

**Bryan Krantz: University of California, Berkeley
MCB 102, Spring 2008, Metabolism Lecture 12**

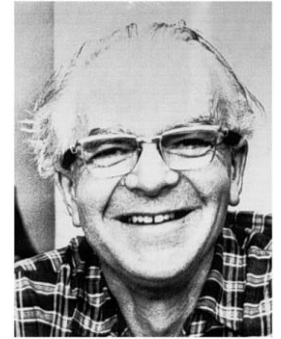
Reading: Ch. 19 of *Principles of Biochemistry*, “Oxidative Phosphorylation & Photophosphorylation.”

OXIDATIVE-PHOSPHORYLATION

- Reduced coenzymes, **FADH₂** / **NADH**, are made; oxidative phosphorylation is the oxidation of these coenzymes coupled to the reduction of oxygen to water.
- ATP was found to be made in a 3:1 ratio for each oxygen atom added to respiring mitochondria.

CHEMIOSMOTIC HYPOTHESIS

- *How might ATP be made?*
- Peter Mitchell answered the question by proposing:
 - [1] Oxidative phosphorylation occurs in a membrane encapsulated organelle.
 - [2] The electron and hydrogen carriers in the membrane are oriented in one direction in the membrane.
 - [3] The electron-hydrogen pairs (or hydrogens) could be transported via a **hydrogen carrier** across a membrane such that the H⁺s would only be released to one side (**p side**); the e⁻s could then move back down on **electron carriers** to pick up protons on a from the other side (**n side**) and travel back drop the H⁺ on the p side. The process repeats until the electrons lose all their energy and reduce oxygen to water.
 - [4] **Proton Motive Force (PMF)** develops; it is composed of chemical and electrical potentials for this proton concentration gradient. **This energy source could then make ATP.**



Peter Mitchell, 1920–1992

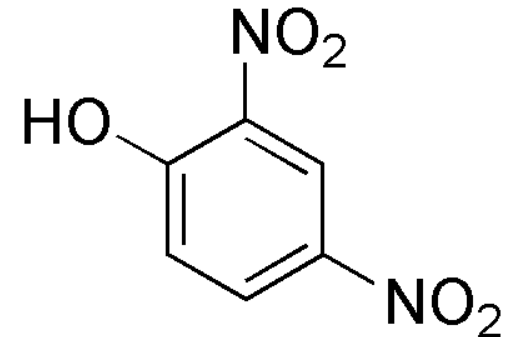
Proof.

[1] Need intact mitochondrial membranes.

[2] Active respiring mitochondria acidify the outside.

[3] **Uncouplers** are compounds that uncouple/decouple the process of oxidation from the process of ATP synthesis. Oxidation keeps going on and oxygen is consumed, but no ATP is made.

- *E.g.*, **2,4-dinitrophenol (DNP)**.
- Substrate-level phosphorylation fails to explain DNP's effect.



[4] **Produce artificial proton gradient.** By acidifying the outside medium, the mitochondria make ATP. Acidification of the outside medium leads to ATP synthesis without oxidation.

[5] **Racker and Stockenius.** The final proof was supplied by a reconstitution experiment of Racker and Stockenius. In pure phospholipid vesicles without any proteins, they added two kinds of proteins. One was **bacteriorhodopsin**, which can pump protons by utilizing light energy. The other was the **F₀F₁ ATPase**, which makes ATP from ADP and Pi. No other proteins from mitochondria were present.

By shining light onto this system, they could show that the ATPase converted the ADP and P_i to ATP without any oxidation or additional carrier proteins or transporters. This experiment sealed the deal.

Proton Motive Force (PMF). PMF is a “battery,” storing energy for eventual ATP synthesis.

[1] The chemical potential.

$\Delta G_{\Delta pH} = \Delta \mu_{\Delta pH} = RT \ln (C_{IN} / C_{OUT}) = 2.3RT \Delta pH$. There is an unequal distribution of the protons so the pH is lower on the outside than on the inside. This is sometimes called ΔpH . Basically, a concentration gradient is a source of potential energy. Equilibrium occurs when the gradient balances, making equal concentrations on either side of the membrane.

[2] The electrical potential .

$\Delta G = -zF \Delta E$. (ΔE is sometimes called $\Delta \Psi$.) The outside of the membrane tends to get charged more positively and the inside is more negative, since a proton is charged. z is the charge (*i.e.*, +1 for H^+).

→ A summed “proton voltage” (PMF) or “proton free energy” (ΔG_{PMF}):

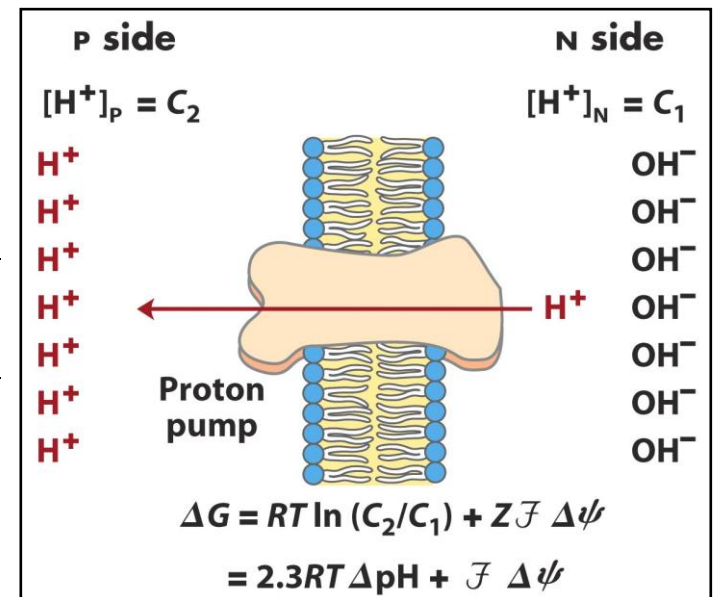
$$PMF = \Delta \Psi + 2.3(RT/F) \Delta pH$$

$$\Delta G_{PMF} = 2.3RT \Delta pH + F \Delta \Psi$$

$$\Delta G_{PMF} = F \times PMF.$$

NOTE: the signs of these eqns. are given by definition of the (+) and (-) sides of mitochondria, *i.e.*, $\Delta \Psi \equiv \Psi_P - \Psi_N$.

- The PMF in the mitochondrion is ~0.2 V.
- In mitochondria, ~70% of the entire proton motive force is due to ΔE . The remaining part is due to the ΔpH .



Theoretical Proof?

Standard state ATP hydrolysis free energy is:



PMF of a typical mitochondrion is **0.2 V**, and **F** is 96.5 kJ/mol/V:

$$\Delta G_{\text{PMF}} = F \times \text{PMF} \quad \Delta G_{\text{PMF}} \sim -20 \text{ kJ/mol.}$$

Another issue to think about carefully, the oxidation of NADH:

The oxidation of NADH to NAD⁺ by oxygen to make water:



$$\Delta G_{\text{OX}} = -2F \times \Delta E^{\circ} \quad \Delta G_{\text{OX}} \sim -220 \text{ kJ/mol.}$$

What if that huge 1.14 V redox potential drove an equivalent number of protons across the membrane during oxidation?

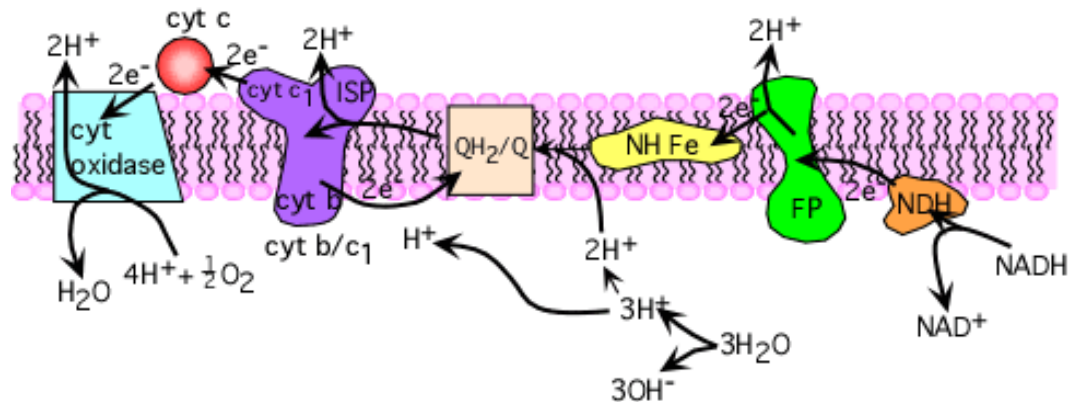
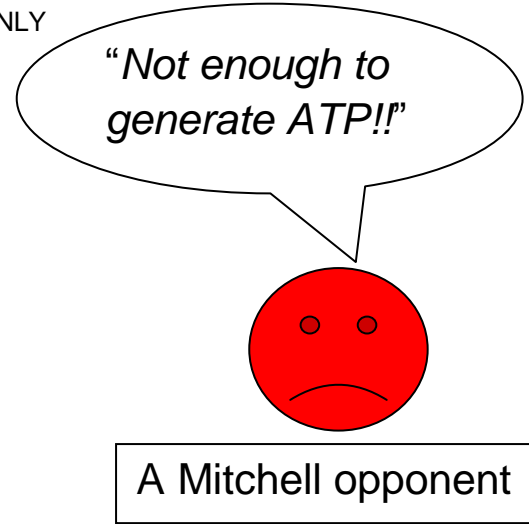
Yes! 10 H⁺ are pumped out per NADH.

Now the math adds up

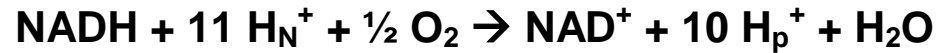
$$10 \times 20 \text{ kJ/mol} = 200 \text{ kJ/mol}$$

→ That'll not only make ATP but make several.

→ 200 kJ / 220 kJ gives the efficiency of oxidation at >90% !



The real equivalence of NADH oxidation to proton gradient generation



Here is the net gain, + 10 H_p⁺ are pumped to form the gradient. (FADH₂ is +6 H_p⁺.)

Multiple protons make each ATP

- ~3 H⁺ per ATP.
- There is a machine in the mitochondrial membrane that does this—the **F₀F₁ ATPase**.

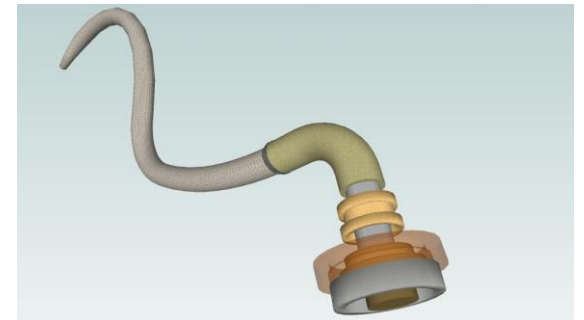
Direct use of PMF to perform work

Before producing ATP, the PMF can be utilized directly as an energy source:

[1] **The bacterial flagellum motor**. The bacteria swim by rotating their flagella. The energy from that does not come from ATP, but from moving protons across a membrane—the PMF.

- Probably it takes more than 20 kJ/mol to swing the flagellum motor around.
- Thus it is perfectly possible that ATP synthesis could take multiple protons to make each ATP.

[2] **Active transport** means that you spend energy transporting metabolites and proteins across the membrane. A proton gradient can be used to pump these molecules “actively” across a membrane.



Transport Examples.

The proton concentration gradient can compensate for an unfavorable concentration gradient of a solute the cell or mitochondrion wants on the inside. For example, consider **Lactose Permease**

| OUTSIDE | | INSIDE |
|---------------------------|--|----------------------------|
| Low lactose | | High lactose |
| High H⁺ | | Lower H⁺ |

Mitochondria make **ATP**, but to make **ATP** requires **P_i** and **ADP**.

[1] Phosphate Symporter. You have to have a system to import P_i from the cytosol to the mitochondrial matrix. This P_i transporter is coupled to the transport of the proton. This system is a **proton symporter**. It is a transporter that couples the movement of something else to the spontaneous movement of proton. Outside the mitochondrion, you have more protons than inside. The protons have a natural tendency to come in from the outside of the mitochondria to inside. You take advantage of that spontaneous tendency to bring in P_i.

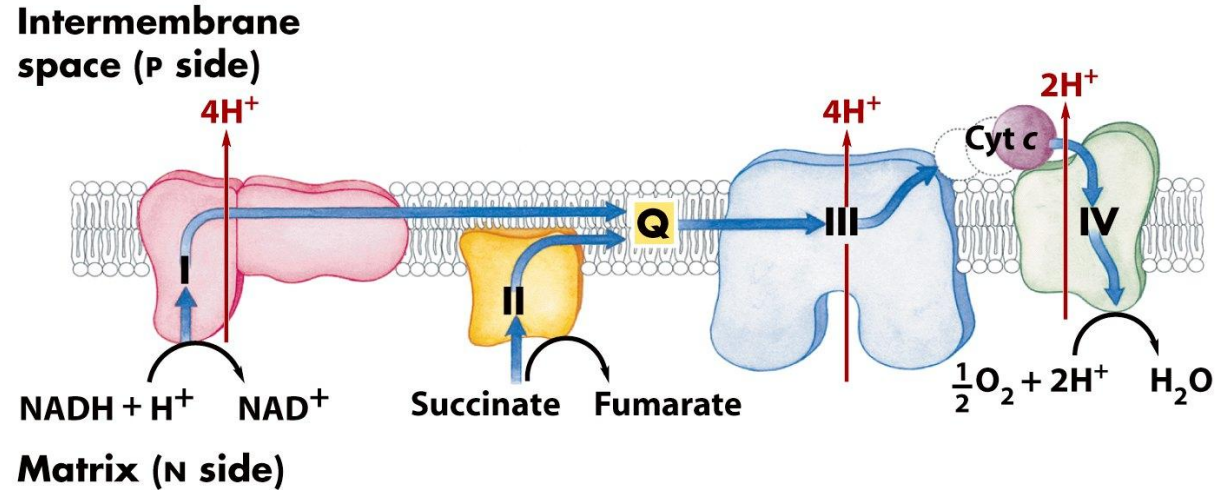
[2] Adenine Nucleotide Translocase. This transporter is an ADP/ATP **exchanger**. The transport of ADP is coupled to the export of the ATP synthesized in the mitochondrion. ATP has four negative charges, but ADP only has three negative charges. Pushing out four negative charges and pulling in three negative charges gives a net movement of one negative charge out of the mitochondrion. This is favorable because the outside surface is more positively charged than the interior surface.

The PMF drives P_i import and ADP/ATP exchange!

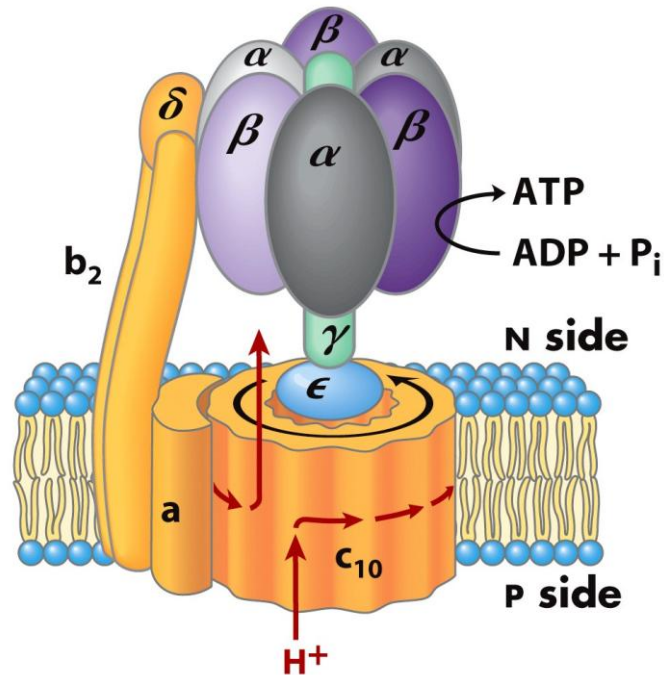
*****For bookkeeping, 1 proton from the PMF is used to get ATP out and ADP and P_i in.*****

What are the Nuts & Bolts of Electron Transport and ATP Synthesis?

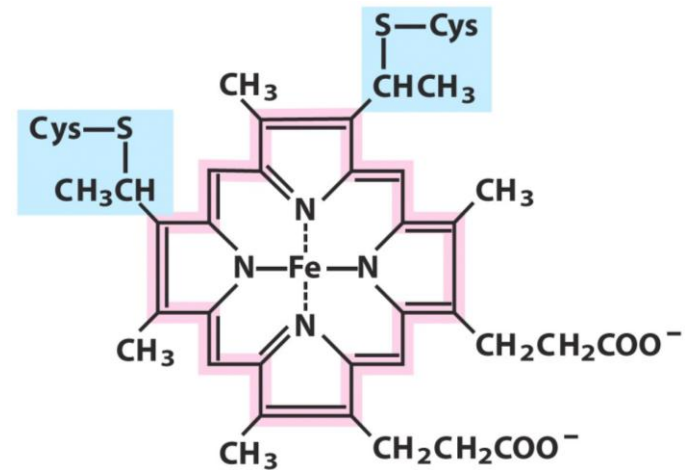
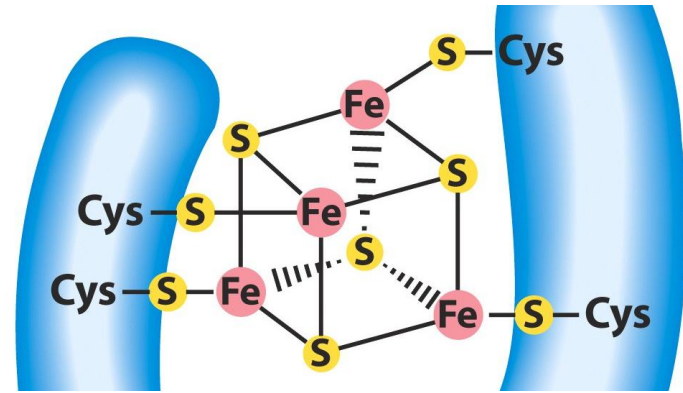
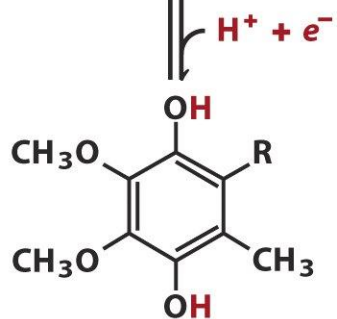
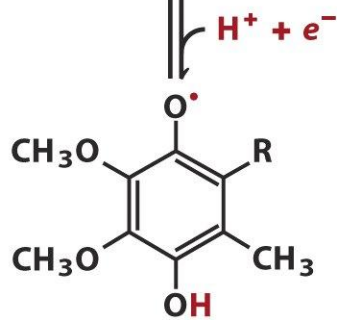
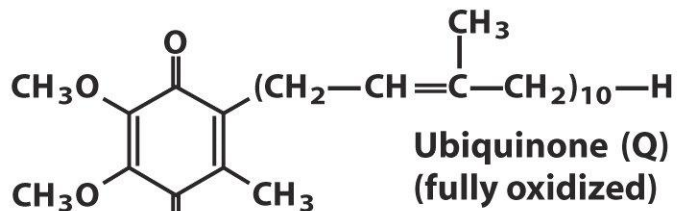
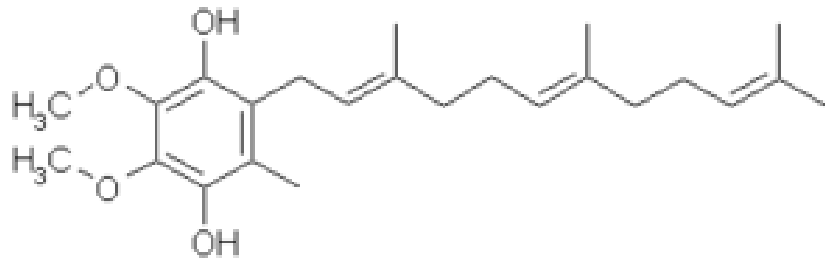
[STEP 1] Electron & proton transport builds PMF.



[STEP 2] PMF drives ATP synthesis.



Proton, Electron and Proton/Electron Carriers



[Complex I] NADH Dehydrogenase.

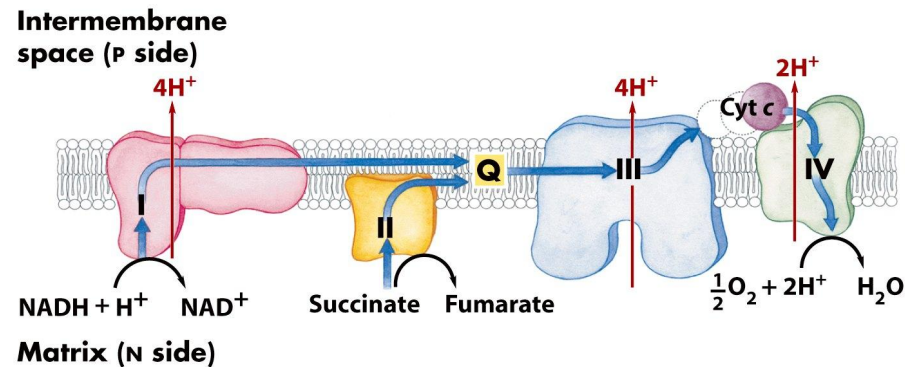
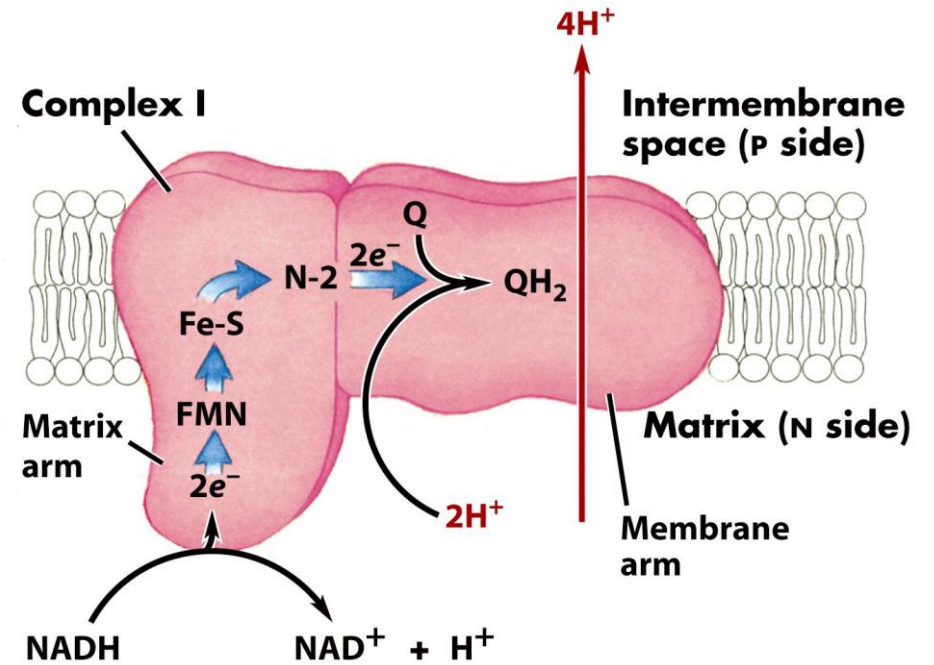


Architecture.

- Massive, 43-protein complex, 850 kDa.
- L-shaped by electron microscopy
- 6 Fe-S centers & FMN cofactor

Mechanism.

- The electrons from the NADH are transferred to FMN and then the Fe-S centers.
- Electron transfer then proceeds via Fe-S centers to the ubiquinone, reducing it to ubiquinol (QH₂).
- The electron transfer leads to a net flux of 4 protons, which move **vectorially** to the P side.
- Complex I is a proton pump energized by electron transfer to Q.



[Complex II] Succinate Dehydrogenase.



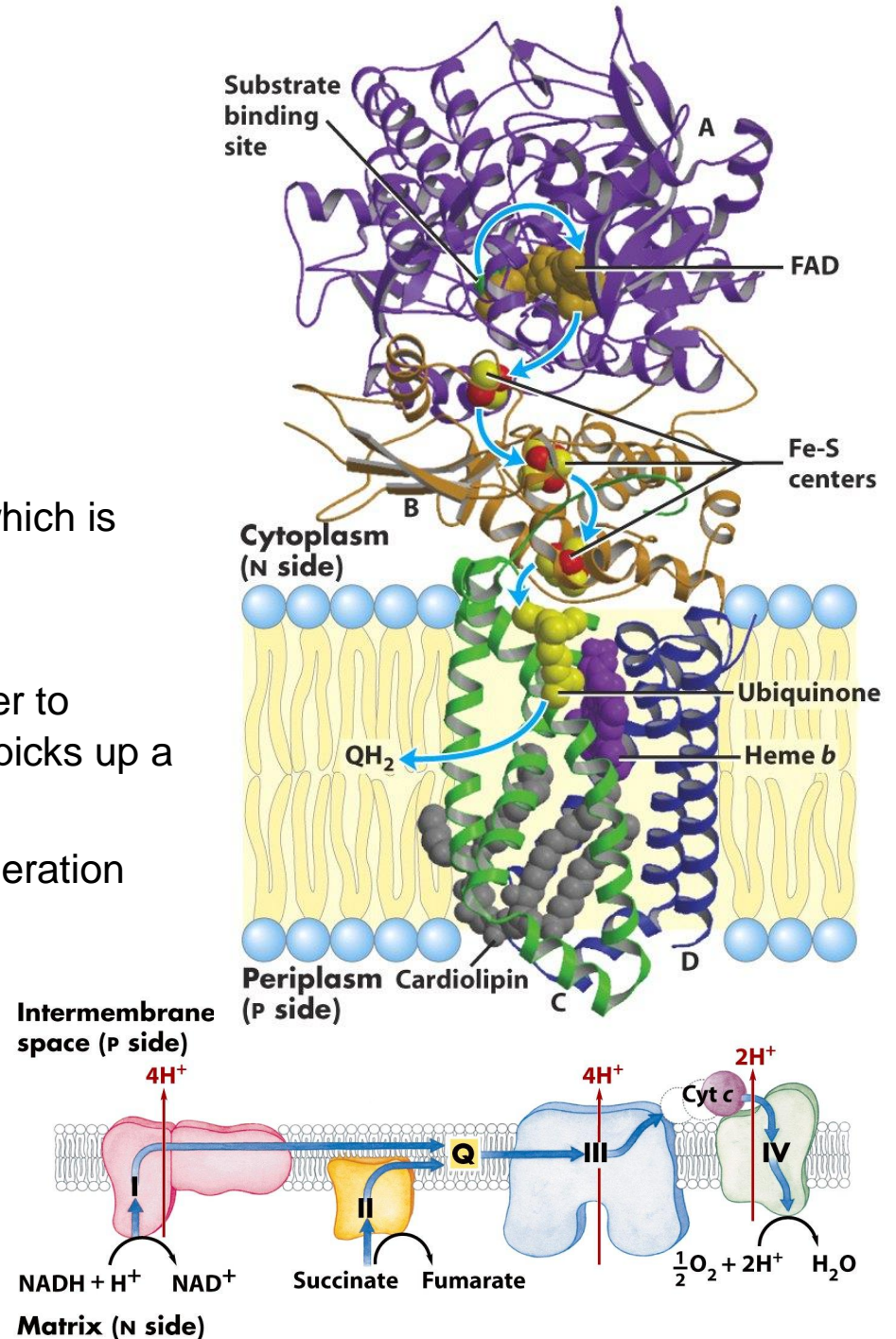
Architecture.

- 3-D structure of a bacterial homolog known.
- 4 protein subunits, 140 kDa.
- 5 prosthetic groups
- Heme, FAD, 3 Fe-S clusters.
- Electron acceptors never more than 11 Å apart, which is optimal for electron transfer.

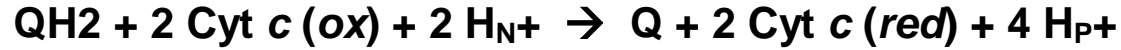
Mechanism.

- Recall the CAC, succinate binds, electrons transfer to bound FAD, then Fe-S clusters, and onto Q, which picks up a pair of protons as usual.
- Heme backstop to prevent superoxide radical generation perhaps when Qs are unavailable.

Analogs. Fatty acid oxidation. Glycerol 3-phosphate dehydrogenase. Flavin proteins are involved that accomplish similar ends.



[Complex III] Ubiquinone: Cytochrome c Oxidoreductase.



Architecture.

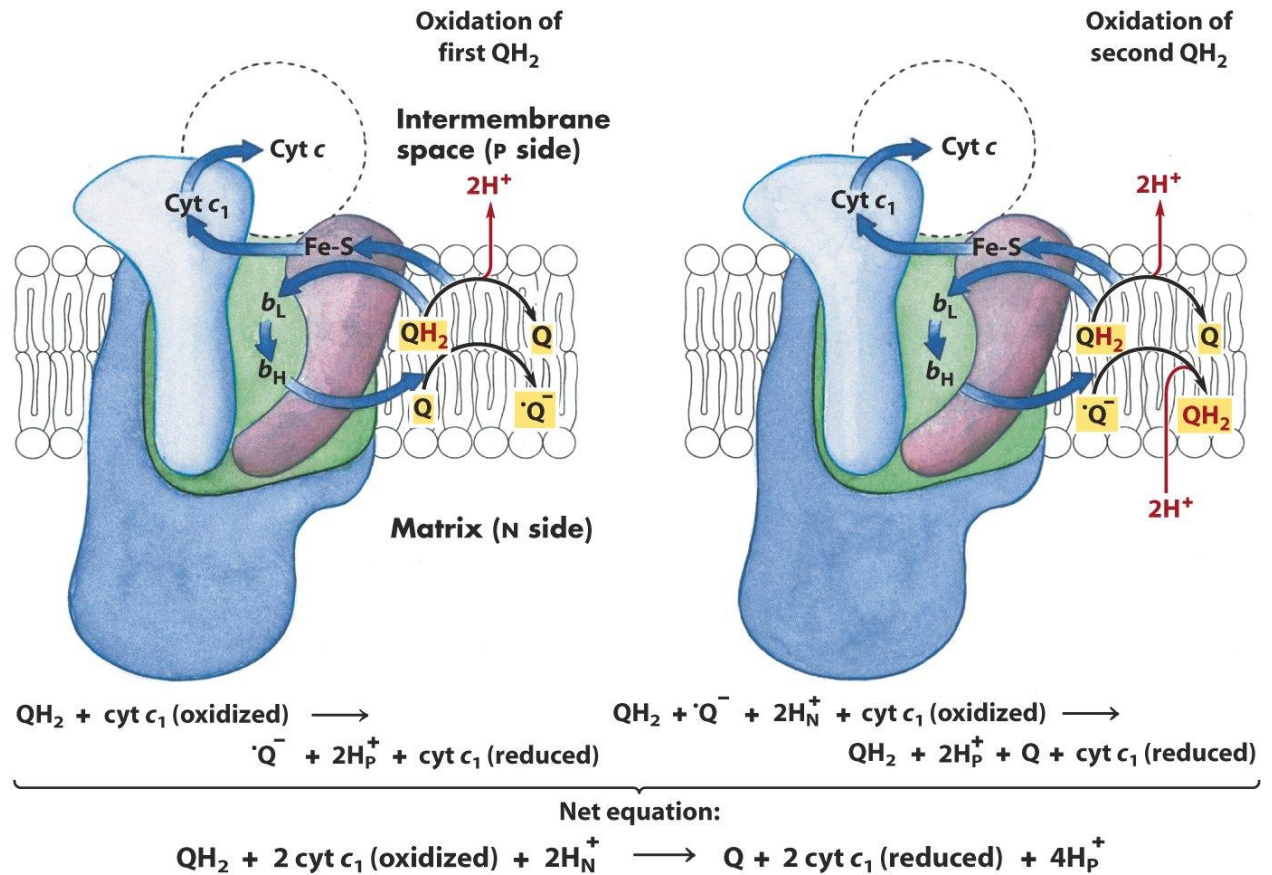
- Big, 11-protein, 250 kDa structure is dimeric—a necessary feature for function.
- Two docking sites for QH₂ and Q.

Mechanism.

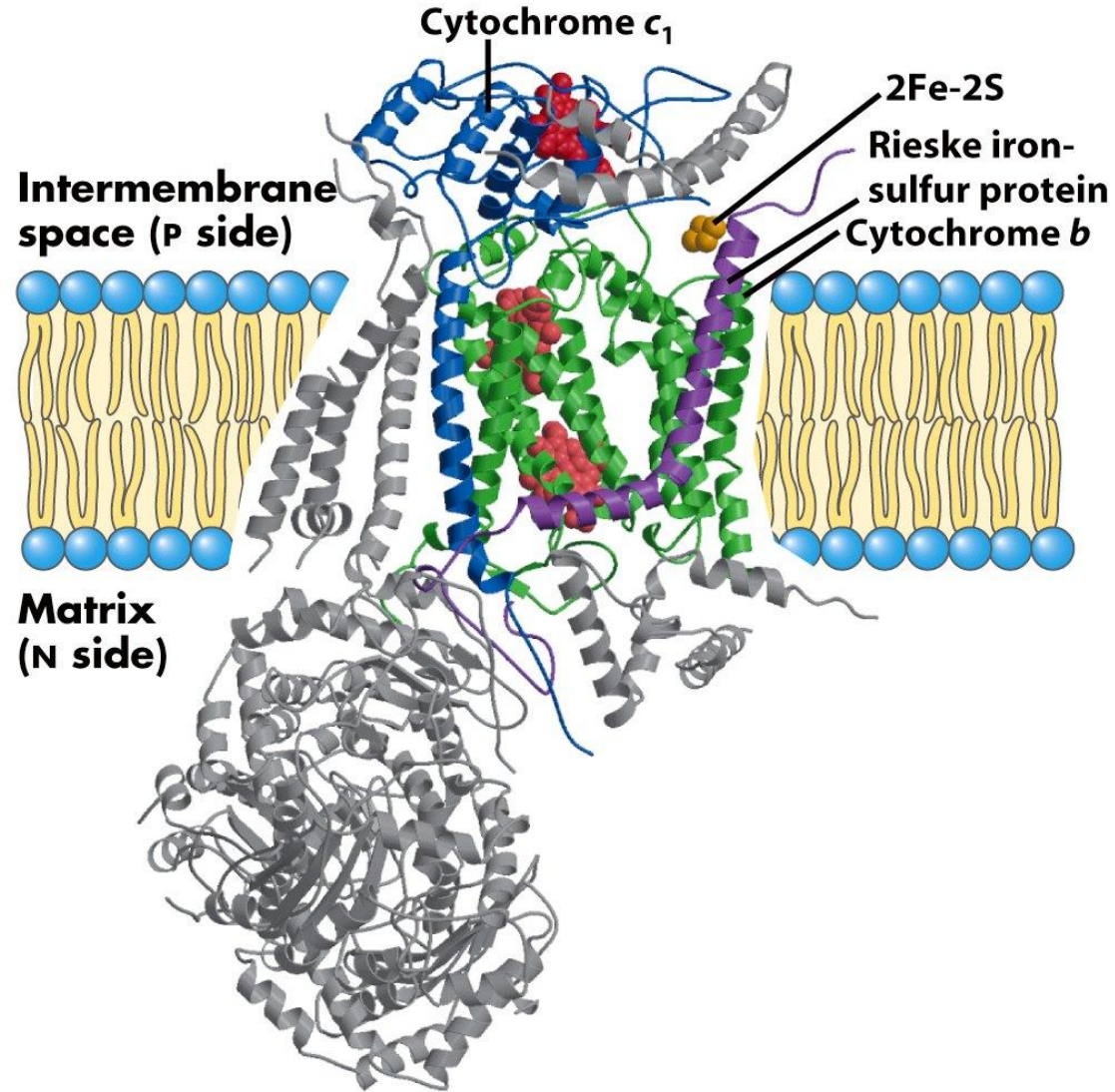
- Need to go from a 2e⁻ carrier, Q, to single e⁻ carrier, Cyt c.
- **Q Cycle**—radical state of Q is formed, **semiubiquinone**. Thus two single e⁻ transfers split to the cytochromes back to a make Q → Q[•] and to Cyt c are possible in one half cycle. In second half cycle, Q[•] → QH₂ after picking up electron after a second split—and so on.

(**Cytochrome** is protein with a Fe-heme group. They only take single electrons on these Fe-hemes, where Fe³⁺ + e⁻ → Fe²⁺.)

- 4 net H⁺ are pumped across the membrane in two half Q cycles of 2 H⁺ each.



Structural Insight on the Split-Electron Path taken in the Q Cycle.



[Complex IV] Cytochrome c oxidase.



Architecture.

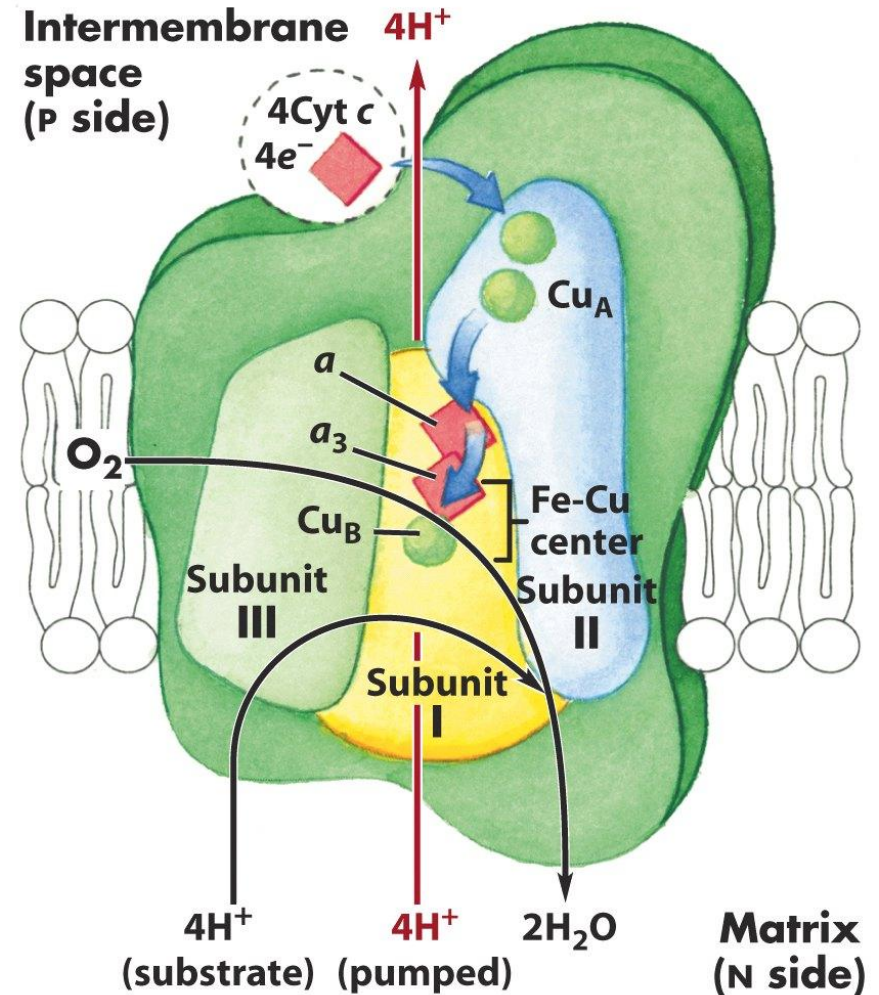
- Large, 204 kDa, 13-protein complex.

Mechanism.

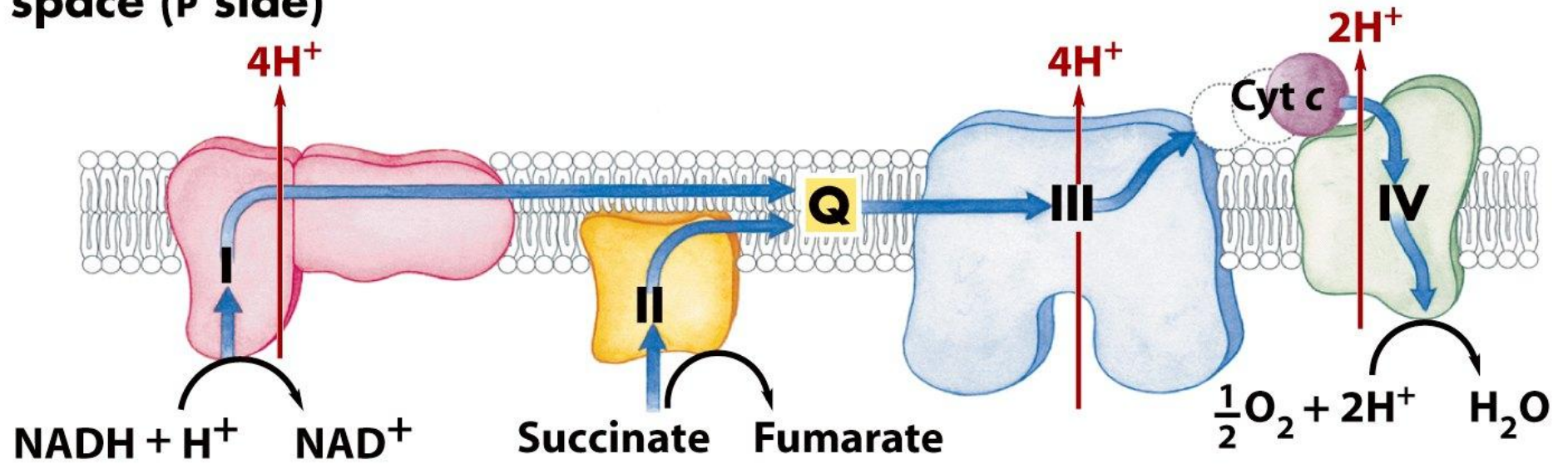
- Cyt c from Complex III must now be oxidized so that its e^- can be transferred to oxygen.
- Electron path contains Fe-heme, Fe-S and copper ions (Cu ion, Cu_B , and an unusual Cu-Cys residue binuclear pair, Cu_A .)
- Path: $\text{Cyt } c \rightarrow \text{Cu}_A \rightarrow \text{Heme} \rightarrow \text{Heme-Cu}_B \rightarrow \text{O}_2$
- 2 net H^+ are pumped across the membrane.
- Oxygen is reduced to water.

Complex IV does not make many mistakes.

Mistakes are probable as the Cyt c transfers are single-electron and O_2 requires four of transfers to make two H_2O . The possibility for **hydrogen peroxide** (H_2O_2) and/or **hydroxy radical** (HO^\bullet) formation is real. Complex IV is a champ!



Intermembrane space (P side)



Matrix (N side)