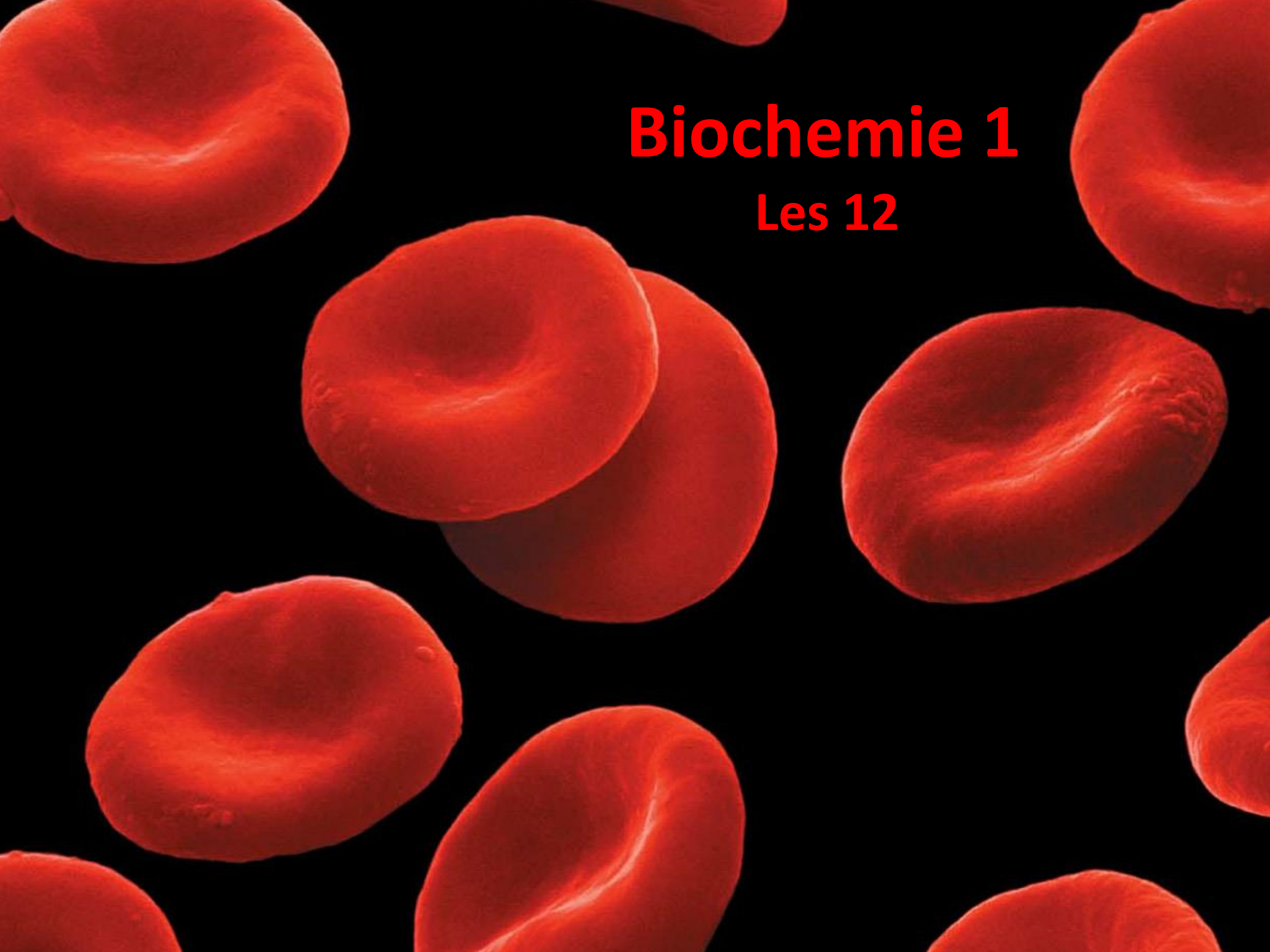


Biochemie 1

Les 12

Vorige les

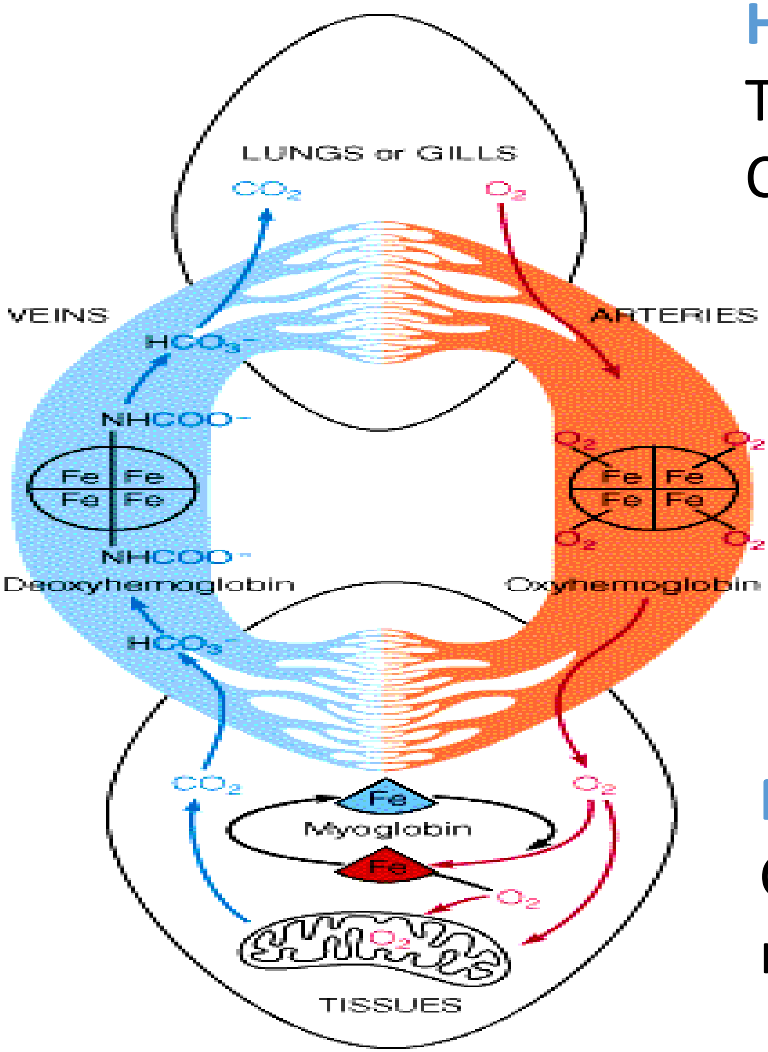
Allosterie

Vandaag

Voorbeeld van een allosterisch eiwit: hemoglobine

Vergelijking tussen hemoglobine en myoglobine

Hemoglobin & Myoglobin



Hemoglobin

Transport O_2 from lungs to tissue and CO_2 from tissue to lungs

Myoglobin

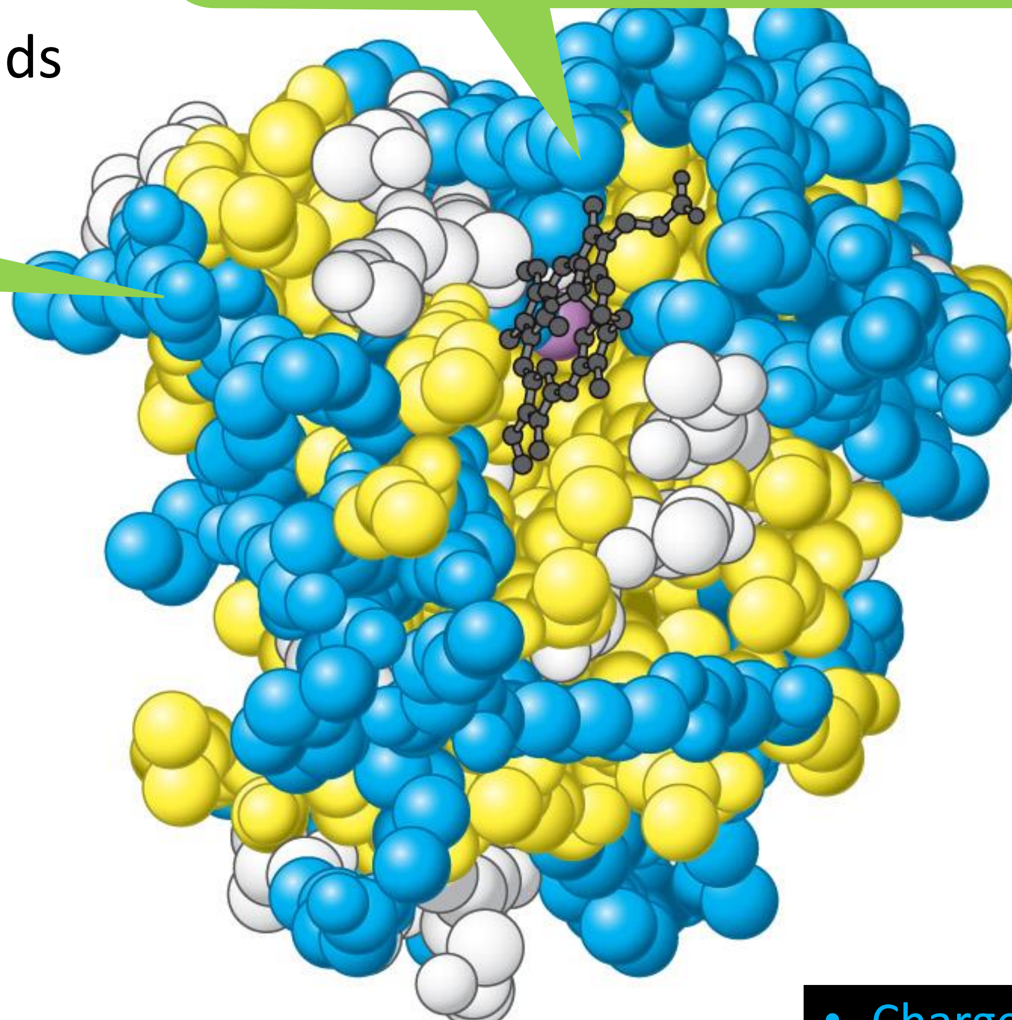
Carries O_2 from cell membrane to mitochondria (in muscle cells)

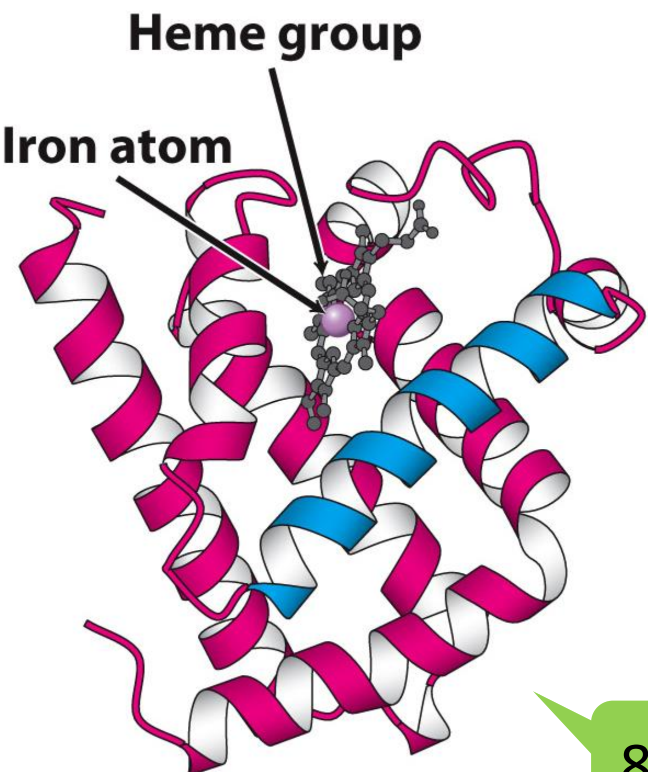
Myoglobin

monomer, 153 amino acids

heme group in hydrophobic pocket, interacts with two His-residues.

most polar side chains on the surface, nonpolar side chains folded to the interior



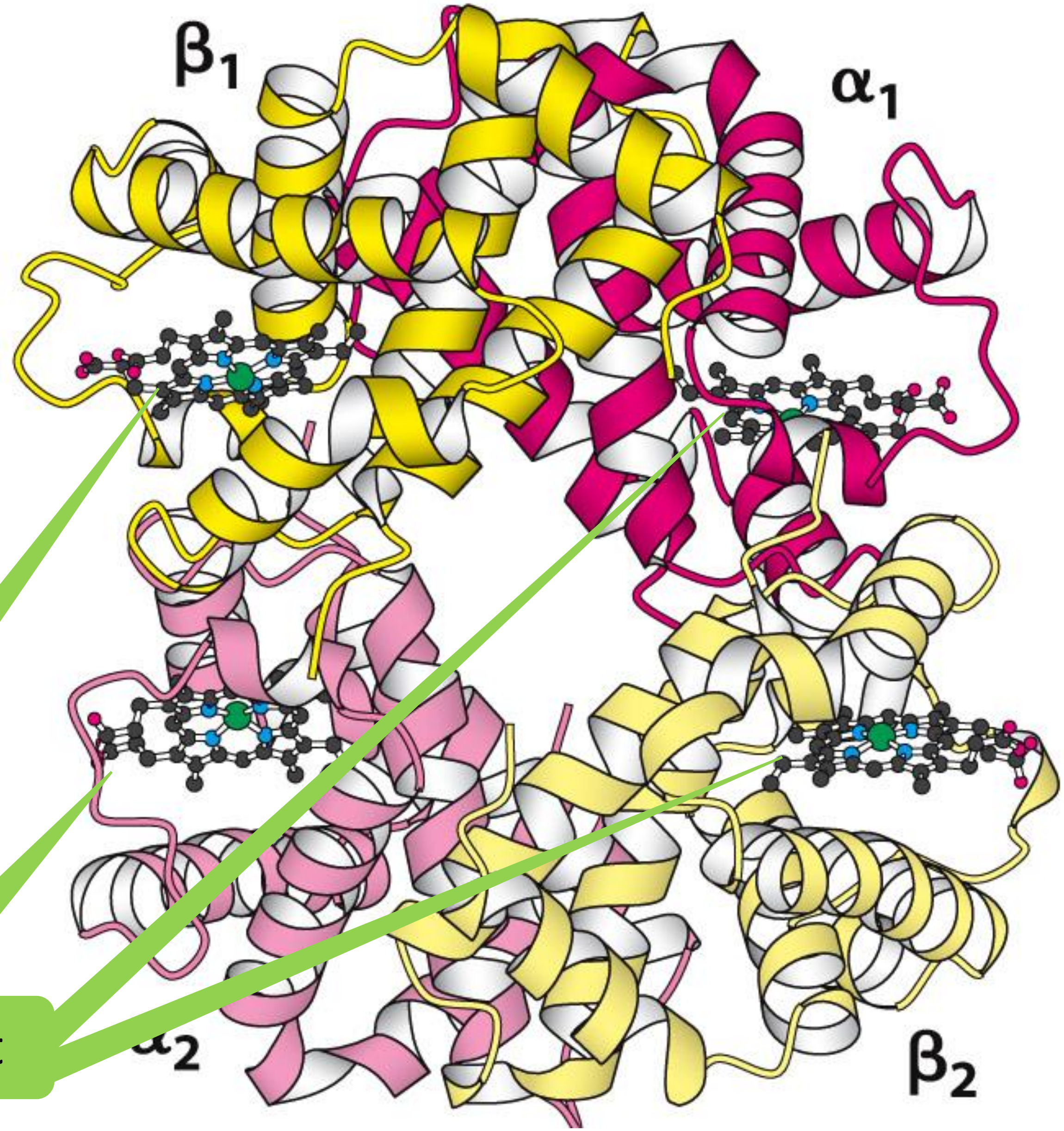


8 α-helices

- Charged
- Hydrophobic
- Polar

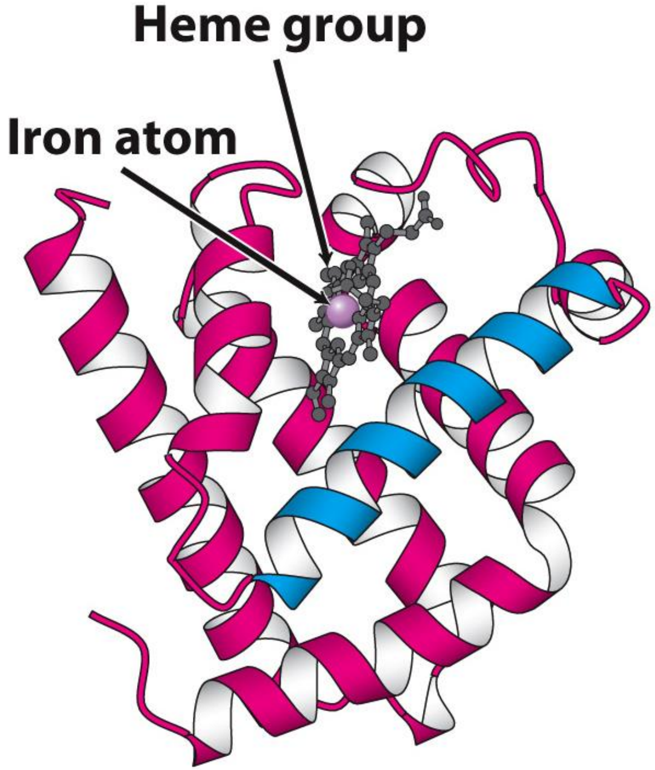
Hemoglobin

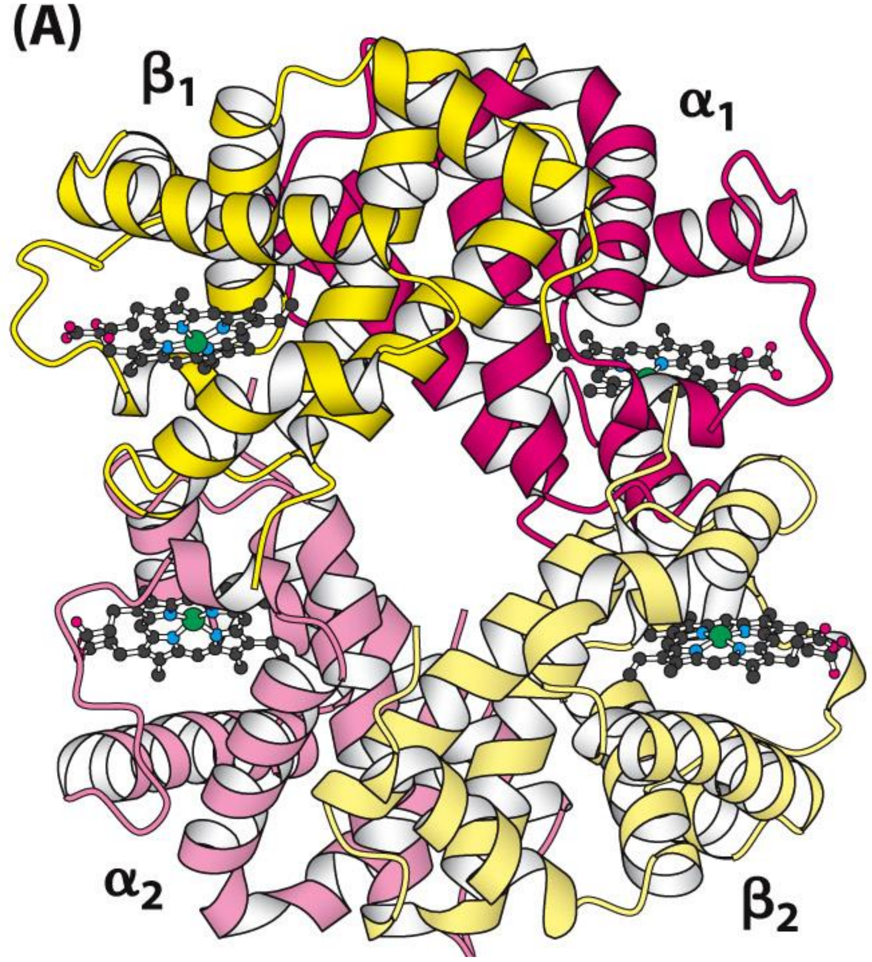
tetramer of two **α-chains** (141 amino acids each) and two **β-chains** (153 amino acids each); $\alpha_2\beta_2$



heme group in each subunit

Quaternary Structures Mb and Hb





Myoglobin is monomeric

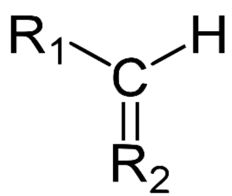
Hemoglobin is tetrameric

Globin fold

Heme group

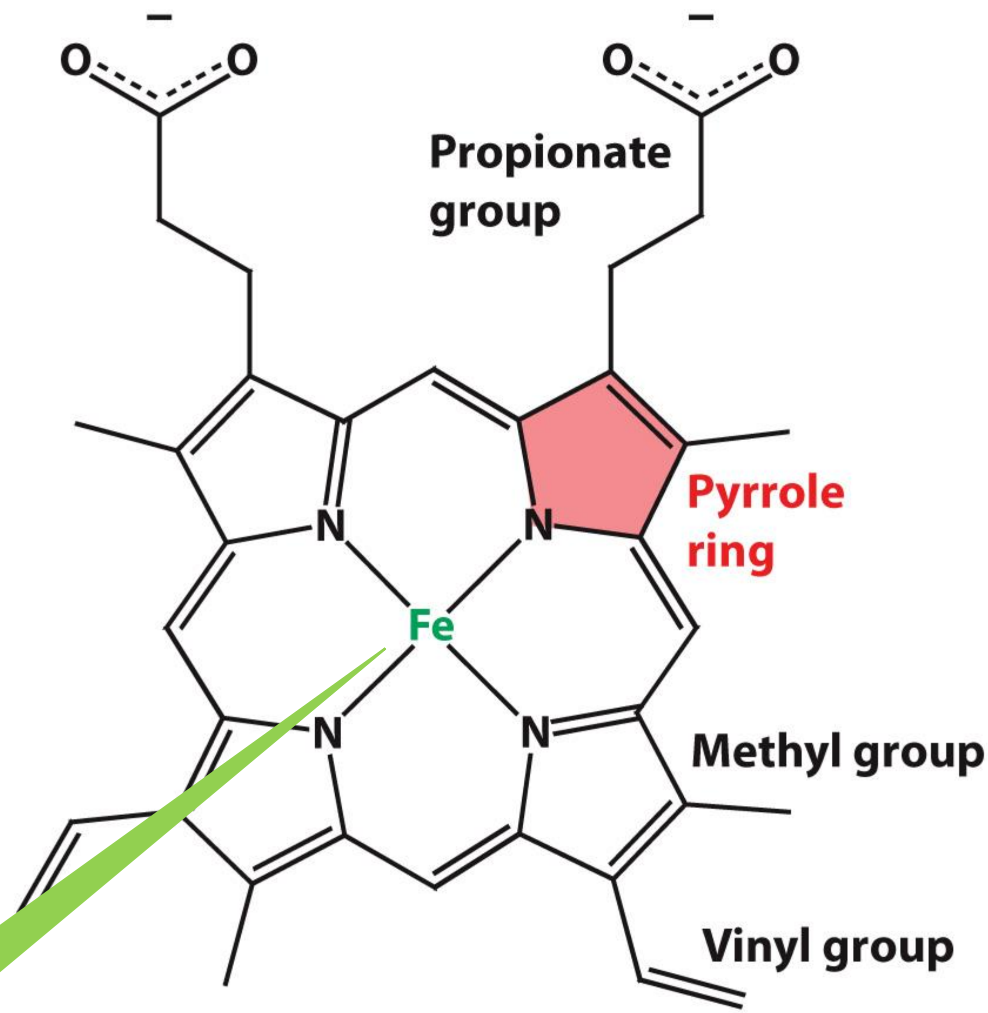
Iron atom + protoporphyrin

Protoporphyrin: 4 pyrrole rings linked by methine bridges (=C-)



Fe^{2+} forms bonds with N atoms of the pyrrole rings

Fe(II): 6 coordination sites. 4 interactions with N's of protoporphyrin → 2 left

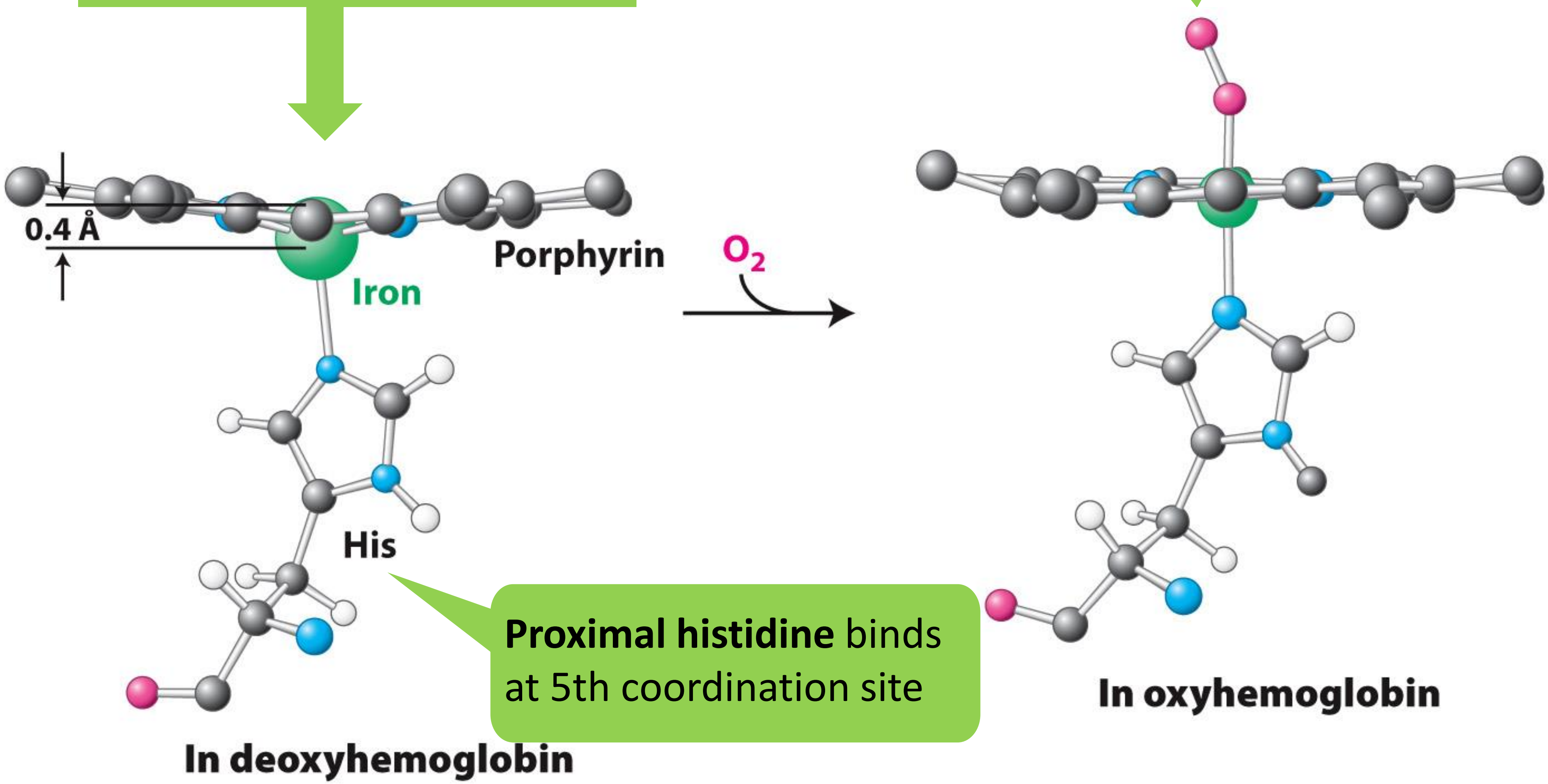


Heme (Fe-protoporphyrin IX)

Heme – oxygen binding

Not shown: distal histidine (prevents oxidation of Fe^{2+})

Oxygen binds at 6th coordination site



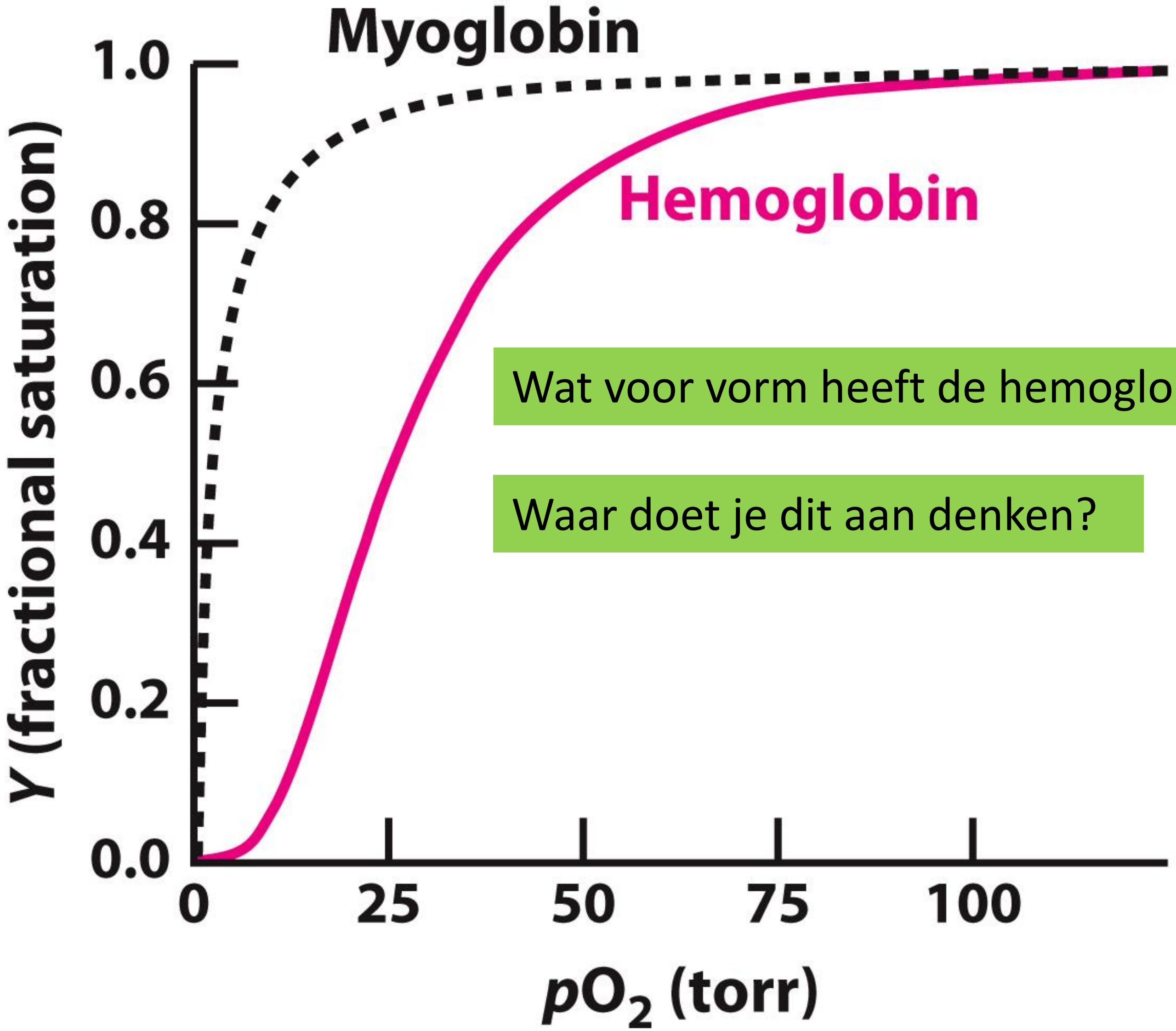
Proximal histidine binds at 5th coordination site

Heme – oxygen binding

Zie ook:

https://pdb101.rcsb.org/motm/41#tabs-2

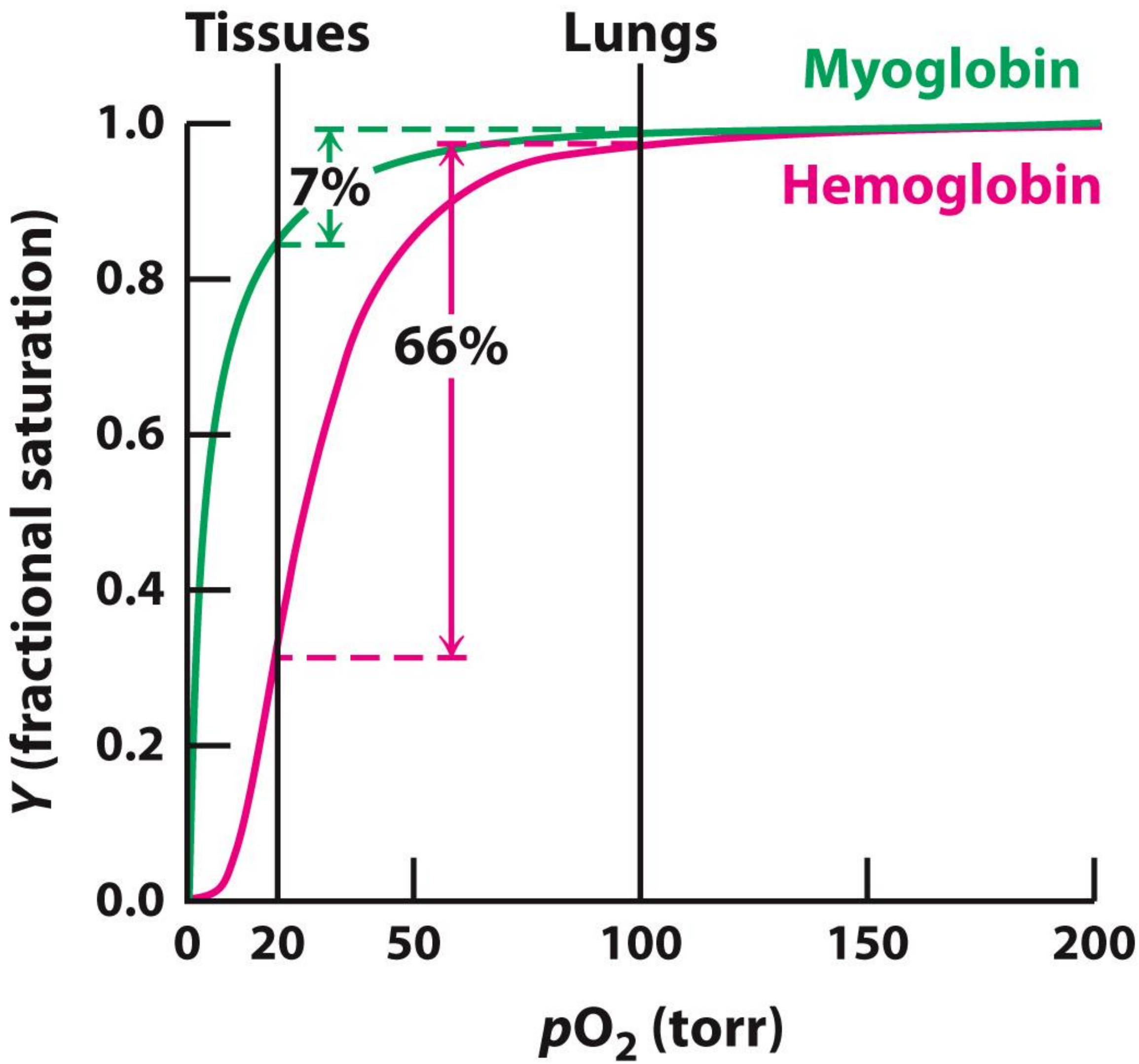
Oxygen binding



Wat voor vorm heeft de hemoglobine curve?

Waar doet je dit aan denken?

Oxygen binding



Oxygen binding to hemoglobin

- each chain has one **heme group**; hemoglobin can bind up to 4 molecules of O_2
- binding is **cooperative**; when one O_2 is bound, it becomes easier for the next O_2 to bind
- Hemoglobin is an **allosteric** protein: binding of O_2 in one subunit brings changes in structure of other subunit(s)

Hemoglobin

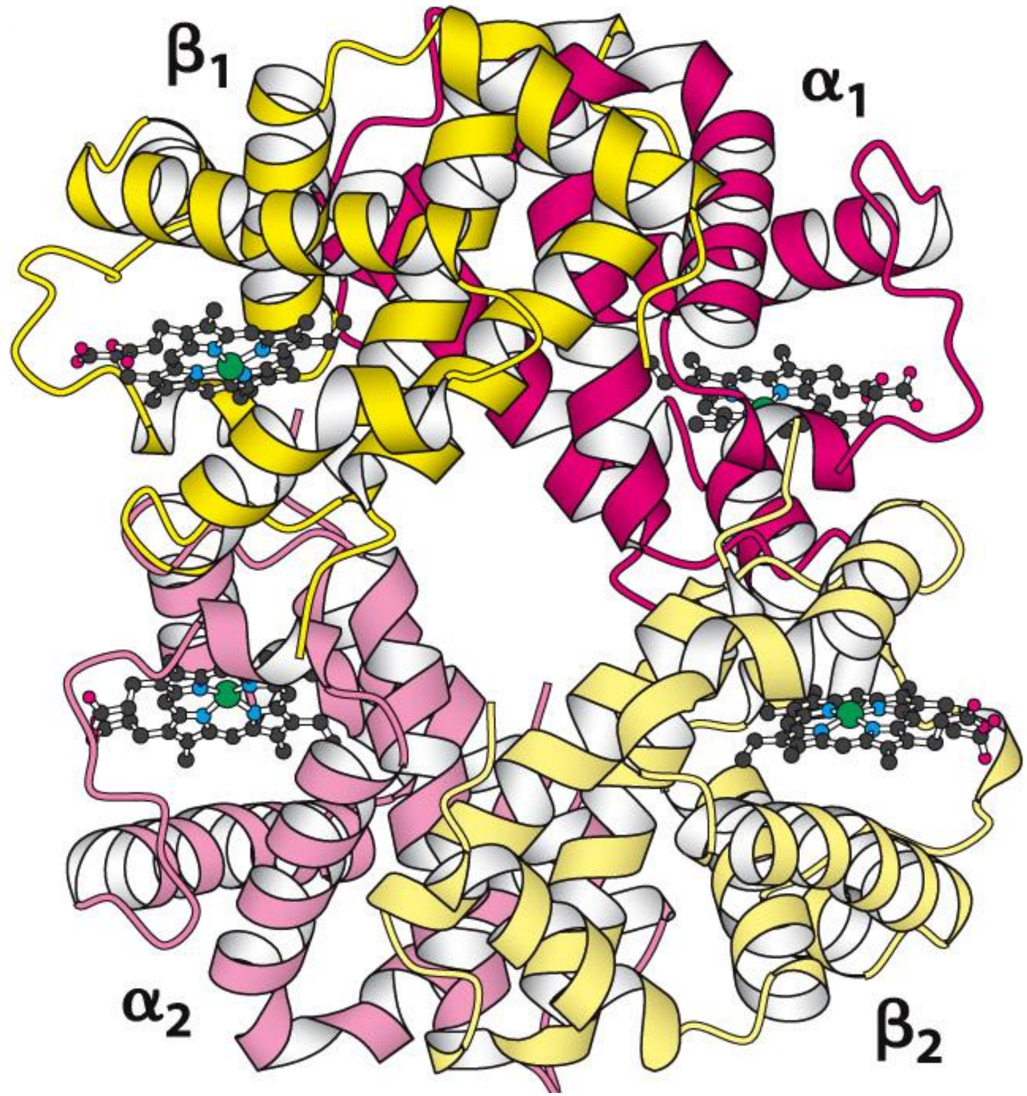
Allosteric protein

Deoxyhemoglobin → T-state

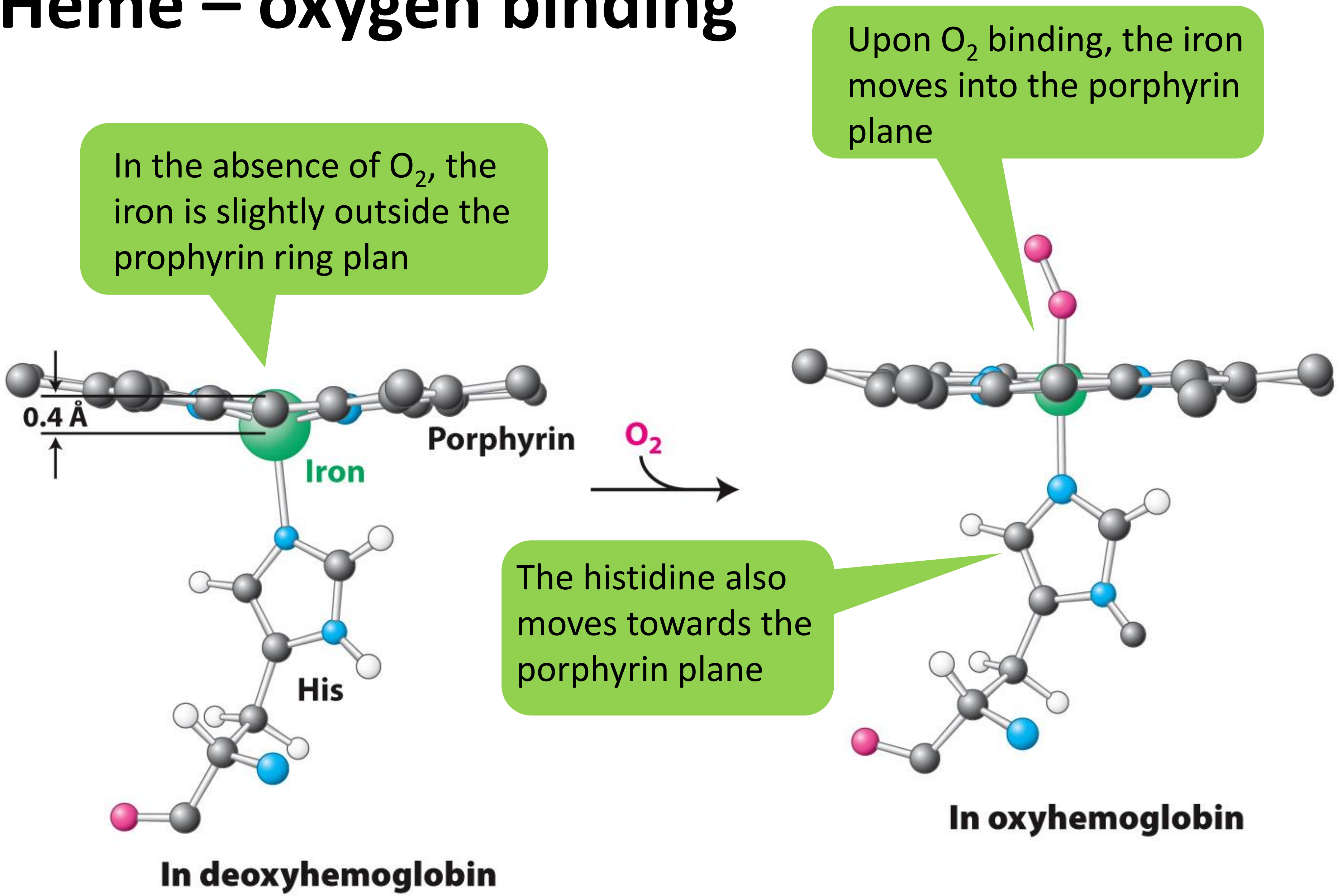
Oxyhemoglobin → R-state

Oxygen binding: T → R

How?



Heme – oxygen binding

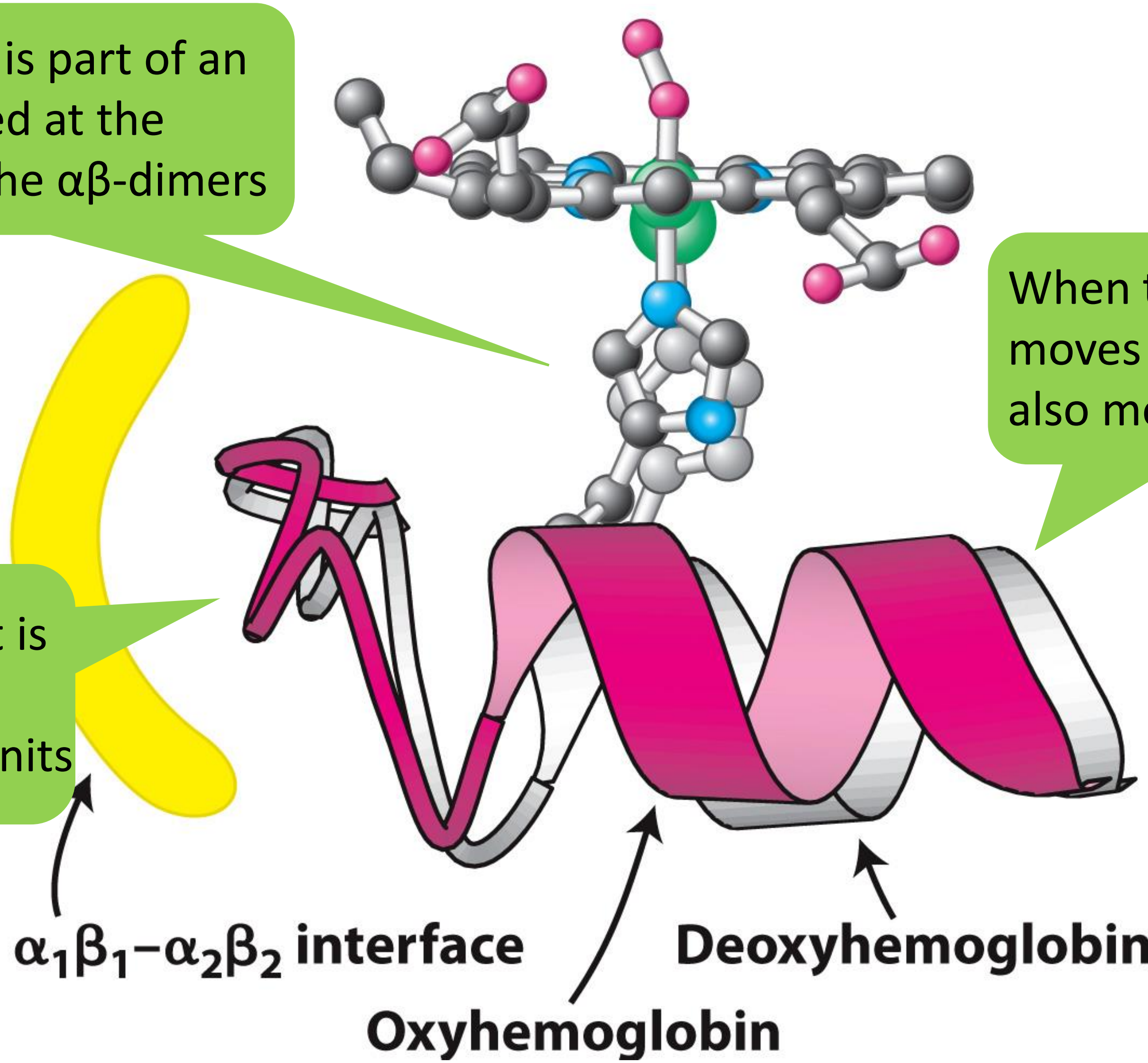


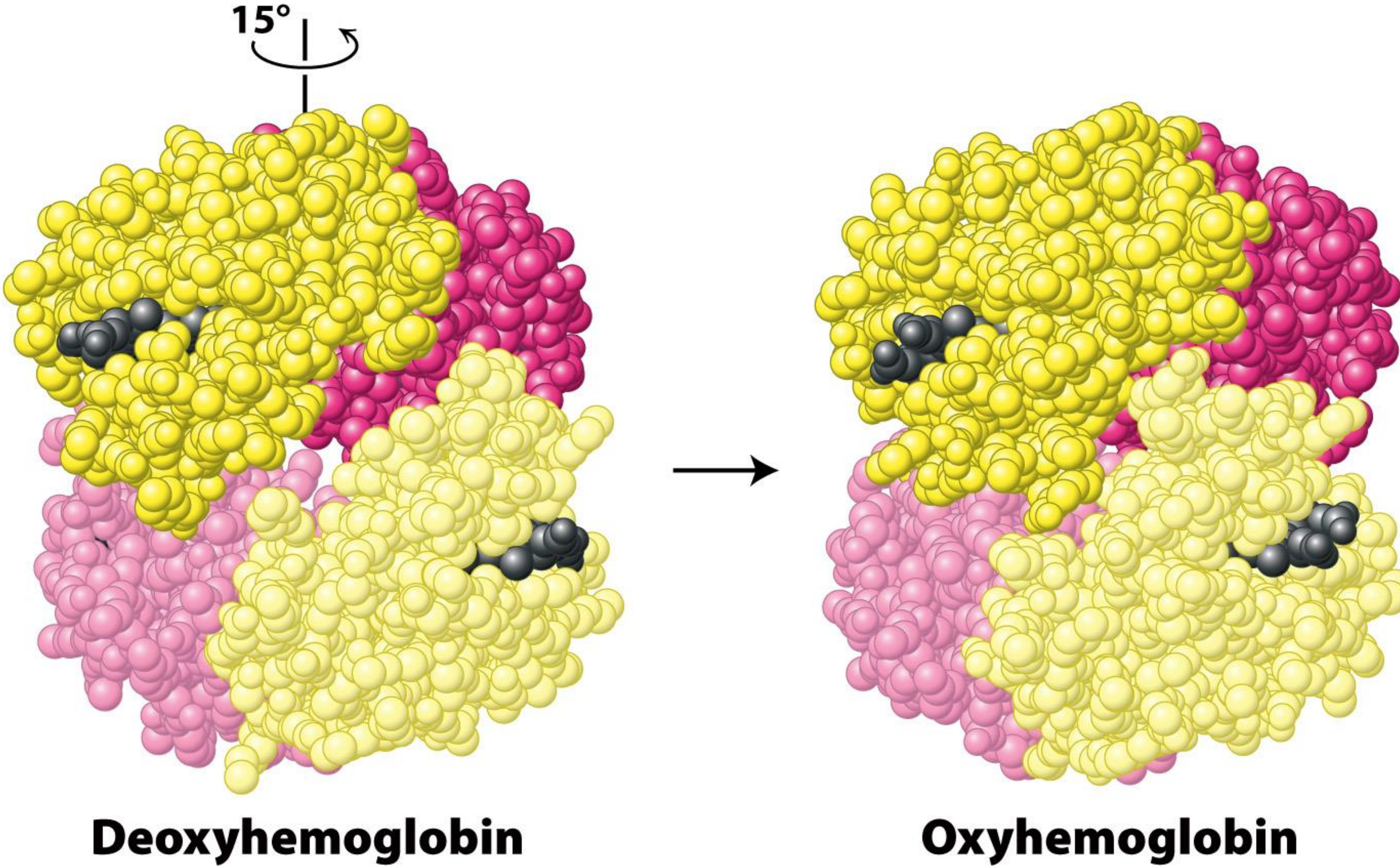
Hemoglobin – oxygen binding

The histidine is part of an α-helix located at the interface of the αβ-dimers

When the histidine moves the α-helix also moves

The movement is transmitted to the other subunits





Zie ook: https://pdb101.rcsb.org/motm/41#tabs-2

Binding of Oxygen by Myoglobin and Hemoglobin

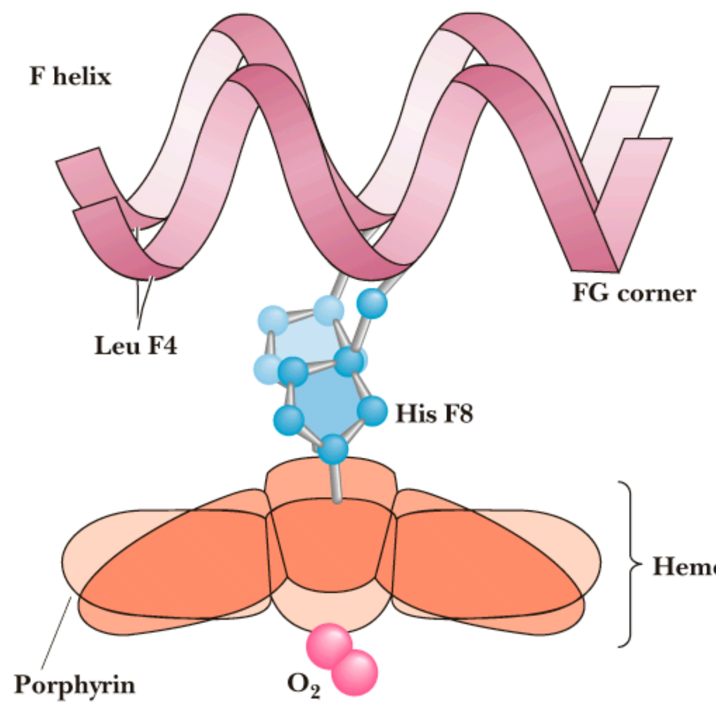
Hemoglobin must bind oxygen in lungs and release it in capillaries. Myoglobin must bind oxygen.

Hemoglobin:

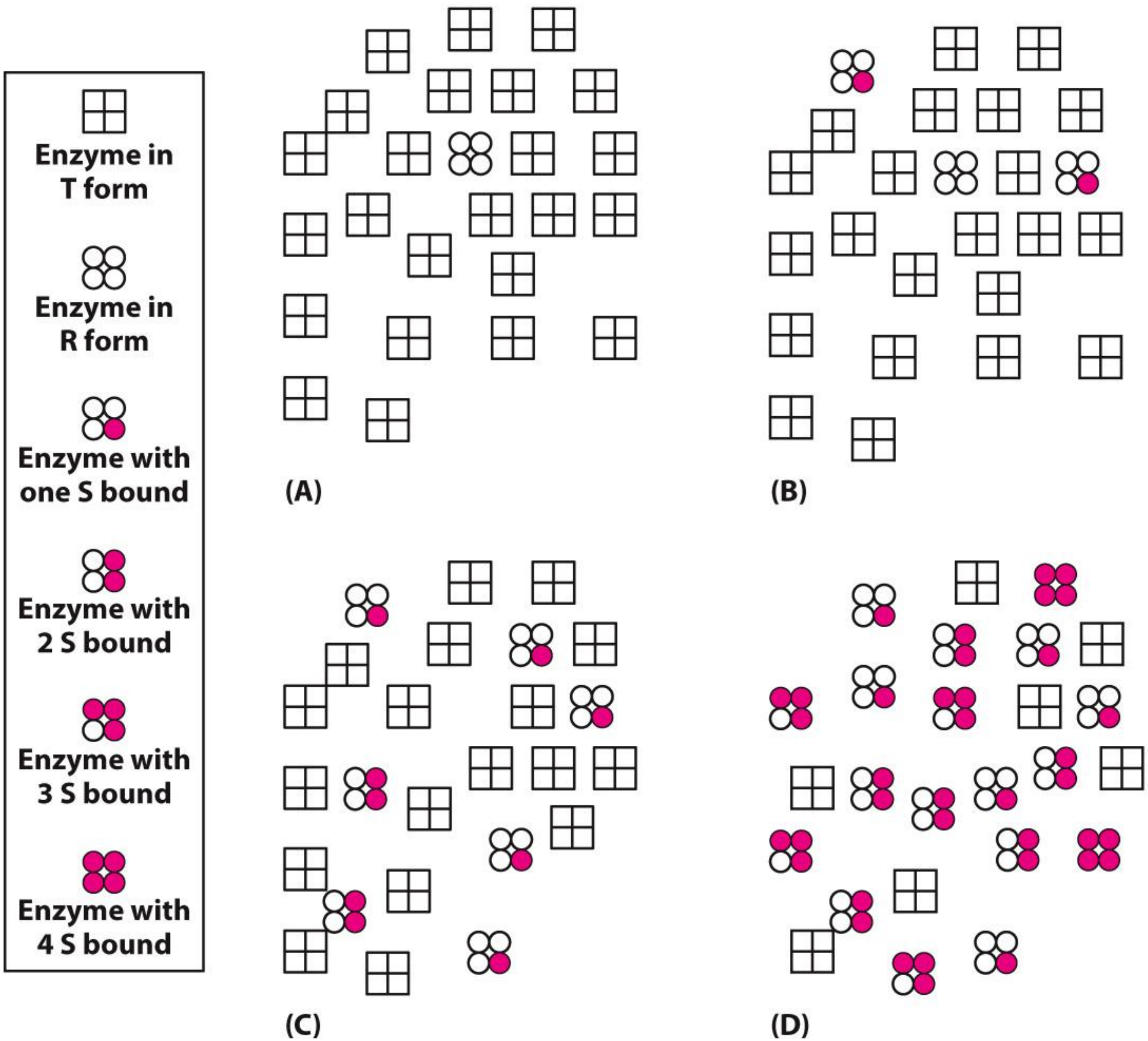
- Adjacent hemoglobin subunits' affinity for oxygen increases
- This is called **positive cooperativity** and does not happen in Myoglobin
- If Hemoglobin behaved like Myoglobin, very little oxygen would be released in capillaries
- The sigmoid, cooperative oxygen binding curve of Hemoglobin makes this possible!

Recap: Hb conformational changes upon O_2 binding

- Without O_2 bound → Fe out of the heme plane
- O_2 binding → Fe pulled into the heme plane → His F8 (proximal histidine) pulled along → F helix moves
- Total movement of Fe is 0.29 Å.
- This change means little to Mb, but lots to Hb!
- Movement of Fe initiates a series of conformational changes to adjacent subunits
- T-to-R-state transition



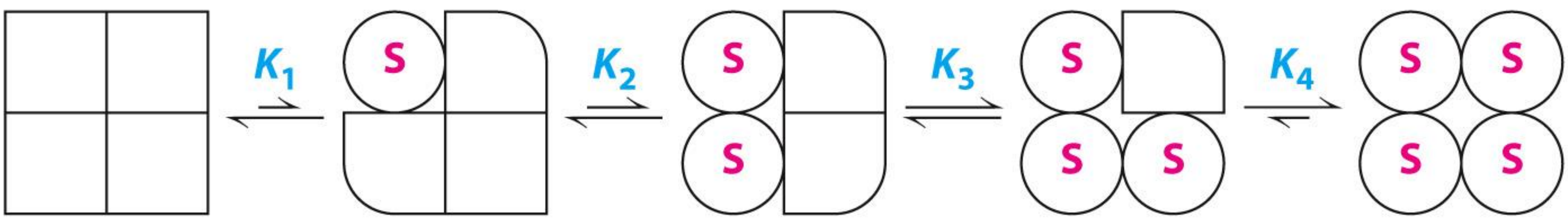
Vorige les: concerted model



Vorige les: sequential model

In het concerted model heeft een enzym 2 toestanden, T en R

Een alternatief is dat binden van S aan één subunit de conformaties van andere subunits verandert, zodat S beter bindt



Onderzoek naar allosterische enzymen suggereert dat veel van deze enzymen werken volgens een combinatie van beide modellen

Hemoglobin

3 sites occupied by oxygen?
→ quaternary structure almost always R state
→ affinity for oxygen of free site 20x higher

Resembles concerted model

1 sites occupied by oxygen?
→ quaternary structure: T state
→ affinity for oxygen of free site 3x higher

Resembles sequential model

Concerted model/ sequential model: idealized cases.

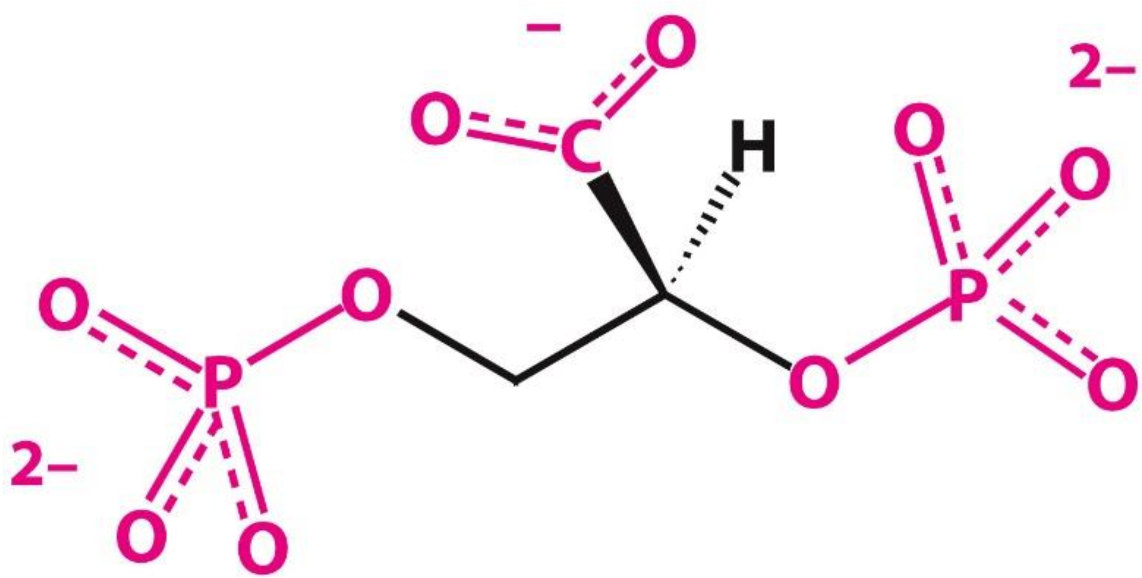
Vorige les: regulatiemoleculen beïnvloeden het evenwicht tussen T en R

- Positieve regulatie:
  - molecuul stabiliseert R
  - concentratie R ↑
  - grotere kans op binding van S

- Negatieve regulatie:
  - molecuul stabiliseert T
  - concentratie T ↑
  - kleinere kans op binding van S

2,3-Bisphosphoglycerate

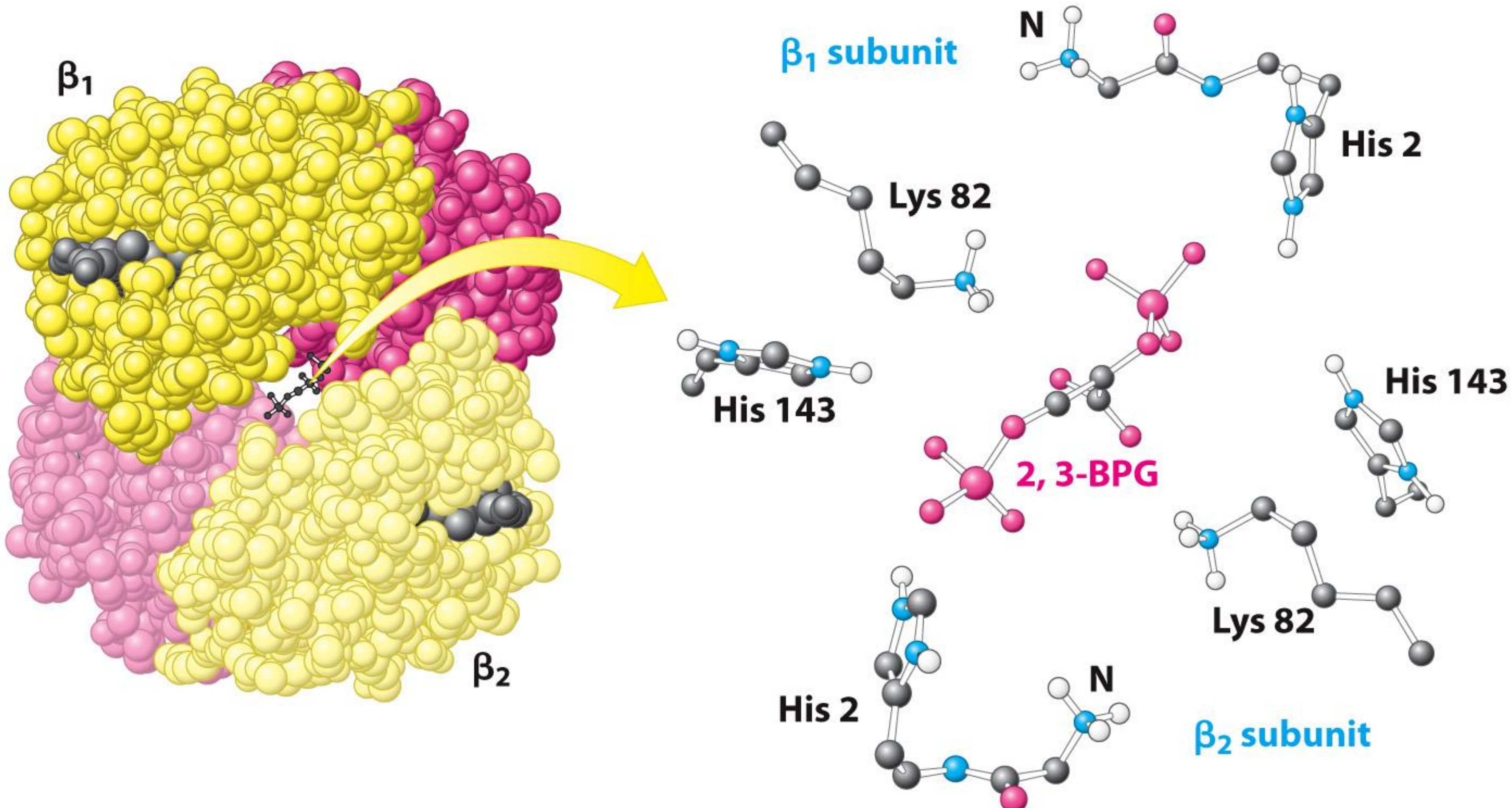
- Hemoglobin in blood is bound to 2,3-BPG



- Highly anionic compound
- Present in red blood cells at ~same concentration as hemoglobin
- 2,3-BPG binds at a site distant from the Fe where oxygen binds → **allosteric effector**

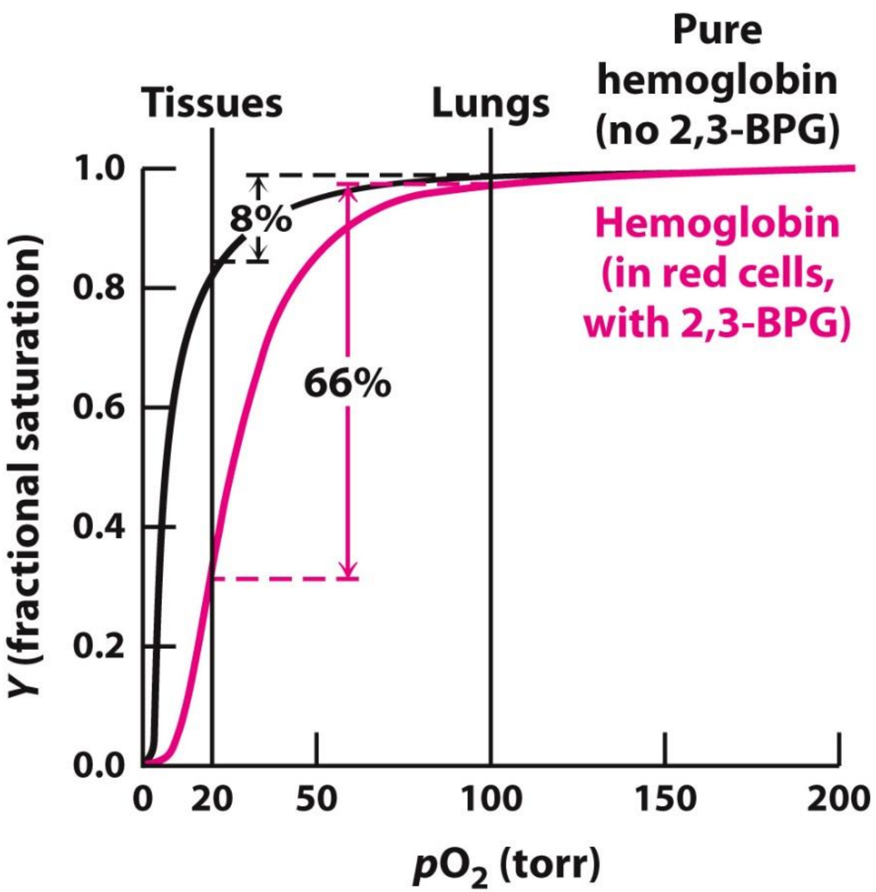
2,3-BPG and hemoglobin

2,3-BPG binds in a pocket only present in the T-form of Hg
Negative charges interact with 2 Lys (β chain), 4 His (each subunit 1 His), 2 N-termini (α chain)



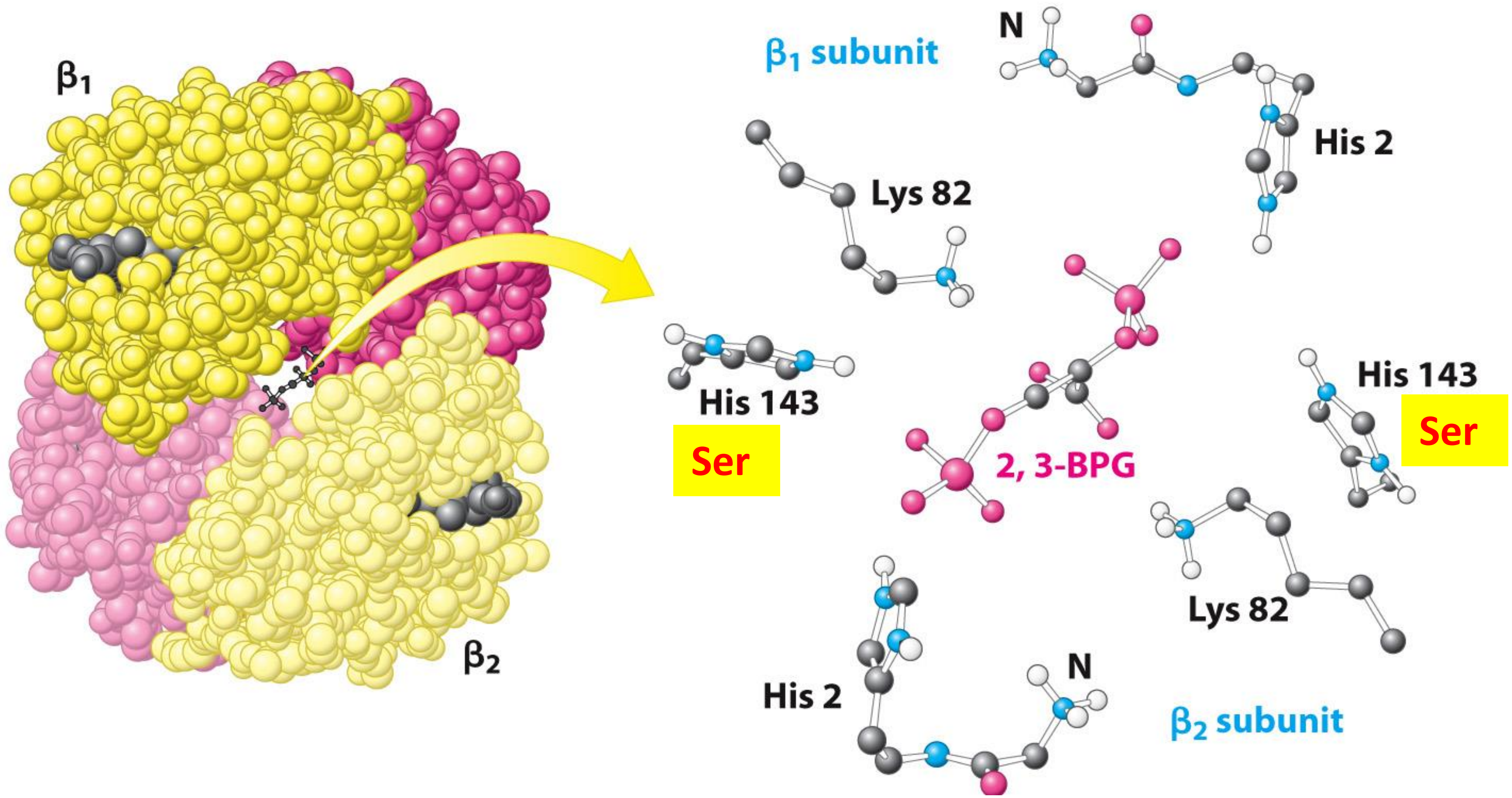
2,3-BPG and hemoglobin

- 2,3-BPG binds preferentially deoxyhemoglobin and stabilizes it
  → 2,3-BPG **reduces** affinity for O_2
- Hemoglobin stripped of 2,3-BPG remains saturated with O_2



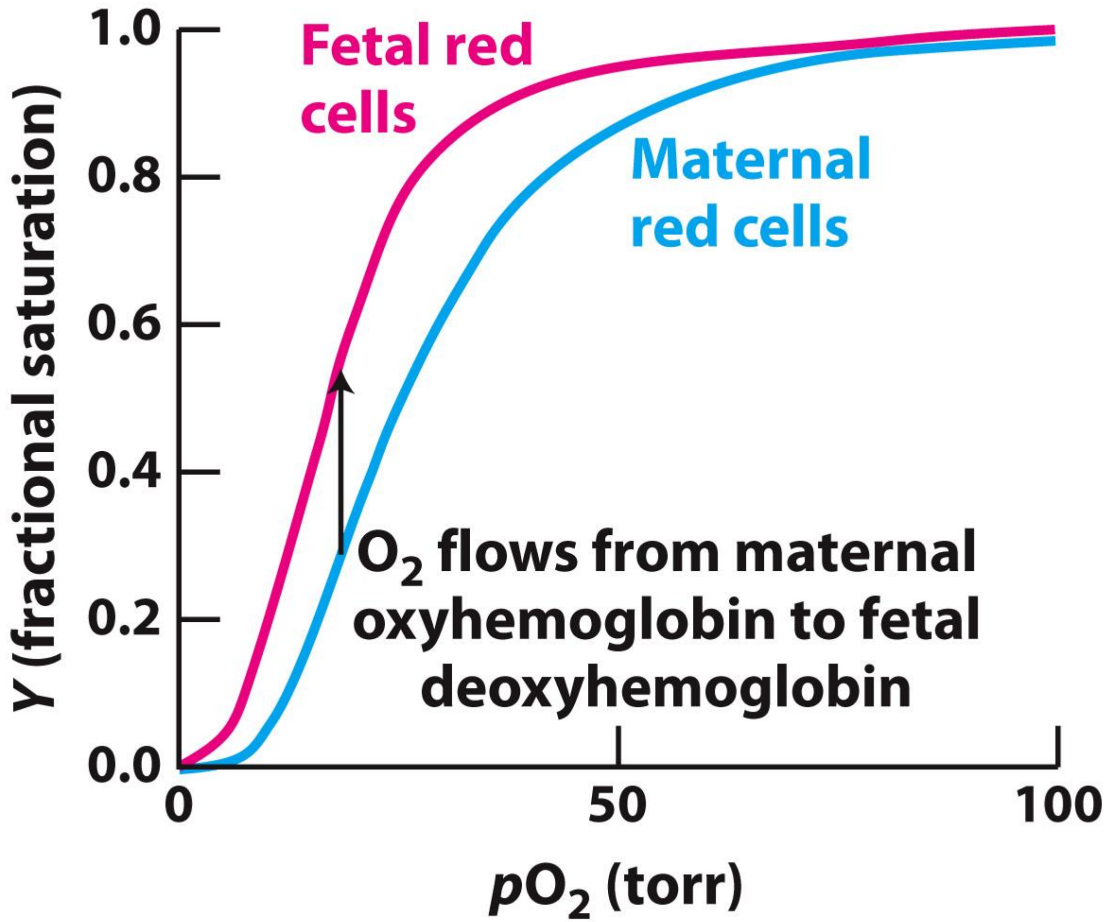
Fetal Hemoglobin

- Two α-chains and two **γ**-chains

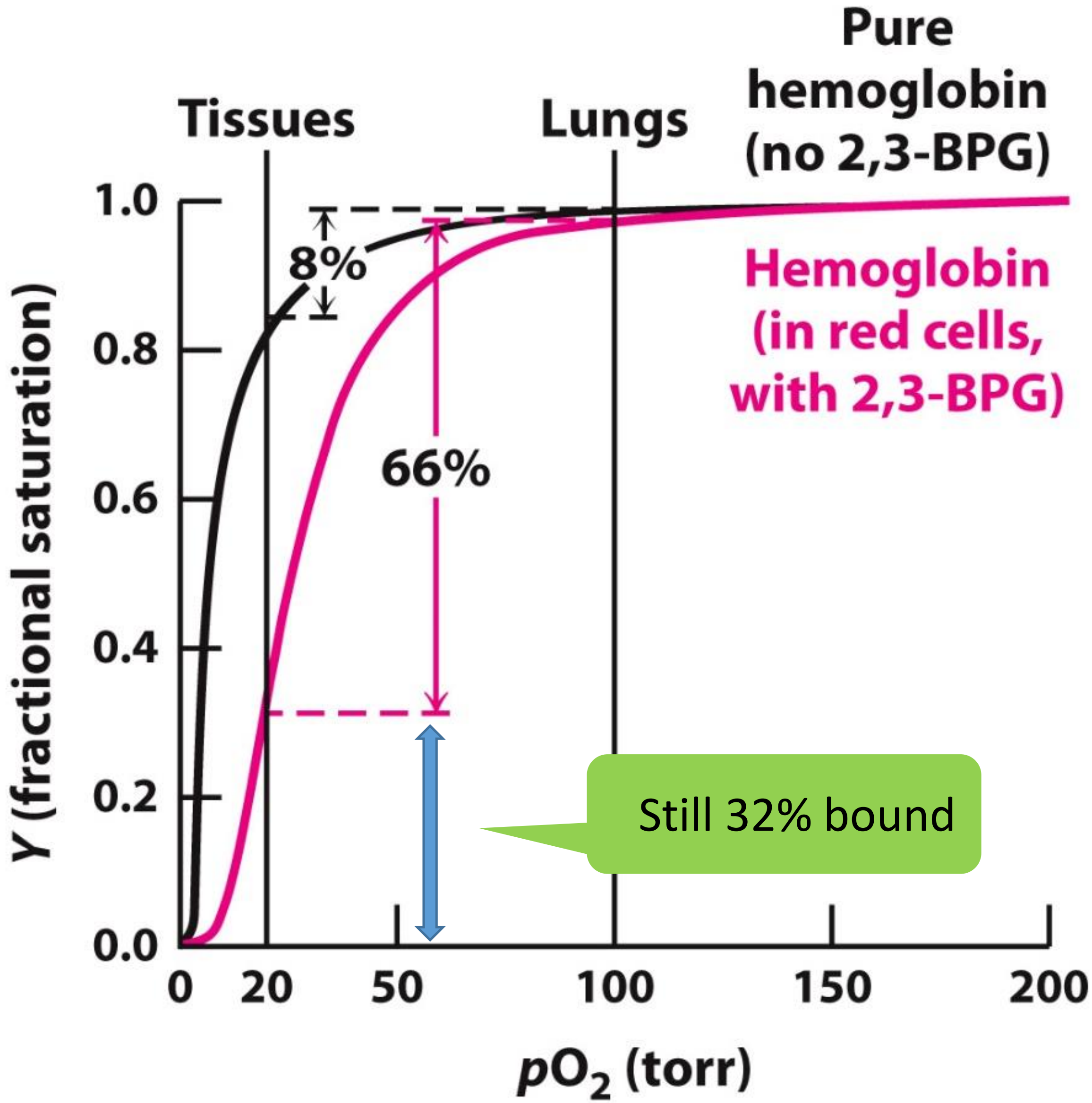


Fetal Hemoglobin

Lower affinity for 2,3-BPG
→ higher affinity for oxygen
→ oxygen from mother Hg to fetal Hg

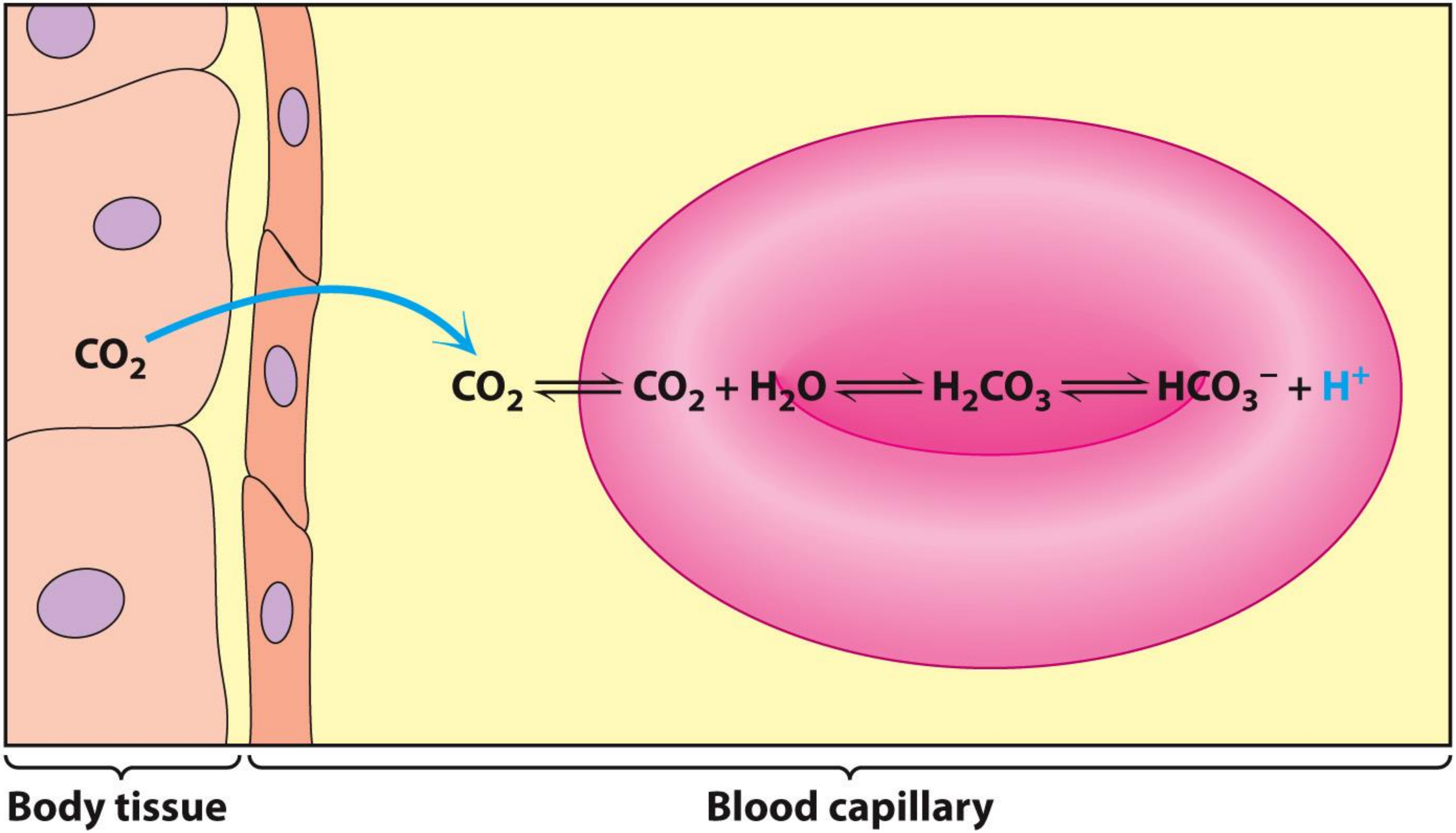


Oxygen binding (so far)



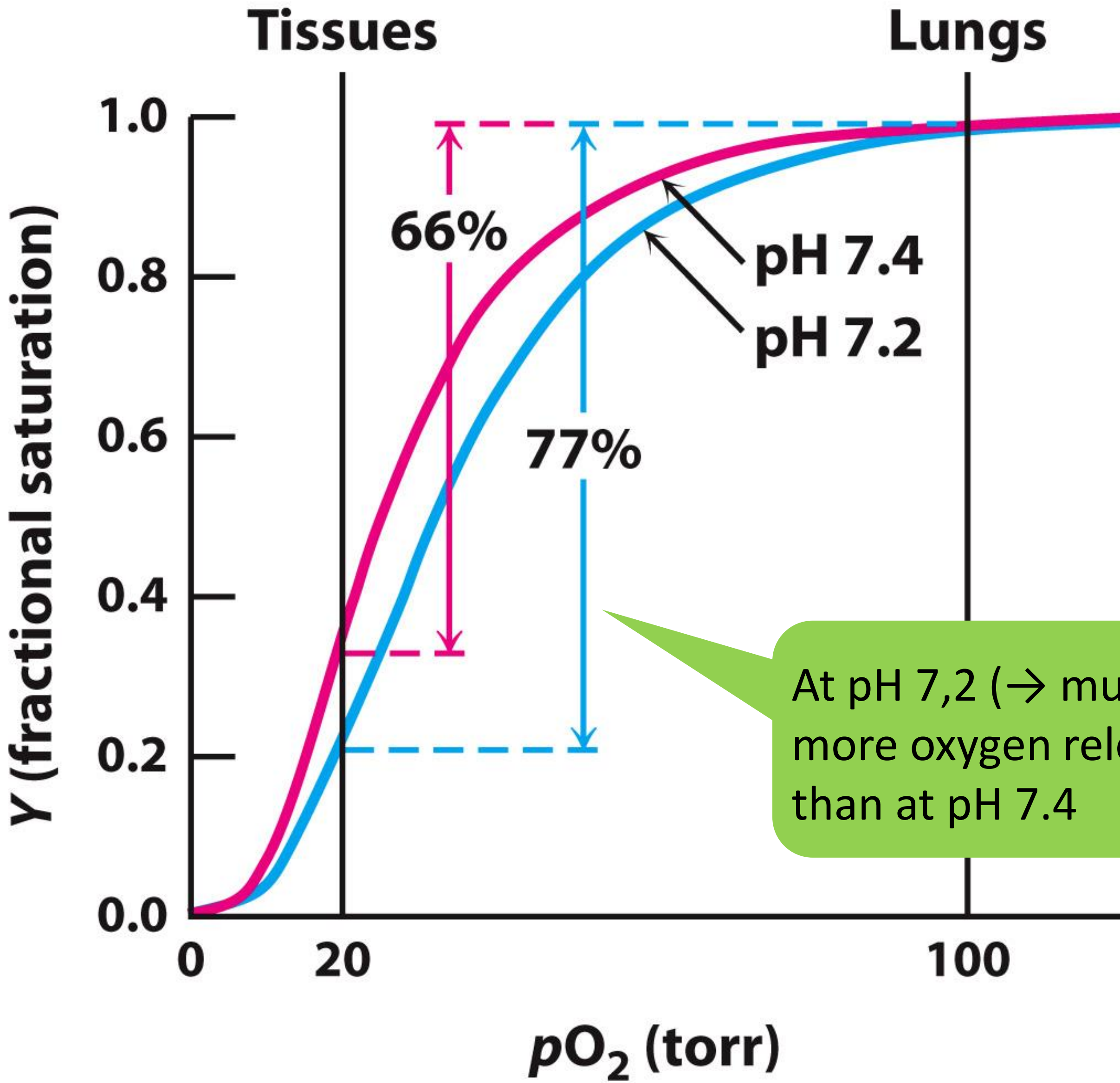
The Bohr Effect

- Regulation of oxygen binding by H^+ and CO_2
- Discovered by Christian Bohr
- Binding of protons and CO_2 diminishes oxygen binding
- Important physiological significance



CO_2
$CO_2 \rightleftharpoons CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$
Body tissue
Blood capillary

Lowering the pH



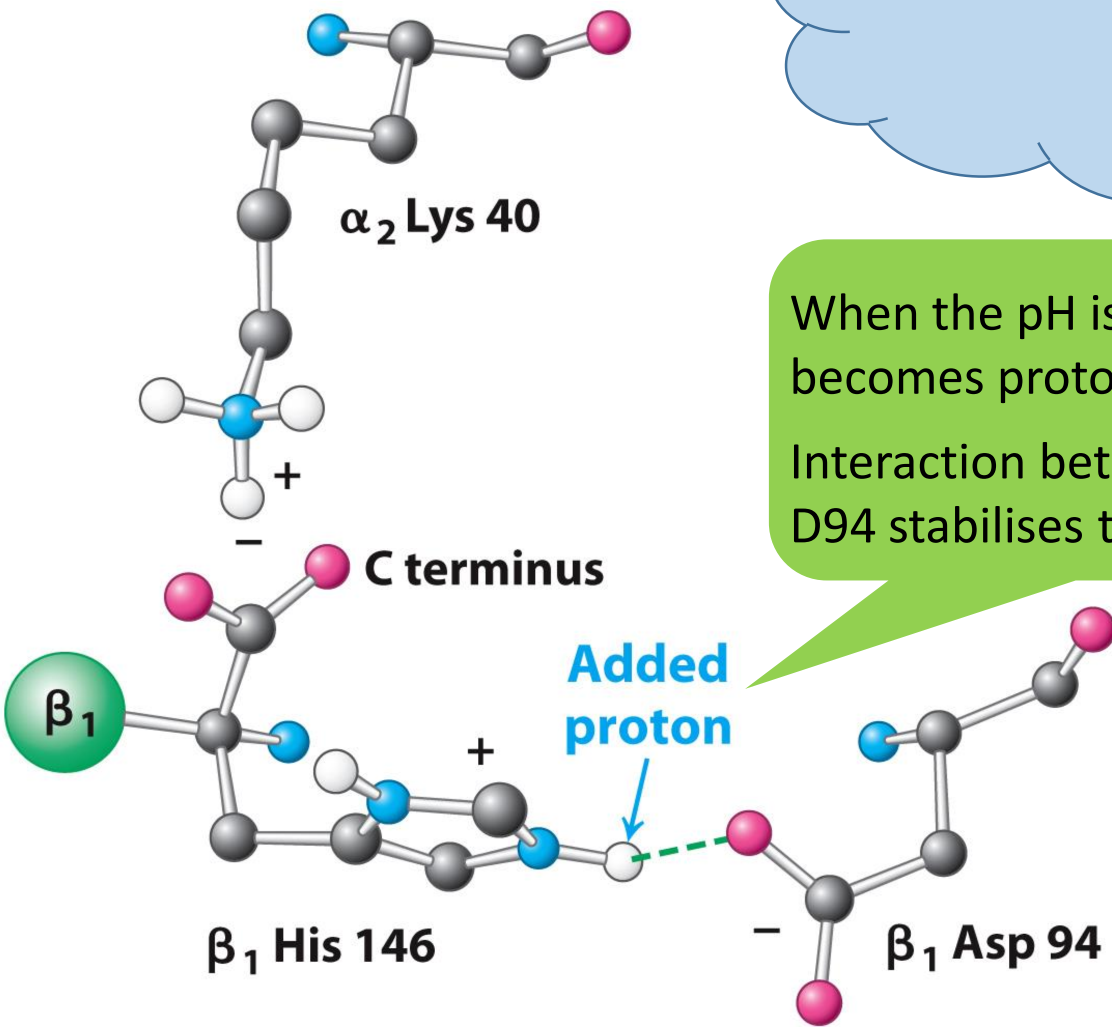
At pH 7,2 (→ muscles) more oxygen released than at pH 7.4

Lowering the pH

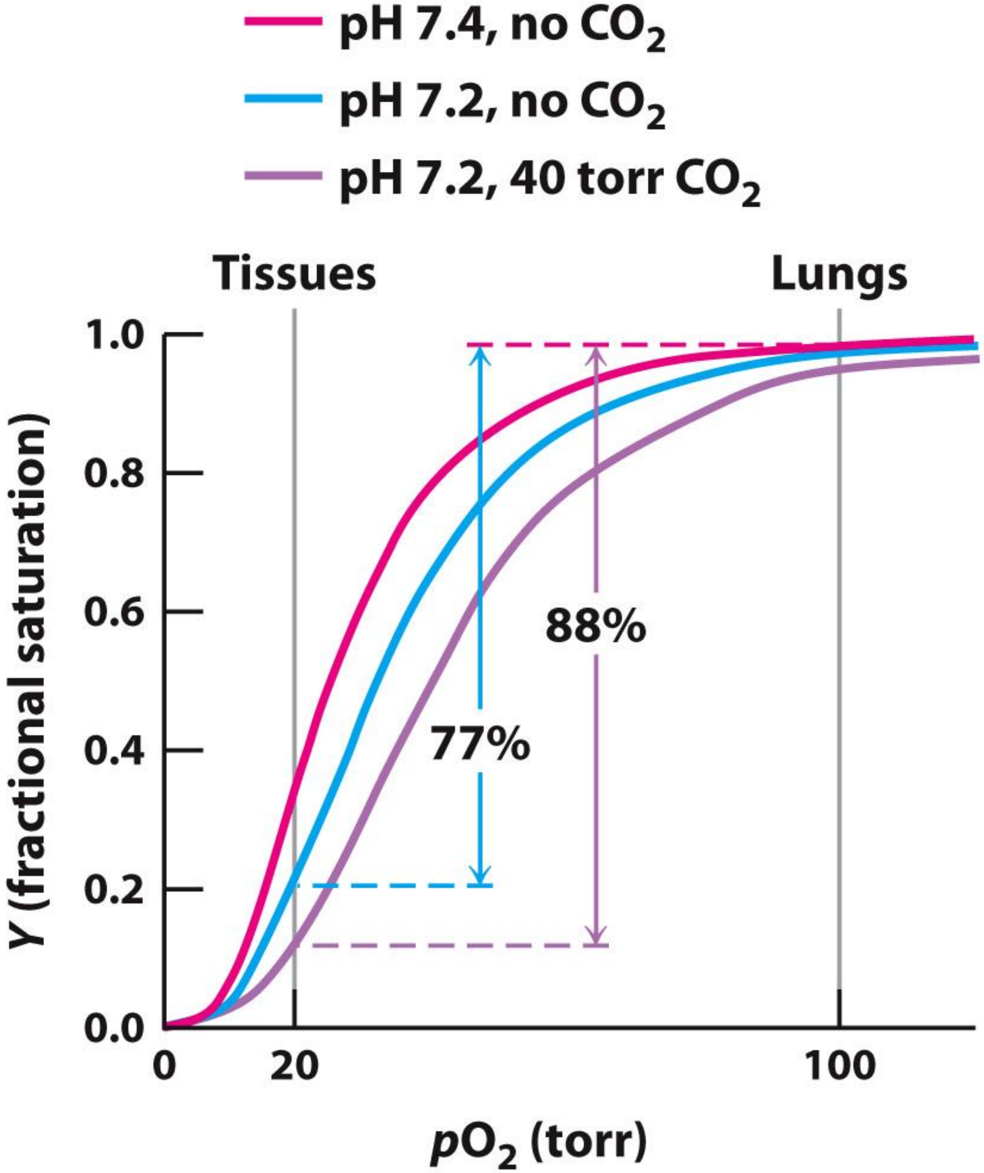
$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

When the pH is lowered, H146 becomes protonated.

Interaction between H146 and D94 stabilises the T-state.

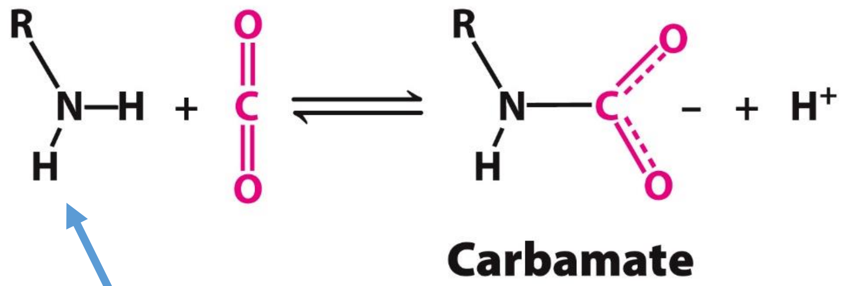


CO_2 effects

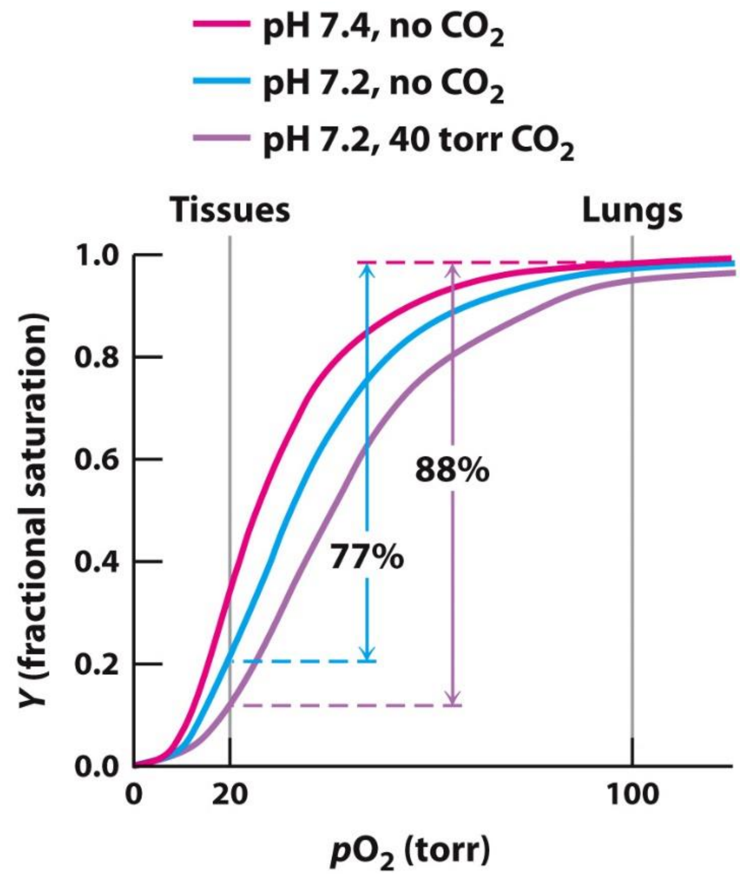


pH 7.4, no CO2
pH 7.2, no CO2
pH 7.2, 40 torr CO2
Tissues
Lungs
1.0
0.8
0.6
0.4
0.2
0.0
Y (fractional saturation)
88%
77%
0
20
100
pO2 (torr)

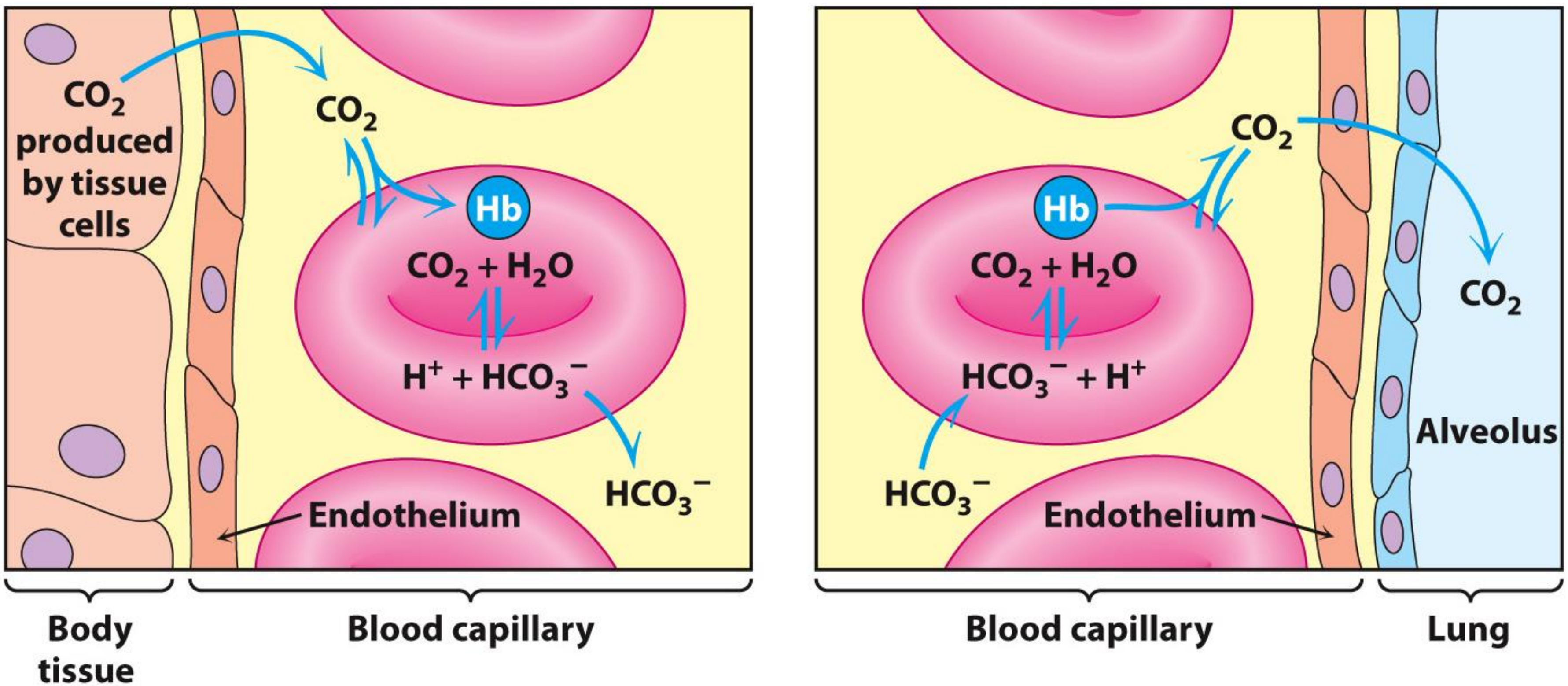
CO_2 effects



aminoterminus
Hemoglobin-monomer



- Amino termini at the interface between αβ dimers
- Negatively charged carbamate groups participate in salt-bridges, characteristic of the T-state structure → deoxyhemoglobin stabilized → release of O_2 favored



CO2 produced by tissue cells
CO2
Hb
CO2 + H2O
H+ + HCO3−
HCO3−
Endothelium
Body tissue
Blood capillary
CO2
Hb
CO2 + H2O
HCO3− + H+
HCO3−
CO2
Alveolus
Endothelium
Blood capillary
Lung

Blackboard:

Extra oefenopgaven over dit hoofdstuk.

Oefententamens

Volgende lessen:

Hoofdstuk 11 en 12

Vragen