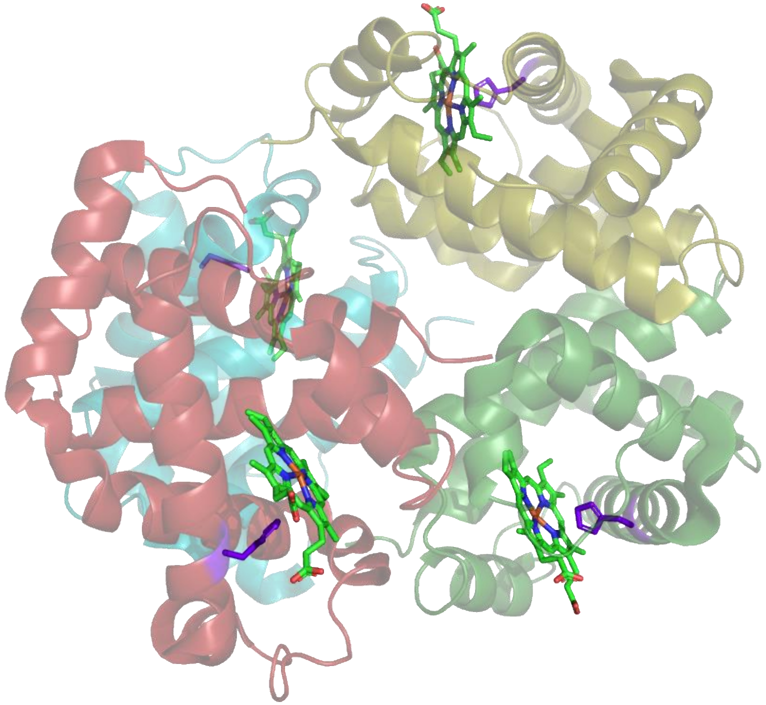
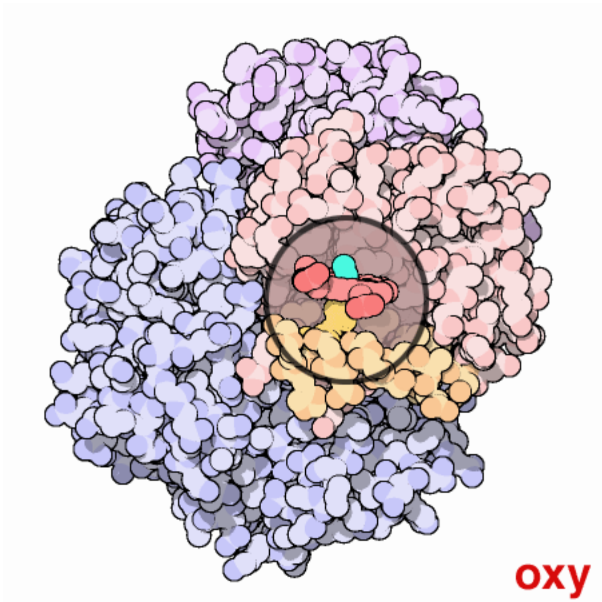
CH 105
Inorganic & Organic Chemistry
Module: Bioinorganic Chemistry





Chemistry Department
IIT Bombay

Figures prepared by using Pymol

Outline

- Relevance of Bioinorganic Chemistry
- O_2 a key player in Bioinorganic Chemistry
- Hemoglobin & reversible O_2 binding
- Trivia

Outline

Relevance of Bioinorganic Chemistry

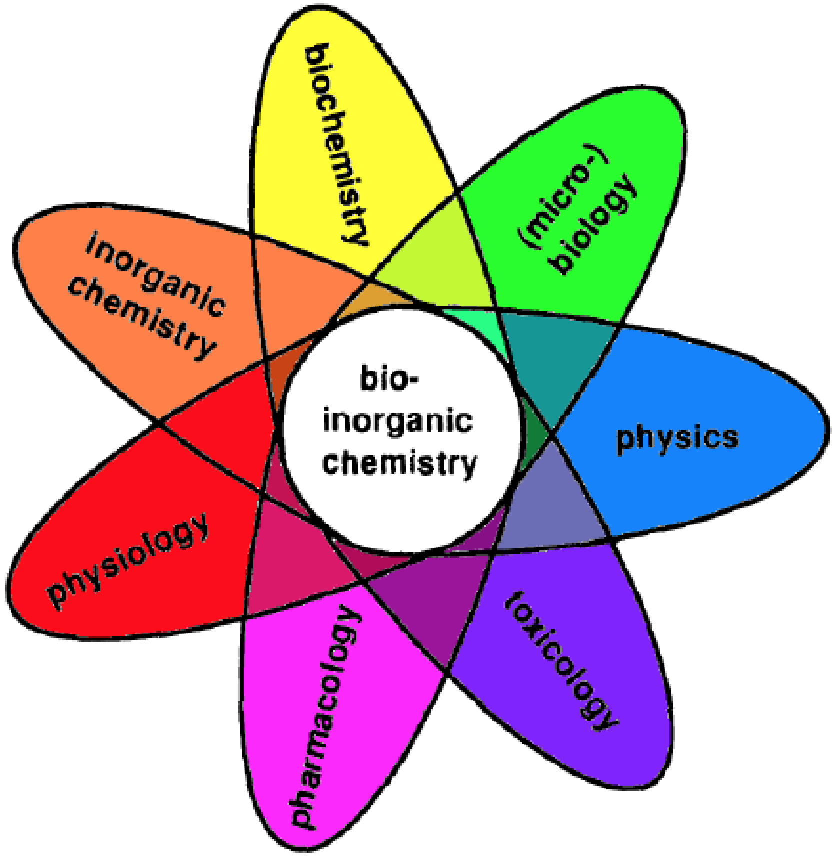
O_2 a key player in Bioinorganic Chemistry

Hemoglobin & reversible O_2 binding

Trivia

Why do we learn Bioinorganic Chemistry ?

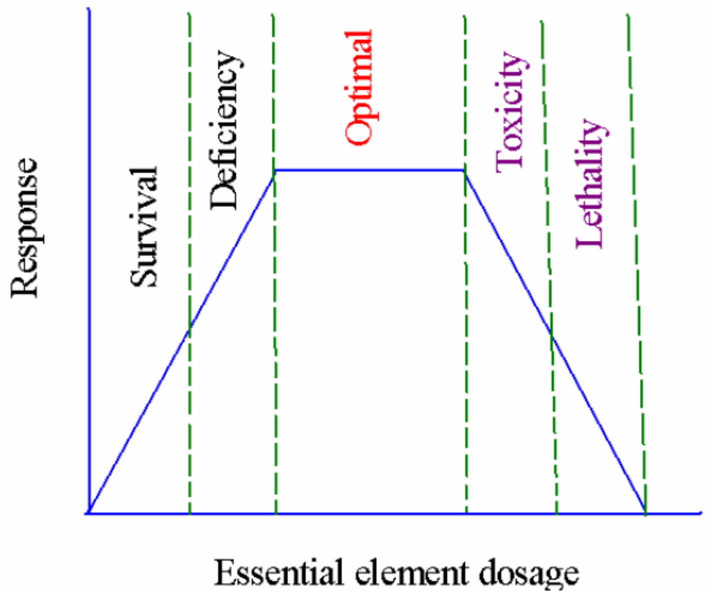
- *A close relation between biology & chemistry*
- *Various elements affects the biochemistry, physiology, micro-biology, toxicology, pharmacology*



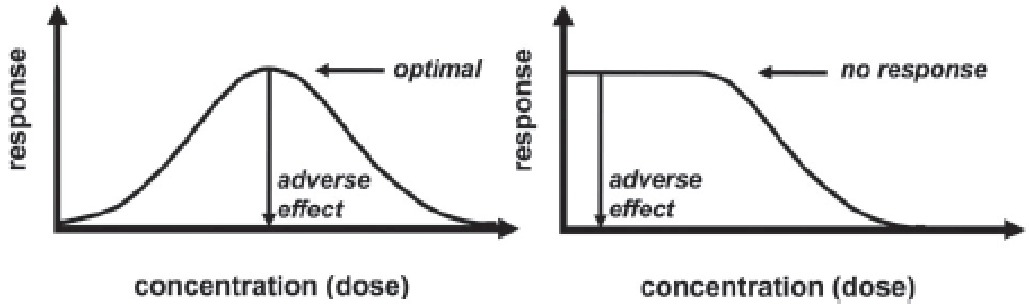
W. Kaim, B. Schwederski, Bioinorganic Chemistry: Inorganic Elements in Chemistry of Life, Wiley, 2013

Why do we learn Bioinorganic Chemistry ?

- *A close relation between biology & chemistry*
- *Various elements affects the biochemistry, physiology, micro-biology, toxicology, pharmacology*
- ***Essential & non-essential elements***



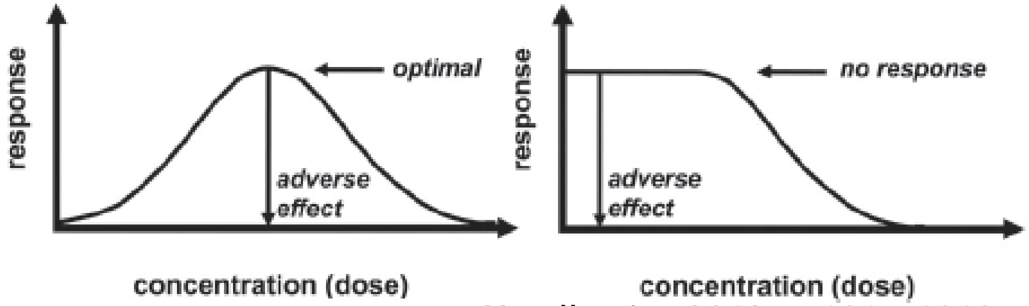
Der Pharma Chemica 4(5):1962-1968



Metallomics, 2012, 4, 1017-1019

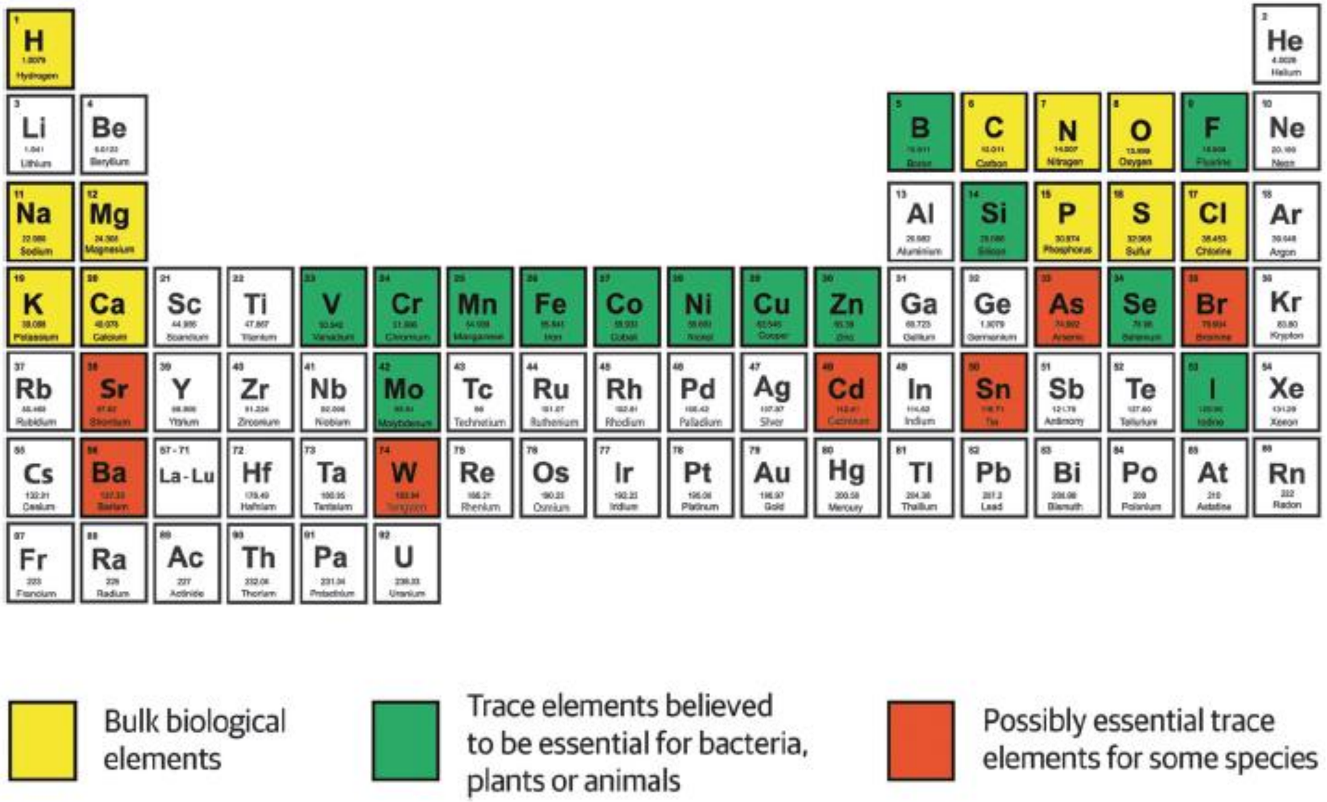
Why do we learn Bioinorganic Chemistry ?

- *A close relation between biology & chemistry*
- *Various elements affects the biochemistry, physiology, micro-biology, toxicology, pharmacology*
- <u>*Essential & non-essential elements*</u>



Metallomics, 2012, 4, 1017-1019

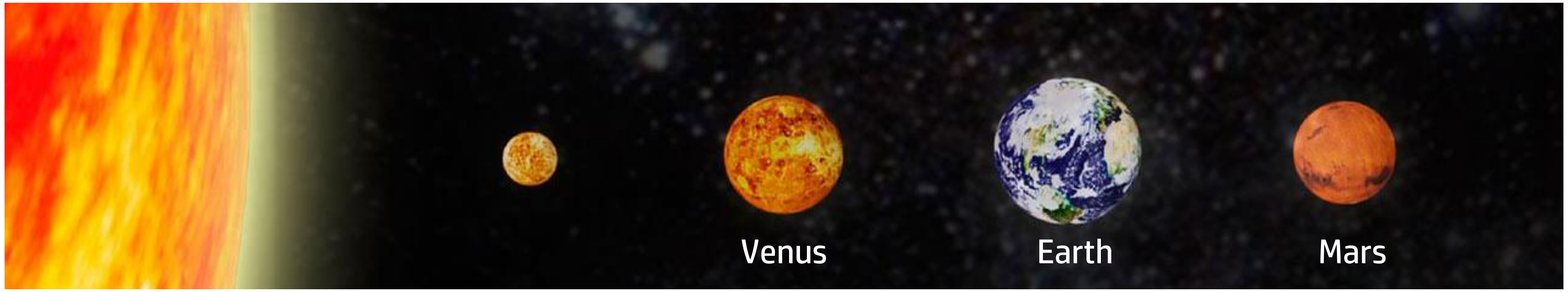
- <u>*Bulk & trace elements*</u>



R. J. P. Williams and R. Rickaby, "A chemical account of evolution", Chem. World, 2012, 6

Bioinorganic Chemistry influenced evolution?

Comparing planetary environments



doi:10.1126/science.aad1784

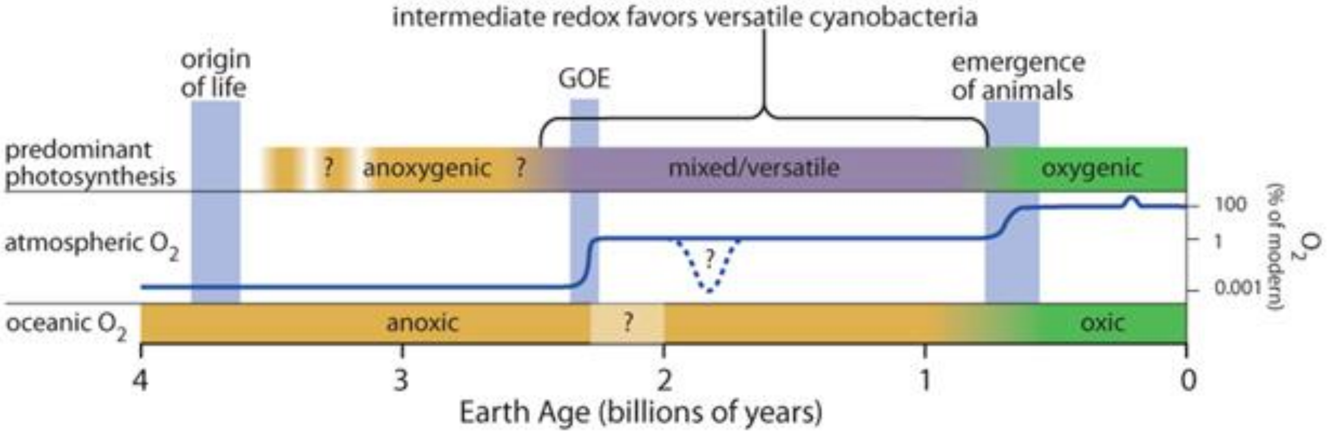
CO_2	96%	0.04%	95%
N_2	3%	78%	3%
O_2	0%	21%	0.2%

In the beginning earth atmosphere was similar to Venus or Mars

Emergence of O_2 occurred at a later stage

Significant contribution from bioinorganic chemistry

Bioinorganic Chemistry influenced evolution?

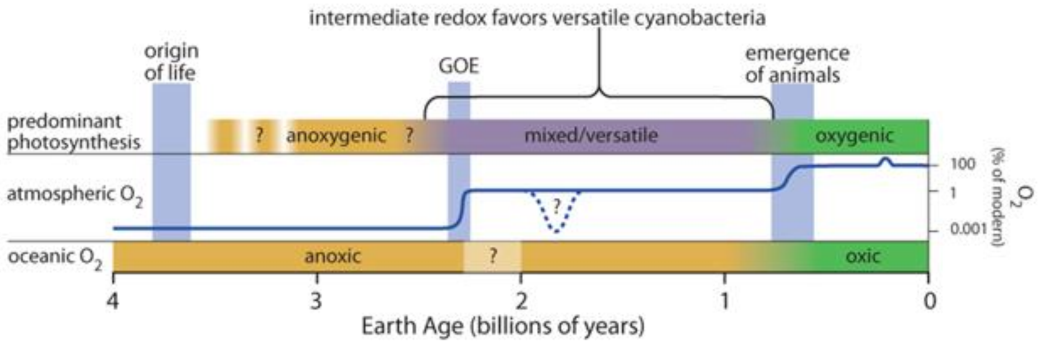


B. Biddanda *et al.*, Nature Education Knowledge, 2012, 3(10):13

In the beginning earth atmosphere was anoxygenic (no O_2)

Emergence of O_2 during the great oxygenation event (GOE)

Bioinorganic Chemistry influenced evolution?



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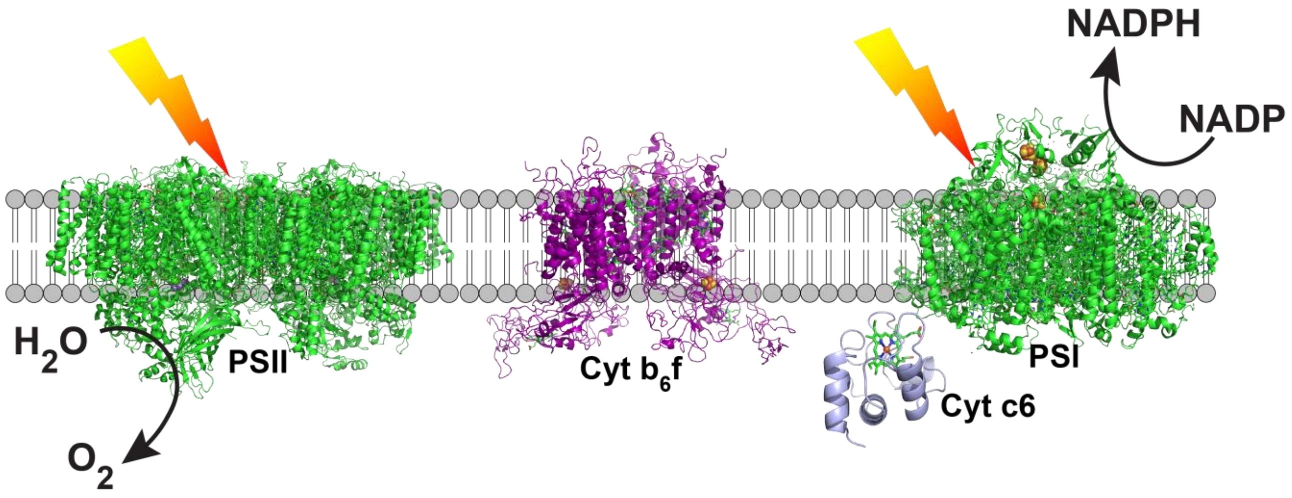


In the beginning earth atmosphere was anoxygenic (no O_2)

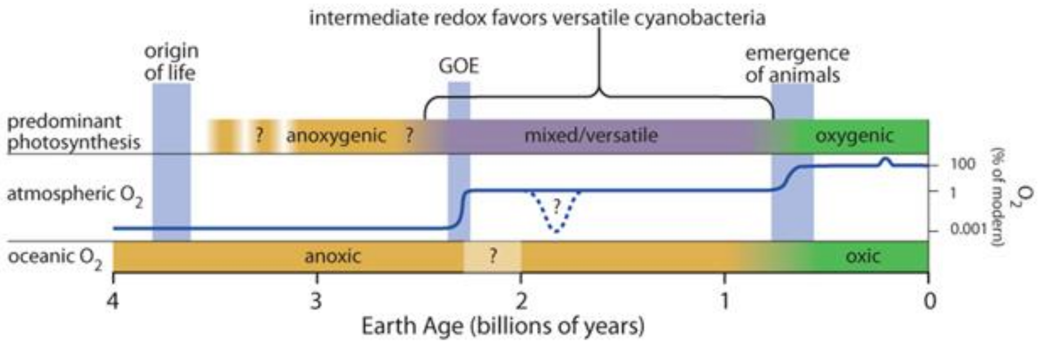
Emergence of O_2 during the great oxygenation event (GOE)

Triggered by photosynthesis

$$CO_2 + H_2O \longrightarrow C_6H_{12}O_6 + O_2$$

H_2O is the source of electron and O_2 is by-product

Bioinorganic Chemistry influenced evolution?



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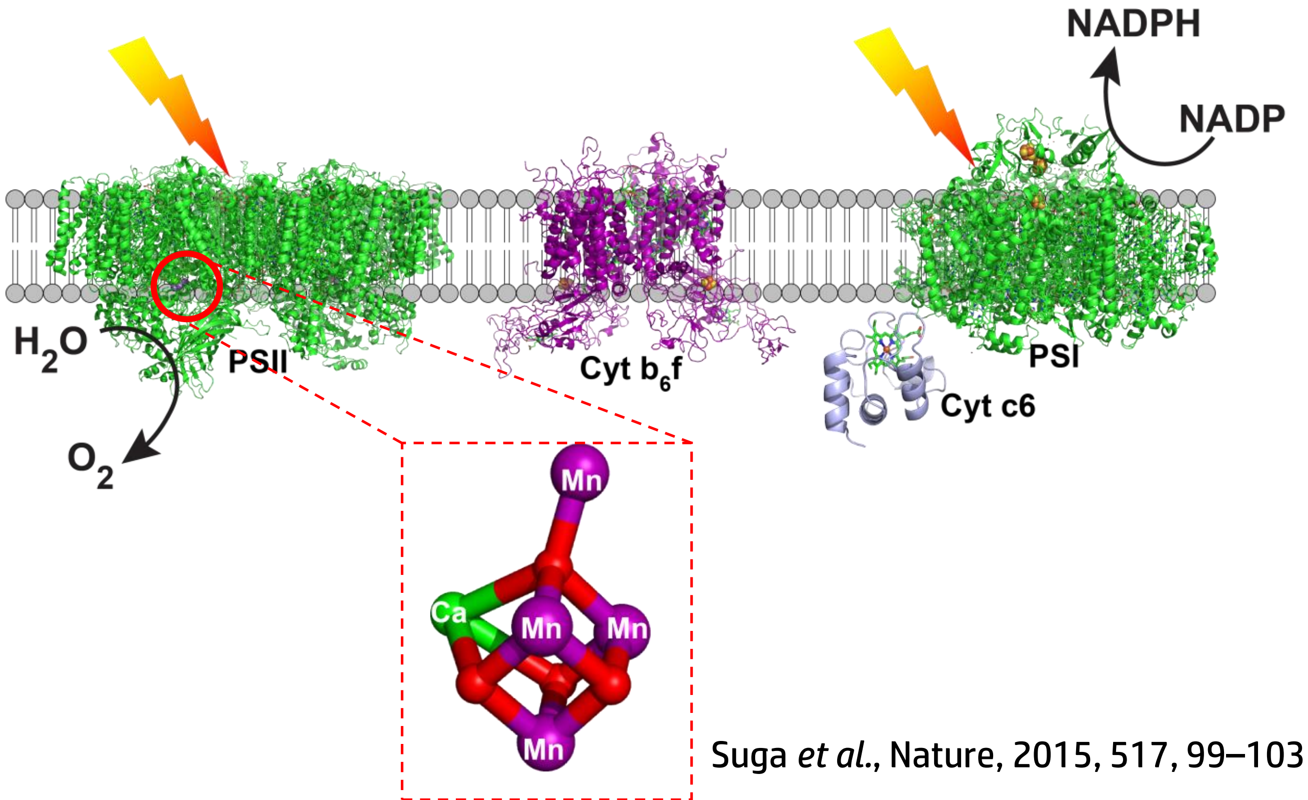


Suga *et al.*, Nature, 2015, 517, 99–103

In the beginning earth atmosphere was anoxygenic (no O_2)

Emergence of O_2 during the great oxygenation event (GOE)

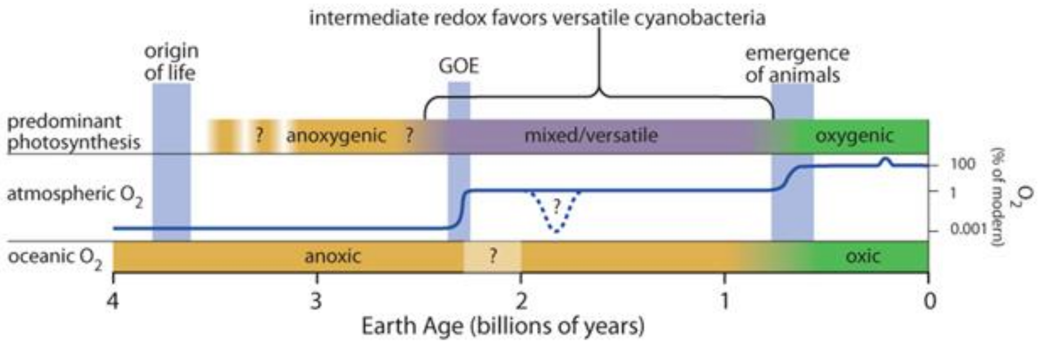
Triggered by photosynthesis

$$CO_2 + H_2O \longrightarrow C_6H_{12}O_6 + O_2$$

H_2O is the source of electron and O_2 is by-product

H_2O oxidation is catalyzed by Mn_4O_4Ca Oxygen Evolving Cluster (OEC)

Bioinorganic Chemistry influenced evolution?



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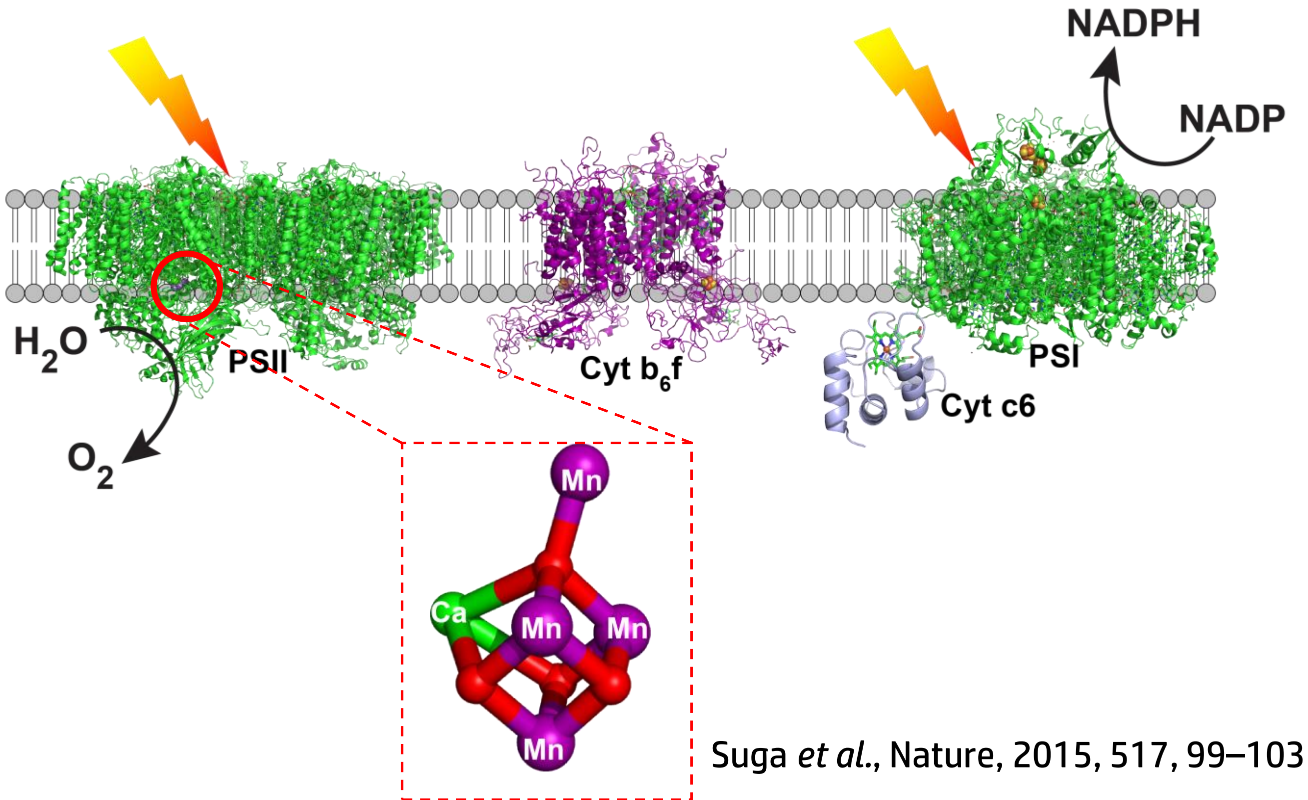


Suga *et al.*, Nature, 2015, 517, 99–103

Photosynthetic O_2 evolution altered the scenario

O_2 is a strong oxidizing agent

Change in the course of evolution

1. *Extinction*
2. *Exiled to anoxic niches*
3. *Adapt to oxygenic environment*

Take Home Messages

O_2 binding to Hemoglobin

- *Inorganic elements are crucial for Biology*
- *Essential/non-essential & bulk/trace elements*
- *Bioinorganic chemistry of photosynthesis*
- *Photosynthetic O_2 production is a game-changer*

Outline

Relevance of Bioinorganic Chemistry

O_2 a key player in Bioinorganic Chemistry

Hemoglobin & reversible O_2 binding

Trivia

Handling O_2 in cellular environments

Generation of ROS in cells can cause oxidative damage (DNA, protein, lipid)

$$O_2 + e^- \longrightarrow O_2^{\bullet-}$$

$$O_2^{\bullet-} + e^- + 2H^+ \longrightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \longrightarrow OH^{\bullet} + H_2O$$

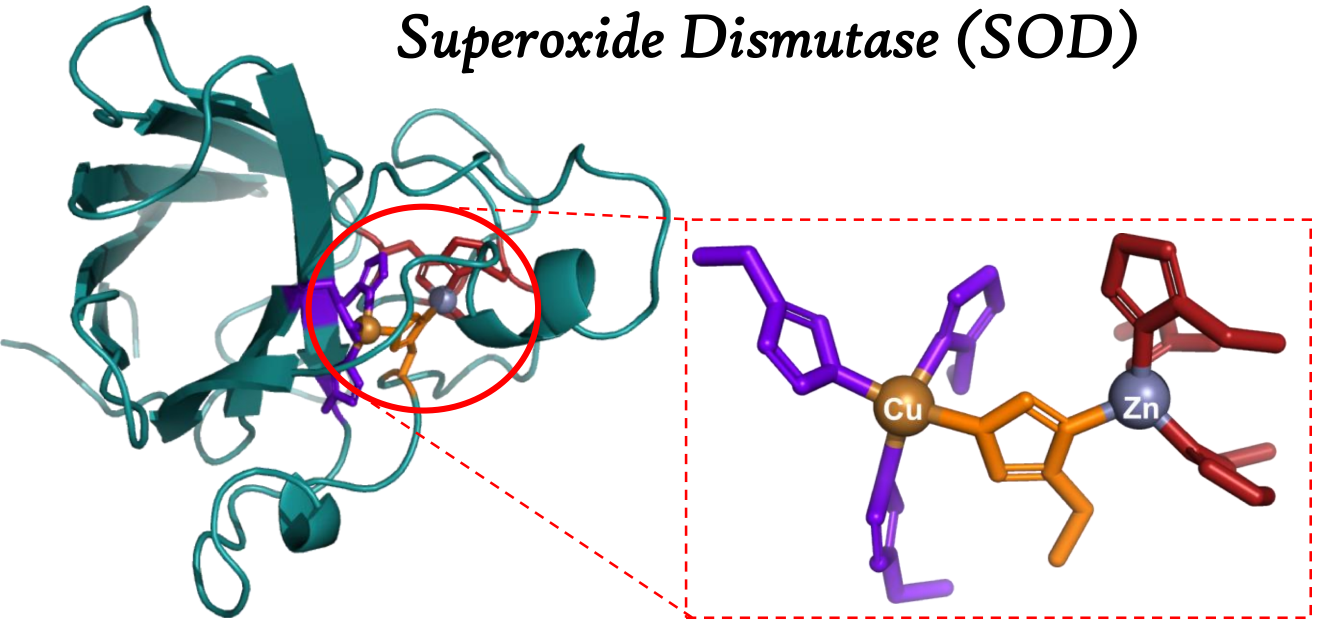
$$OH^{\bullet} + e^- + H^+ \longrightarrow H_2O$$

ROS: Reactive Oxygen Species

$O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$

Handling O_2 in cellular environments

Superoxide Dismutase (SOD)



PDB 1SXZ

$$2O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Dismutation reaction

Mn-, Fe-, and Ni-SOD also exist

H_2O_2 still an issue

Generation of ROS in cells can cause oxidative damage (DNA, protein, lipid)

$$O_2 + e^- \longrightarrow O_2^{\bullet -}$$

$$O_2^{\bullet -} + e^- + 2H^+ \longrightarrow H_2O_2$$

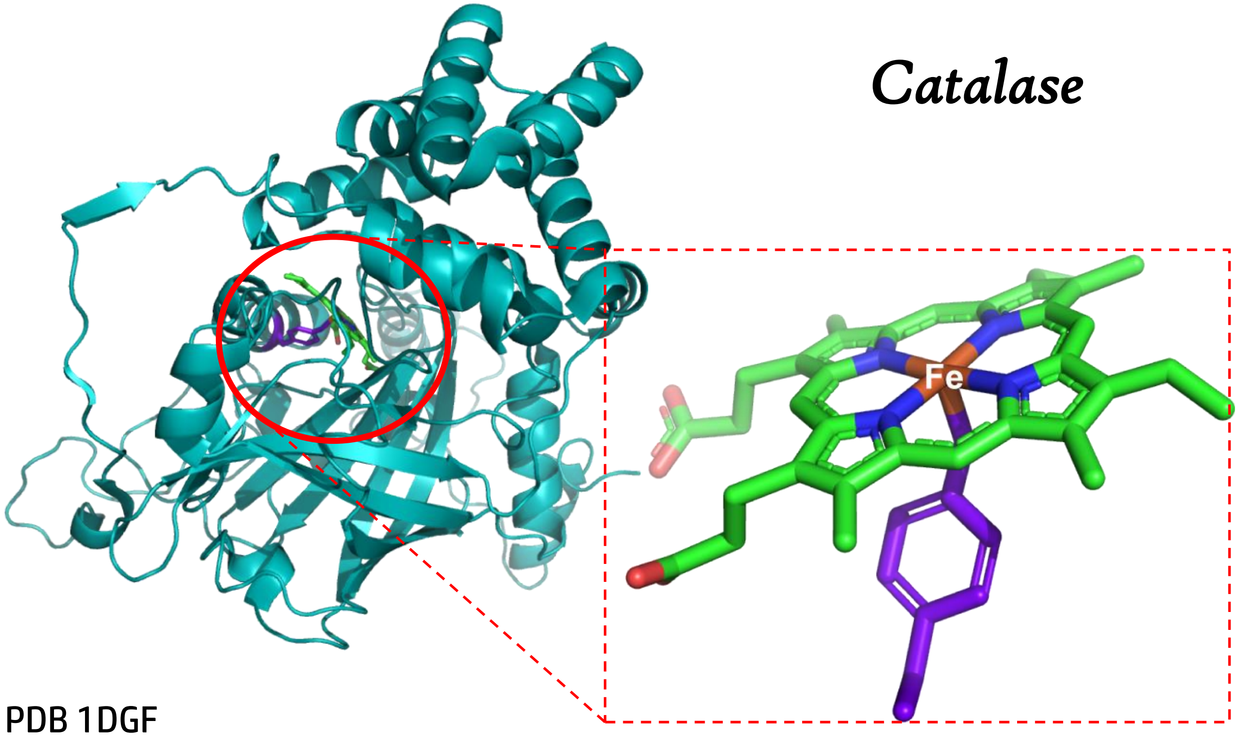
$$H_2O_2 + e^- + H^+ \longrightarrow OH^\bullet + H_2O$$

$$OH^\bullet + e^- + H^+ \longrightarrow H_2O$$

Anti-oxidants: Defense system of biology

- *Superoxide Dismutase (SOD)*

Handling O_2 in cellular environments



PDB 1DGF

Catalase

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Dismutation reaction

Removal of toxic H_2O_2

Generation of ROS in cells can cause oxidative damage (DNA, protein, lipid)

$$O_2 + e^- \longrightarrow O_2^{\bullet -}$$

$$O_2^{\bullet -} + e^- + 2H^+ \longrightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \longrightarrow OH^\bullet + H_2O$$

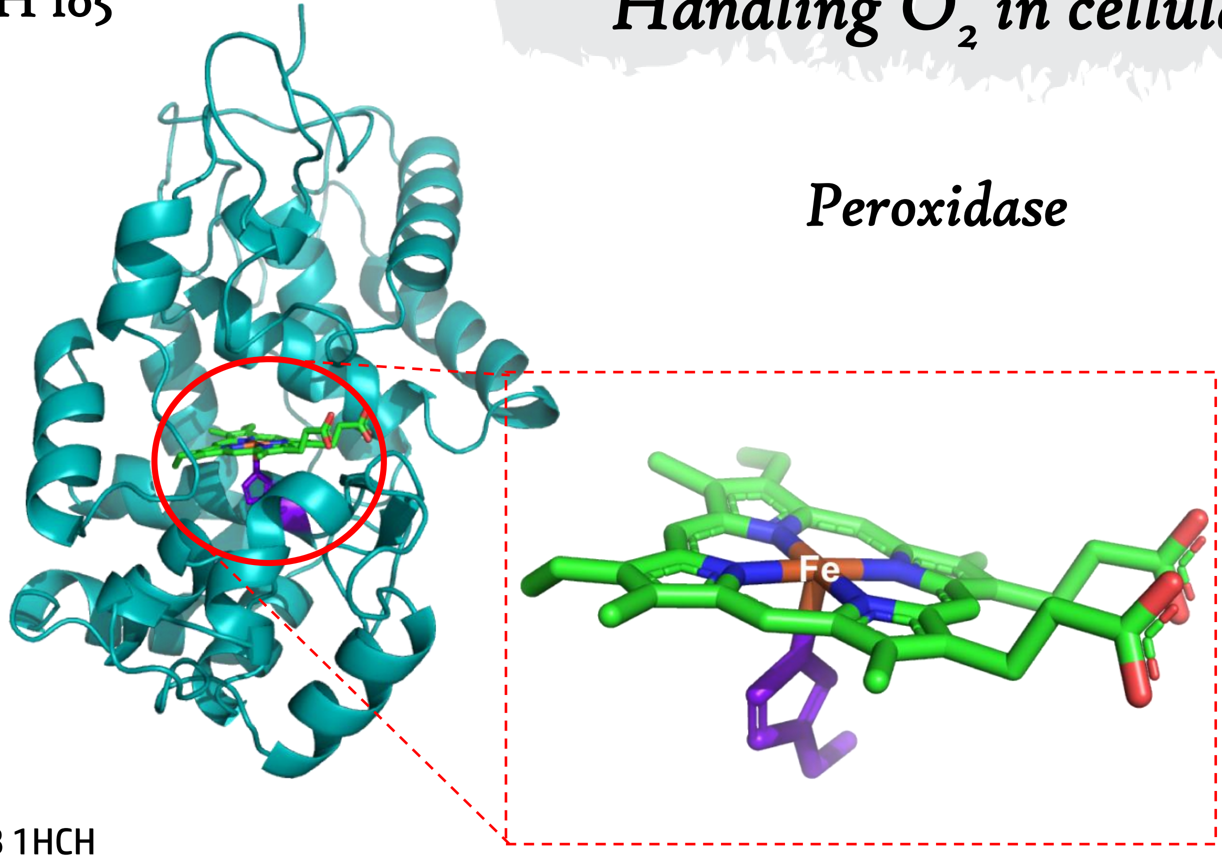
$$OH^\bullet + e^- + H^+ \longrightarrow H_2O$$

Anti-oxidants: Defense system of biology

- *Superoxide Dismutase (SOD)*
- *Catalase*

Handling O_2 in cellular environments

Peroxidase



PDB 1HCH

$$H_2O_2 + 2\ Sub(red) \longrightarrow 2H_2O + 2\ Sub(ox)$$

Controlled utilization of the oxidizing power of H_2O_2

Generation of ROS in cells can cause oxidative damage (DNA, protein, lipid)

$$O_2 + e^- \longrightarrow O_2^{\bullet -}$$

$$O_2^{\bullet -} + e^- + 2H^+ \longrightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \longrightarrow OH^{\bullet} + H_2O$$

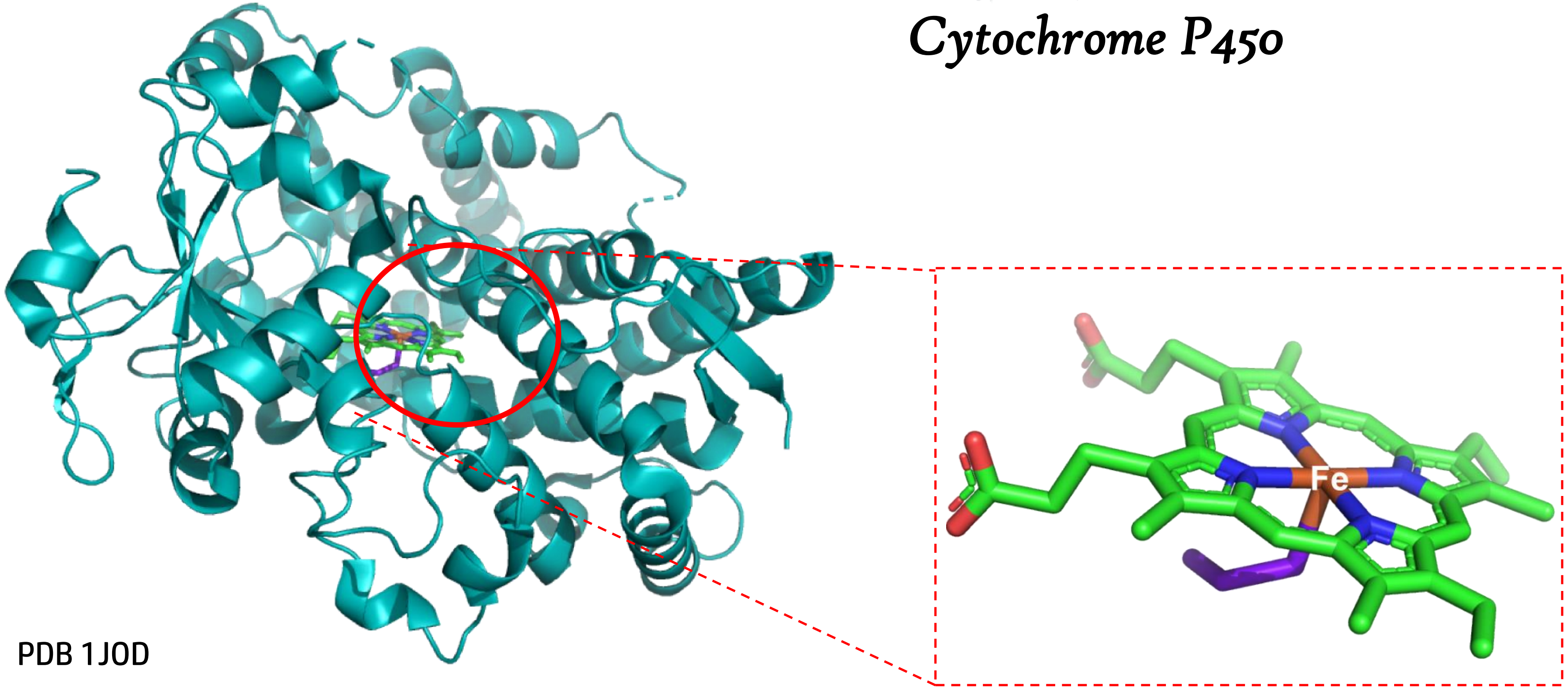
$$OH^{\bullet} + e^- + H^+ \longrightarrow H_2O$$

Anti-oxidants: Defense system of biology

- *Superoxide Dismutase (SOD)*
- *Catalase & Peroxidase*

Handling O_2 in cellular environments

Cytochrome P450



PDB 1JOD

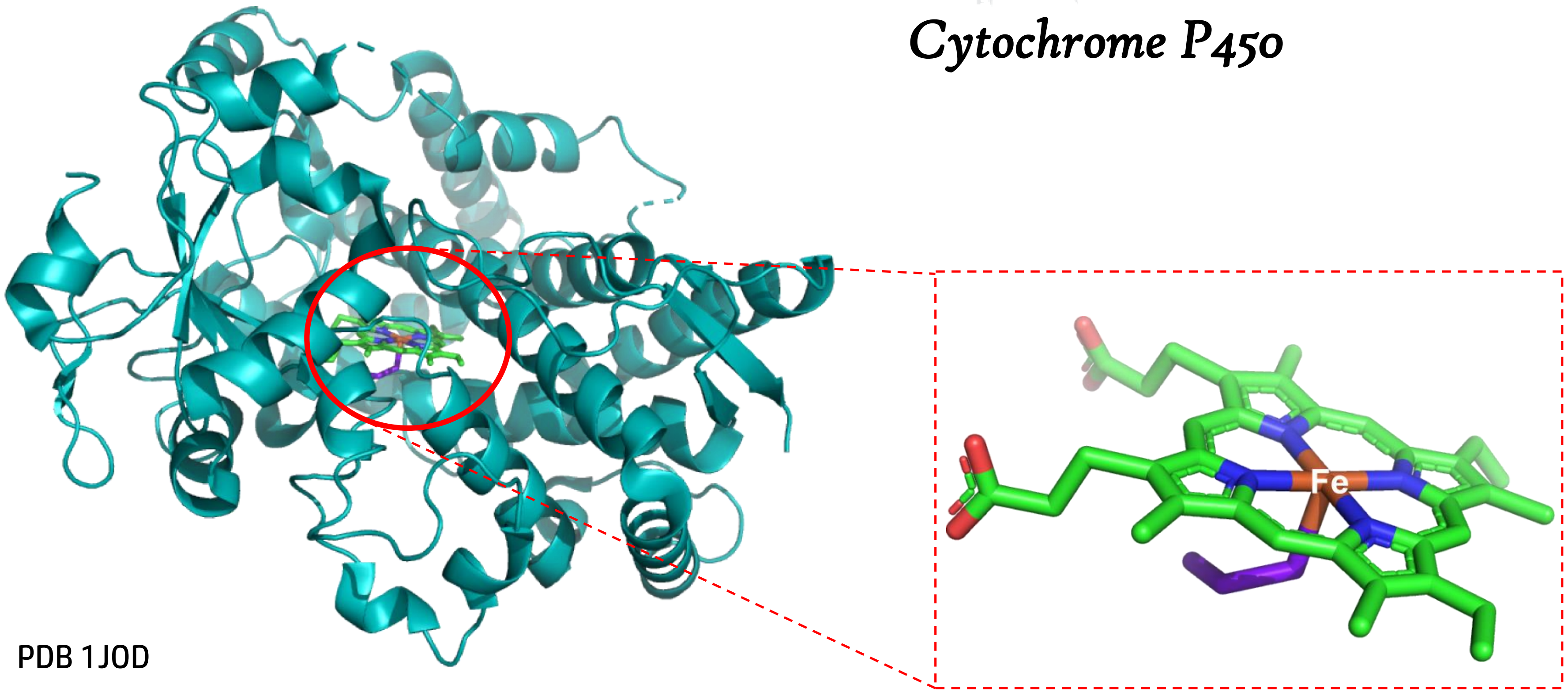
$$O_2 + Sub(red) + 2e^- + 2H^+ \rightarrow H_2O + 2\ Sub(ox)$$

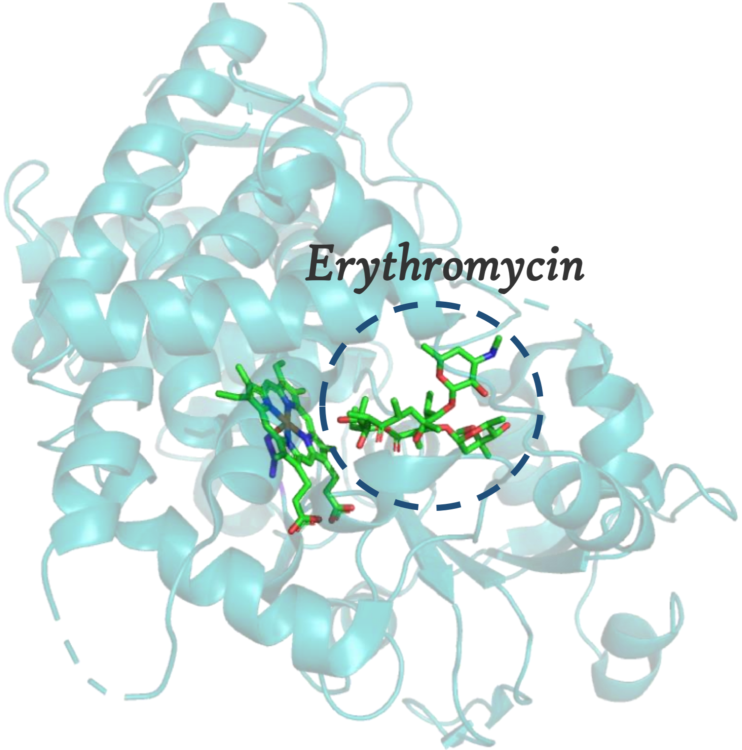
Poison O_2 becomes lifeline

Harnessing the oxidizing power of O_2

Handling O_2 in cellular environments

Cytochrome P450





$O_2 + Sub(red) + 2e^- + 2H^+ \rightarrow H_2O + 2\ Sub(ox)$

Poison O_2 becomes lifeline

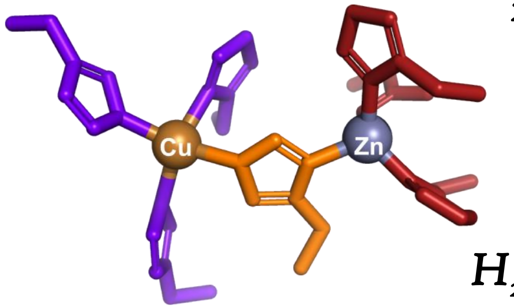
Harnessing the oxidizing power of O_2
Critical for pharmaceutical metabolism

Requires controlled O_2 transport in biology

Take Home Messages

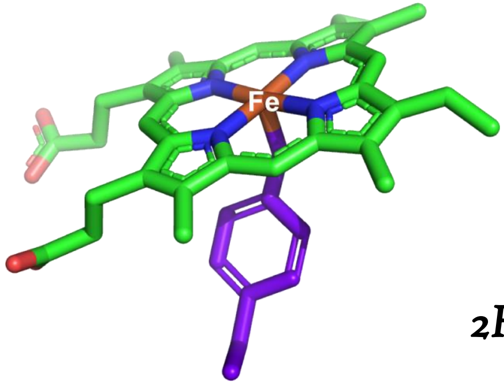
O_2 a key player in Bioinorganic Chemistry

- *Oxidizing agent O_2 can produce ROS [$O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$]*
- *Transition metal containing enzymes as defense system*



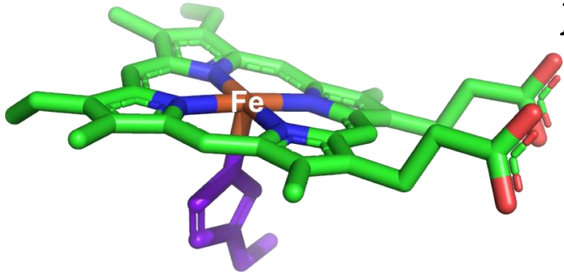
$$2O_2^{\bullet-} \longrightarrow H_2O_2 + O_2$$

Superoxide Dismutase



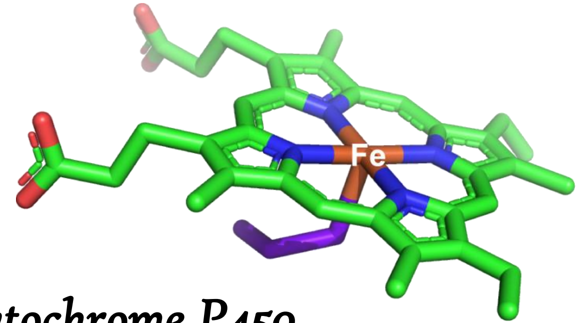
$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase



$$H_2O_2 \xrightarrow{Sub(red) \rightarrow Sub(ox)} 2H_2O$$

Peroxidase



Cytochrome P450

$$O_2 \xrightarrow{Sub(red) \rightarrow Sub(ox)} H_2O$$

- *Use of H_2O_2 for controlled oxidation (peroxidase)*
- *Further advancement: Use of O_2 as an oxidant (cytochrome P450)*

Outline

Relevance of Bioinorganic Chemistry

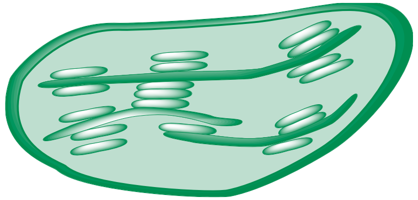
O_2 a key player in Bioinorganic Chemistry

Hemoglobin & reversible O_2 binding

Trivia

Hemoglobin & reversible O_2 binding

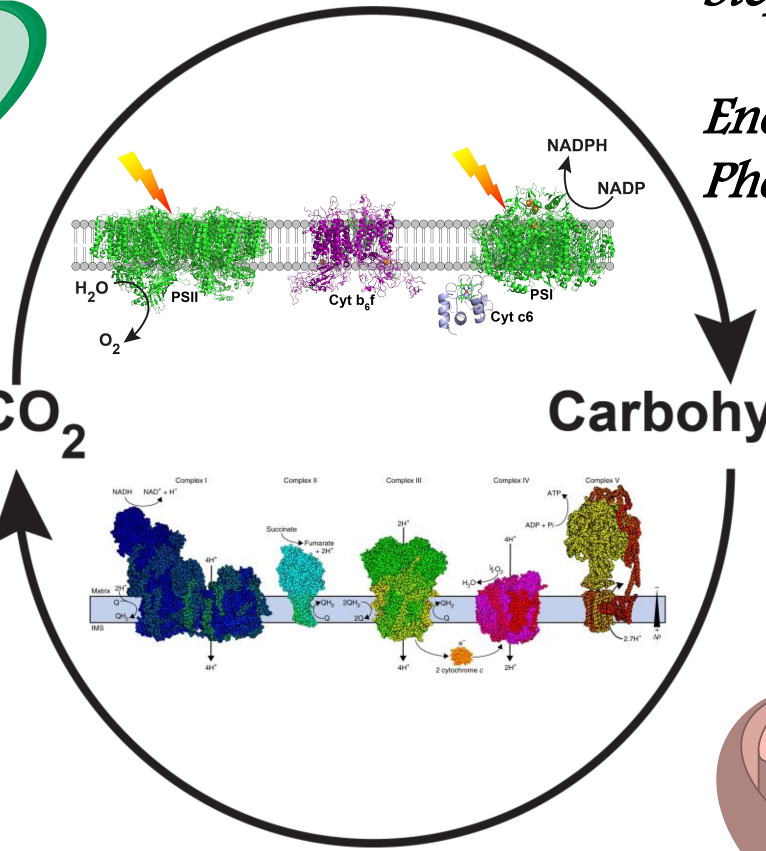
Biological Energy Conversion Scheme



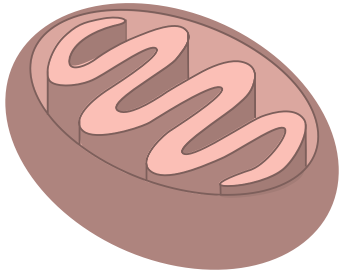
Chloroplast

Step 1.

Energy Storage: Photosynthesis



H_2O + CO_2 Carbohydrate + O_2



Mitochondria

Step 2.

Energy Utilization: Respiration

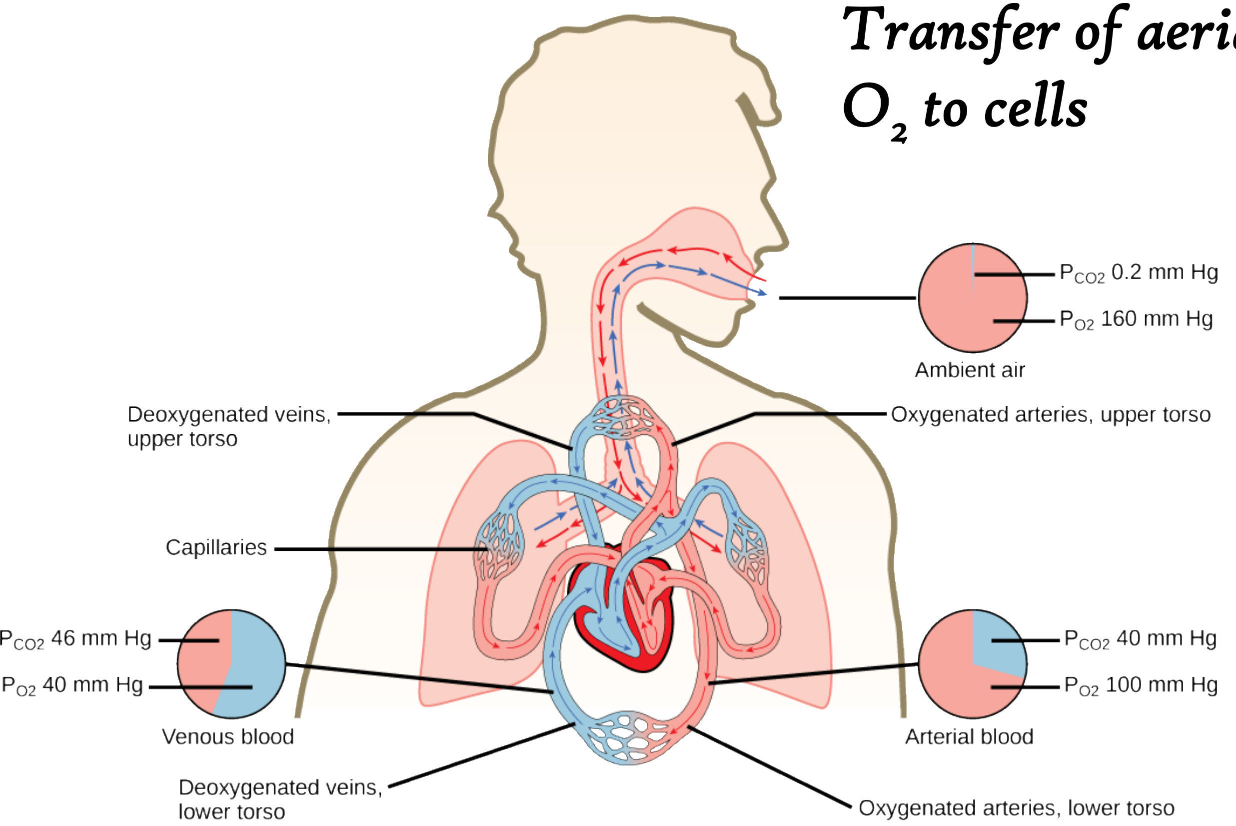
O_2 a key player in the energy transduction process

Respiration liberates stored chemical energy

Regulated O_2 supply in the cells is vital

Hemoglobin & reversible O_2 binding

Transfer of aerial O_2 to cells

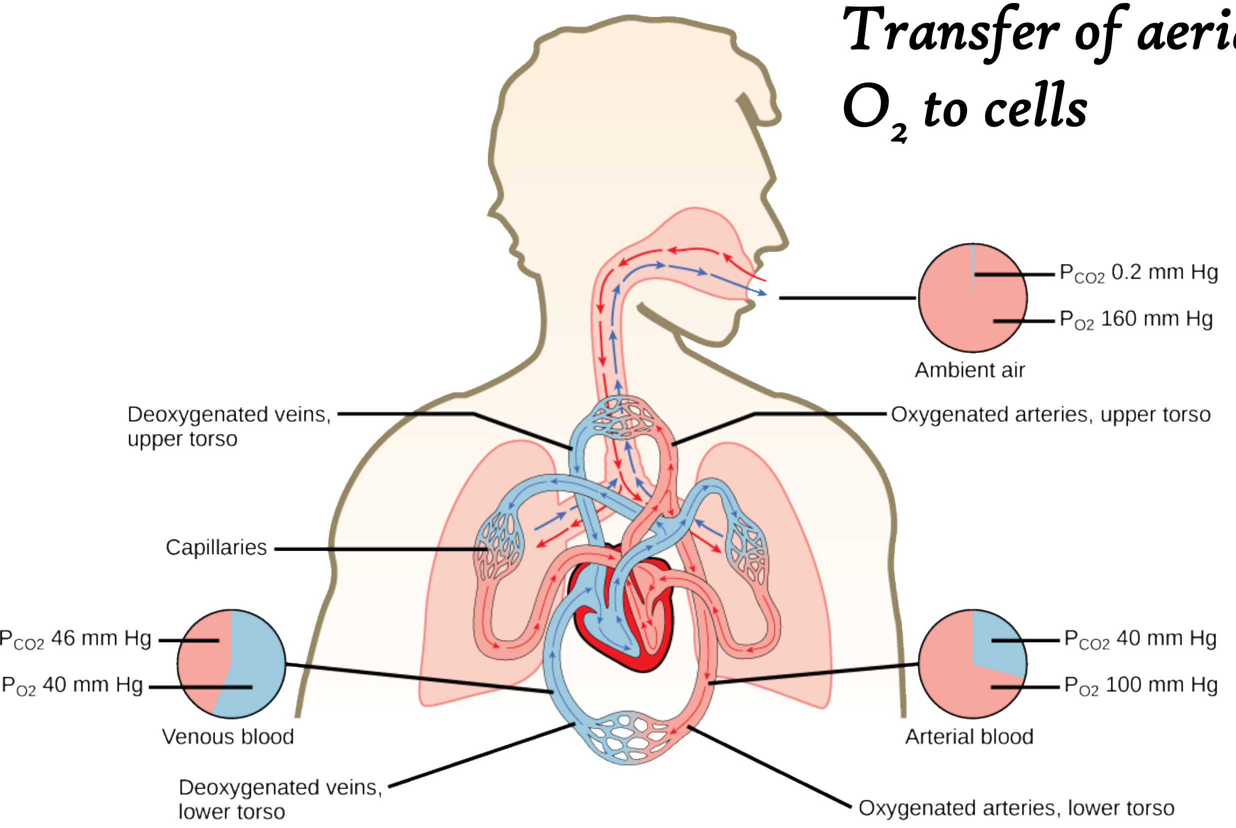


http://organismalbio.biosci.gatech.edu/

Air	Breathing	Lungs	Transporter	Cells
$P(O_2)$ (160 mm of Hg)	$\rightleftharpoons$	$P(O_2)$ (100 mm of Hg)	$\rightleftharpoons$	$P(O_2)$ (20-40 mm of Hg)
				Storage

Hemoglobin & reversible O_2 binding

Transfer of aerial O_2 to cells



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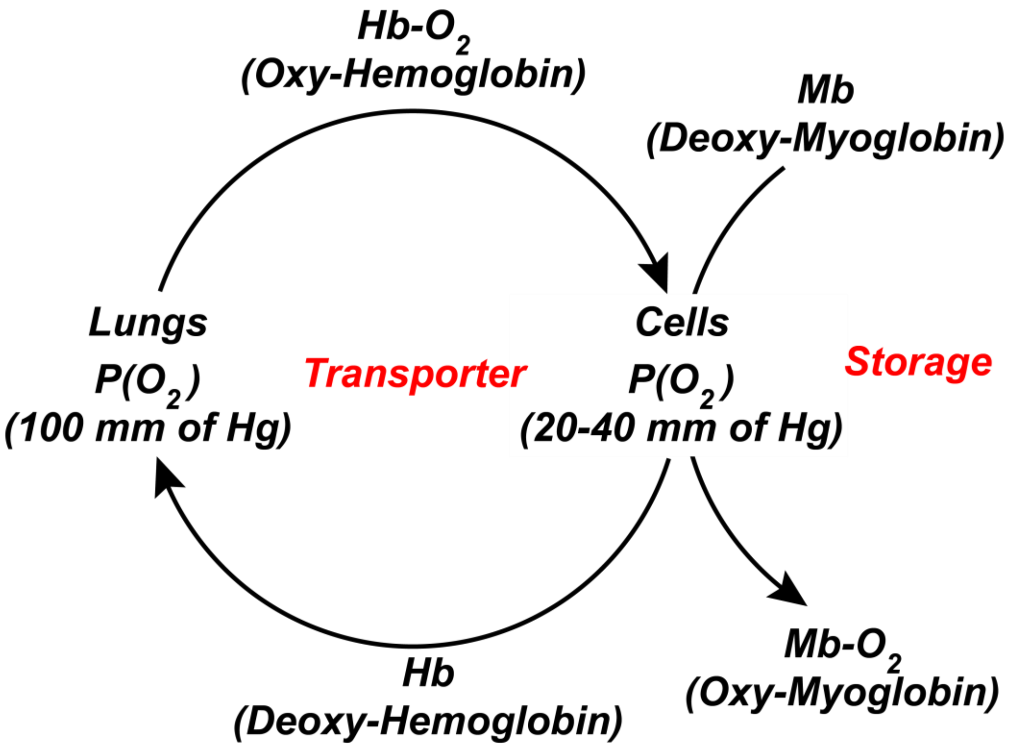
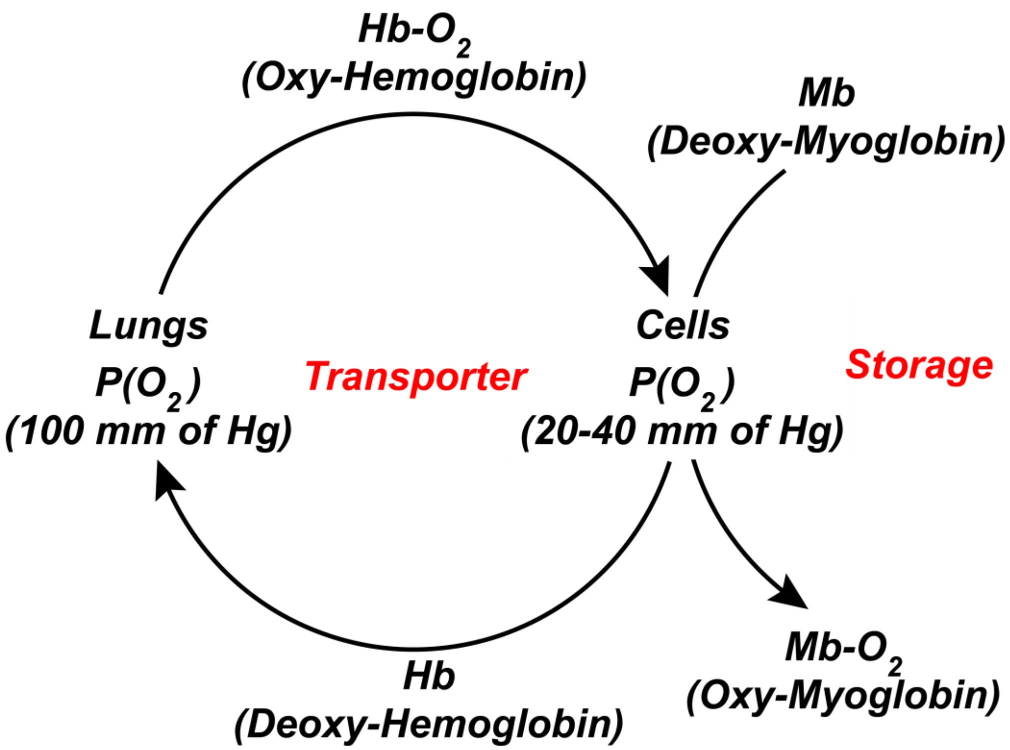
Air $P(O_2)$ (160 mm of Hg) $\xrightleftharpoons{\text{Breathing}}$ **Lungs** $P(O_2)$ (100 mm of Hg) $\xrightleftharpoons{\text{Transporter}}$ **Cells** $P(O_2)$ (20-40 mm of Hg) **Storage**

Need an O_2 transporter
Hemoglobin (Hb)

and an O_2-storage system
Myoglobin (Mb)

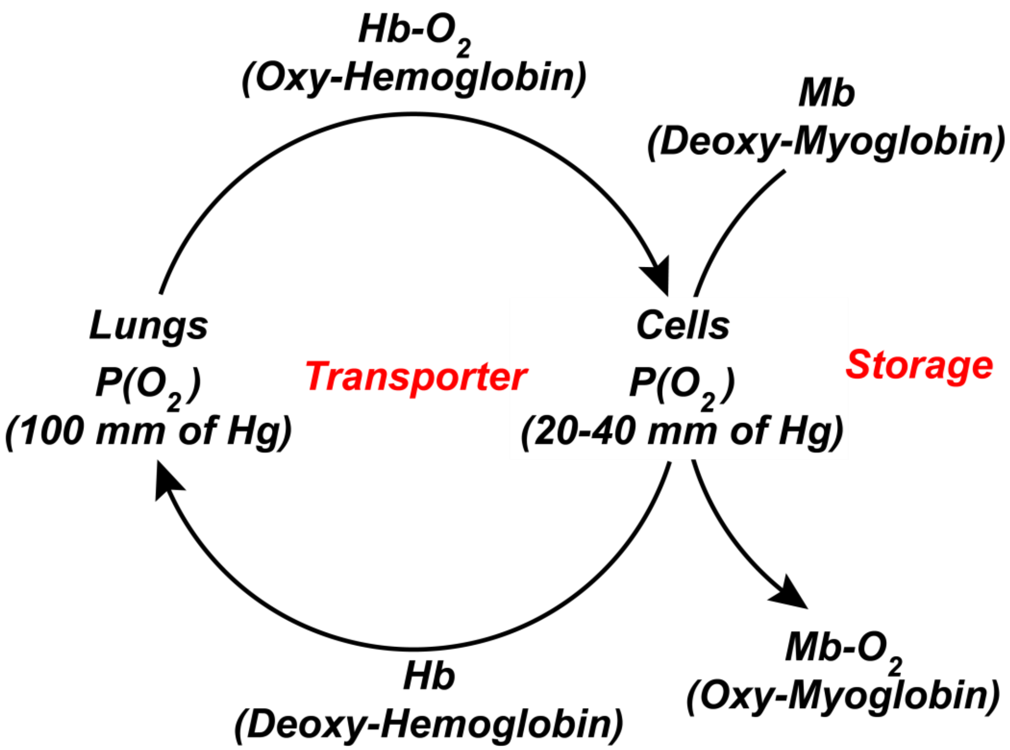


Hemoglobin & reversible O_2 binding



Differential O_2 binding ability of Hb & Mb is crucial

Hemoglobin & reversible O_2 binding



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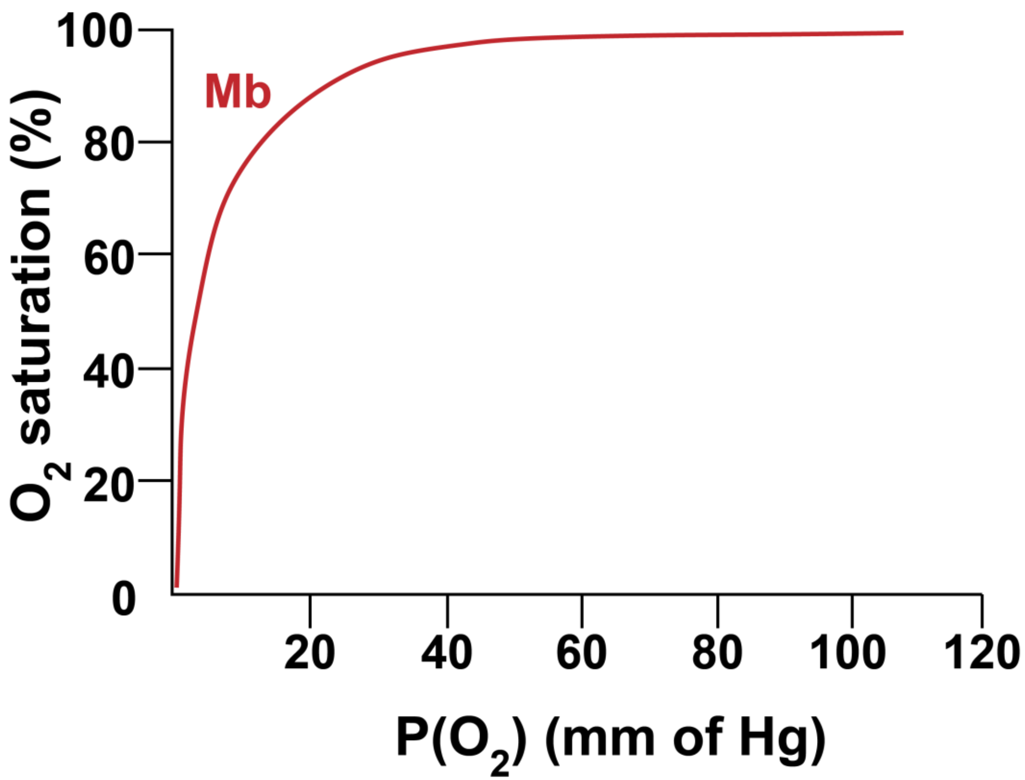
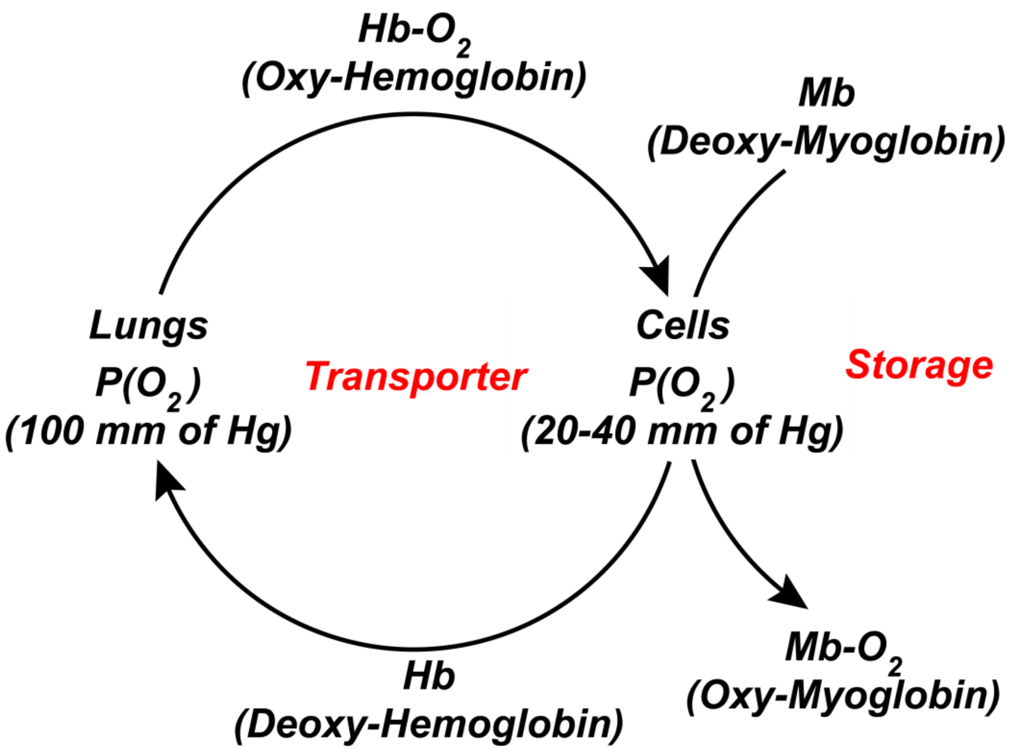


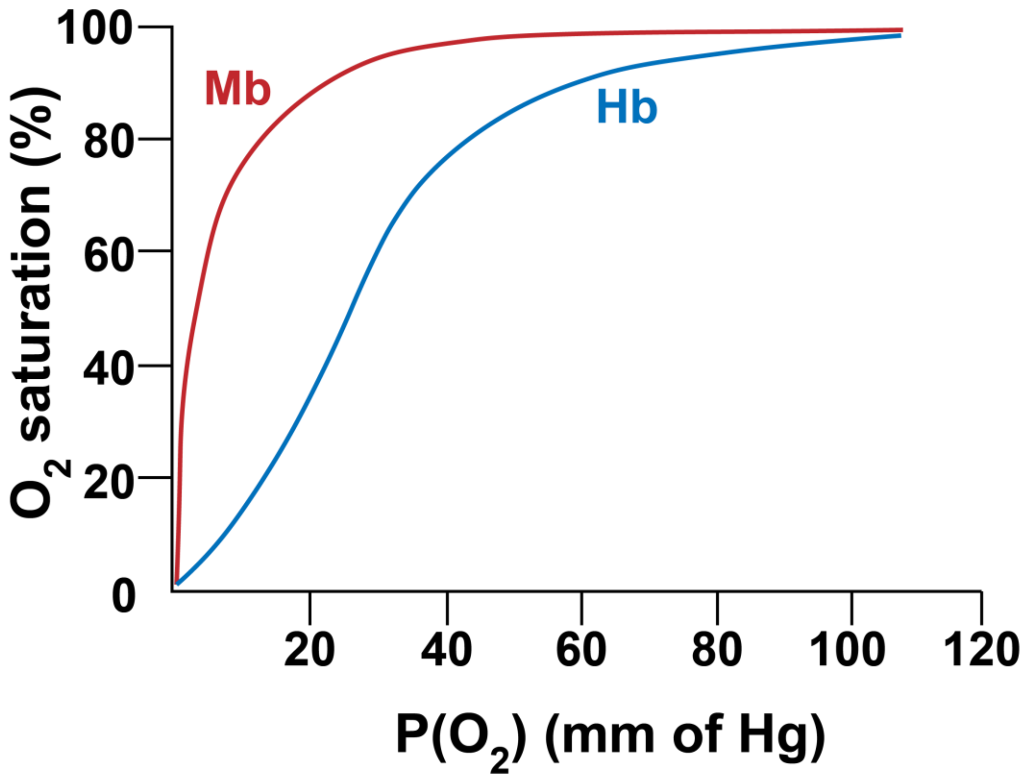
O_2 binding curve: Notice x- and y-axes

Mb follows a hyperbolic O_2 binding

Binds O_2 strongly even at low O_2 conc.

Hemoglobin & reversible O_2 binding





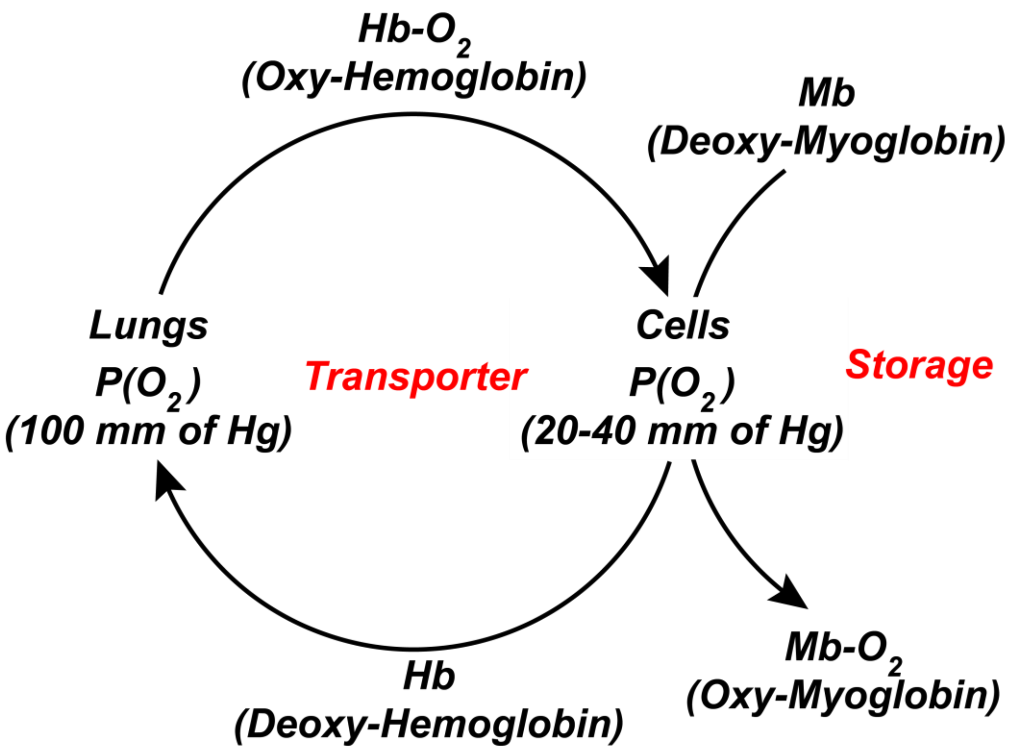
Differential O_2 binding ability of Hb & Mb is crucial

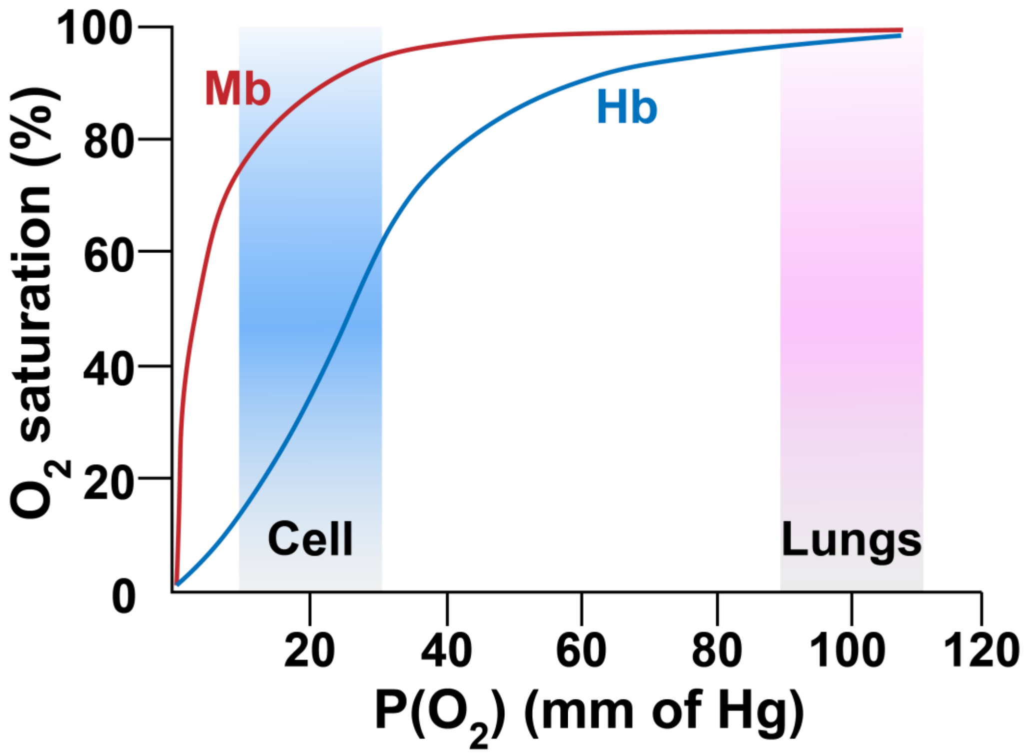
Mb follows a hyperbolic O_2 binding

Hb follows a sigmoidal trend

Binds O_2 strongly only at higher O_2 conc.

Hemoglobin & reversible O_2 binding





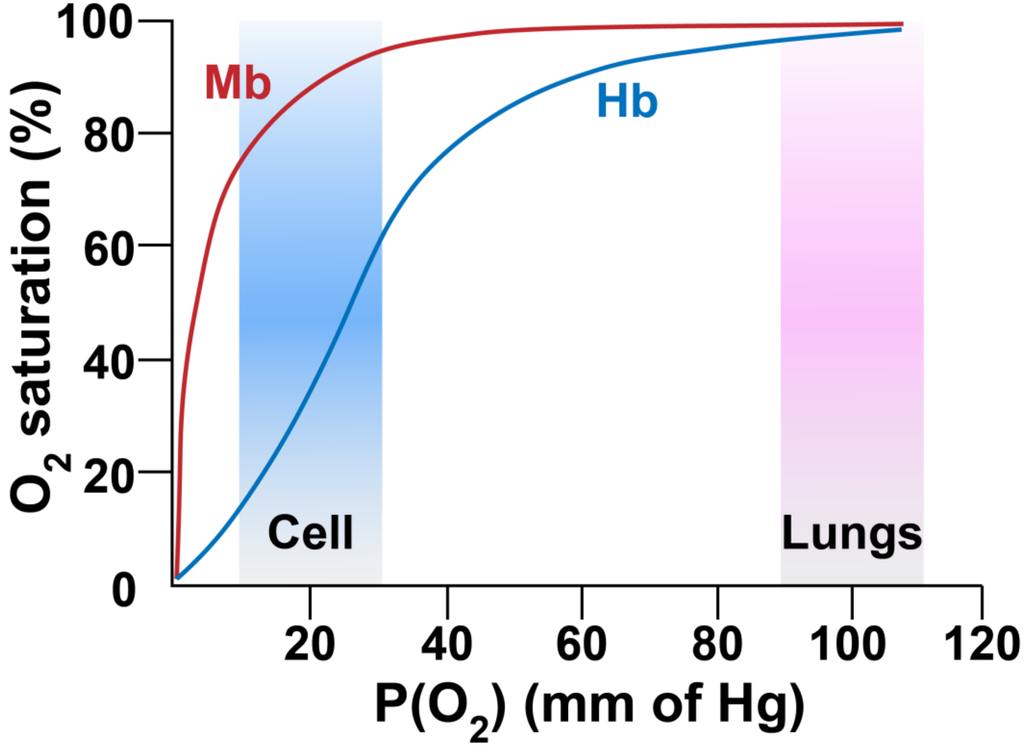
Differential O_2 binding ability of Hb & Mb is crucial

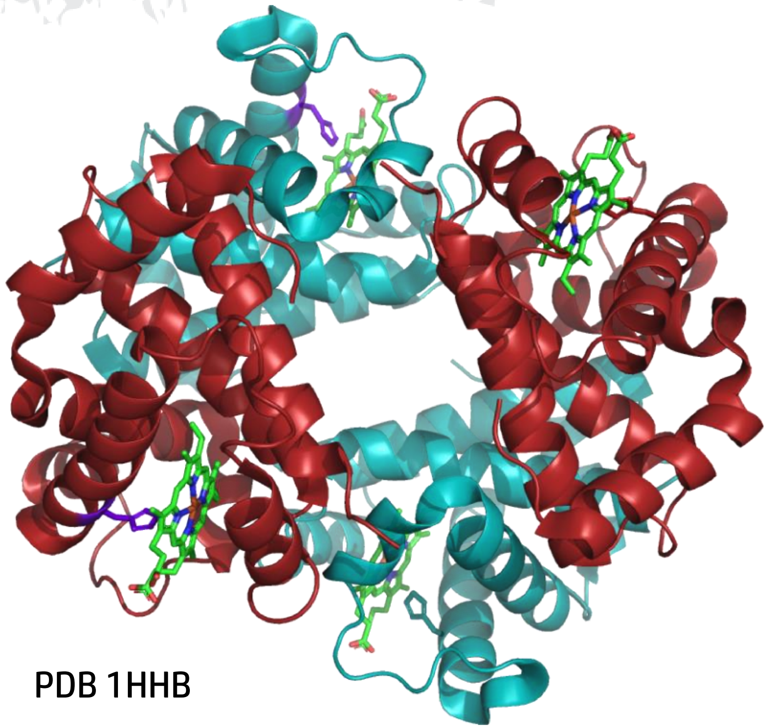
In cells/tissues Mb binds O_2 better than Hb

Hb binds O_2 better in lungs

Reasons behind such differential O_2 binding?

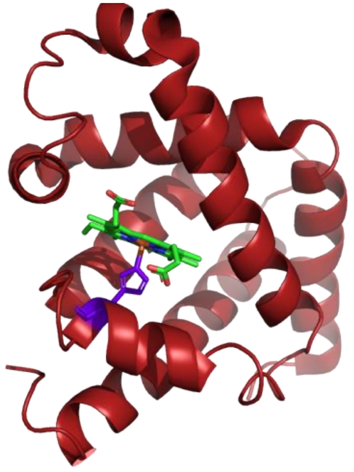
Hemoglobin & reversible O_2 binding





PDB 1HHB

Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

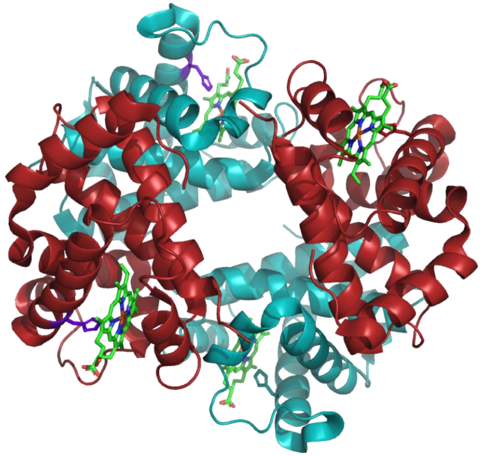


PDB 1MBN

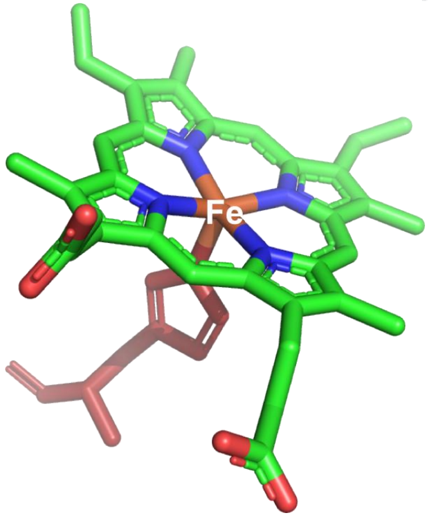
Myoglobin (Mb)
Monomeric structure

Deciphering the relationship between differential O_2 binding & structure for Mb/Hb

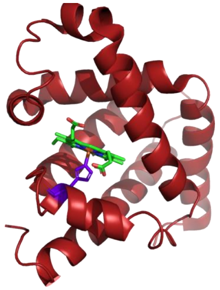
Hemoglobin: structure-function relationship



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

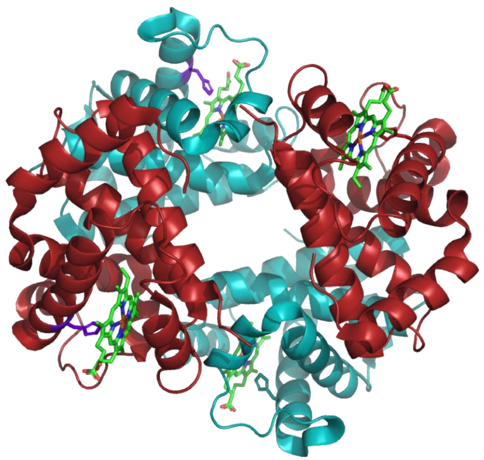


Fe-containing heme core

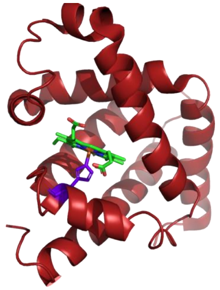


Myoglobin (Mb)
Monomeric structure

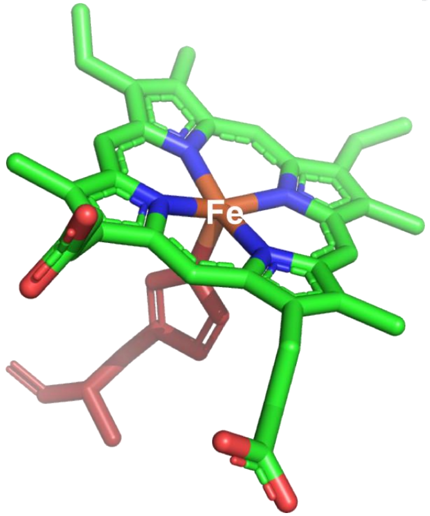
Hemoglobin: structure-function relationship



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

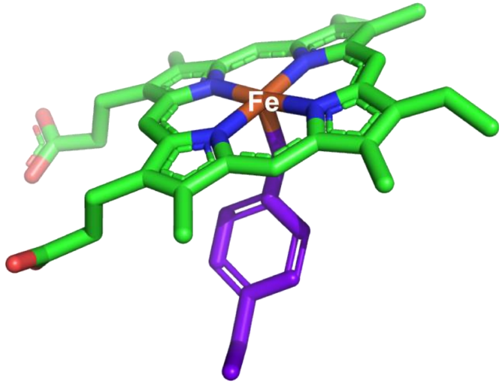


Myoglobin (Mb)
Monomeric structure

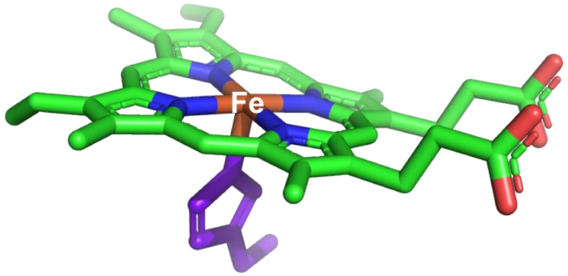


Fe-containing heme core

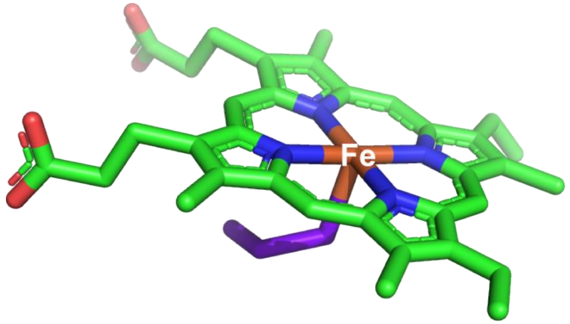
Analogous to catalase, peroxidase, cyt-P450



Catalase

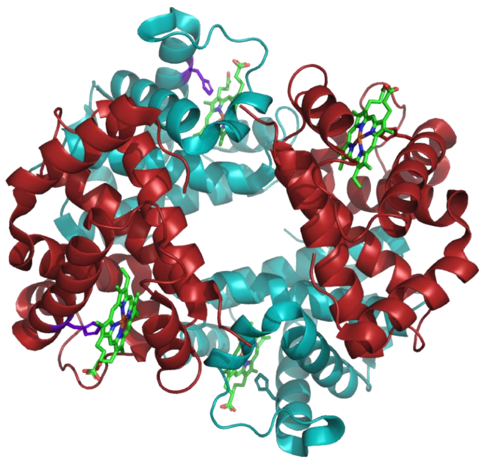


Peroxidase

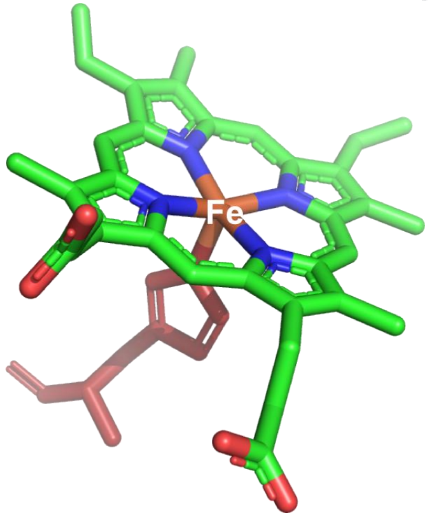


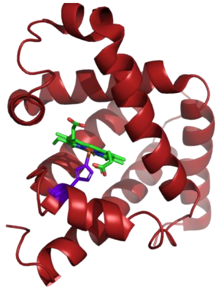
Cytochrome P450

Hemoglobin: structure-function relationship



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

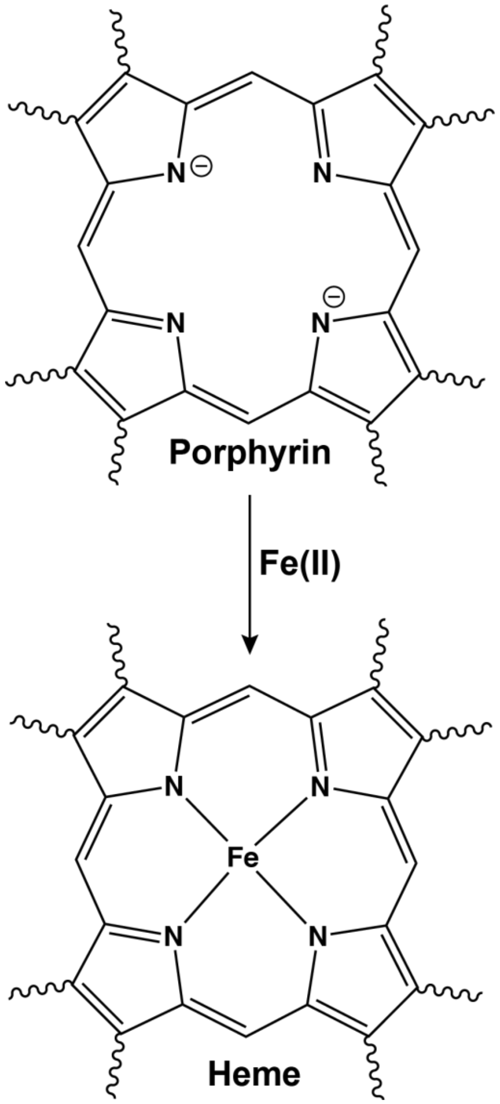




Myoglobin (Mb)
Monomeric structure

Fe-containing heme core

Analogous to catalase, peroxidase, cyt-P450



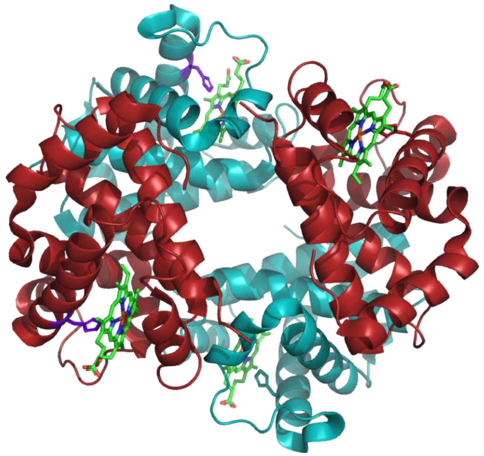
Porphyrins are macrocyclic tetrapyrrole ligands

Variation in the substituents on porphyrin tunes its electronic properties

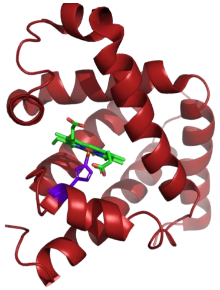
Creates a planar N_4-coordination for metal binding

Anchored to protein via axial ligation

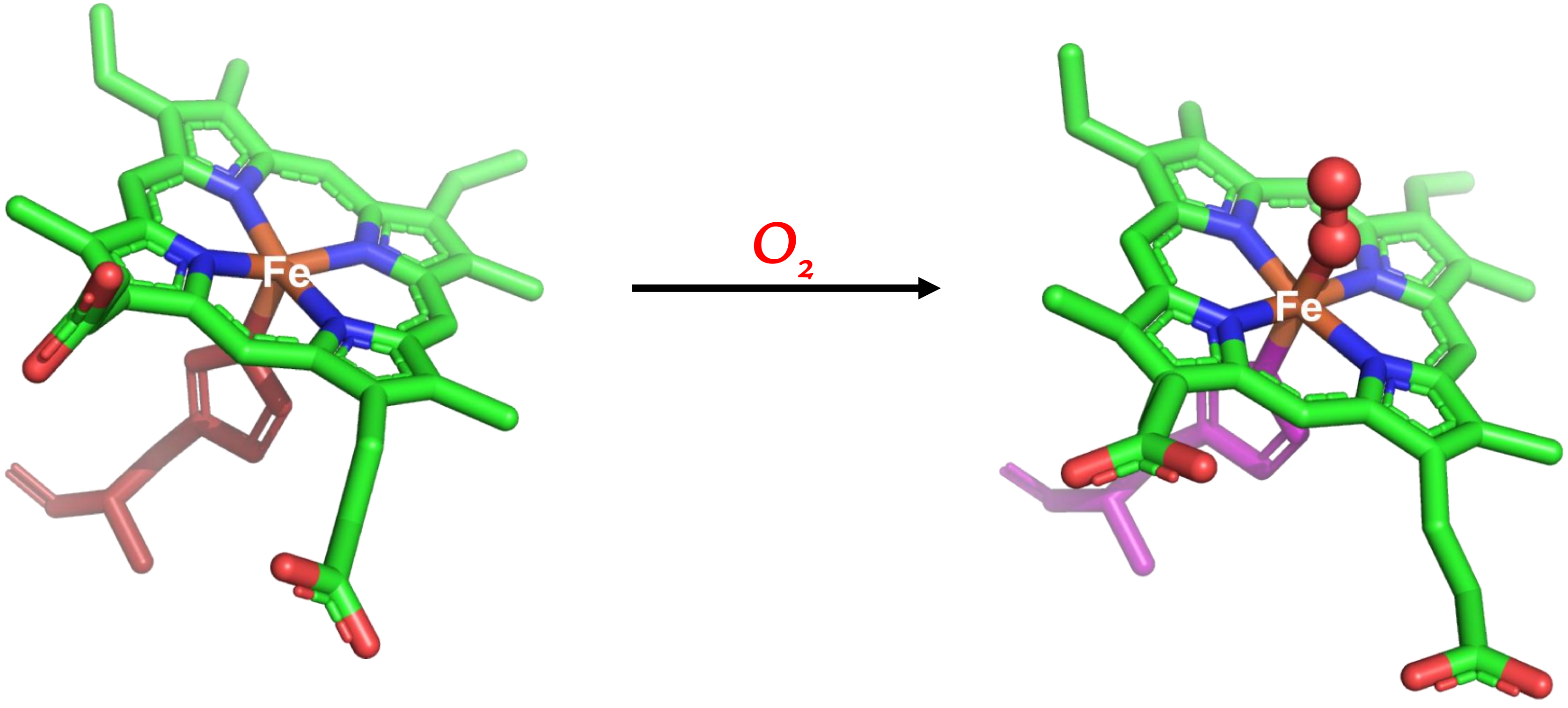
Hemoglobin: structure-function relationship



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure



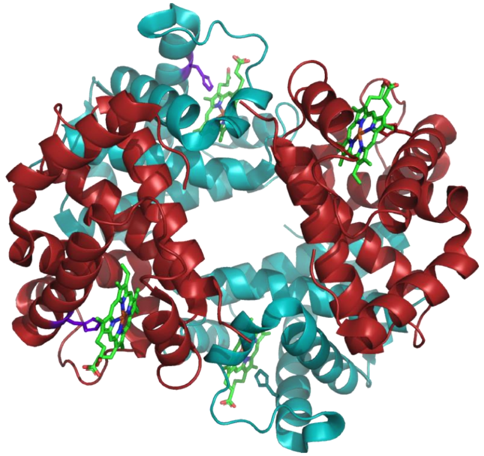
Myoglobin (Mb)
Monomeric structure



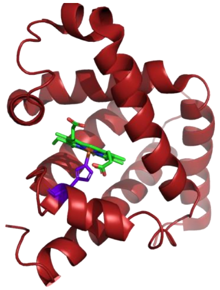
Heme core-originated O_2-binding reactivity

Each heme core binds to one molecule of O_2

Hemoglobin: structure-function relationship



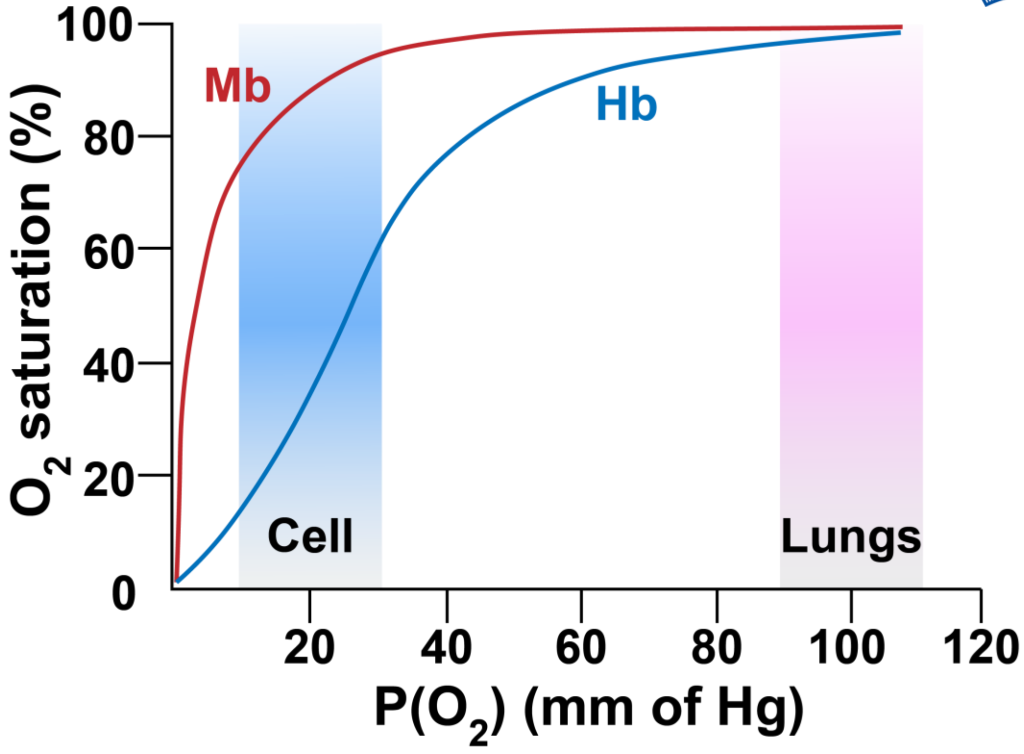
Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure



Myoglobin (Mb)
Monomeric structure

Hb can bind 4 O_2

Mb binds only one O_2



Mb (Deoxy) + O_2 $\longrightarrow$ Mb-O_2 (oxy)

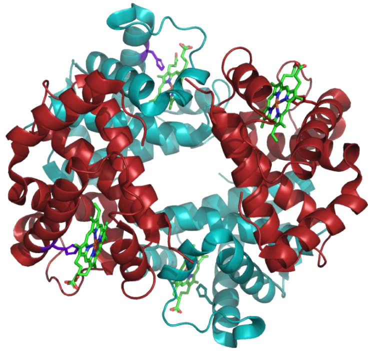
Hb (Deoxy) + xO_2 $\longrightarrow$ Hb-$(O_2)_x$(oxy)

Not all the O_2 binds at the same time to four hemes at Hb

x = 1-4

Binding of O_2 in one subunit of Hb affect others: <u>Cooperativity effect</u>

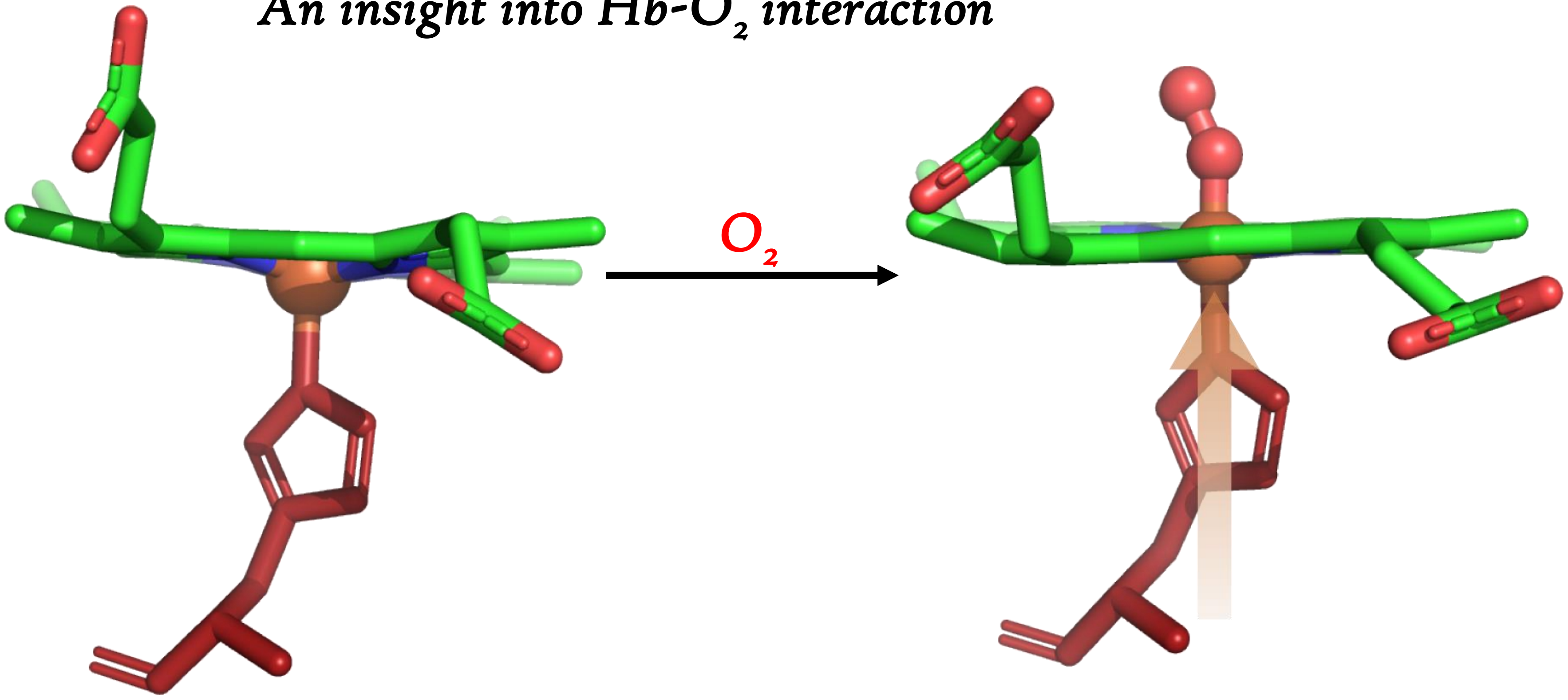
Hemoglobin: Cooperativity Effect



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

O$_2$ binding <u>pulls</u> the Fe center & proximal histidine towards the porphyrin plane

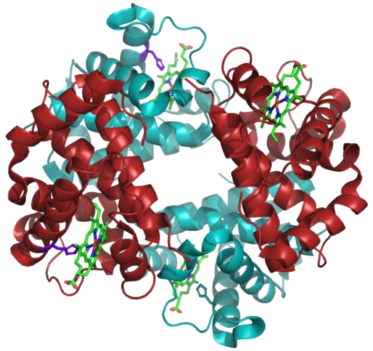
An insight into Hb-O$_2$ interaction



<u>Deoxy-Hb</u>
Fe(II) high-spin system
Too big to fit in the porphyrin cavity
(78 pm)

<u>Oxy-Hb</u>
Fe(III) low-spin system
Smaller size fits better
(55 pm)

Hemoglobin: Cooperativity Effect



Hemoglobin (Hb)
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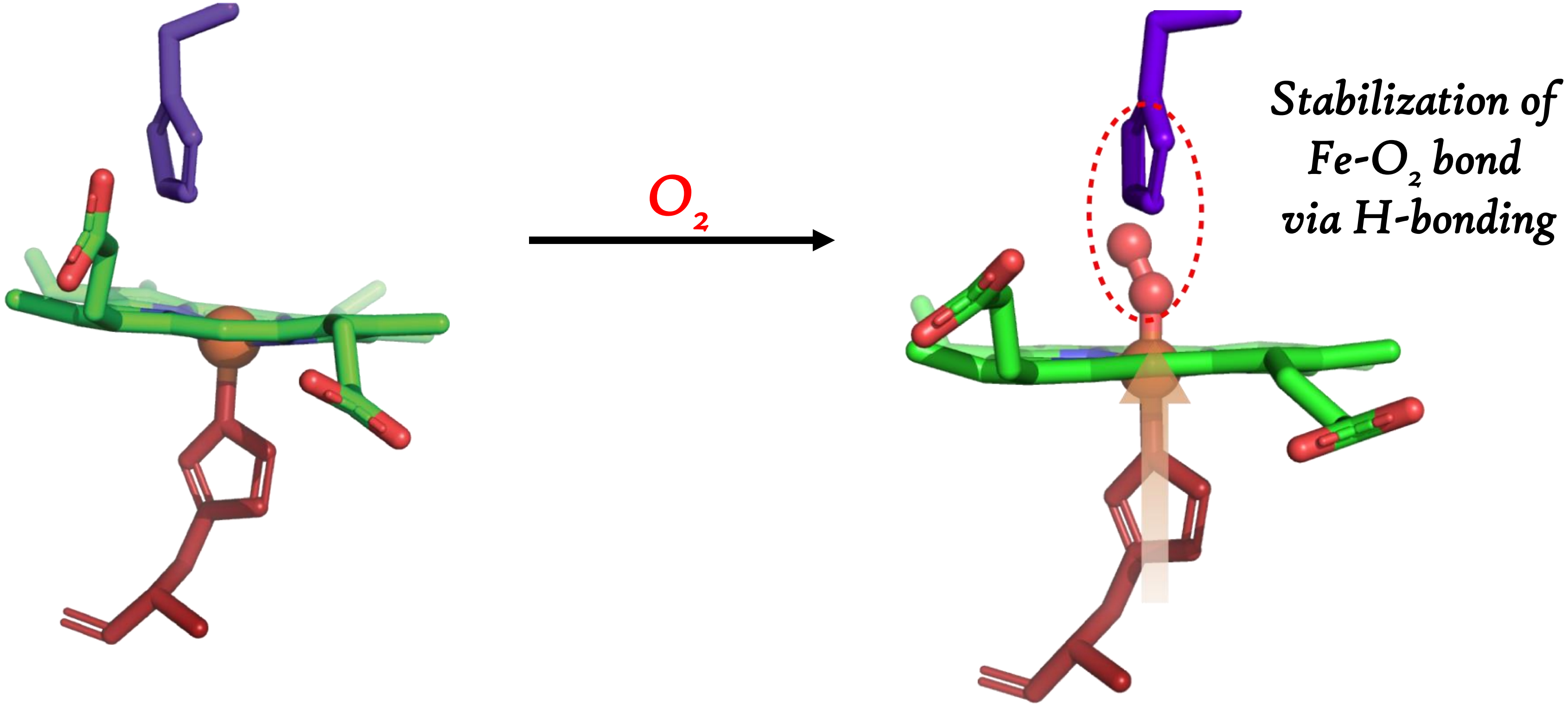

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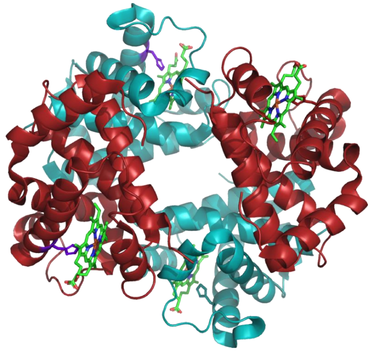
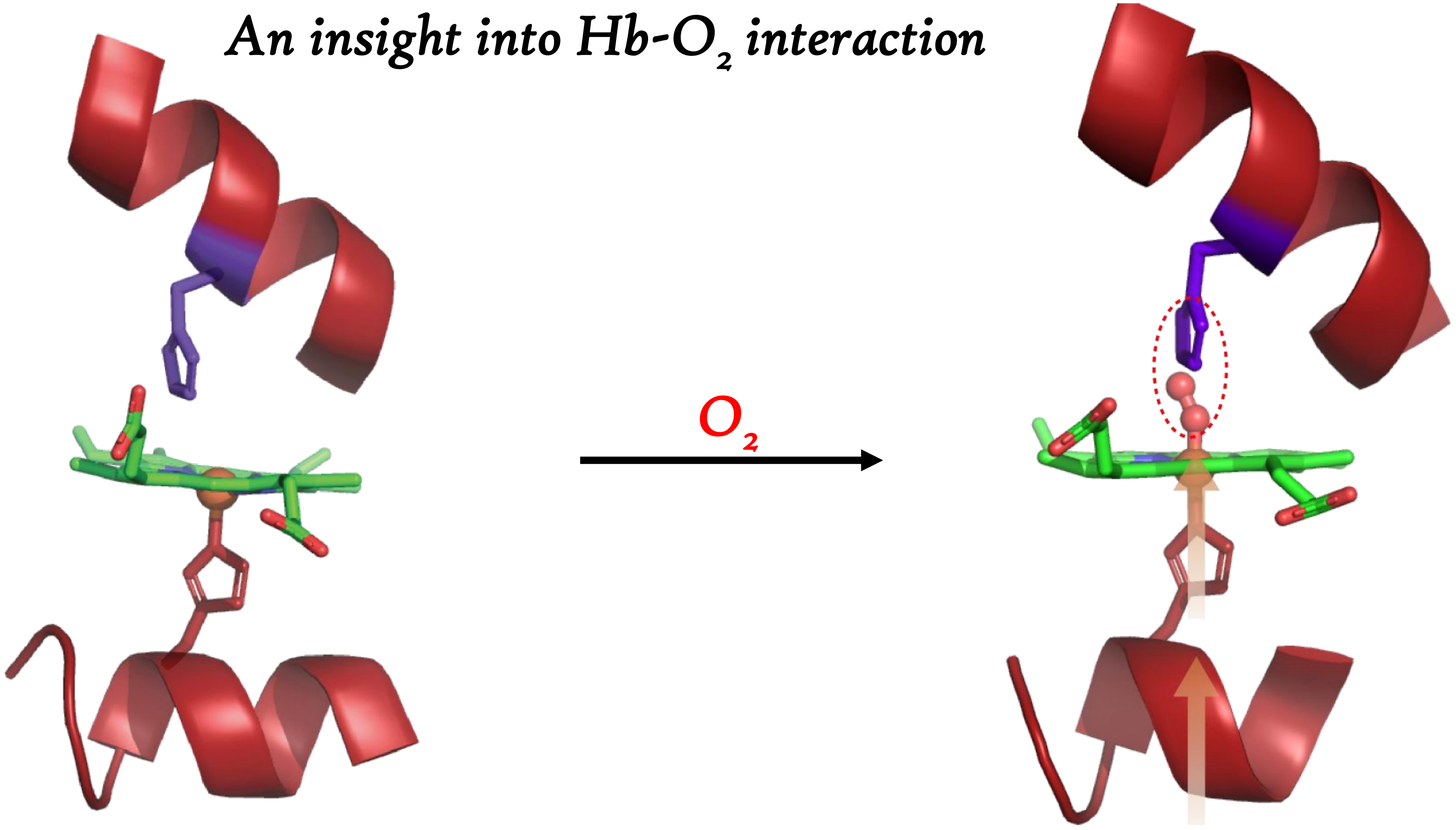
Hemoglobin: Cooperativity Effect



Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric structure

An insight into Hb-O_2 interaction



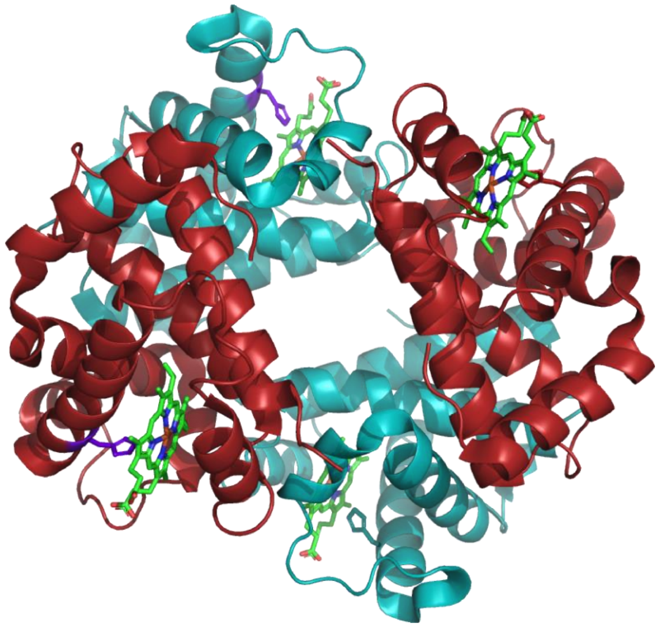
Deoxy-Hb
Fe(II) high-spin system

Oxy-Hb
Fe(III) low-spin system

O_2 binding pulls the Fe center & proximal histidine towards the porphyrin plane

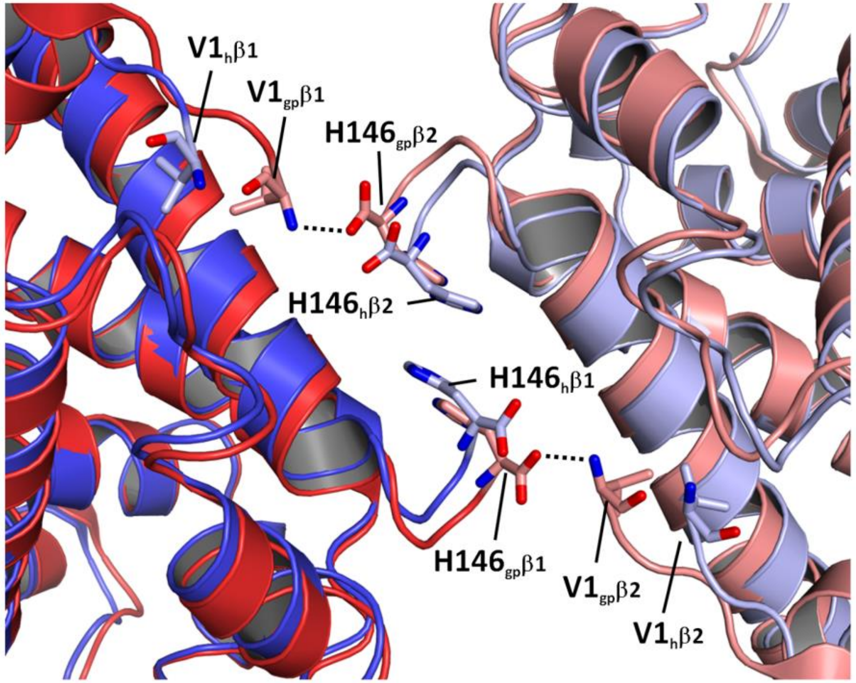
O_2 binding pulls the proximal histidine and the protein scaffold

Hemoglobin: Cooperativity Effect



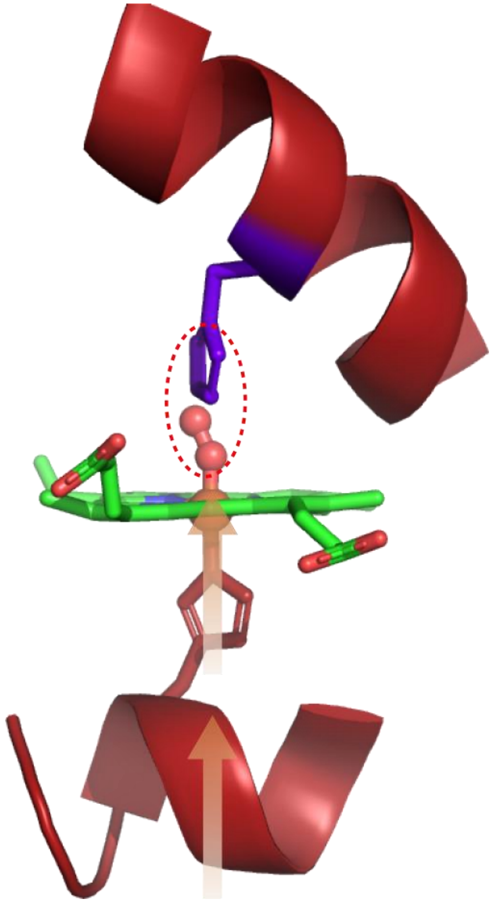
Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric Structure

Hb-O_2 interaction & structural dynamics



doi.org/10.1371/journal.pone.0012389

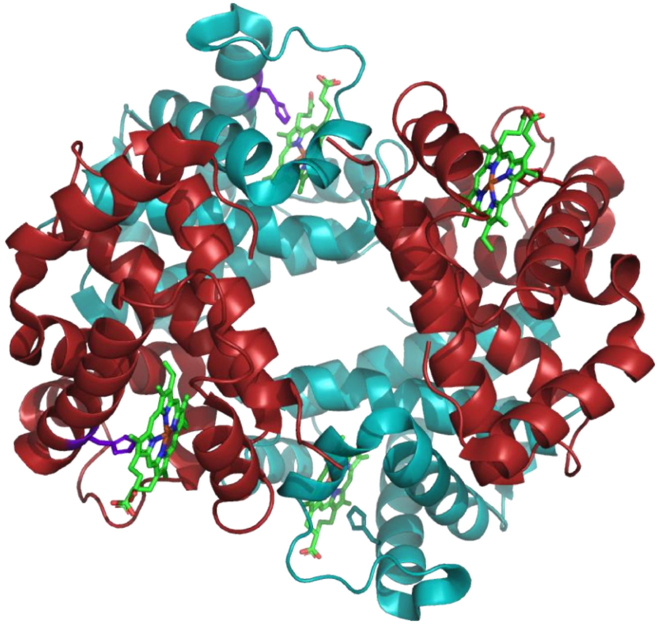
H-bonding & salt-bridge interaction between residues on the subunit borders



Oxy-Hb

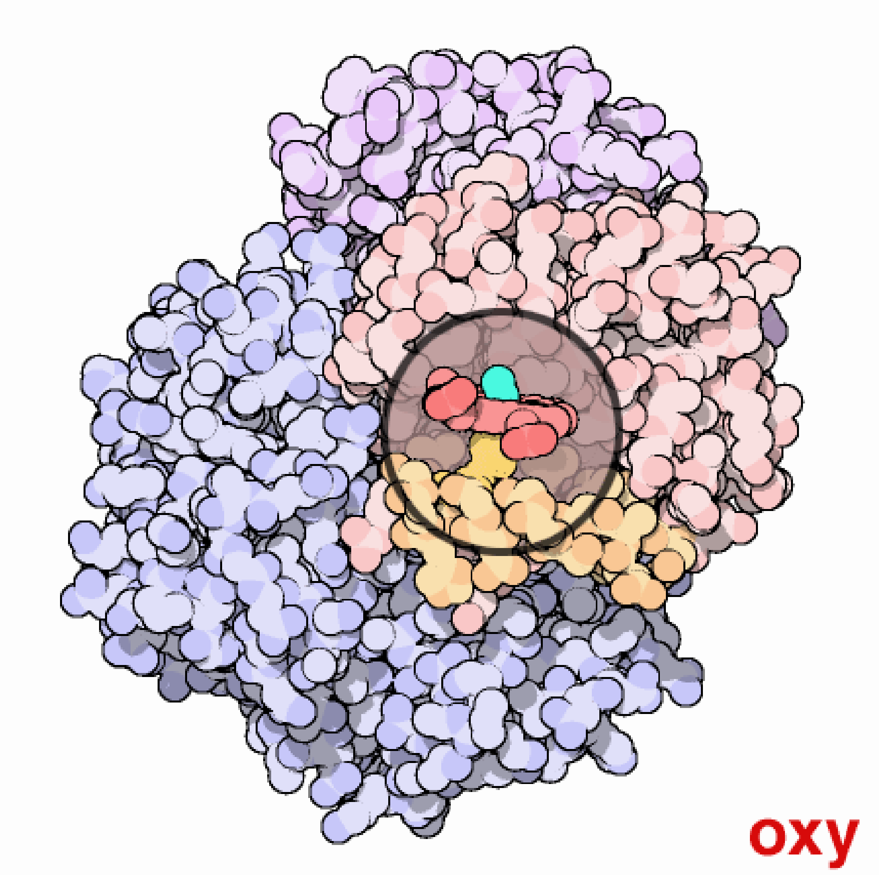
The Hb-O_2 binding pulls off the protein chain and strains the sub-unit interactions

Hemoglobin: Cooperativity Effect

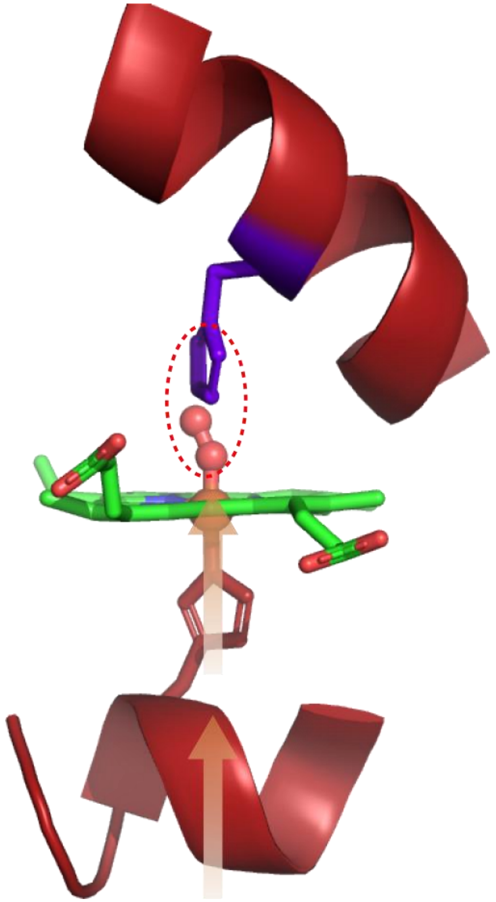


Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric Structure

Hb-O_2 interaction & structural dynamics



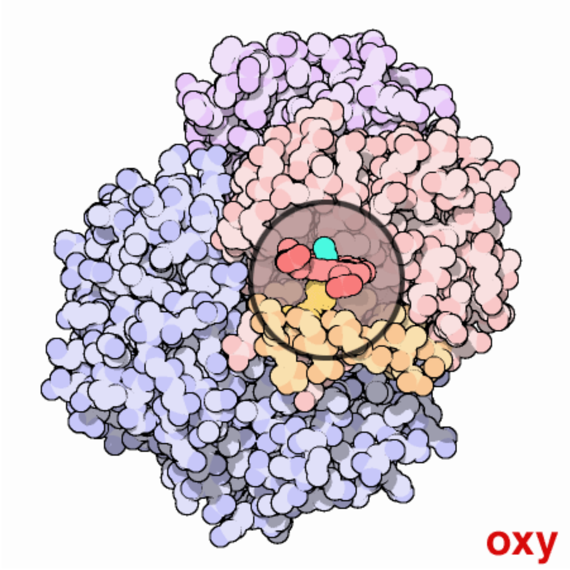
https://pdb101.rcsb.org/motm/41



Oxy-Hb

The Hb-O_2 binding induces fluxionality in Hb structure

Hemoglobin: Cooperativity Effect

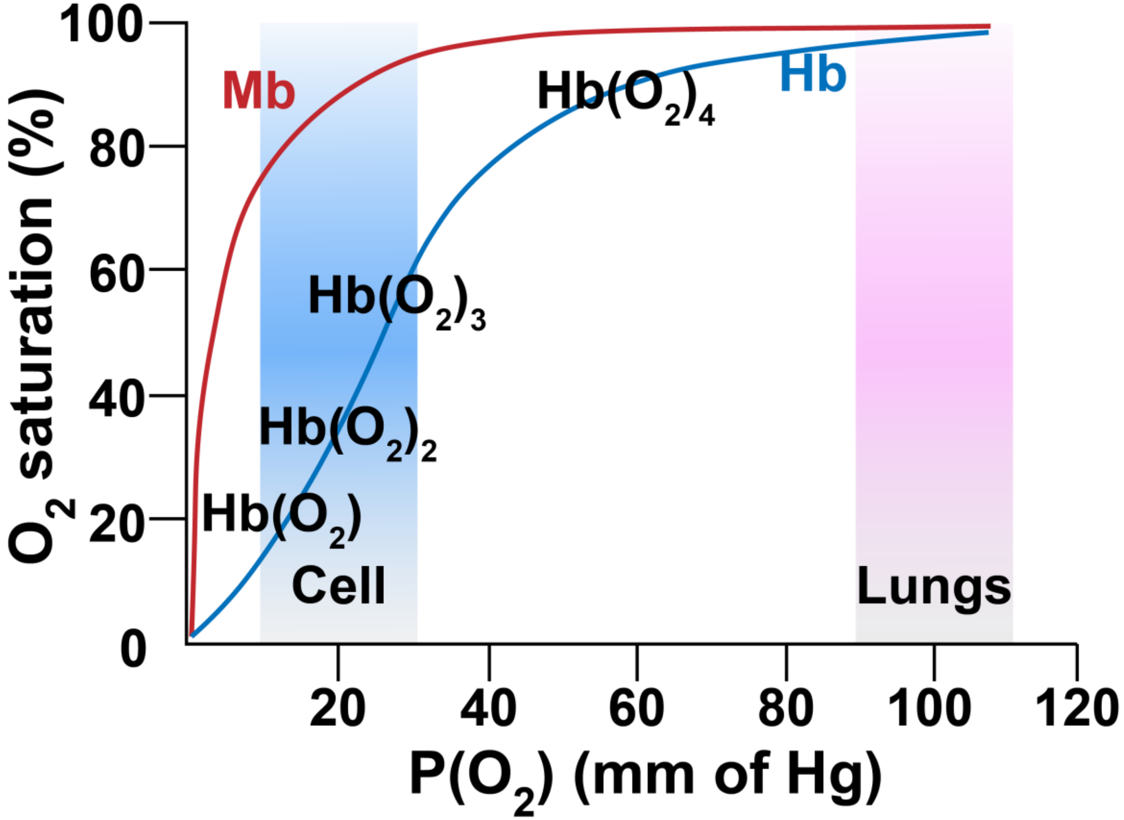


https://pdb101.rcsb.org/motm/41

Hemoglobin (Hb)
$\alpha_2\beta_2$ hetero-tetrameric Structure

Competition between Hb-O_2 binding & breaking of bonding interactions between sub-units

1. *First O_2:* *Unfavorable (Tensed)*
2. *Second O_2:* *Unfavorable (Tensed)*
3. *Third O_2:* *Tilts the bar (Relaxed)*
4. *Fourth O_2:* *Favorable (Relaxed)*

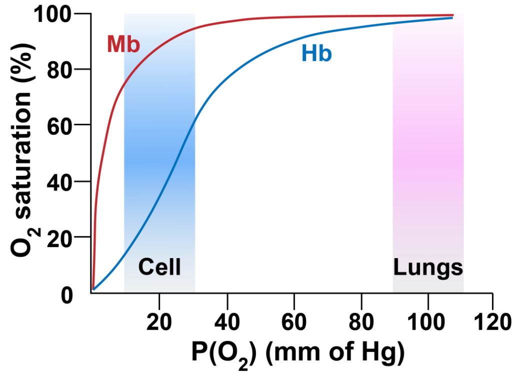


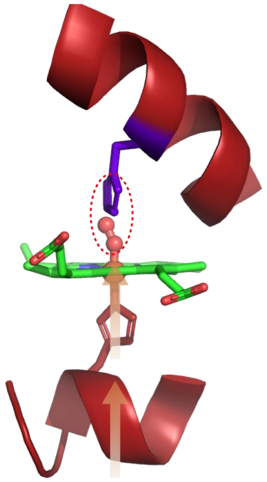
Binding of O_2 to one-subunit effect others: Cooperativity or Allosteric Effect
Only in Hb not in Mb

Take Home Messages

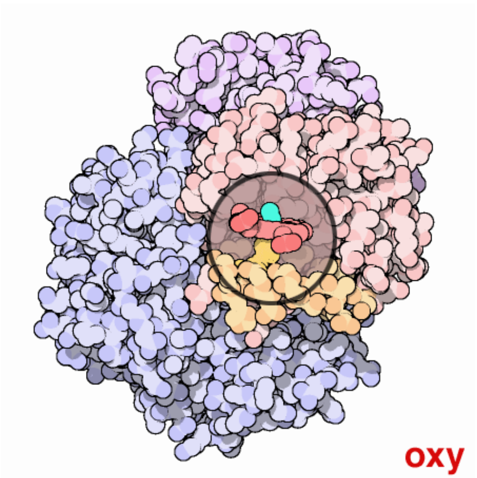
O_2 a key player in Bioinorganic Chemistry

- Differential O_2 binding for Hb & Mb





- O_2-binding to heme pulls Fe-center and proximal histidine towards porphyrin plane
- This movement affects the H-bonding and salt-bridge interactions between subunits in Hb (not in monomeric Mb)
- Cooperativity effect observed in Hb



Outline

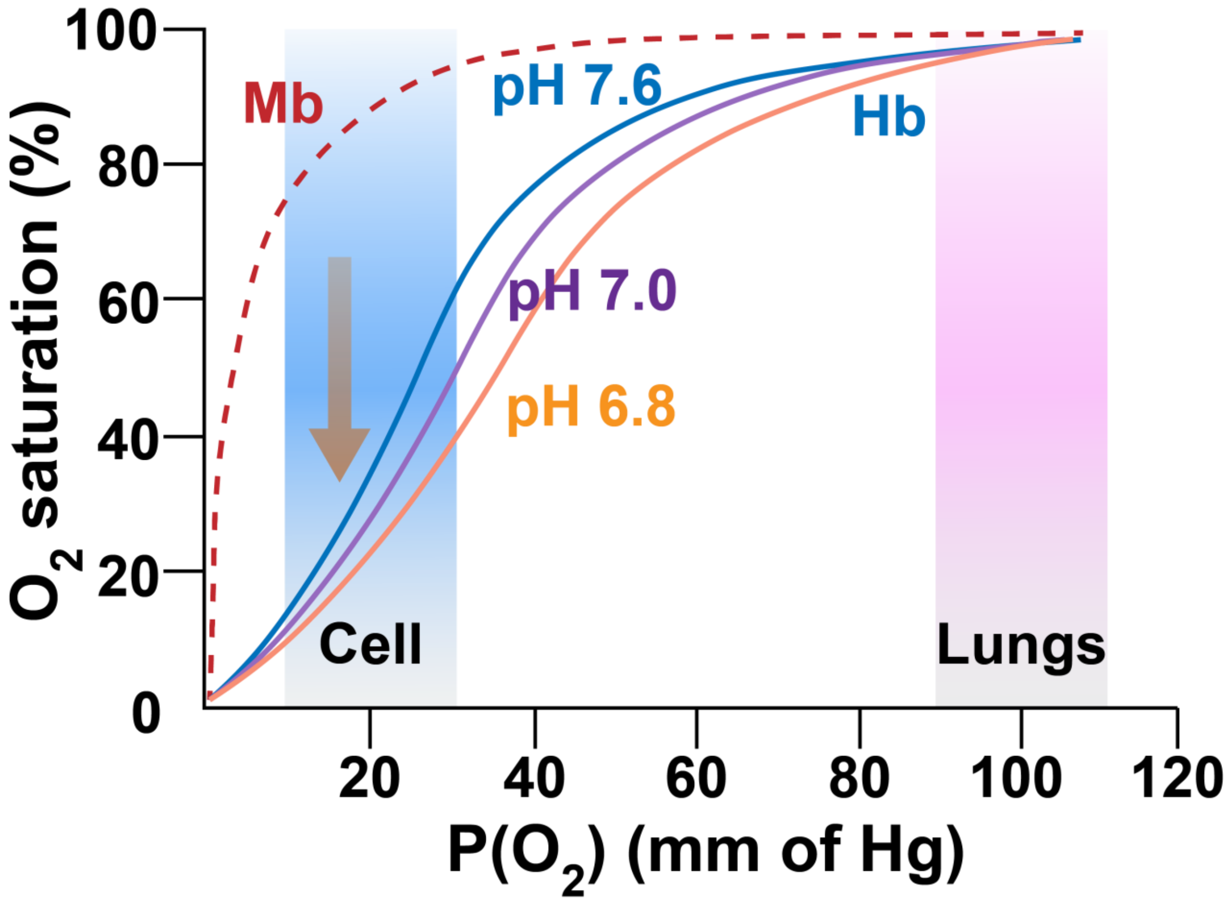
Relevance of Bioinorganic Chemistry

O_2 a key player in Bioinorganic Chemistry

Hemoglobin & reversible O_2 binding

Trivia

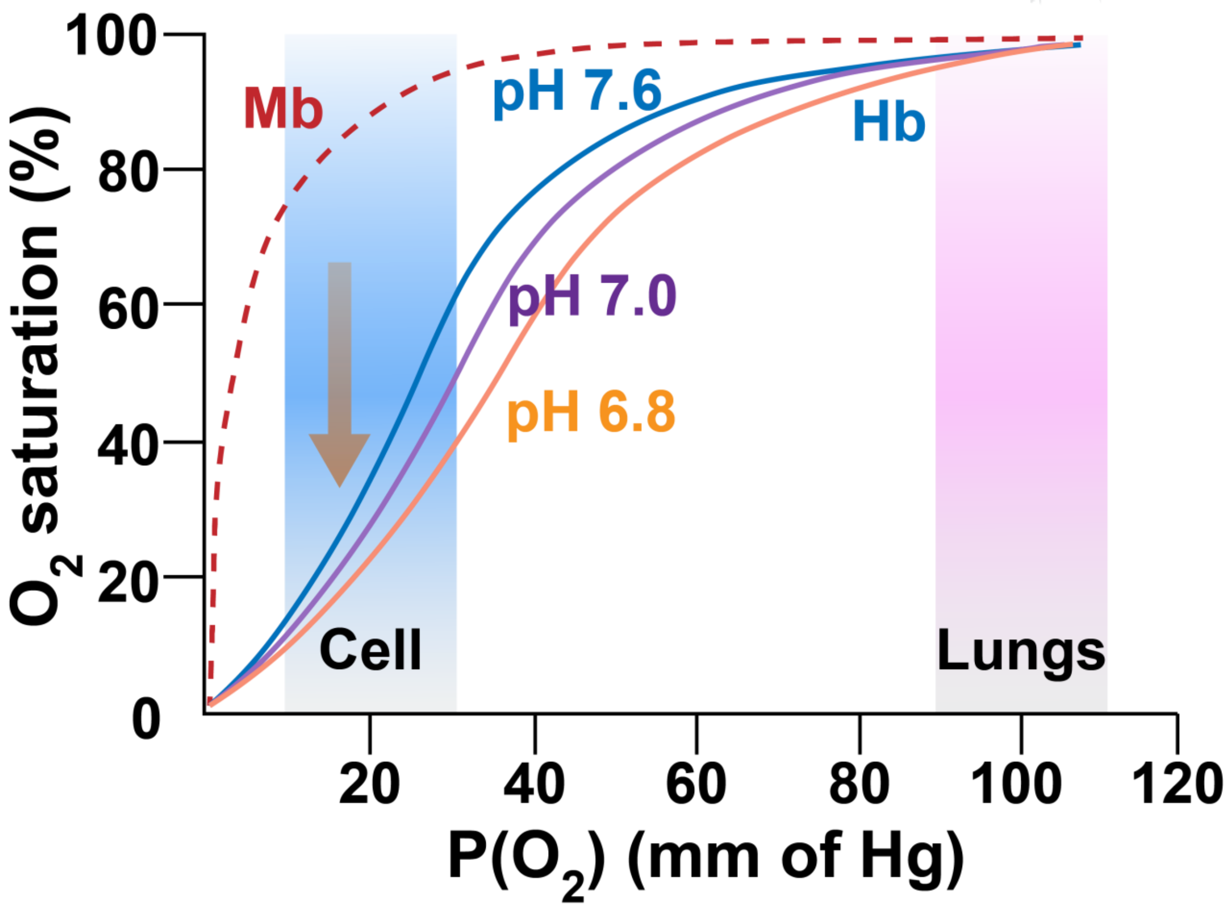
Effect of pH on Hb-O_2 binding



The O_2-binding ability for Hb decreases at lower conc. of O_2 with increasing acidity

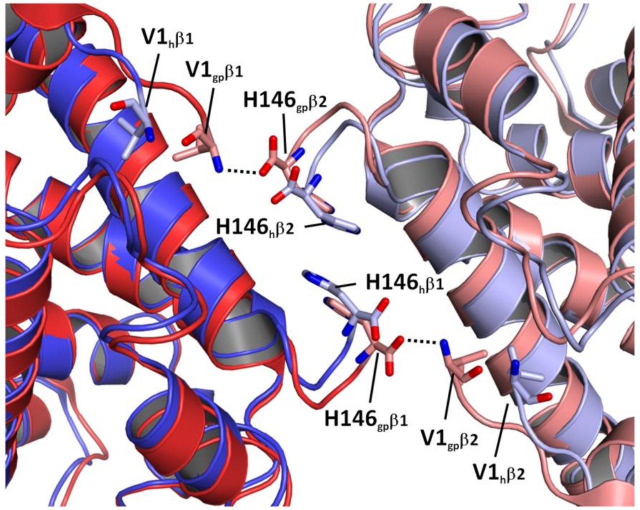
Why such effect?

Effect of pH on Hb-O_2 binding



The O_2-binding ability for Hb decreases at lower conc. of O_2 with increasing acidity

Bohr effect



doi.org/10.1371/journal.pone.0012389

H-bonding & salt-bridge interaction between subunits improves in acidic conditions

Protonation of histidine (pK_a ~7.5)

Needs more energy to break them

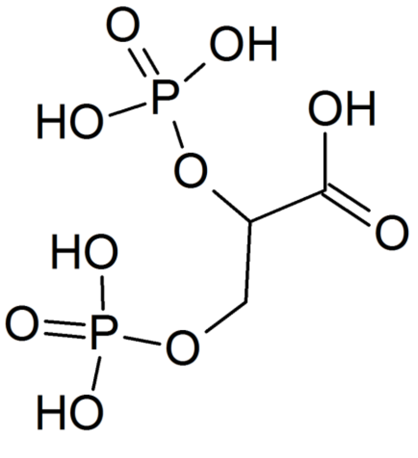
CO_2 rich condition

Better O_2 delivery in fatigued cells

Hb delivers higher amount of O_2 than normal to the cells/tissues after exercise or at higher altitude

Why?

Better O_2 delivery in fatigued cells



2,3-Biphosphoglyceric acid (2,3-BPG)

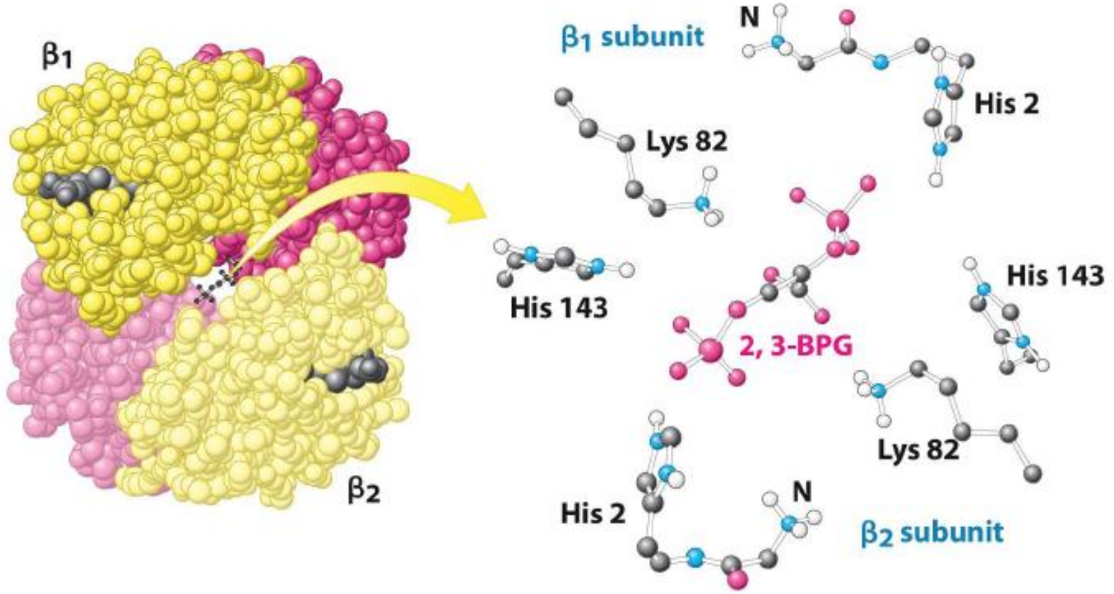


Figure 7.17
Biochemistry, Eighth Edition
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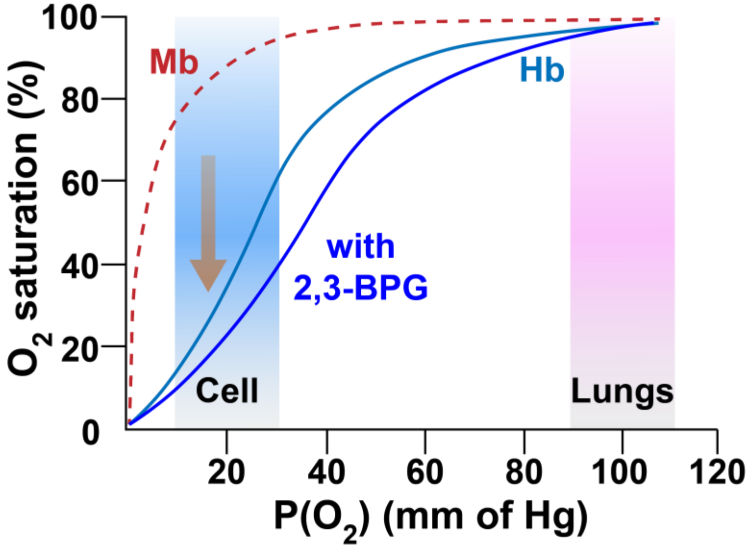


Cells release 2,3-BPG under stress

2,3-BPG facilitate strong interaction between subunits

2,3-BPG is known as an <u>allosteric effector</u>

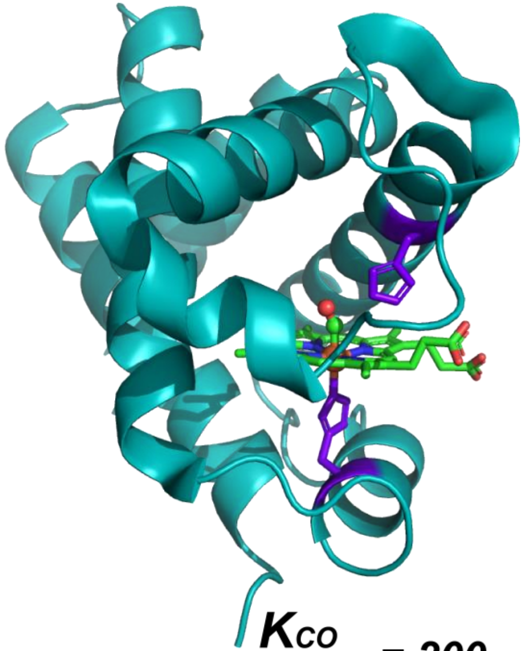
Hb structure saves us from CO poisoning?

In free heme CO binds Fe-center 25000 times strongly compared to O_2

But in Hb, CO binds only 200 times strongly than O_2

Why?

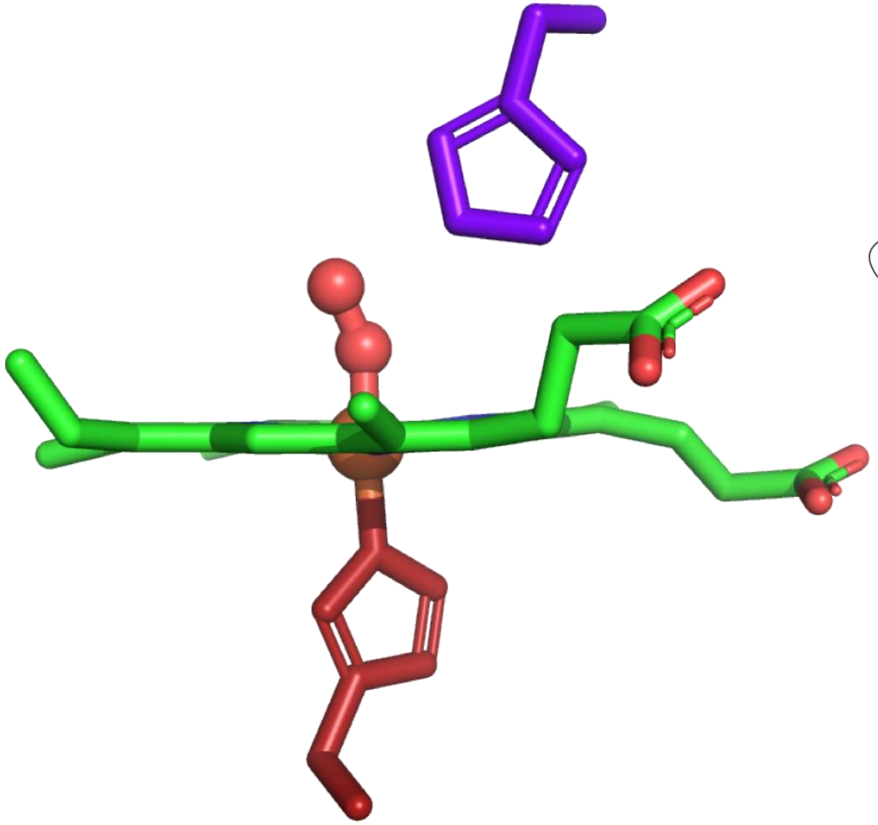
$$\frac{K_{CO}}{K_{O_2}} = 25000$$

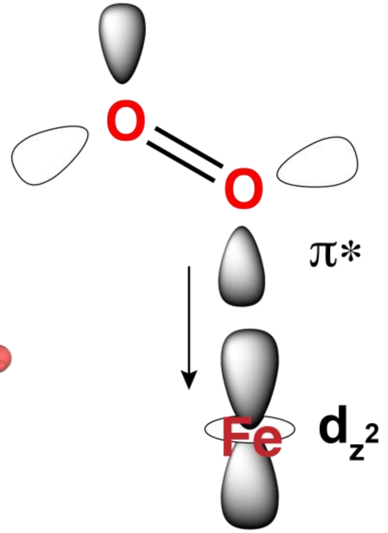


$$\frac{K_{CO}}{K_{O_2}} = 200$$

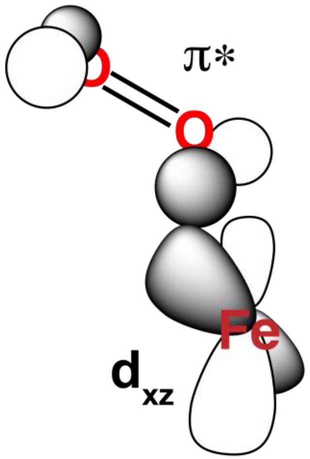
Hb structure saves us from CO poisoning?

Hb binds O_2 in bent mode





σ–interaction

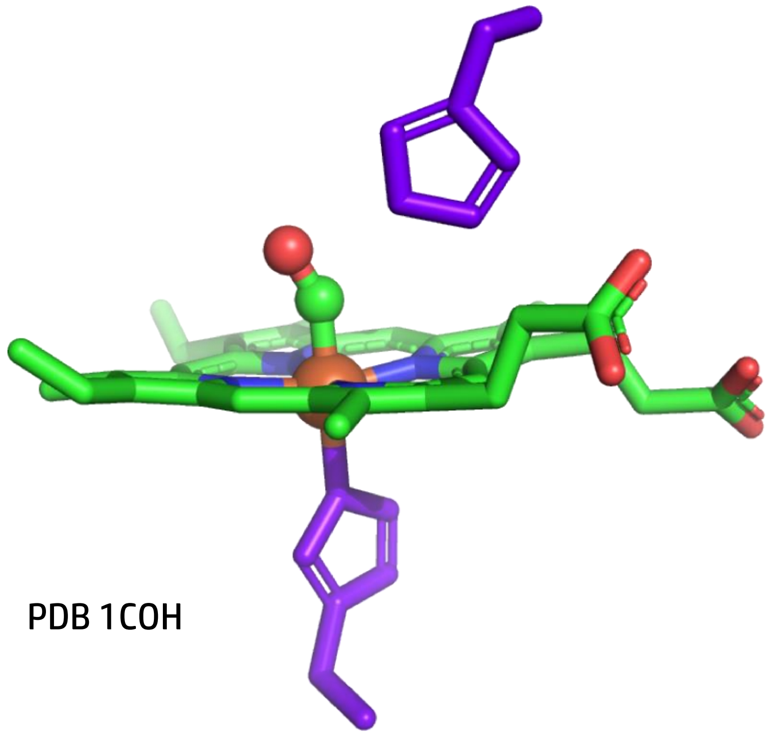


π–interaction

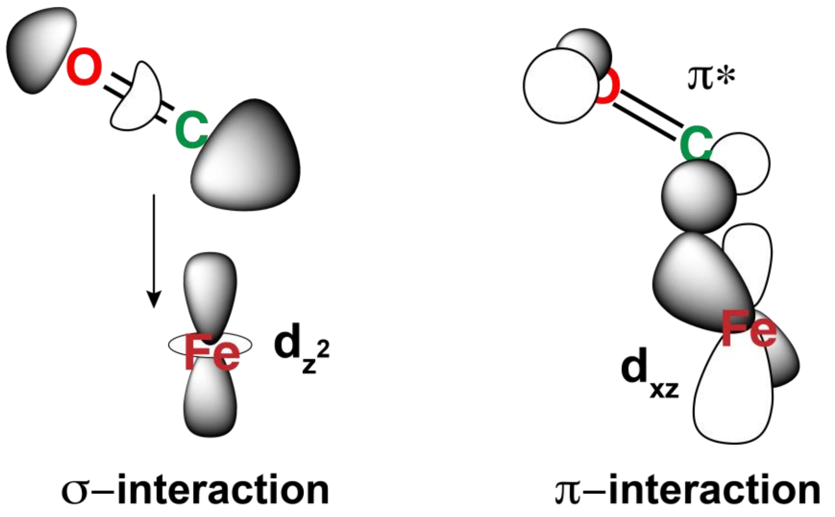
1. *Interaction with distal histidine*
2. *Favored σ- and π-bonding interactions between O_2 π* and Fe d-orbitals*

Hb structure saves us from CO poisoning?

Hb binds CO in bent mode



PDB 1COH



Toxic dose of CO 20-30%
Lethal dose > 60%

1. *Steric interaction with distal histidine*
2. *Disfavored σ - bonding interactions between CO_2 σ and Fe dz^2-orbital*