BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Exam-2 material

Fermentations
Pyruvate
- pyruvate dehydrogenase (ox-decarbox; S-ester)

Krebs' Cycle
- How did he figure it out?
- Overview
- 8 Steps
  - Citrate Synthase (C-C)
  - Aconitase (=, -OH)
  - Isocitrate dehydrogenase (ox-decarbox; =O)
  - Ketoglutarate dehydrogenase (ox-decarbox; S-ester)
  - Succinyl-CoA synthetase (sub-level phos)
  - Succinate dehydrogenase (=)
  - Fumarase (-OH)
  - Malate dehydrogenase (=O)
- Energetics
- Regulation
- Summary

See *Achieve*:
Ch19: Case Study: The Narrow Window

Oxidative Phosphorylation
- Energetics (−0.16 V needed for making ATP)
- Mitochondria
- Transport (2.4 kcal/mol needed to transport H^+ out)
- Electron transport
  - Discovery
  - Four Complexes
    - Complex I: NADH → $CoQH_2$
    - Complex II: Succinate → $CoQH_2$
    - Complex III: $CoQH_2$ → Cytochrome C (Fe^{2+})
    - Complex IV: Cytochrome C (Fe^{2+}) → H_2O

Electron Transport

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

Enzyme complex/protein	Mass (kDa)	Number of subunits[a]	Prosthetic group(s)	Reduction potential (E_o' V)	Binding sites for:	Inhibited by:
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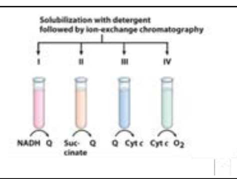
II Succinate dehydrogenase	140	4	FAD-E, Fe-S	0.09 0.05 (CoQ)	Succinate, CoQ	malonate
III Ubiquinone: cytochrome *c* oxidoreductase[b]	250	11	Hemes b, c_1, Fe-S	0.17	CoQ, Cytochrome c	antimycin a
Cytochrome *c*[c]	13	1	Heme	0.25 (Cyt c)		
IV Cytochrome oxidase[b]	204	13 (3–4)	Hemes a, a_3; Cu_A, Cu_B	0.57 0.82 (O_2)	Cytochrome c, O_2	Cyanide, azide, CO

[a]Number of subunits in the bacterial complexes in parentheses.
[b]Mass and subunit data are for the monomeric form.
[c]Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

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

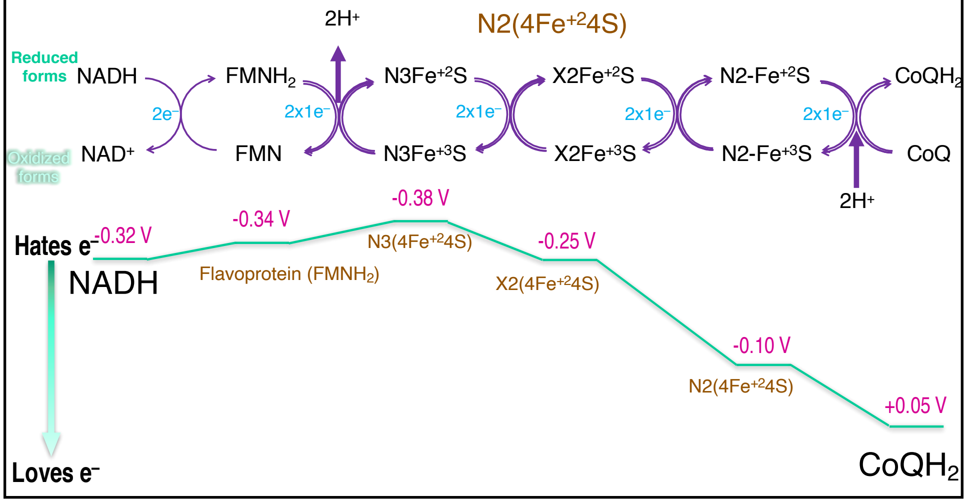
$\Delta E_o'$ of Complex I = –0.41 v
$\Delta E_o'$ of Complex III = –0.2 v
$\Delta E_o'$ of Complex IV = –0.25 v



Electron Transport

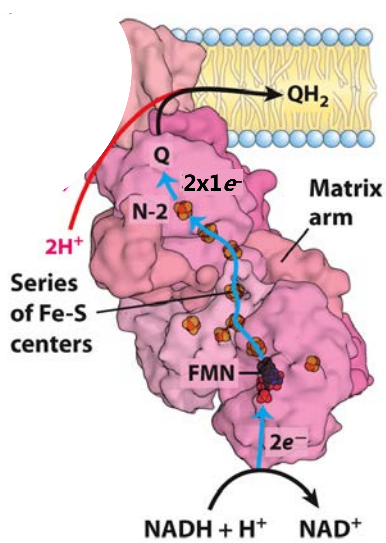
NADH:Ubiquinone Oxidoreductase, a.k.a. Complex I

Flavoprotein ($FMNH_2$)
N3($4Fe^{+2}4S$)
X2($4Fe^{+2}4S$)
N2($4Fe^{+2}4S$)

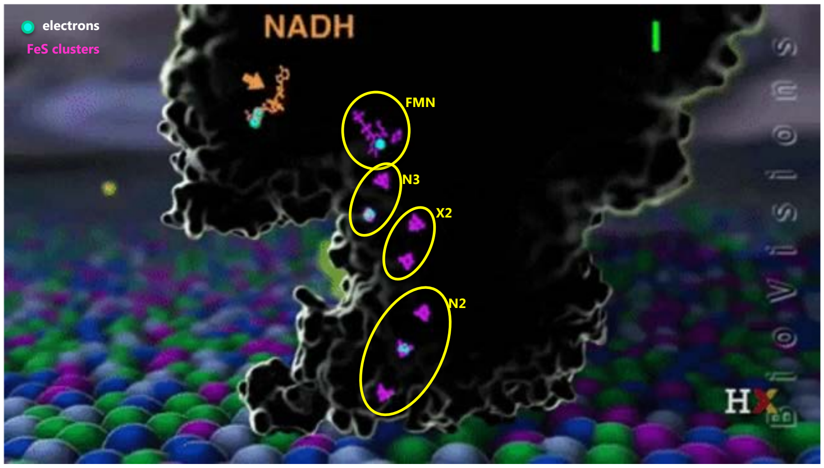


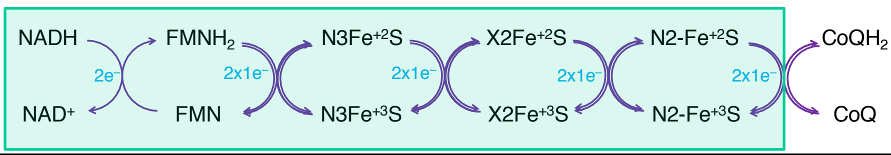
Electron Transport: Complex I

- One of the largest macro-molecular assemblies in the mammalian cell
- Over 40 different polypeptide chai encoded by both nuclear and mitochondrial genes
- NADH binding site in the matrix 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.



Electron Transport: Complex I





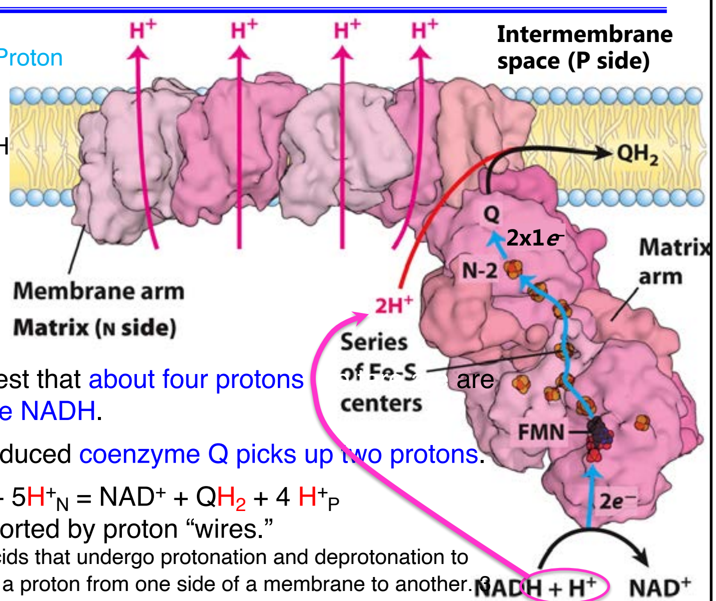
Electron Transport: Complex I

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.

$$NADH + Q + 5H^+_N = NAD^+ + QH_2 + 4\ H^+_P$$

- 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+ antiporters, the 4th seems to control them all and uses the energy from the oxidation of NADH



Electron Transport

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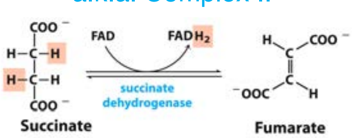
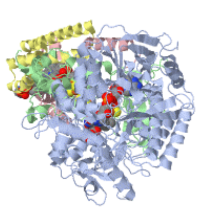
Electron Transport: Complex II

Succinate Dehydrogenase, a.k.a. Complex II

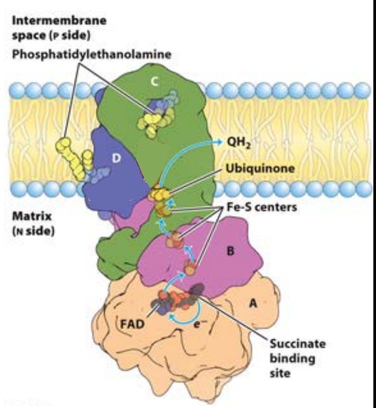


- FAD accepts two electrons from succinate.
- Electrons are passed, one at a time, via iron-sulfur centers to ubiquinone, which becomes reduced $CoQH_2$.
- Does not transport protons



Jmol

- 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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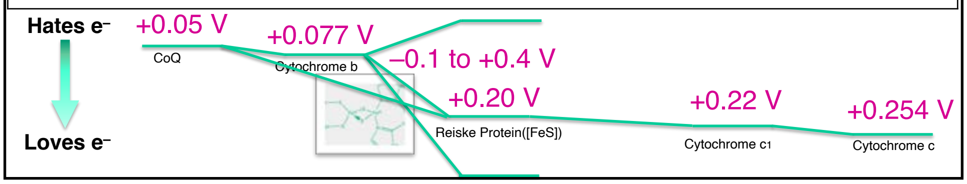
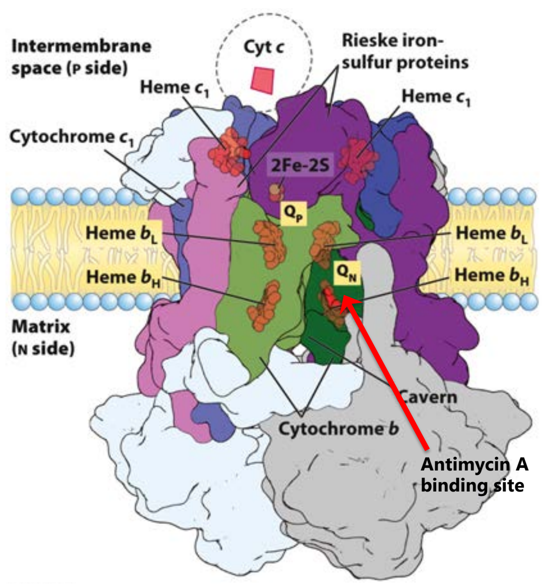


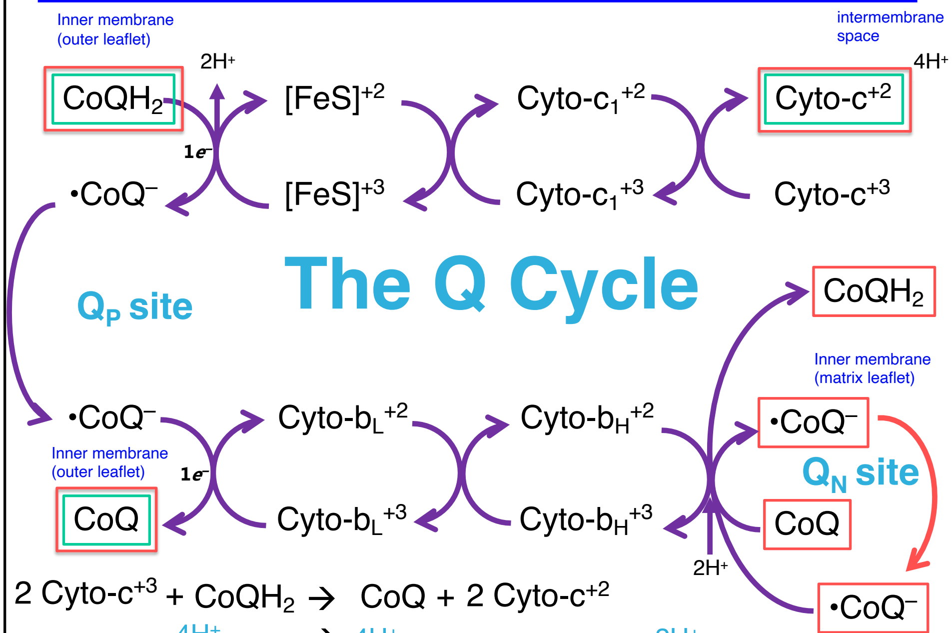
Electron Transport: Complex III

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

- Uses two electrons from $CoQH_2$ to reduce two molecules of cytochrome *c*
- Additionally contains iron-sulfur clusters (Rieske protein), two different cytochrome *b's*, and a cytochrome c_1
- 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 $CoQH_2$ and get one electron at a time into cytochrome c?



Electron Transport: Complex III

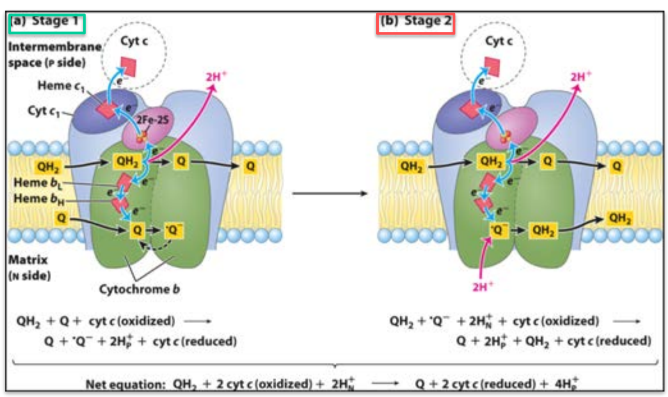


$2\ \text{Cyto-c}^{+3} + CoQH_2 \rightarrow CoQ + 2\ \text{Cyto-c}^{+2}$

$4H^+_{(\text{matrix(N)})} \rightarrow 4H^+_{(\text{intermembrane space (P)})} - 2H^+_{(\text{matrix(N)})}$

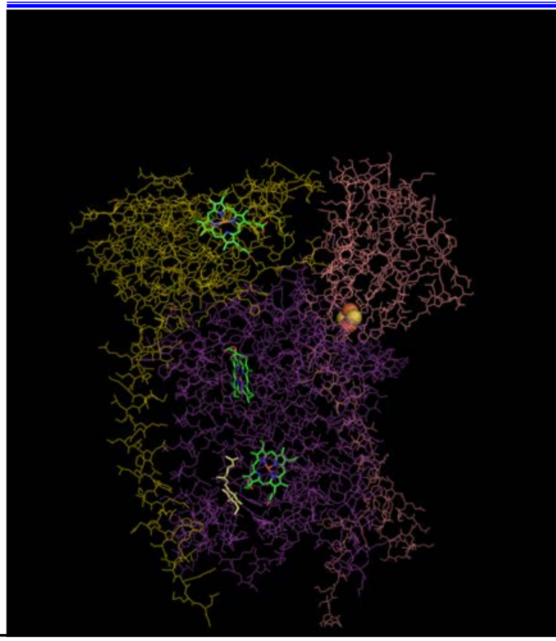
Electron Transport: Complex III

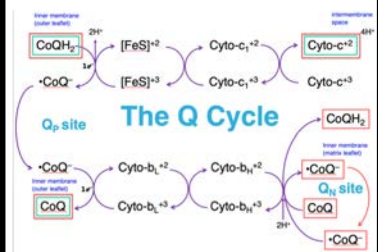
The Q Cycle



- Clearance of electrons from the reduced quinones via the Q-cycle results in translocation of four protons to the inter-membrane 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 $CoQH_2$ 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 CoQ adds to the membrane potential.

Electron Transport: Complex III





Electron Transport

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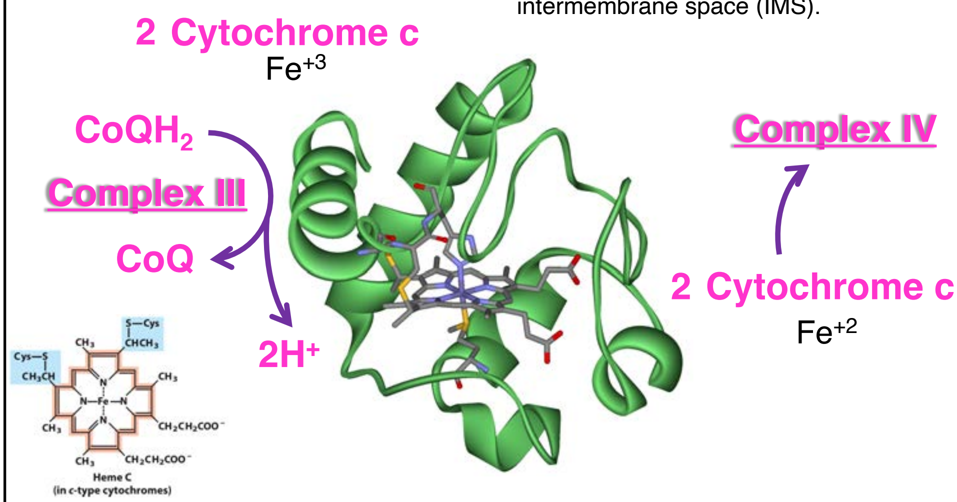
Electron Transport

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



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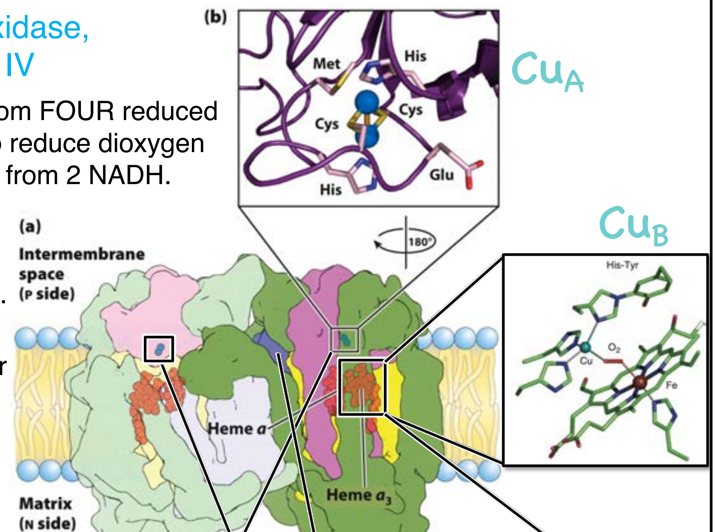
Electron Transport: Complex IV

Cytochrome Oxidase,
a.k.a. Complex IV

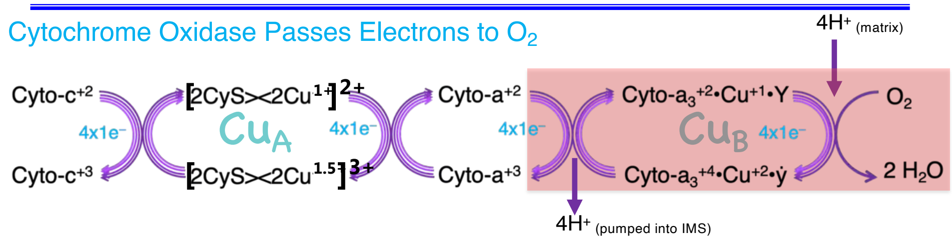
- Uses electrons from FOUR reduced cytochrome *c's* to reduce dioxygen (O_2) to waters (2) from 2 NADH.
- This reaction is among the most difficult chemically.
- The bovine complex is a dimer of 13 subunits (26 proteins). Three main proteins for each of the redox centers.



- Contains two copper proteins: one with a Cu-Cys cluster, and one with two different cytochrome a's (a & a_3) 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

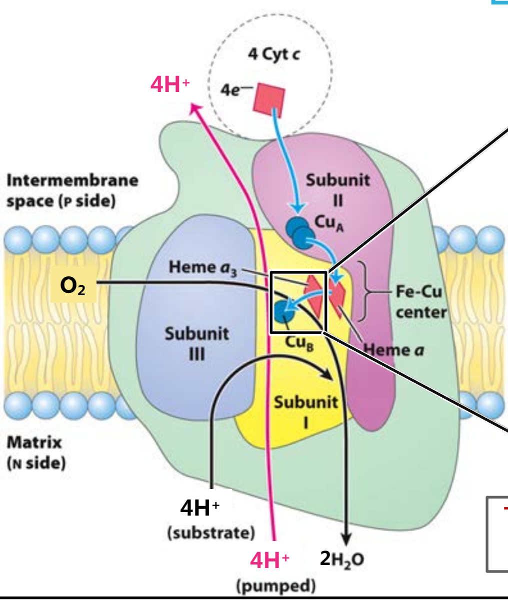


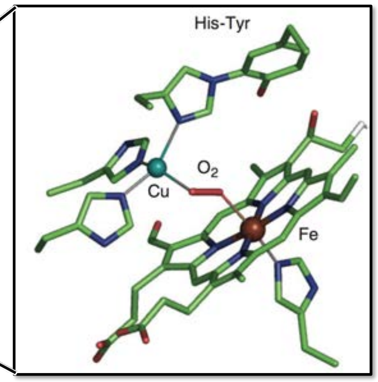
- Contains 6 copper ions per complex (3/monomer$_{13}$)
- There are two types/sites for the copper:
  - Cu_A: two ions that accept electrons from cyt *c* (looks like a 2Fe-2S cluster)
  - Cu_B: bonded to heme a_3, forming a binuclear center that transfers four electrons to oxygen.
- Four electrons are 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 $4e^-$ coming down from **2** NADH or **2** $FADH_2$

Electron Transport: Complex IV

Electron Flow Through Complex IV

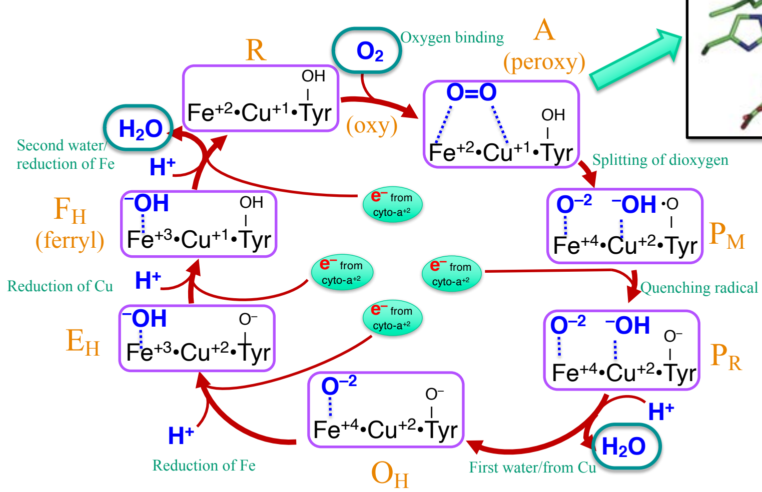


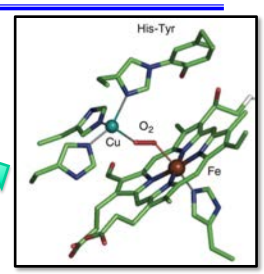


This scheme is for 4e⁻ coming down from 2 NADH or 2 $FADH_2$

Electron Transport: Complex IV

Electron Flow Through Complex IV: **Chemistry**

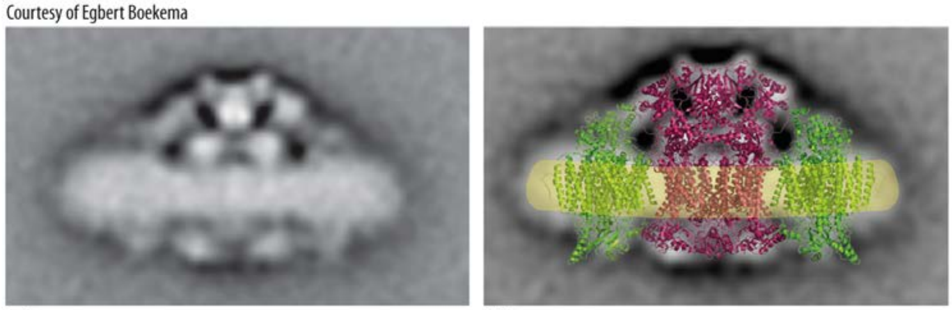




Electron Transport

Multiple Complexes Associate Together to Form a "Respirasome"

Courtesy of Egbert Boekema

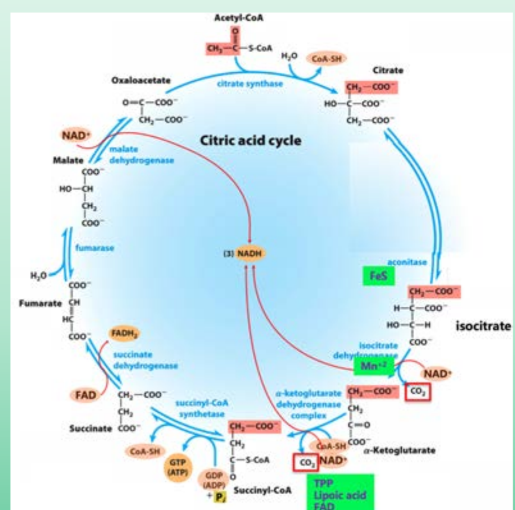


Complex III and **Complex IV**

Clinical Correlations

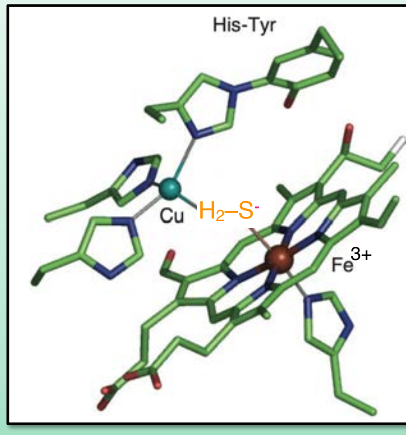
Rare Fumarase Deficiency

- 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, α-ketoglutarate, citrate, and malate.
- Humans with this disease have neurological impairment, encephalomyopathy (brain/muscle malady), dystonia (muscle teaching)



Clinical Correlations

Cyanide poisoning



- CN gas or KCN ingestion causes rapid loss of mitochondrial function and death.
- Cyanide works by inhibition of cytochrome oxidase by binding tightly to the Fe^{3+} of heme-a_3. Mitochondrial respiration, and energy production ceases, and cell death rapidly follows.
- Cyanide is one of the most potent and rapidly acting poisons known. Other poisons do the same thing: CO, H_2S, N_3^-
- If detected early enough, the antidote is to offer the CN more Fe^{3+} sites to bind and titrate it off of the heme-a_3.
- Creation of "metHb" by oxidation of Hb using various nitrates ($Fe^{2+} \rightarrow Fe^{3+}$) can work due to vast amounts of Hb.