What makes this proton motive force?

Electron Transport

ATP Synthesis $\gtrsim$ 0.16V

H^+ 2.4 Kcal/mole (~3 protons/ATP)

P/O ratio of NADH ~3 (3 ATP per $2e \rightarrow \frac{1}{2}O_2$)

Electron Transport

Electron-Transport Chain Complexes Contain a Series of Electron Carriers

- When it was realized that isolated mitochondria are capable of respiration (oxygen consumption when provided fuels), biochemists began purifying components.
- The first things purified were redox compounds and small stable proteins:
  - NADH
  - flavin mononucleotide (FMN)
  - flavin adenine dinucleotide (FAD)(bound to protein; flavoproteins)
  - iron-sulfur clusters
  - Coenzyme Q (Ubiquinol)
  - cytochromes *a*, *b*, or *c*

- Once purified, they were analyzed by measuring their $E^{o\prime}$.
- Order of transfer of electrons is dependent on $E^{o\prime}$:

TABLE 19-2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

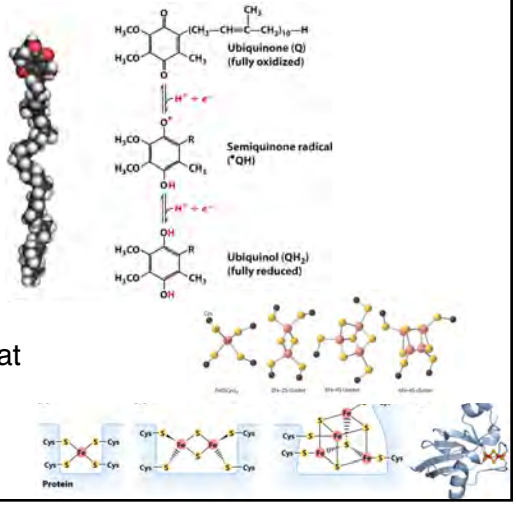
Redox reaction (half-reaction)	E'^{o} (V)
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
FAD-E + 2 H^+ + 2e- → $FADH_2$-E	−0.02
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$) FeS	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome*c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.817

Big Drop! Big Drop! Big Drop!

NADH ⟶ Q ⟶ Cyt *b* ⟶ Cyt c_1 ⟶ Cyt *c* ⟶ Cyt ($a + a_3$)

Electron Transport

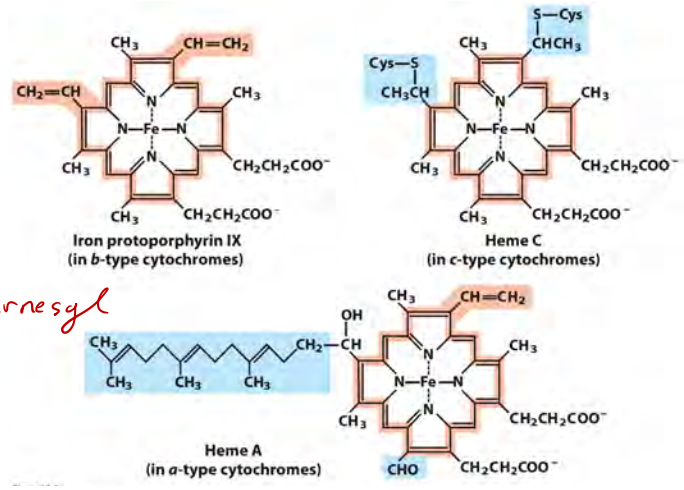
- NAD^+, FMN, and FAD accept electrons. The flavin nucleotides can accept one or two electrons, and can also donate one electron at a time to acceptors that can only accept single electrons
- Ubiquinone, also called Coenzyme Q, is an isoprene lipid that readily accepts electrons. Upon accepting two electrons, it picks up two protons to give an alcohol, ubiquinol ($CoQH_2$). Its found IN the inner membrane.
- Iron-sulfur complexes (Fe-S) that can only carry one electron at a time (role is different than in aconitase).



Electron Transport

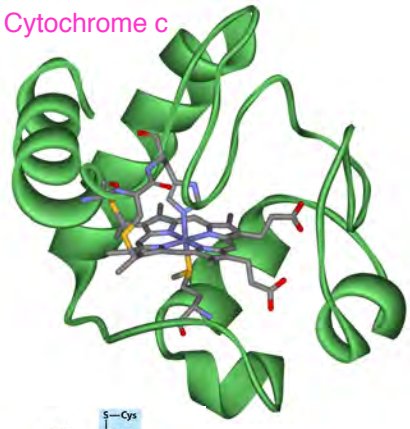
- Small one-electron carrier proteins
- Iron coordinating porphyrin ring derivatives
- *b*/b_1, *c, or a*/a_3 differ by ring additions

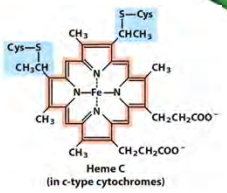
Cytochromes



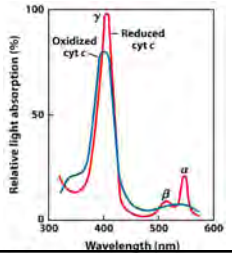
Electron Transport

Cytochrome c





- Mobile electron carrier; a peripheral membrane protein
  - Cytochrome *c* moves through the intermembrane space.
- A soluble heme-containing protein
- Heme iron can be either ferrous (Fe^{2+}, reduced) or ferric (Fe^{3+}, oxidized).
- Cytochrome *c* carries a single electron.
- The two redox forms have different spectra:

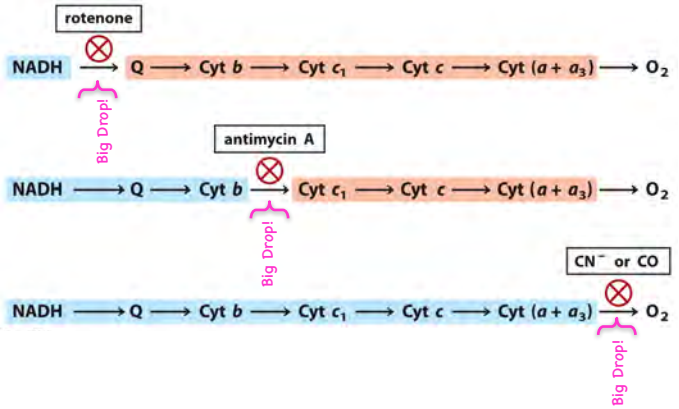


- Intense Soret band near 400 nm absorbs blue light and gives cytochrome *c* an intense red color.

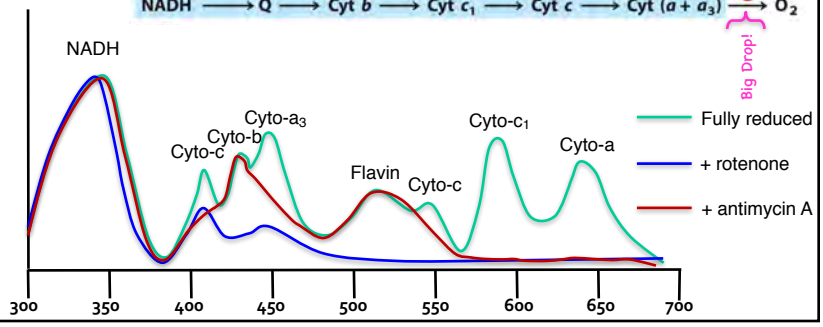
Electron Transport

Inhibitors of Electron Transport

- Inhibitors all stop ET and ATP synthesis: very toxic!

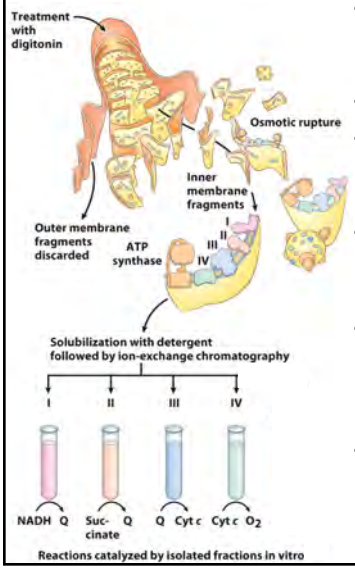


Spectral work



Electron Transport

Electron-Transport Chain Complexes Contain a Series of Electron Carriers



- Better techniques for isolating and handling mitochondria, and isolated various fractions of the inner mitochondrial membrane
- Measure $E^{o'}$
- They corresponded to these large drops and they contained the redox compounds isolated previously.
- When assayed for what reactions they could perform, they could perform certain redox reactions and not others.
- When isolated, including isolating the individual redox compounds, and the $E^{o'}$ measured for each, it was clear that an electron chain was occurring; like a wire!
- Lastly, when certain inhibitors were added, some of the redox reactions could be inhibited and others not. Site of the inhibition could be mapped.

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 (ΔE'° V)	Binding sites for:	Inhibted by:
I NADH dehydrogenase	850	45 (14)	FMN, Fe-S	0.36	NADH, CoQ	amytal, rotenone
II Succinate dehydrogenase	140	4	FAD-E, Fe-S	0.09	Succinate, CoQ	
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			
IV Cytochrome oxidase[b]	204	13 (3–4)	Hemes a, a_3; Cu_A, Cu_B	0.57	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

Electron Transport

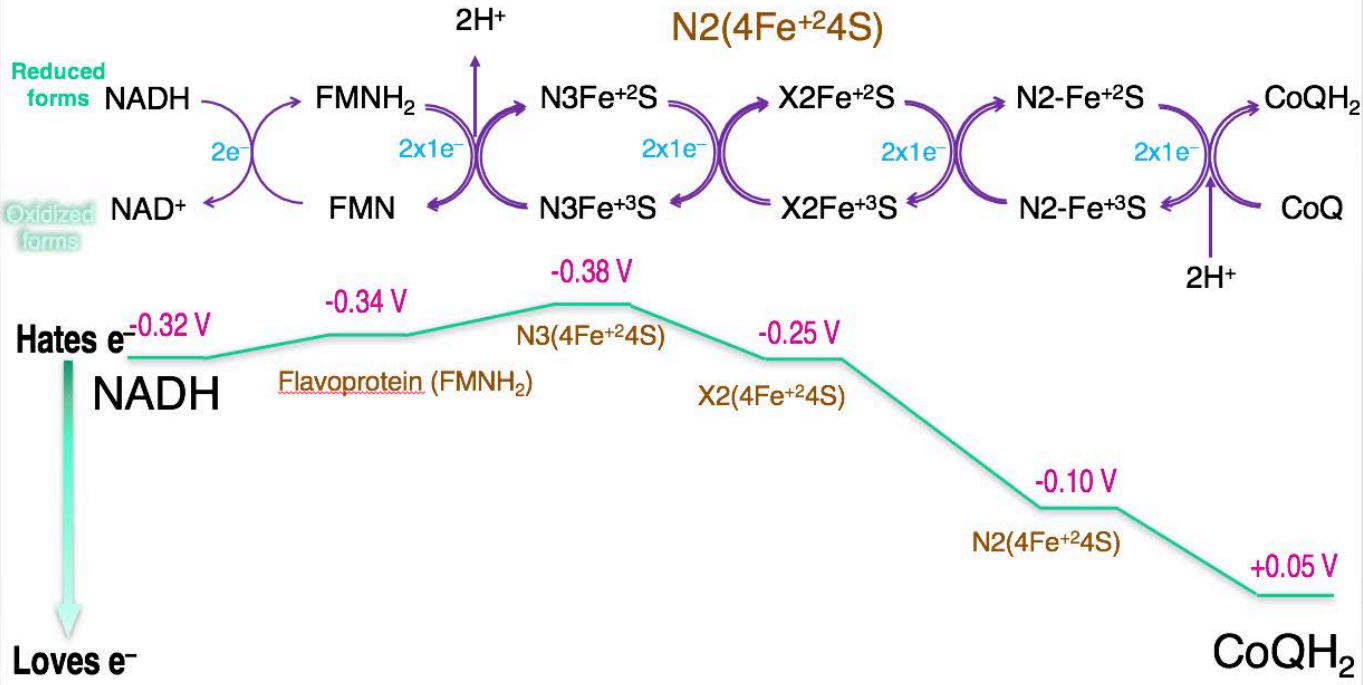
NADH:Ubiquinone Oxidoreductase, a.k.a. Complex I

Flavoprotein ($FMNH_2$)

N3($4Fe^{+2}4S$)

X2($4Fe^{+2}4S$)

N2($4Fe^{+2}4S$)



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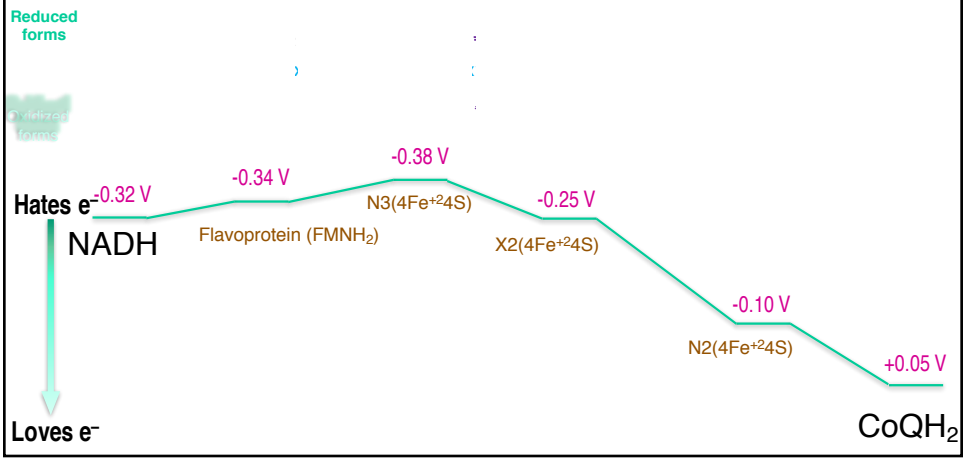
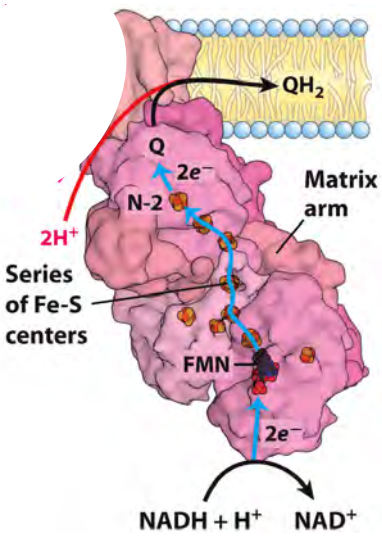


Electron Transport: Complex I

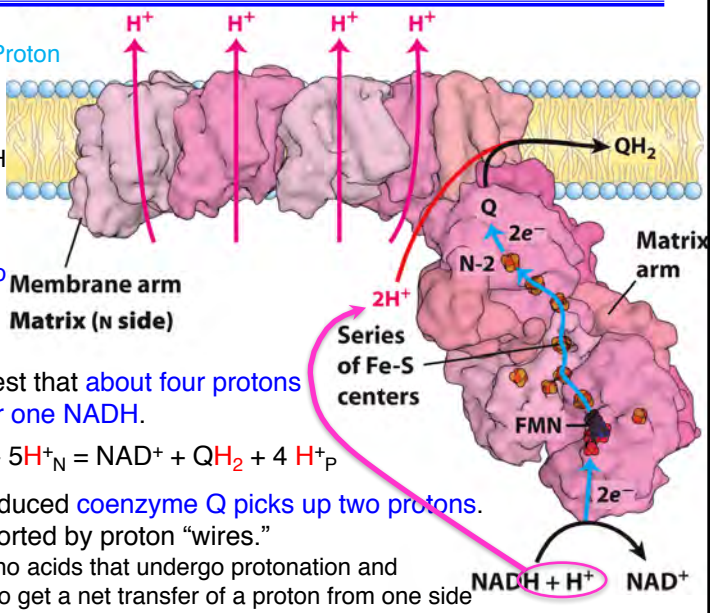
- One of the largest macro-molecular assemblies in the mammalian cell
- Over 40 different polypeptide chai encoded by both nuclear and mitochondrial genes
- NADH binding site in the matrix side
- Noncovalently bound flavin mononucleotide (FMN) accepts two electrons from NADH.
- Several iron-sulfur centers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



Electron Transport: Complex I

NADH:Ubiquinone Oxidoreductase Is a Proton Pump

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

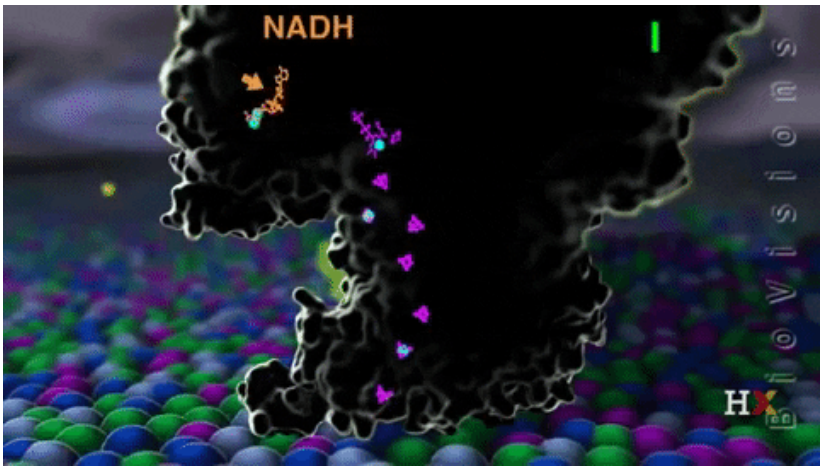


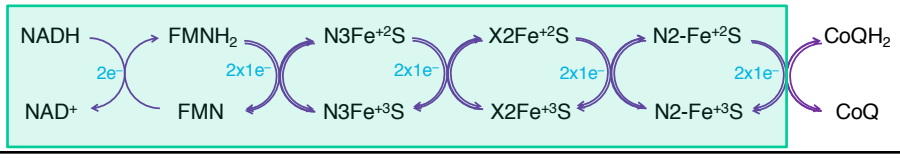
- Experiments suggest that about four protons are transported per one NADH.

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

- 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

Electron Transport: Complex I





Electron Transport

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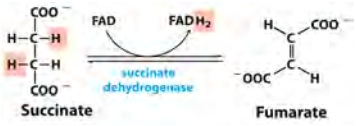
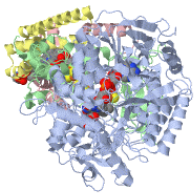
[a]Number of subunits in the bacterial complexes in parentheses.
[b]Mass and subunit data are for the monomeric form.
[c]Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

Electron Transport: Complex II

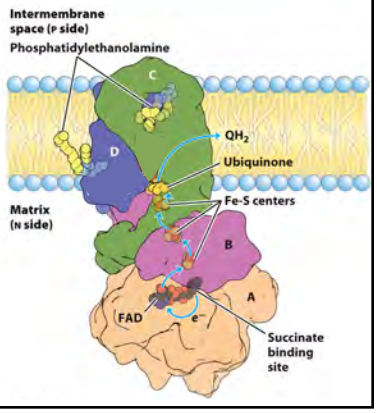
Succinate Dehydrogenase, a.k.a. Complex II



- 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



- 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



Jmol

Electron Transport

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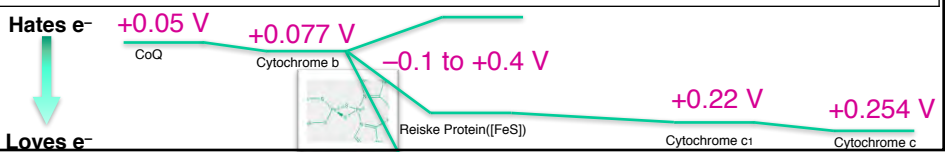

Electron Transport: Complex III

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.
- 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 without any flavin cofactors?