Porphyrins:

Chemistry and Biology

20.109 Lecture 6

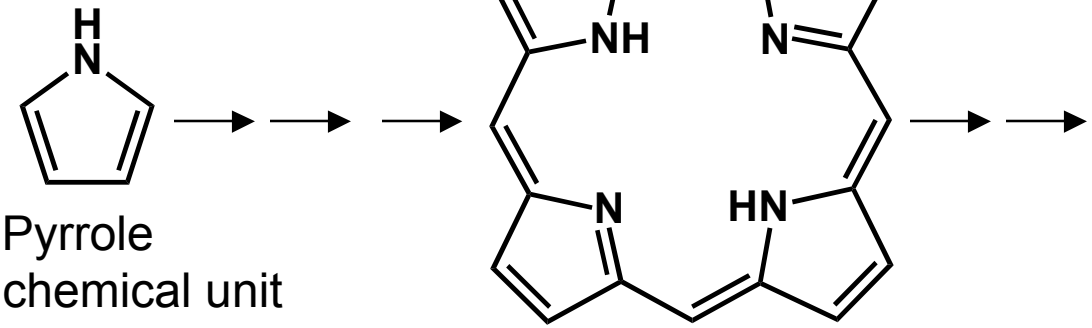
25 February, 2010

Goals

- Explore some of the essential roles heme plays in biology
- Appreciate how Nature has used the same basic cofactor to achieve diverse functions
- Gain basic chemical insight into how the cofactor properties can be tuned by its macromolecular environment

Porphyrin structure

Porphyrins are “tetrapyrroles”



Pyrrole
Basic chemical unit

Porphine
= simplest porphyrin

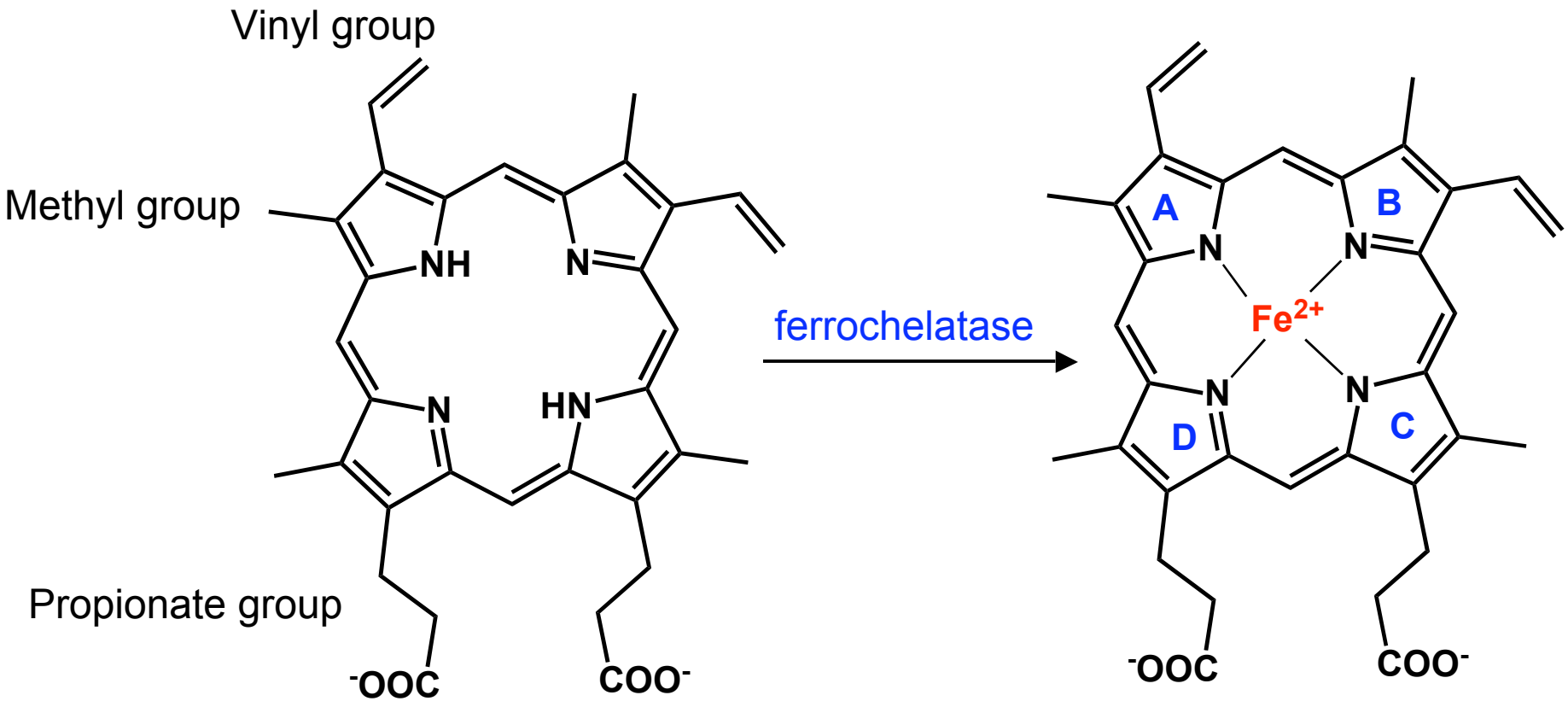
Features distinguishing porphyrins

1. Functional groups elaborated from this basic tetrapyrrole structure;

2. Identity of the coordinated metal ion

Porphyrin structure

A biologically relevant porphyrin

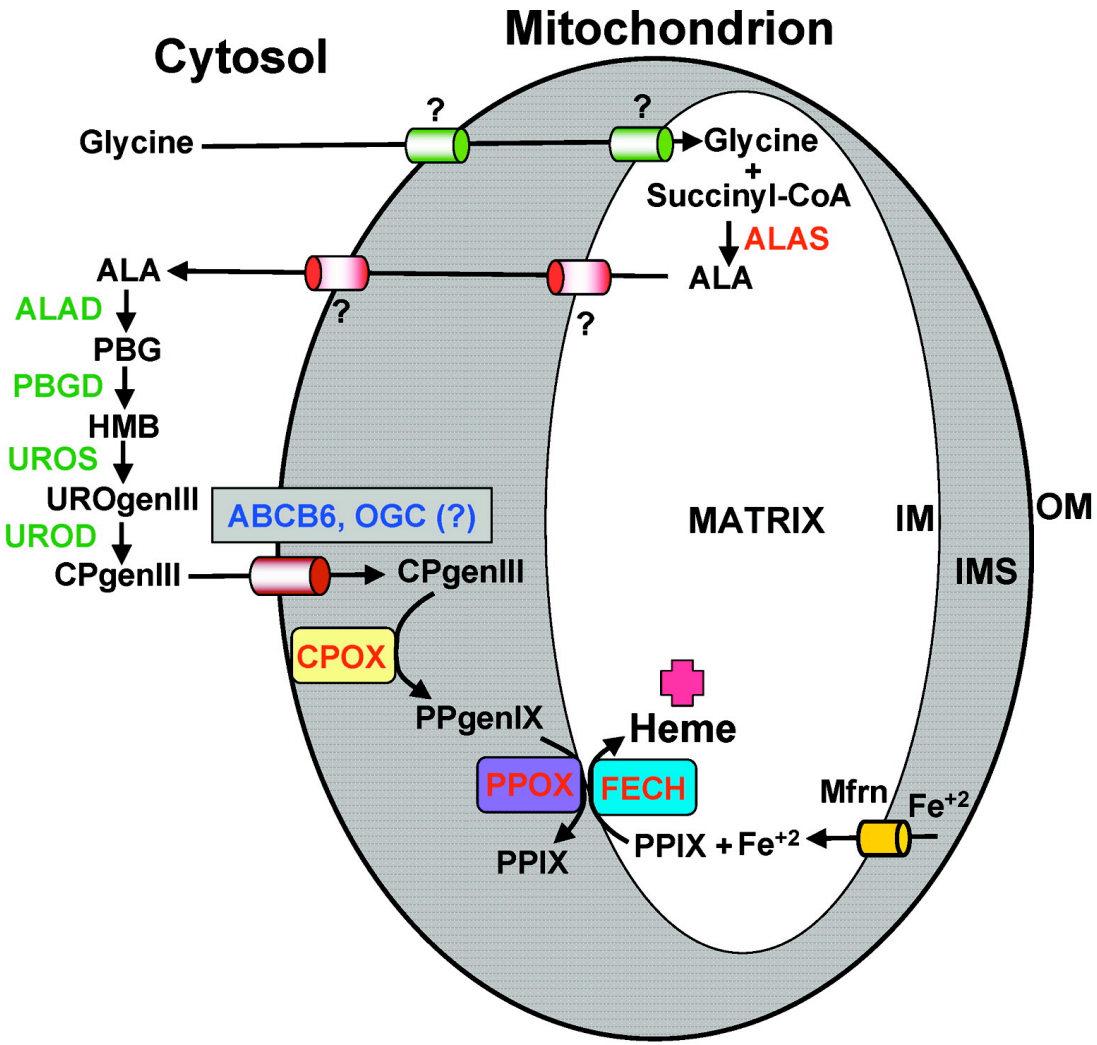


Protoporphyrin IX

Iron protoporphyrin IX (heme b)

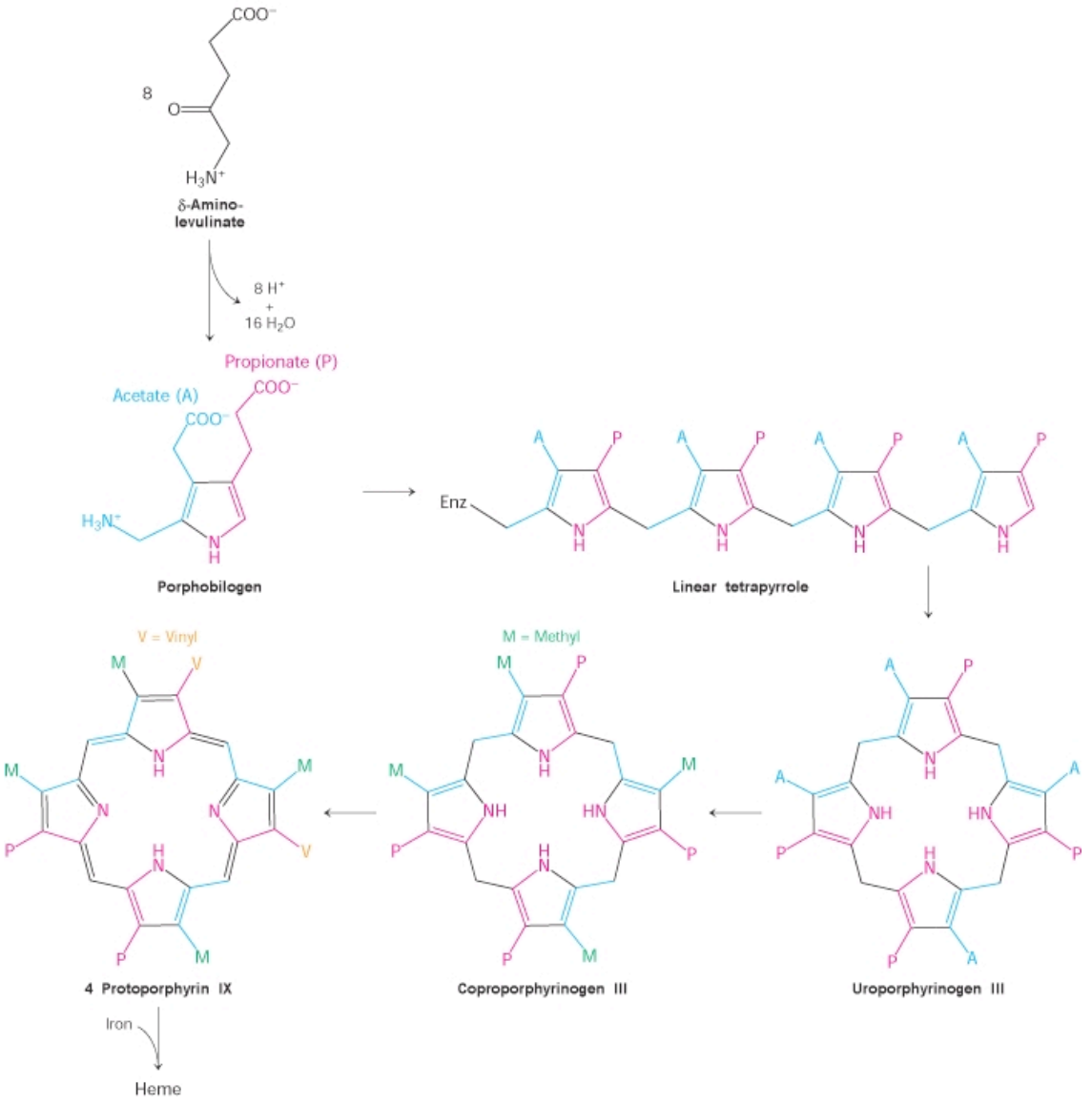
Heme biosynthesis

- Complex, multistep process
  - Several enzymes
    - Mitochondrial
    - Cytosolic
  - Uses amino acid (glycine) and Kreb's cycle intermediate (succinyl CoA) as initial substrates
  - Terminal step involves inserting Fe^{2+} into protoporphyrin IX skeleton to make heme *b*



Severance S. & Hamza I, ***Chemical Reviews***, 109(10):4596-4616 (2009)

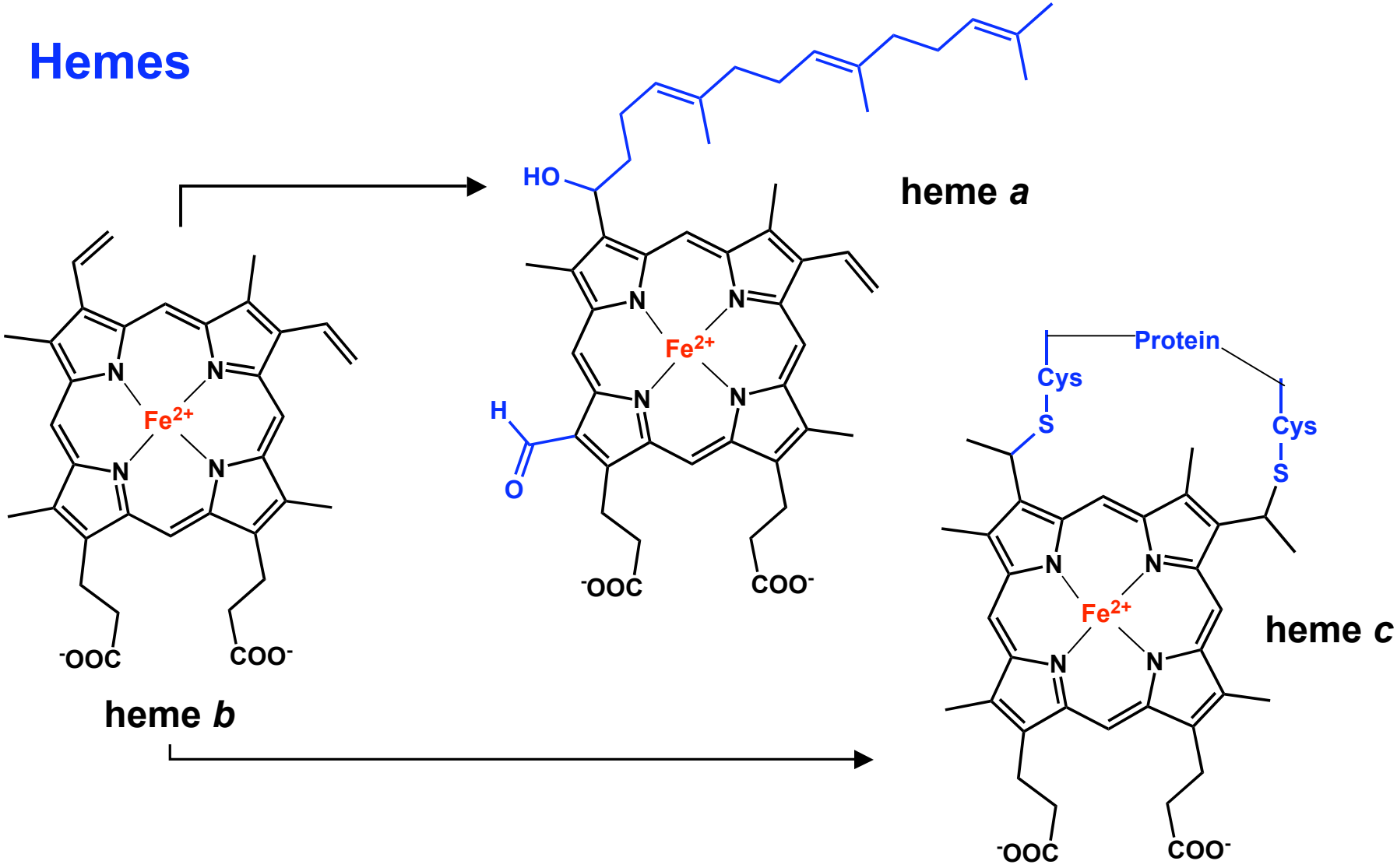
Heme biosynthesis



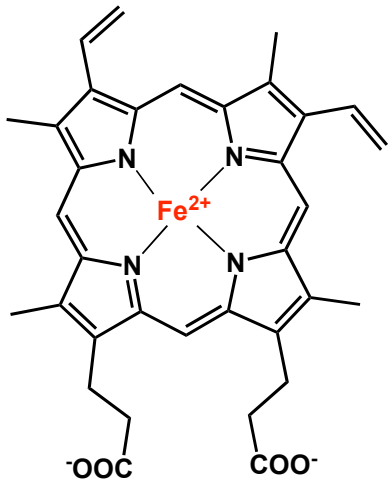
Biochemistry (Stryer, 5th Ed)

Some biologically relevant porphyrins

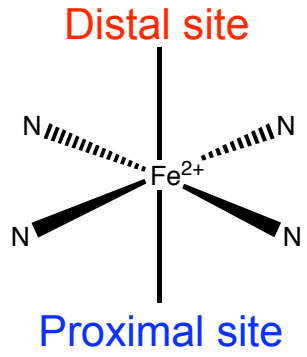
Hemes



Some heme properties correlated with function



heme *b*

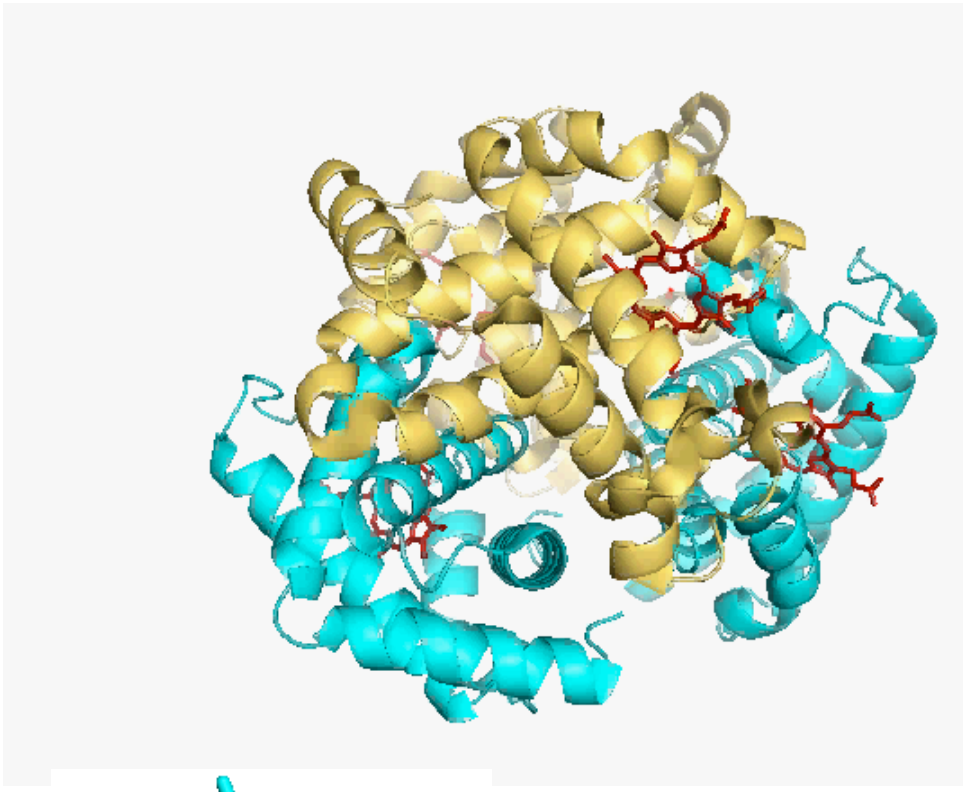


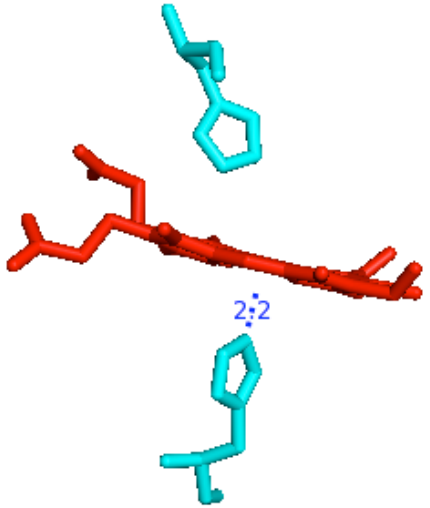
Heme coordination sites

- Resting redox state of iron (Fe^{2+} v. Fe^{3+})
- Affinity for non-protein derived ligand
  - Impacted by iron redox state
  - Some ligands bind Fe^{2+} better than Fe^{3+}
- Identity of the protein-derived ligand
  - Amino acid (e.g. histidine, cysteine, methionine, tyrosine) side chain
- Shape of the heme cofactor

Hemoproteins and their functions

- **Function: Oxygen transport**
- **Hemoglobins**
  - *Non-protein ligand*: O_2
  - *Cofactor*: heme *b*
  - *Resting redox state:* Fe^{2+}
  - *Protein ligand to heme*: histidine
  - Tetrameric protein
    - 2α chains
    - 2 β chains
  - Each monomeric chain binds one heme *b* molecule
    - 4 hemes/tetramer
  - Each heme can bind one O_2 atom

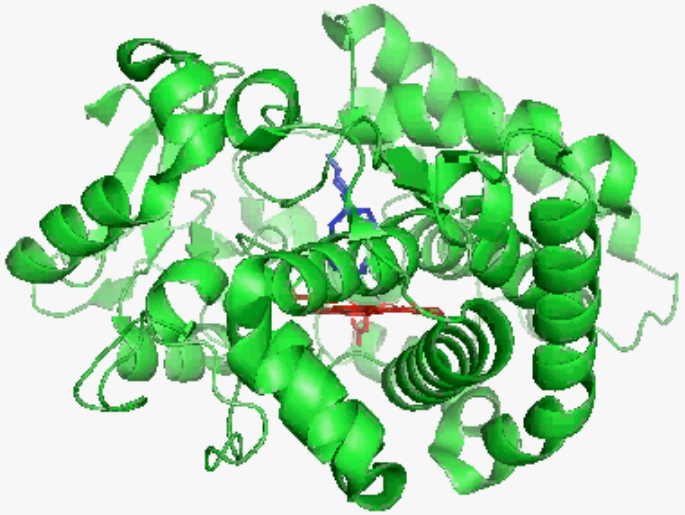




PDB

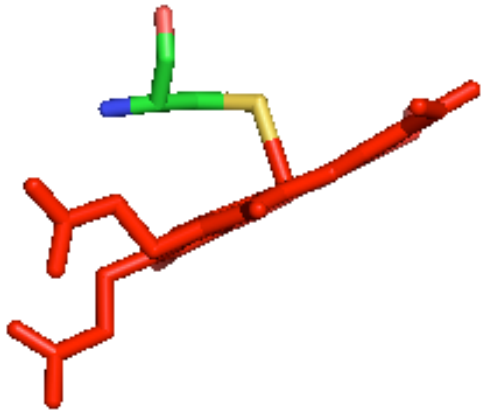
Hemoproteins and their functions

- **Enzymatic activity**
  - **Cytochrome P450s**



- *Non-protein ligand*: O_2 (upon iron reduction to Fe^{2+} during catalytic cycle)
- *Cofactor*: heme *b*
- *Resting redox state:* Fe^{3+}
- *Protein ligand to heme*: cysteine

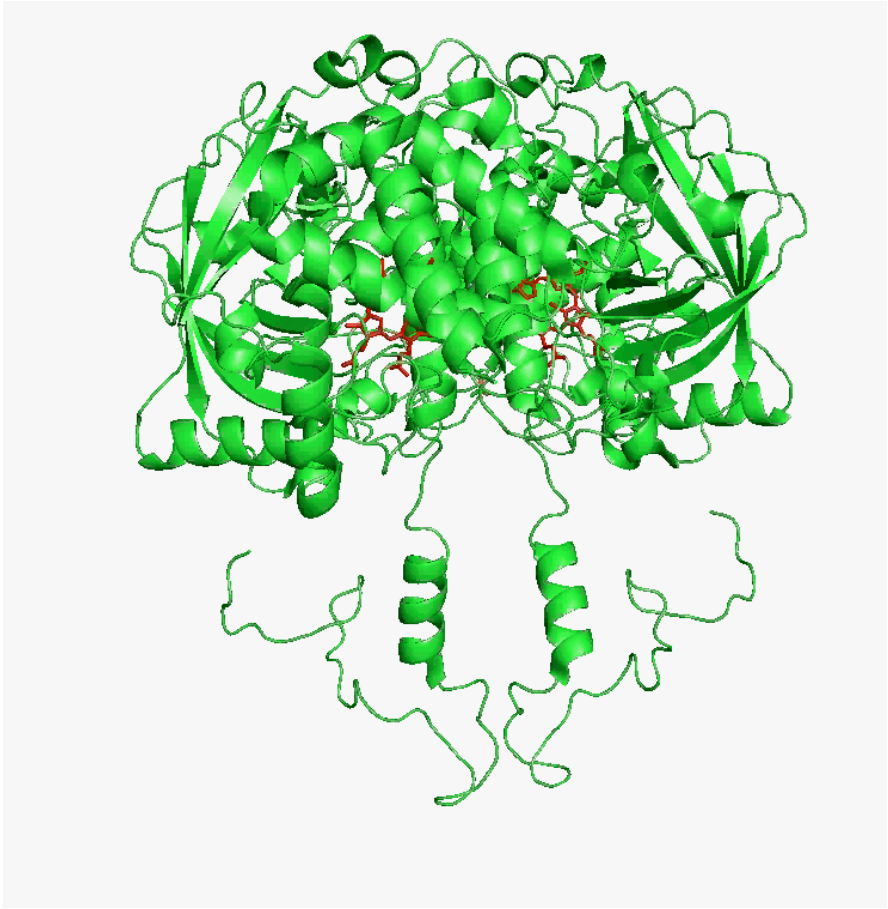
- **Function:**
  - Detoxify xenobiotics = foreign compounds
    - E.g. medications; environmental toxicants
  - Catalyze reactions such as: substrate oxidations



PDB

Hemoproteins and their functions

- **Enzymatic activity**
- **Catalase**:
  - *Non-protein ligand*: H_2O_2
  - *Cofactor*: heme *b*
  - *Resting redox state:* Fe^{3+}
  - *Protein ligand to heme*: tyrosine



- **Function**
  - Protects against hydrogen peroxide-induced oxidative damage
  - Breaks down hydrogen peroxide

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Catalase *from H. Pylori* (PDB accession: 2IQF)

Hemoproteins and their functions

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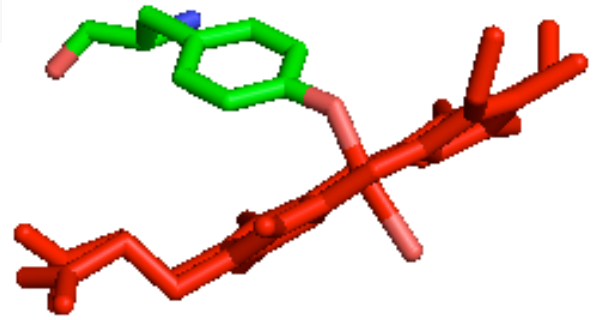
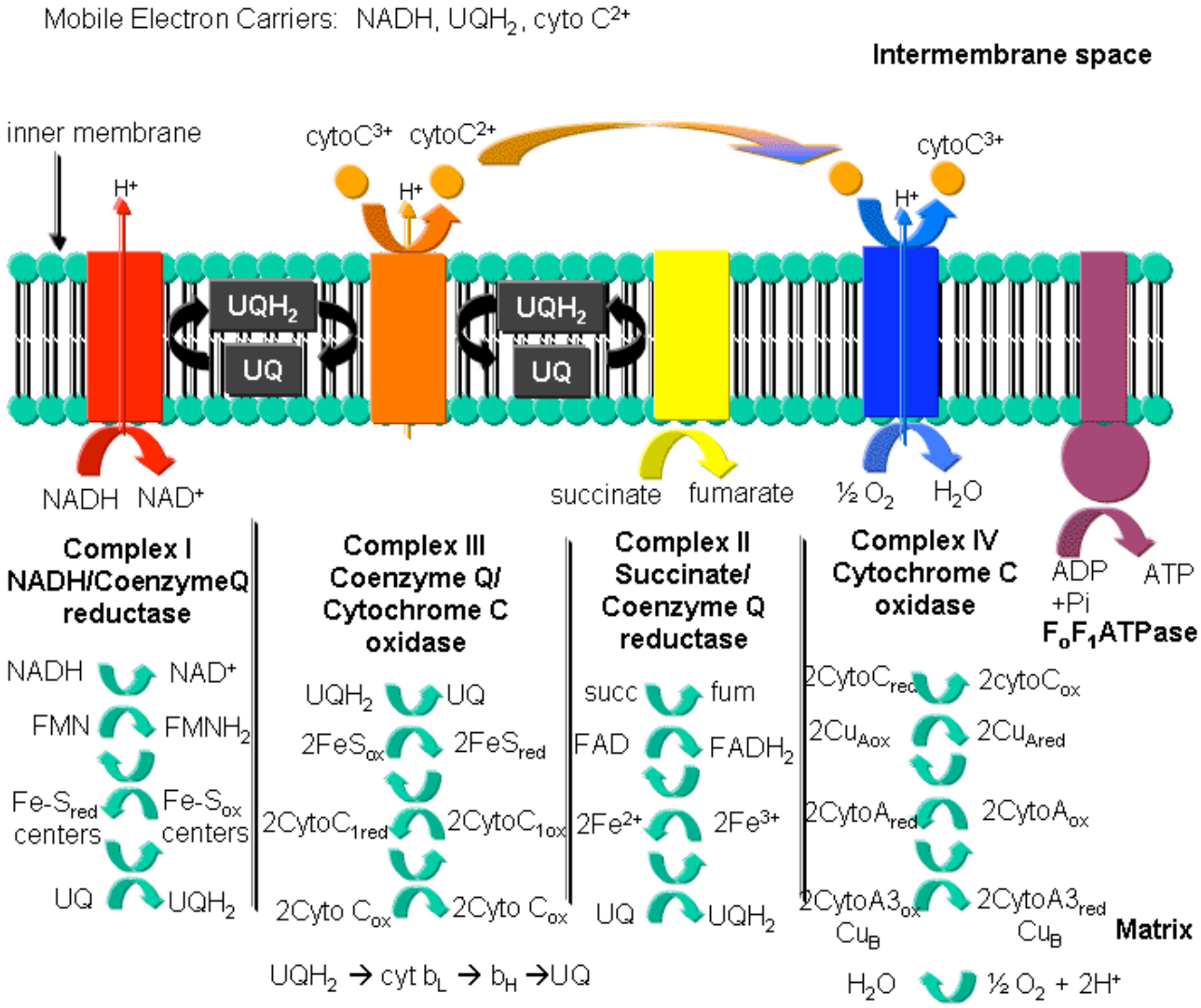


Catalase from H. Pylori (PDB accession: 2IQF)

Hemoproteins and their functions

Electron transport chain: cytochromes



Electron transport summary

Hemoproteins and their functions

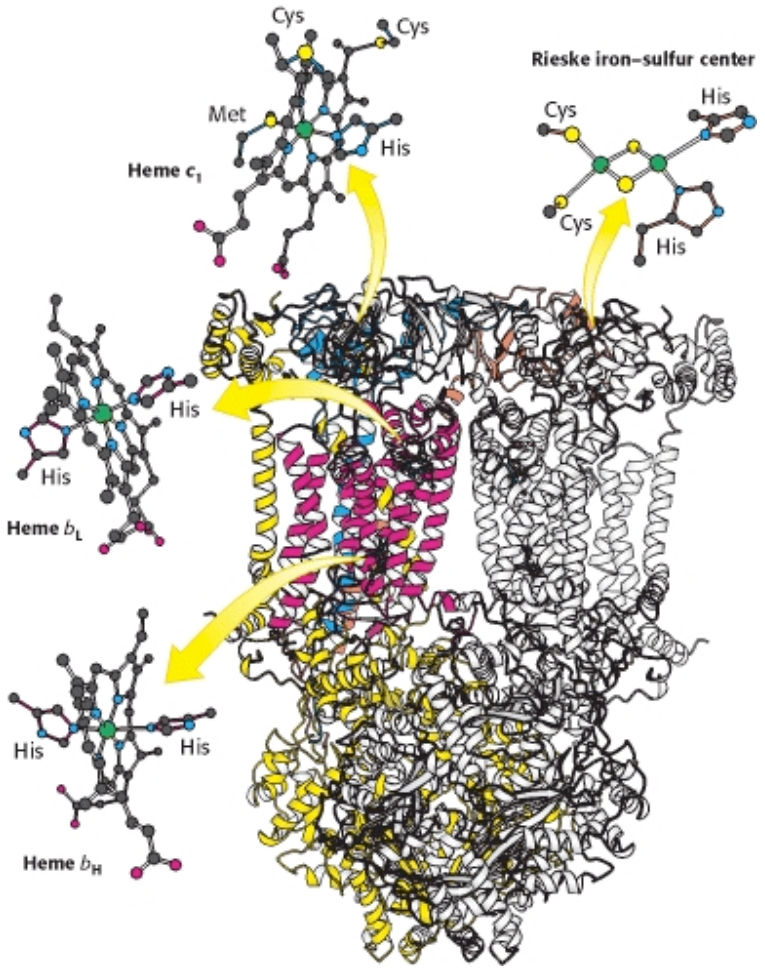
- **Electron transport chain: cytochromes**

- **Cytochrome *bc*1**
  - *Non-protein ligand*: None
  - *Cofactors*: 2 heme *b* + *1 heme* c
  - *Resting redox state:* Fe^{3+}
  - *Protein ligand to heme*: 2 histidines

- **Function**

  - Electron transfer (*not O_2 binding*) is the main function of the heme

  - Bis-histidyl ligation prevents ligand binding



Cytochrome c oxidoreductase
(Complex III)

Hemoproteins and their functions

- **Electron transport chain: cytochromes**

- **Cytochrome *c***
  - *Non-protein ligand*: None
  - *Cofactors*: *1 heme* c
  - *Resting redox state:* Fe^{3+}
  - *Protein residue binding heme*: 2 histidines

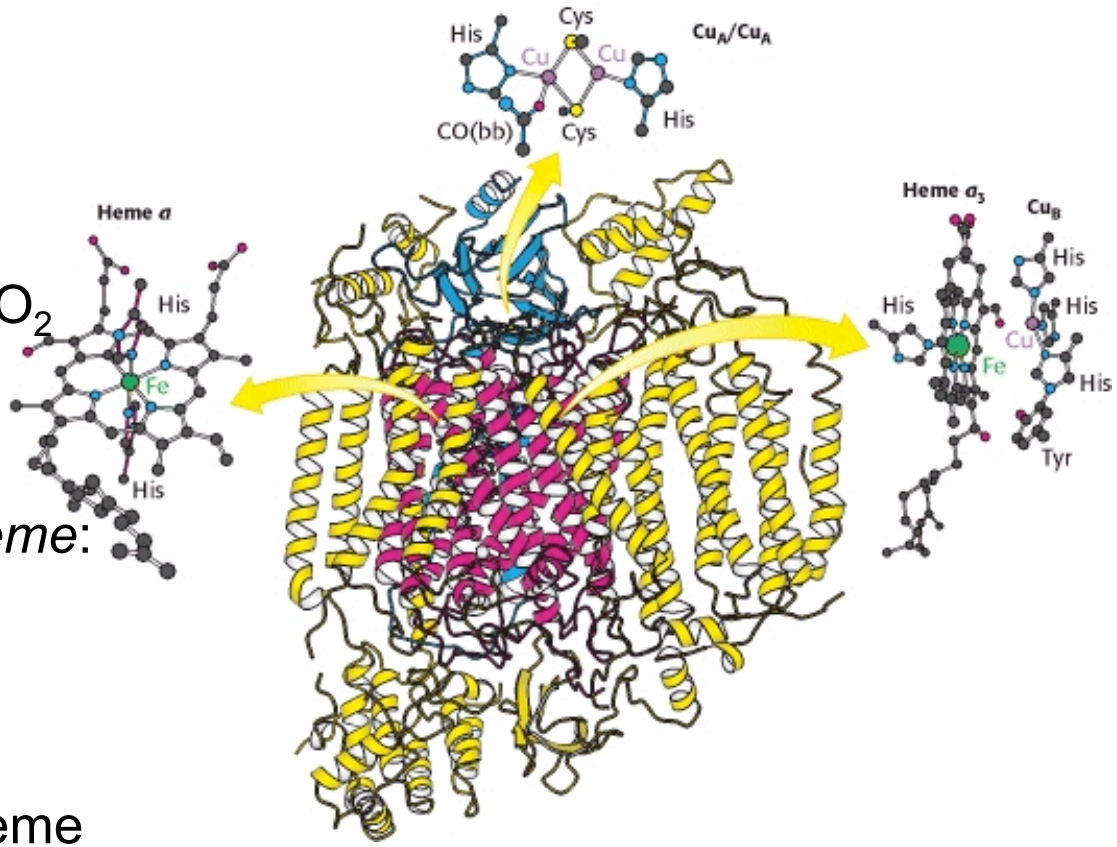
- **Function**:
  - Electron transfer
    - Shuttles electrons from Complex III to Complex IV
    - Bis-histidyl ligation excludes non-protein ligand binding

Hemoproteins and their functions

- **Electron transport chain: cytochromes**

- **Cytochrome *c* oxidase**
  - *Non-protein ligand*: None/O_2
  - *Cofactors*: *2 heme a*
  - *Resting redox state:* Fe^{3+}
  - *Protein residue binding heme*: 1 or 2 histidines

- **Function**
  - Electron transport only (heme *a* – *2 histidine ligands*)
  - Electron transport **AND** O_2 reduction (heme *a*3 – one histidine ligand)

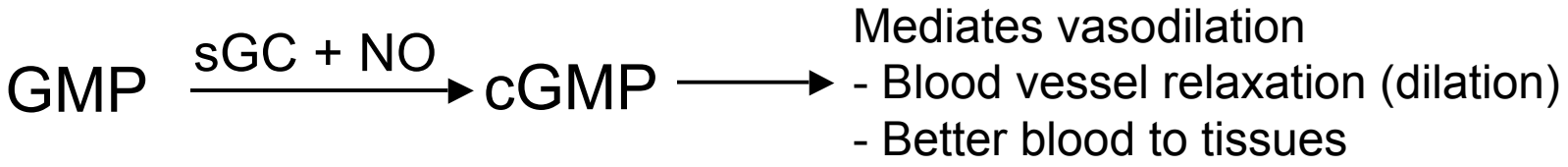


Cytochrome c oxidase (Complex IV)

Hemoproteins and their functions

- **Allosteric regulation of enzymatic activity:**
- **Soluble guanylate cyclase (sGC)**
  - *Non-protein ligand*: NO (nitric oxide)
  - *Cofactors*: *heme b*
  - *Resting redox state:* Fe^{2+}
  - *Protein residue binding heme*: 1 histidine

- **Function**
  - NO binding to heme stimulates sGC activity



Summary of heme cofactor properties

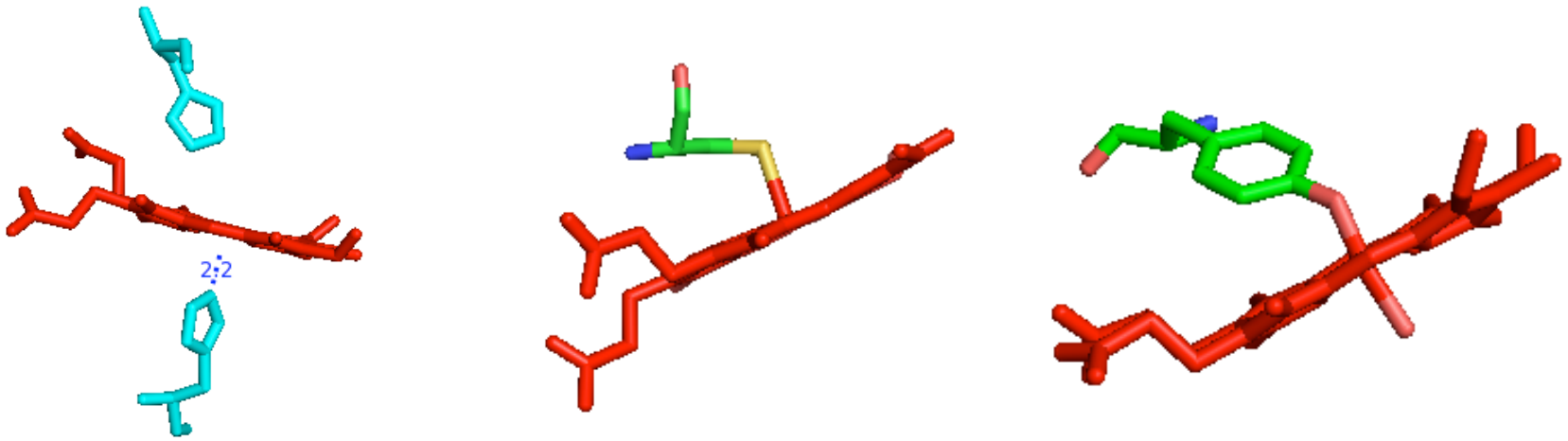
heme *a* cofactor	*Non-protein ligand*	*Ligand fate*
– Cytochrome c Oxidase	O_2 (heme *a3*)	– Reduced to H_2O
	None (heme *a*)	– Pure electron transport
heme *c* cofactor		
– Cyctochrome *c*	None	– Pure electron transport
– Cytochrome *c1*	None	– Pure electron transport

Summary of heme cofactor properties

heme *b* cofactor	*Non-protein ligand*	*Ligand fate*
– Hemoglobin	O_2	– Transported intact
– Cytochrome P450	O_2	– Incorporated into product
– Catalase	H_2O_2	– Degraded
– sGC	NO	– Unchanged by sGC

- Same cofactor, yet VERY different ligand binding properties
  - How might this be achieved?
  - How can the identity of the ligand binding the cofactor be tuned?

- Identical interacting ligand, yet VERY distinct outcomes possible!
  - How might this be achieved?



- Iron oxidation status
  - Fe^{2+} (O_2, NO, CO binding favored)
  - Fe^{3+} (H_2O, H_2O_2, CN^- (cyanide), N_3^- (azide)
- Identity of the side chains close to distal pocket
  - Block access of certain ligands
  - Stabilize bound ligand (e.g. H-bonding)
- Electron distribution in heme cofactor
  - Protein derived side chain identity
  - Heme distortion

Studying hemoproteins

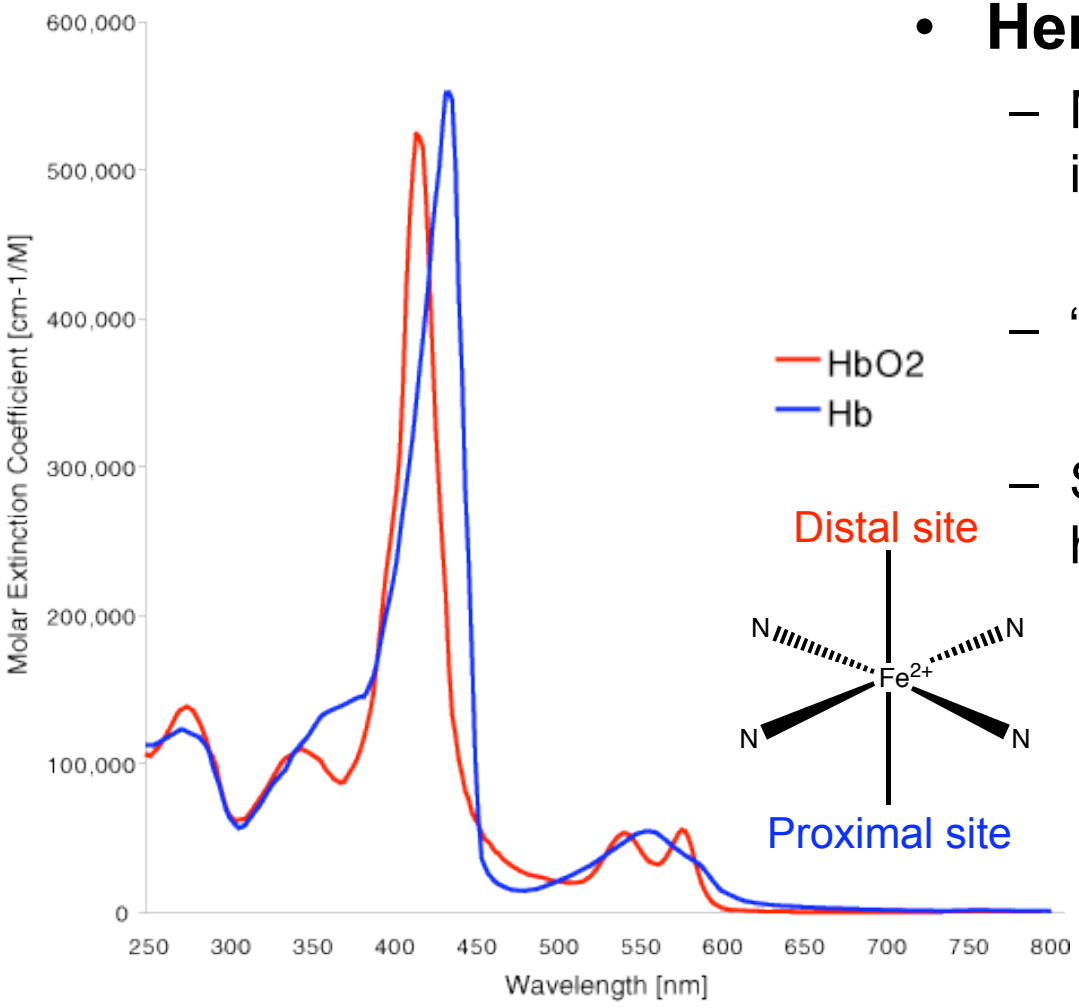
- Gaining insight into hemoprotein biochemistry
  - Ligand binding status
  - Oxidation state
  - Porphyrin ring distortion

- X-ray crystallographic data not always available
  - Even when available, cannot distinguish iron oxidation states

Studying hemoproteins

- Frequently used techniques:
  - Electronic absorption spectroscopy (UV-vis)
    - Iron coordination status (e.g. 5 versus 6 coordinate)
    - Iron oxidation state
  - Electron paramagnetic resonance (EPR)
    - Iron oxidation state
      - Spin state (presence of paired versus unpaired outer shell electrons)
  - Resonance Raman & Infrared spectroscopy (vibrational spectroscopy)
    - Insight into heme distortion

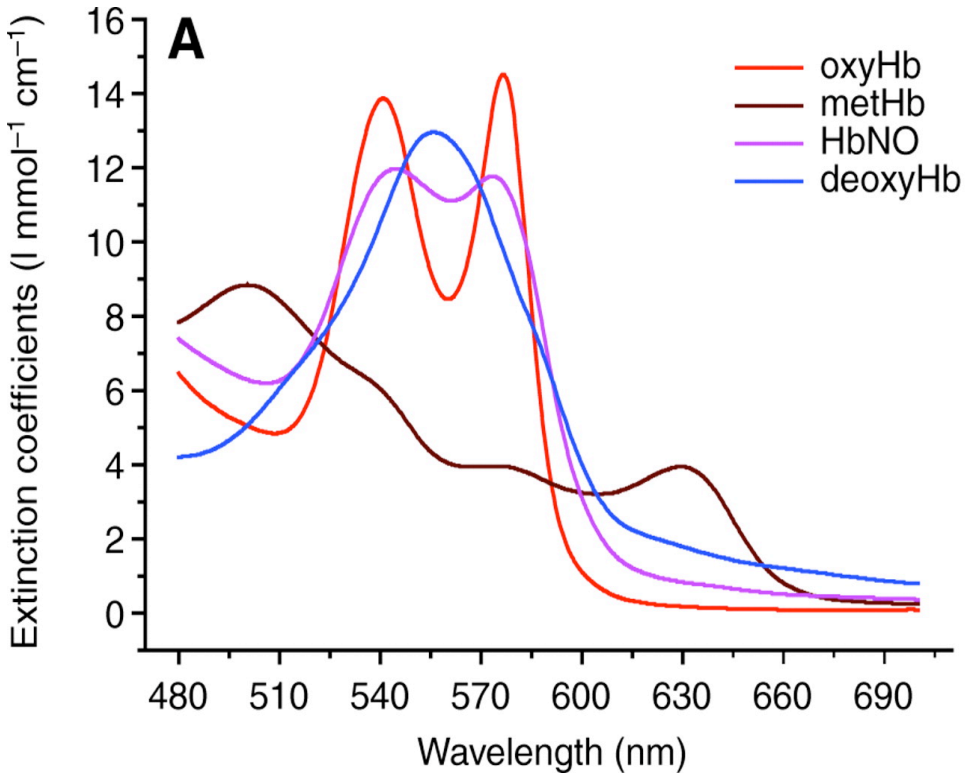
Sample electronic absorption spectra



- **Hemoglobin**
  - Maximum absorbance intensity in the 414-432 nm range
  - "**Soret**" peak
  - Soret maximum is sensitive to heme environment
    - Ligand present versus absent
    - HbO_2 (6 coordinate iron) ~ 414 nm Soret
    - Hb (5-coordinate) ~ 432 nm Soret

Sample electronic absorption spectra

- Think of absorption spectrum as "fingerprint" for the hemoprotein state
- Absorption in this wavelength range is sensitive to the:
  - Iron oxidation state (MetHb = Fe^{3+})
  - Iron coordination state (Hb versus HbO_2)
  - Coordinated ligand (O_2 versus NO)



Jensen, F. B. *J Exp Biol,* 210:3387-3394 (2007)

Summary

- Nature uses the same basic cofactor to achieve many distinct functions:
  - Electron transfer
  - Ligand transport
  - Enzyme catalysis
  - Allosteric regulation

- These distinct functions are only possible because the chemical properties of heme can be precisely tuned by its macromolecular environment
  - Nature uses several strategies to achieve the desired tuning