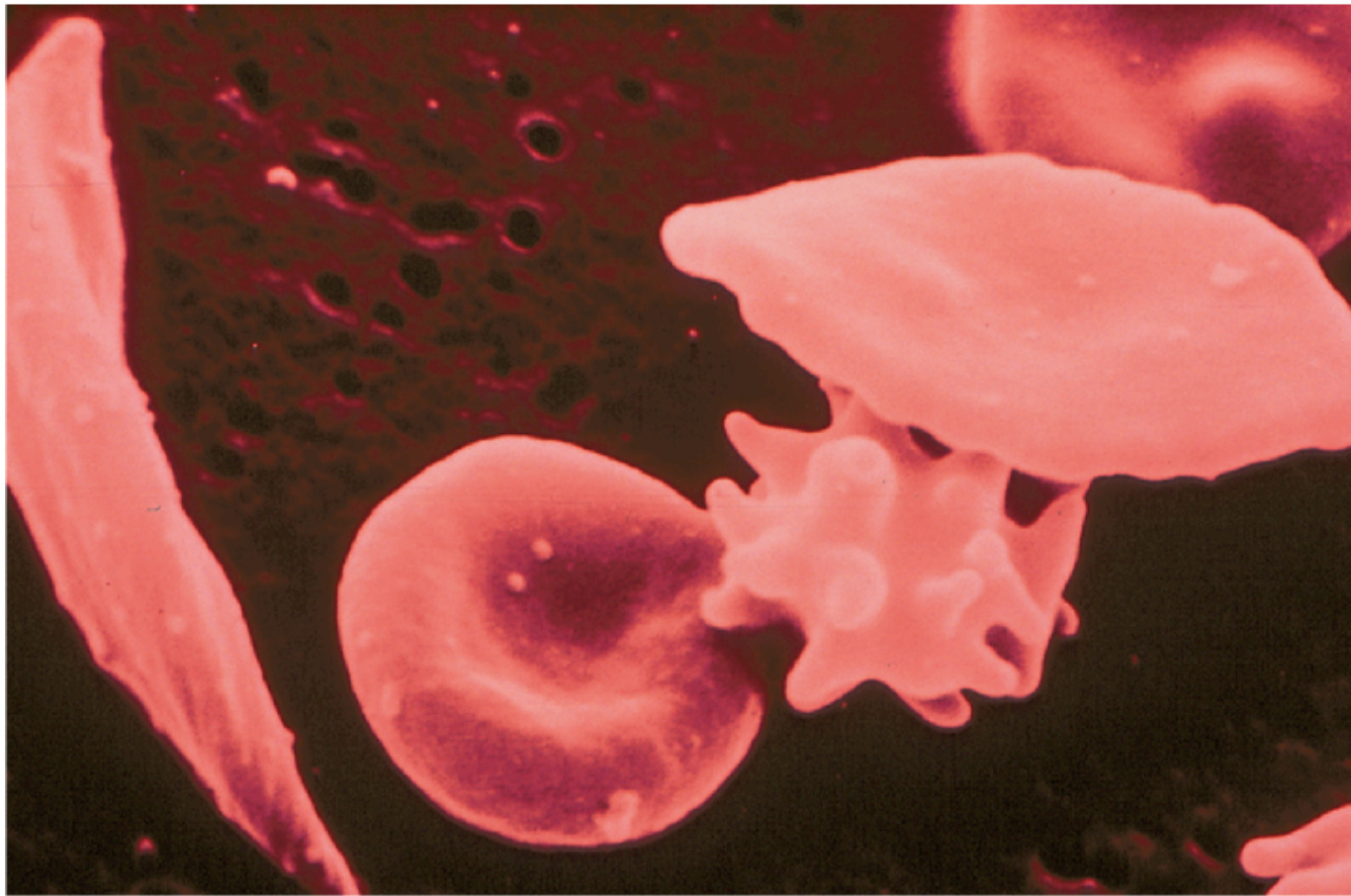
Strand formation



**Alignment and crystallization
(fiber formation)**

(b)





(b)

