

# BB 422/622

## OUTLINE:

Introduction and review

Transport

Glycogenolysis

Glycolysis

Introduction & overview; 2 phases

Phase I

Phase II

Summary: logic, energetics, labeling studies

Other sugars

Pasteur: Anaerobic vs Aerobic

Fermentations: anaerobic fates of

pyruvate

Lactate-lactate dehydrogenase

Exam-1 material Acetoacetate decarboxylase

Exam-2 material Ethanol-pyruvate decarboxylase & alcohol dehydrogenase

Pyruvate oxidation:

aerobic fates of pyruvate

pyruvate dehydrogenase

complex

Krebs' Cycle

How did he figure it out?

Overview

See *Achieve*:

Ch19: [Case Study: The Narrow Window](#)

8 Steps

Citrate Synthase

Aconitase

Isocitrate dehydrogenase

Ketoglutarate dehydrogenase

Succinyl-CoA synthetase

Succinate dehydrogenase

Fumarase

Malate dehydrogenase

Energetics; Regulation

Summary

Oxidative Phosphorylation

Energetics

Mitochondria

Transport of protons out

Electron transport

Discovery

Four Complexes

Complex I:  $\text{NADH} \rightarrow \text{CoQH}_2$

Complex II:  $\text{Succinate} \rightarrow \text{CoQH}_2$

Complex III:  $\text{CoQH}_2 \rightarrow \text{Cytochrome C (Fe}^{2+})$

Complex IV:  $\text{Cytochrome C (Fe}^{2+}) \rightarrow \text{H}_2\text{O}$

Chemiosmotic theory

ATP synthesis

## Electron Transport

**TABLE 19-3** The Protein Components of the Mitochondrial Respiratory Chain

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| II Succinate dehydrogenase                               | 140        | 4                               | FAD-E, Fe-S                                                 | 0.09                            | 0.05 (CoQ)<br>Succinate, CoQ | malonate           |
| III Ubiquinone: cytochrome c oxidoreductase <sup>b</sup> | 250        | 11                              | Hemes b, c <sub>1</sub> , Fe-S                              | 0.17                            | CoQ, Cytochrome c            | antimycin a        |
| Cytochrome c <sup>c</sup>                                | 13         | 1                               | Heme                                                        | 0.25 (Cyt c)                    |                              |                    |
| IV Cytochrome oxidase <sup>b</sup>                       | 204        | 13 (3-4)                        | Hemes a, a <sub>3</sub> ; Cu <sub>A</sub> , Cu <sub>B</sub> | 0.57                            | Cytochrome c, O <sub>2</sub> | Cyanide, azide, CO |

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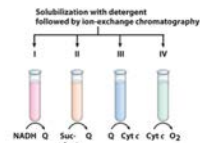
" $\Delta E_o'$ " of ATP synthesis = -0.16 v

$\Delta E_o'$  of Complex I = -0.41 v

$\Delta E_o'$  of Complex III = -0.2 v

$\Delta E_o'$  of Complex IV = -0.25 v

" $\Delta E_o'$ " from 2e<sup>-</sup> needed for proton pumping = 0.053 V/proton



# Electron Transport

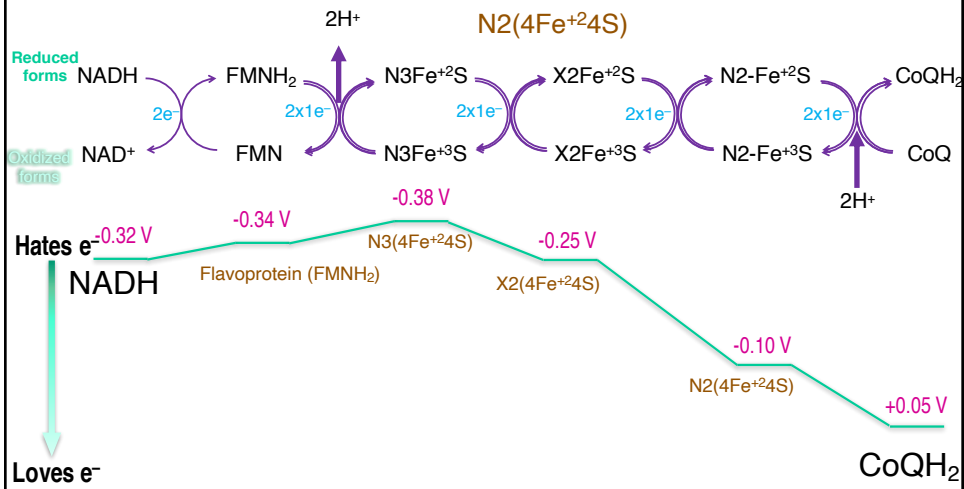
NADH:Ubiquinone Oxidoreductase,  
a.k.a. Complex I

Flavoprotein (FMNH<sub>2</sub>)

N3(4Fe<sup>2+</sup>4S)

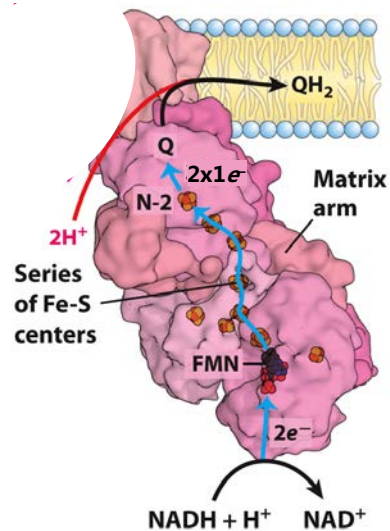
X2(4Fe<sup>2+</sup>4S)

N2(4Fe<sup>2+</sup>4S)

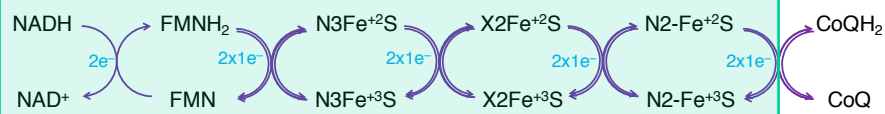
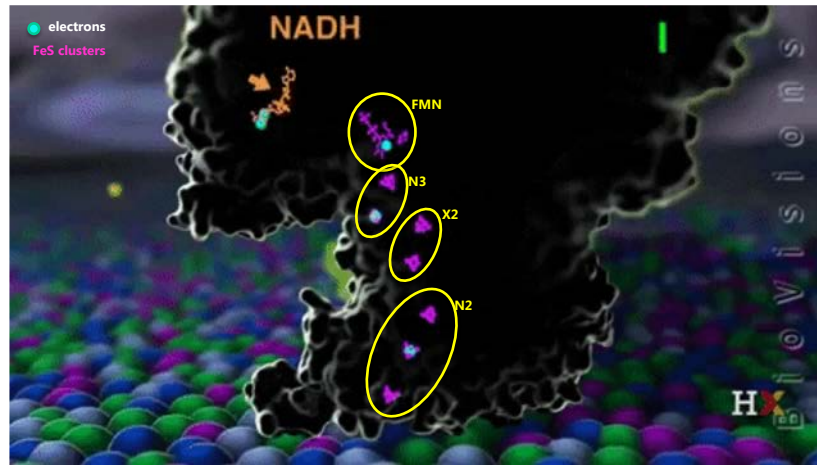


## Electron Transport: Complex I

- One of the largest macro-molecular assemblies in the mammalian cell
- Over 40 different polypeptide chains encoded by both nuclear and mitochondrial genes
- NADH binding site in the matrix side (N-side); on the flavoprotein subunit
- Non-covalently bound **flavin mononucleotide (FMN)** accepts two electrons from NADH.
- Several **iron-sulfur centers** pass one electron at a time toward the **ubiquinone** binding site (in membrane).



# Electron Transport: Complex I



# Electron Transport: Complex I

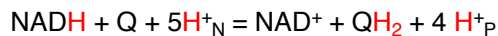
NADH:Ubiquinone

Oxidoreductase Is a Proton Pump

- The transfer of two electrons from NADH to ubiquinone is accompanied by a transfer of multiple protons from the matrix (N) to the intermembrane space (P).

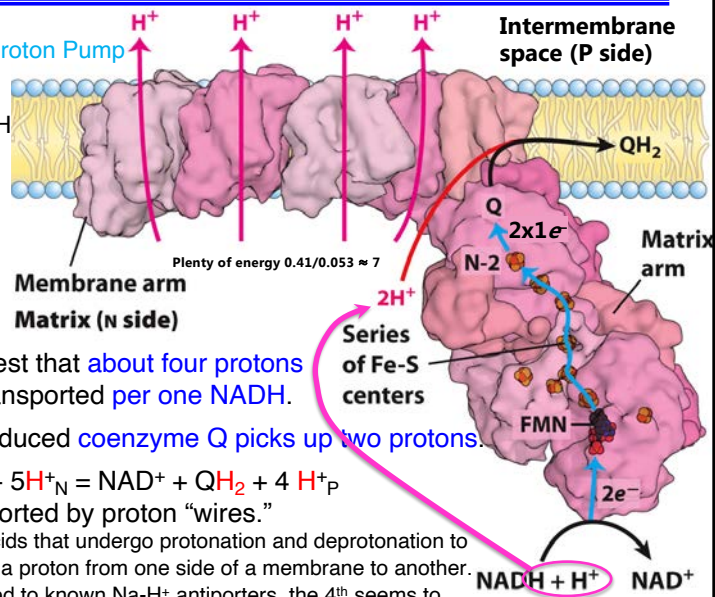
- Experiments suggest that about four protons are transported per one NADH.

- Additionally, the reduced coenzyme Q picks up two protons.



- Protons are transported by proton "wires."

- a series of amino acids that undergo protonation and deprotonation to get a net transfer of a proton from one side of a membrane to another. 3 subunits are related to known Na-H<sup>+</sup> antiporters, the 4<sup>th</sup> seems to control them all and uses the energy from the oxidation of NADH



# Electron Transport

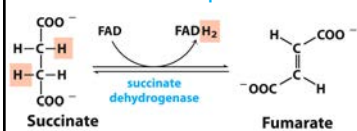
**TABLE 19-3** The Protein Components of the Mitochondrial Respiratory Chain

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| III Ubiquinone: cytochrome c oxidoreductase <sup>b</sup> | 250        | 11                              | Hemes b, c <sub>1</sub> , Fe-S                              | 0.17                            | CoQ, Cytochrome c            | antimycin a        |
| Cytochrome c <sup>c</sup>                                | 13         | 1                               | Heme                                                        |                                 |                              |                    |
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## Electron Transport: Complex II

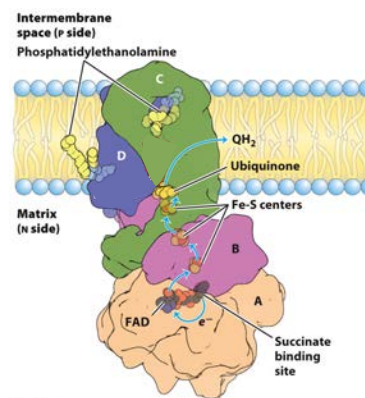
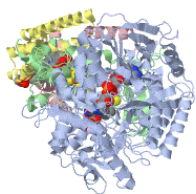
Succinate Dehydrogenase, a.k.a. Complex II



- FAD accepts two electrons from succinate.
- Electrons are passed, one at a time, via iron-sulfur centers to ubiquinone, which becomes reduced CoQH<sub>2</sub>.
- Does not transport protons

• Succinate dehydrogenase is a single enzyme with dual roles:

- convert succinate to fumarate in the citric acid cycle
- donate those electrons in the electron transport chain

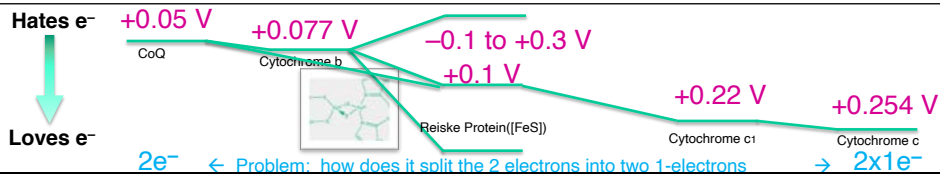


# Electron Transport

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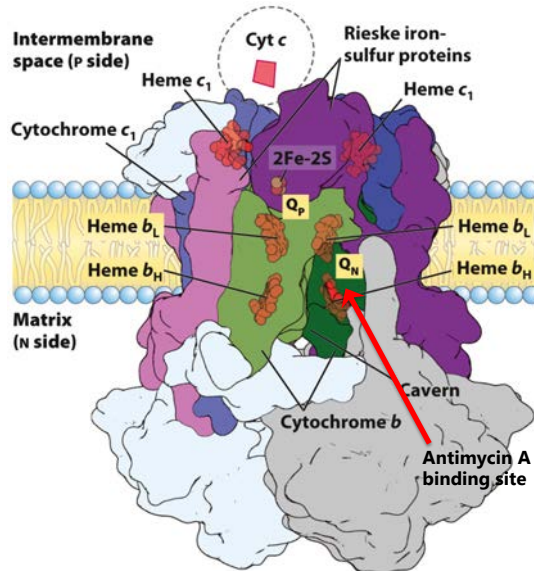
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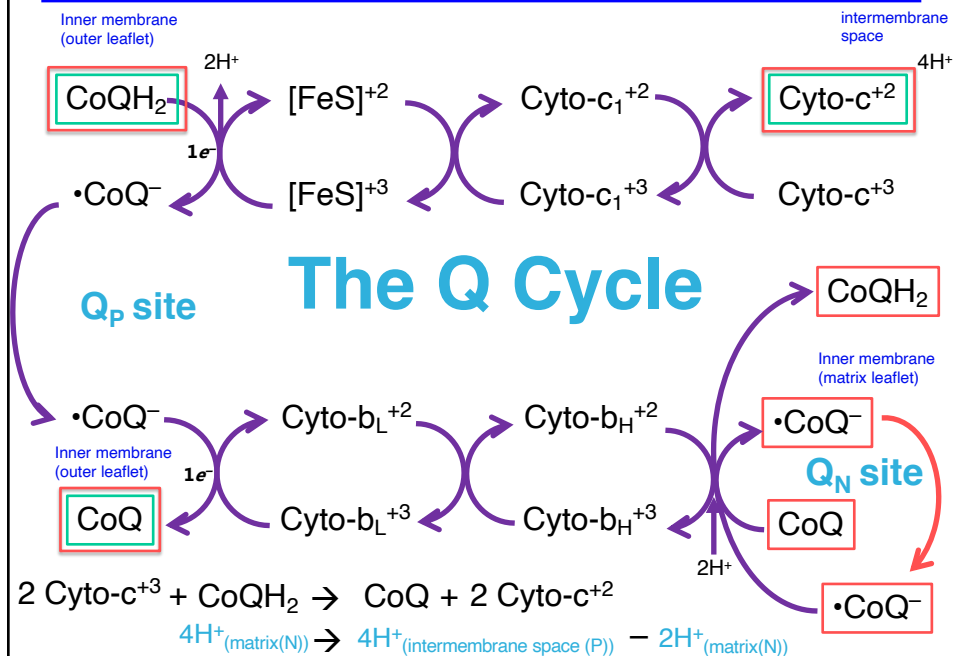
## Electron Transport: Complex III

Ubiquinone: Cytochrome c Oxidoreductase, a.k.a. Complex III

- Uses two electrons from  $\text{CoQH}_2$  to reduce two molecules of cytochrome c
- Additionally contains iron-sulfur clusters (Rieske protein), two different cytochrome b's, and a cytochrome c<sub>1</sub>
- It's a dimer of 11 subunits (22 proteins). Three main proteins for each of the redox centers (gray, green, purple).
- Dimers create a central cavern with TWO CoQ binding sites
- Problem: How do we take the 2-electron  $\text{CoQH}_2$  and get one electron at a time into cytochrome c?



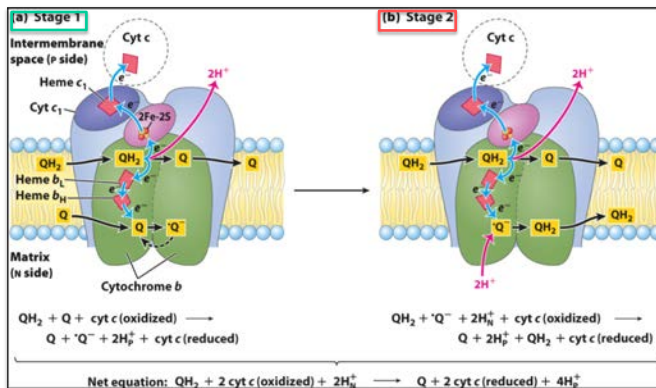
## Electron Transport: Complex III



## Electron Transport: Complex III

### The Q Cycle

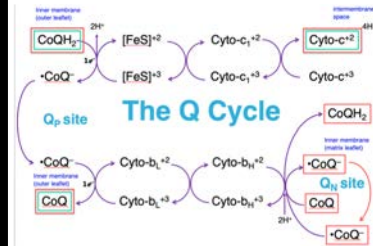
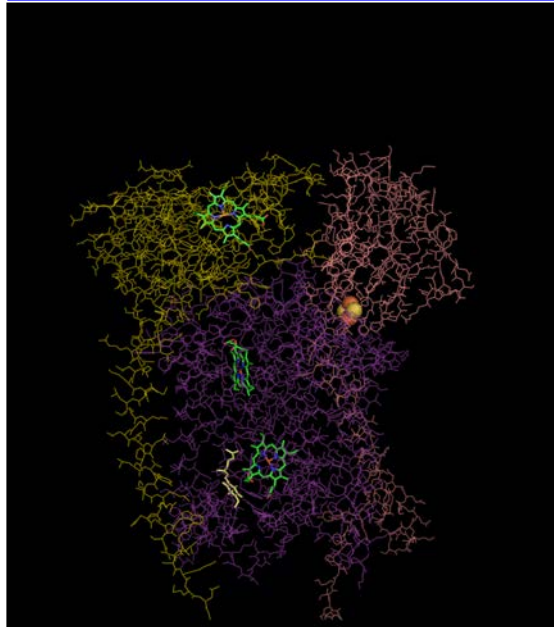
- Clearance of electrons from the reduced quinones via the **Q-cycle** results in translocation of **four** protons to the intermembrane space.
- This cycle matches experimental evidence that **four** protons are transported across the membrane per two electrons that reach cyt c.



- The Q cycle provides a good model that explains how two additional protons are picked up from the matrix.
  - Two molecules of  $\text{CoQH}_2$  become oxidized, releasing protons into the IMS.
  - One molecule becomes re-reduced, thus a net transfer of **four protons per reduced coenzyme Q**, plus a loss of protons from the matrix into the re-reduced  $\text{CoQ}$  adds to the membrane potential.



## Electron Transport: Complex III



## Electron Transport

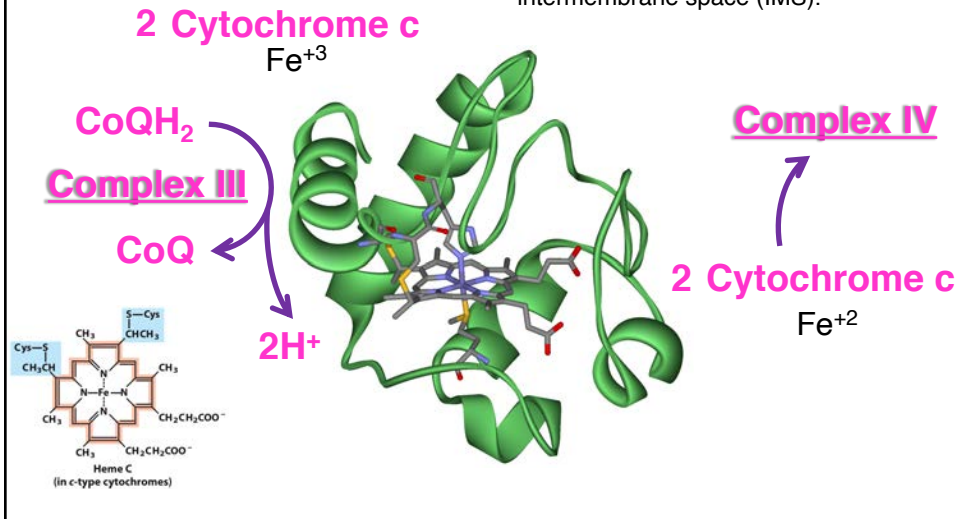
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## Electron Transport

- Mobile electron carrier; a peripheral membrane protein
  - Cytochrome *c* moves through the intermembrane space (IMS).



## Electron Transport

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| Cytochrome <i>c</i> <sup>c</sup>                                | 13         | 1                               | Heme                                           |                                 |                                    |                    |
| IV Cytochrome oxidase <sup>b</sup>                              | 204        | 13 (3–4)                        | Hemes a, $a_3$ ; $\text{Cu}_A$ , $\text{Cu}_B$ | 0.57                            | Cytochrome <i>c</i> , $\text{O}_2$ | Cyanide, azide, CO |

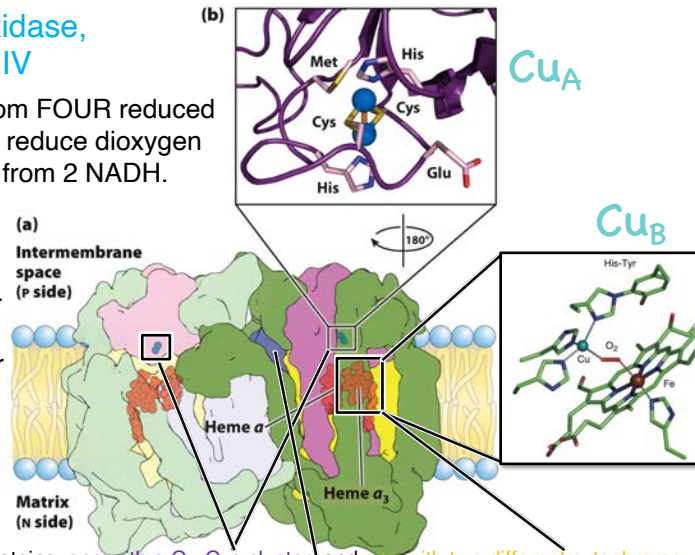
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## Electron Transport: Complex IV

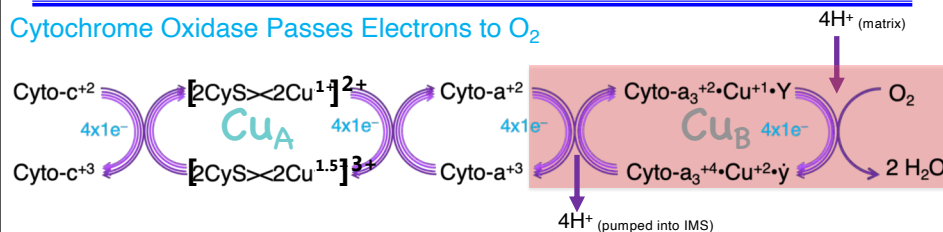
### Cytochrome Oxidase, a.k.a. Complex IV

- Uses electrons from FOUR reduced cytochrome *c*'s to reduce dioxygen ( $O_2$ ) to waters (2) from 2 NADH.
- This reaction is among the most difficult chemically.
- The bovine complex is a dimer of 13 subunits (26 proteins). Three main proteins for each of the redox centers.
- Contains two copper proteins: **one with a Cu-Cys cluster**, and **one with two different cytochrome *a*'s (*a* & *a*<sub>3</sub>) and Cu**. The third main protein **moves protons from the matrix to the second Cu-cofactor**. Function of the other 10 are unknown, although undoubtedly involved as a proton pump.



## Electron Transport: Complex IV

### Cytochrome Oxidase Passes Electrons to $O_2$

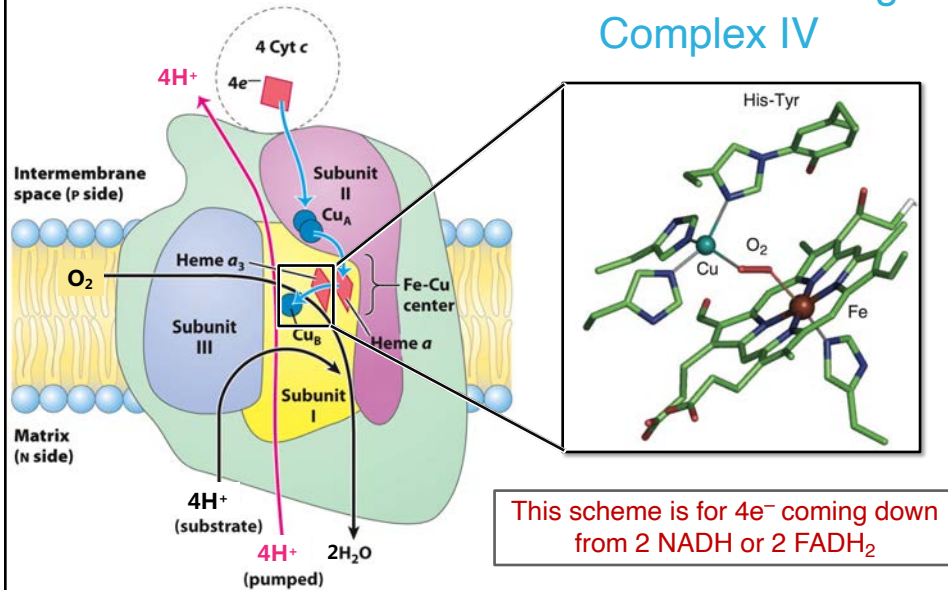


- Contains 6 copper ions per complex (3/monomer<sub>13</sub>)
- There are two types/sites for the copper:
  - $Cu_A$ : two ions that accept electrons from cyt *c* (looks like a 2Fe-2S cluster)
  - $Cu_B$ : bonded to heme *a*<sub>3</sub>, forming a binuclear center that transfers four electrons to oxygen.
- Four electrons are used to **reduce one oxygen molecule** into **two** water molecules.
- Four **protons are picked up from the matrix** in this process.
- **Four** additional protons are pumped from the matrix to the intermembrane space.

This scheme is for 4e<sup>-</sup> coming down from 2 NADH or 2 FADH<sub>2</sub>

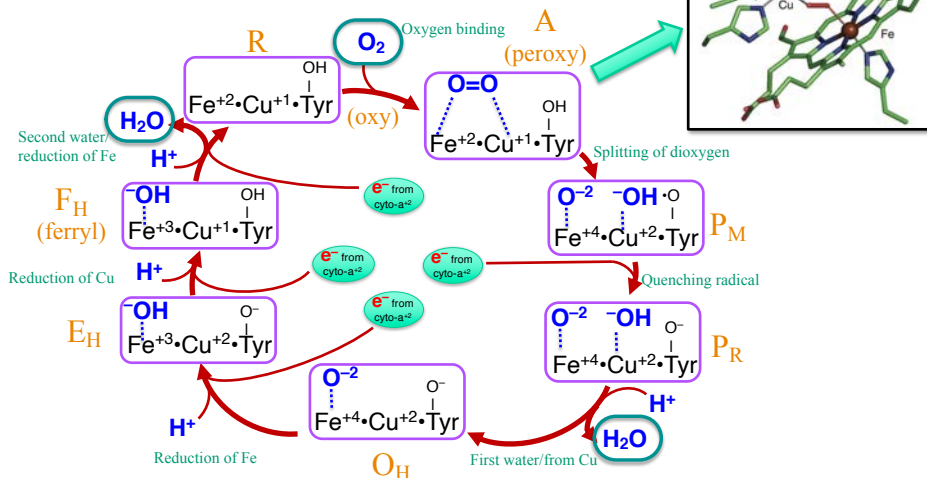
# Electron Transport: Complex IV

## Electron Flow Through Complex IV



# Electron Transport: Complex IV

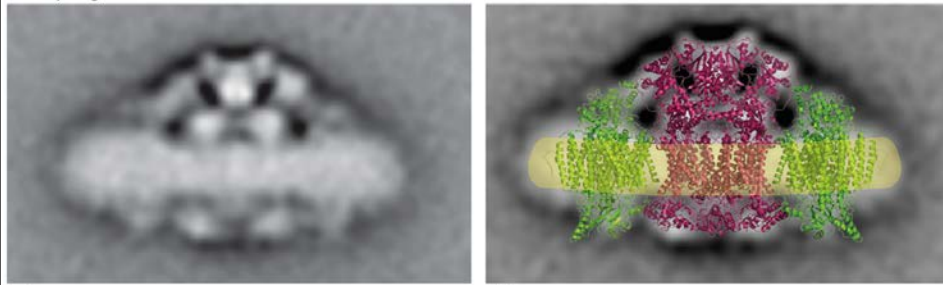
## Electron Flow Through Complex IV: Chemistry



# Electron Transport

## Multiple Complexes Associate Together to Form a “Respirasome”

Courtesy of Egbert Boekema



Complex III and Complex IV

# Clinical Correlations

## Hemolytic Anemia: Deficiencies in Glycolytic Enzymes

- Red-blood cells do not have nuclei or mitochondria. They still need to maintain their membrane potential using the Na/K pump and membrane shape using actin microfilaments. Both use glycolysis as the sole source of ATP.
- Any glycolytic enzyme that is impaired sufficiently will affect the efficiency of the entire pathway. Without ATP, the cells swell and lyse, which is a condition called non-spherocytic hemolytic anemia.
- The most common form is from a deficiency in pyruvate kinase. This form is more tolerable than a form from a deficiency of aldolase A. This is likely because there is a buildup of intermediates behind the block at PK, including 2,3-bisphosphoglycerate, the negative heterotropic allosteric effector of Hb. This causes Hb to release more O<sub>2</sub>.
- The precise defect in aldolase A that causes hemolytic anemia lead to a deeper understanding of protein stability and cellular function.



# Clinical Correlations

## Rare Fumarase Deficiency

- Loss of activity in TCA enzymes is rare as it is lethal to the cell. However, a few very rare cases have been described.
- One such case was a severe deficiency of fumarase.
- Urine and blood have high levels of fumarate, succinate,  $\alpha$ -ketoglutarate, citrate, and malate.
- Humans with this disease have neurological impairment, encephalomyopathy (brain/muscle malady), dystonia (muscle twitching)

