BioChem 330 - Course Outline

- **Metabolism and Bioenergetics (II)**
  - ENZYME CATALYSIS:
    - kinetic constants k_{cat}, K_m
    - Catalytic strategies, the serine proteases
  - CATABOLISM (*breakdown*)
    - Carbohydrates
      - Glycolysis
      - Tricarboxylic Acid Cycle
      - Electron Transport
      - Chemiosmosis and ATPase
    - Fatty acids and amino acids

Overview for Today, November 15, 2011

- Electron Transport Players
- Diagram of the Chain
- Complex 1….
  - NADH oxidase/ubiquinone reductase
- Complex III ……
- ubiquinol oxidase/cyc c reductase

A reminder, things above reduce things below…

Table 19–2

Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

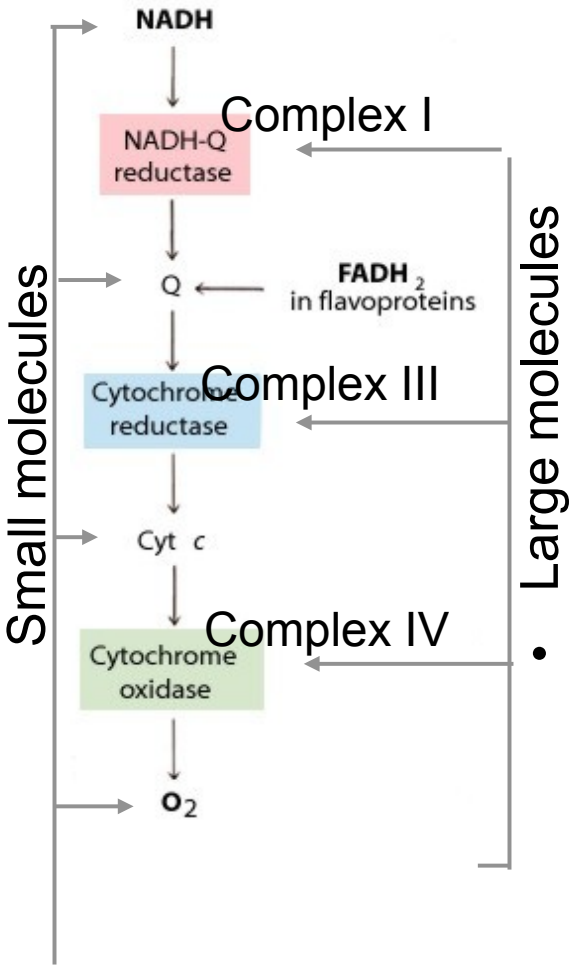
Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ 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+})	0.55
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816

Note sign change

- So:
  - NADH → $NAD^+ + H^+ + 2e^-$ $E_o' = +0.32$ V
  - $UbiQ + 2H^+ + 2e^- \rightarrow UbiQH_2$ $E_o' = +0.045$ V
  - NADH + UbiQ + H+ → NAD+ UbiQH2 $\Delta E_o' = +0.365$ V

- And $\Delta G^{o'} = - nF\Delta E^{o'} = - (2)(23.1 \text{ kcal/V*mol})(.365) = -17$ kcal/mole

An overview of what happens to NADH…



Electrons are transferred from NADH to O_2 through a chain of three large protein complexes called *NADH-Q reductase*, *cytochrome reductase*, and *cytochrome oxidase*. Electron flow within these transmembrane complexes leads to the pumping of protons across the inner mitochondrial membrane [and thereby to the synthesis of ATP].

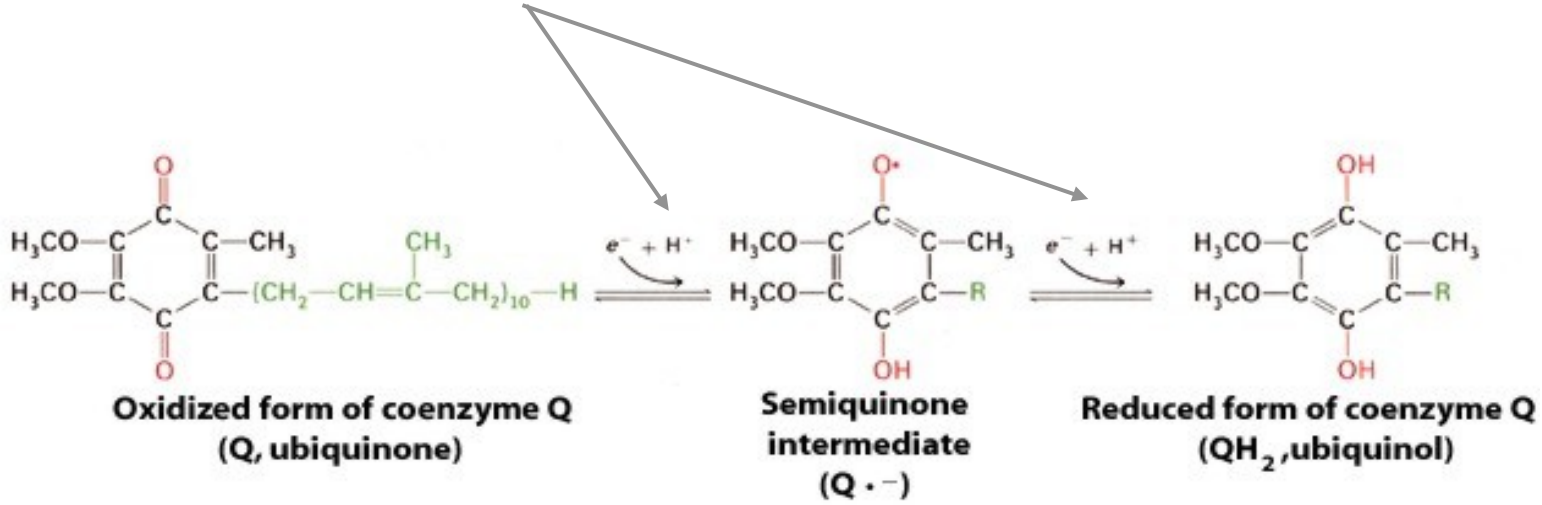
Table 21-2
Components of the mitochondrial electron transport chain

Enzyme complex	*Mass (kd)*	*Subunits*	*Prosthetic group*	*Oxidant or reductant*		
				Matrix side	*Hydrocarbon core*	*Cytosolic side*
NADH-Q reductase	880	≥34	FMN Fe-S	NADH	Q	
Succinate-Q reductase	140	4	FAD Fe-S	Succinate	Q	
Cytochrome reductase	250	10	Heme *b*-562 Heme *b*-566 Heme c_1 Fe-S		Q	Cyt *c*
Cytochrome oxidase	160	10	Heme *a* Heme a_3 Cu_A and Cu_B			Cyt *c*

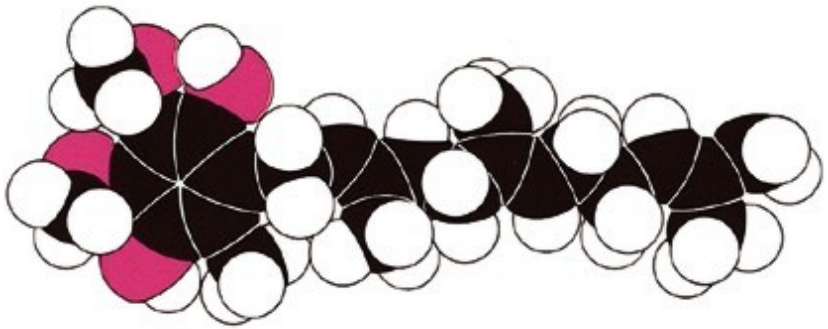
Sources: J.W. DePierre and L. Ernster, *Ann. Rev. Biochem.* 46(1977):215, Y. Hatefi, *Ann. Rev. Biochem.* 54(1985):1015, and J.E. Walker, *Quart. Rev. Biophys.* 25(1992):253.

Ubiquinone

Note that two protons need to be added to convert Q to QH_2



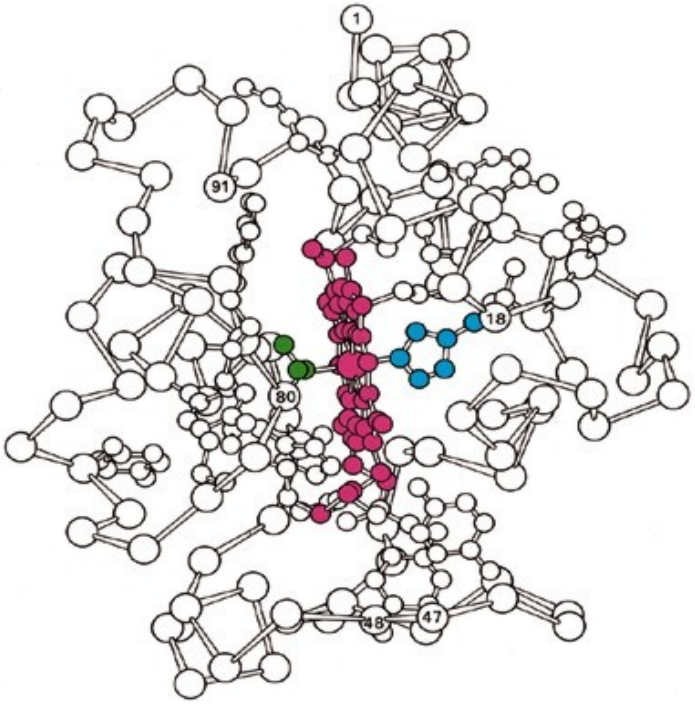
- Small molecule
- Hydrophobic, soluble in the mitochondrial membrane
- Carrier of both electrons/ $2H^+$

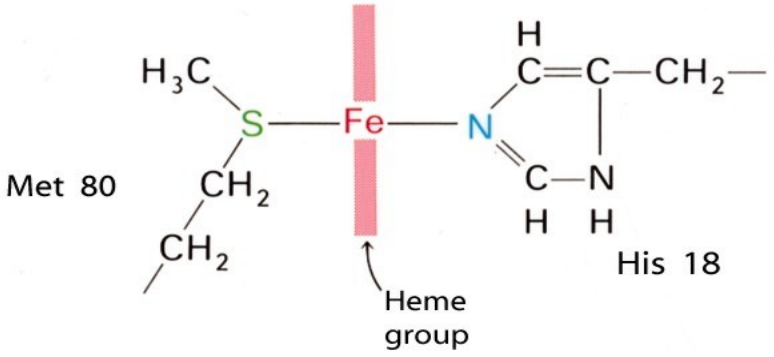


…+ 7 more isoprenoid units

Cytochromes…

(carry only 1 electron)





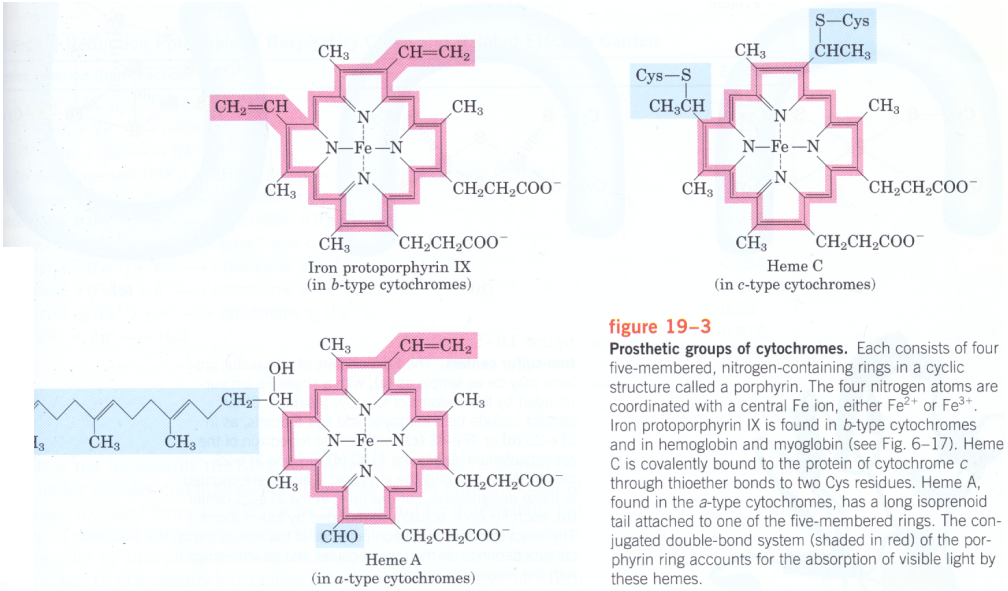


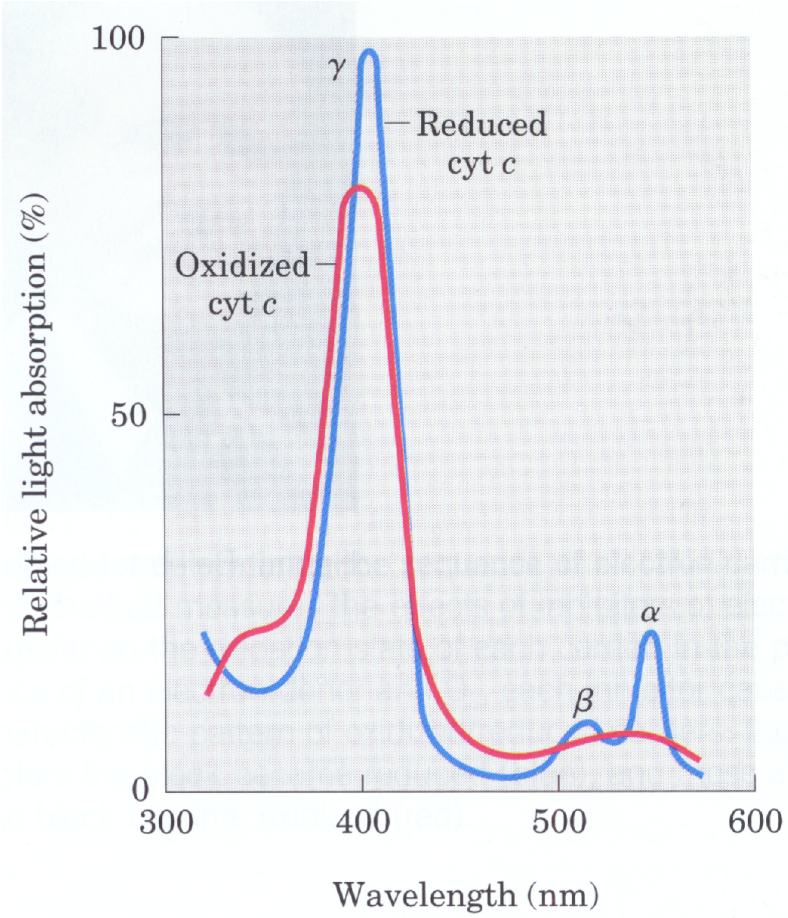
figure 19–3

Prosthetic groups of cytochromes. Each consists of four five-membered, nitrogen-containing rings in a cyclic structure called a porphyrin. The four nitrogen atoms are coordinated with a central Fe ion, either Fe^{2+} or Fe^{3+}. Iron protoporphyrin IX is found in *b*-type cytochromes and in hemoglobin and myoglobin (see Fig. 6–17). Heme C is covalently bound to the protein of cytochrome *c* through thioether bonds to two Cys residues. Heme A, found in the *a*-type cytochromes, has a long isoprenoid tail attached to one of the five-membered rings. The conjugated double-bond system (shaded in red) of the porphyrin ring accounts for the absorption of visible light by these hemes.

Differences in the protein environment of the heme group in various cytochromes make it more or less costly to remove an electron: [$Fe^{2+} \rightarrow Fe^{3+} + 1\ e^-$]

- Differences in that energy cost correspond to differences in E^{o}'.
- Cytochrome C is shown at left.

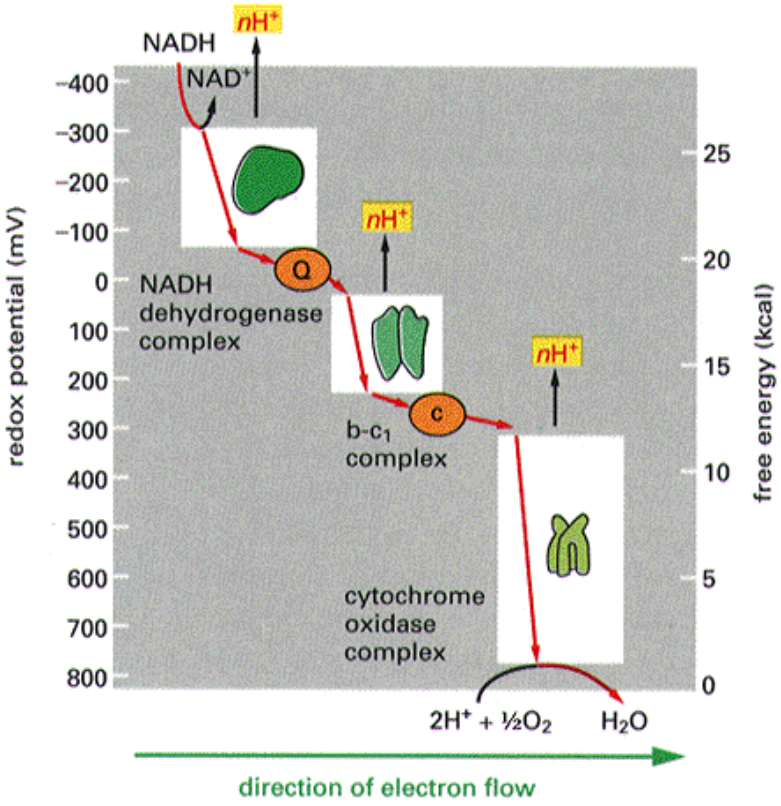
How we know what we do about the chain…



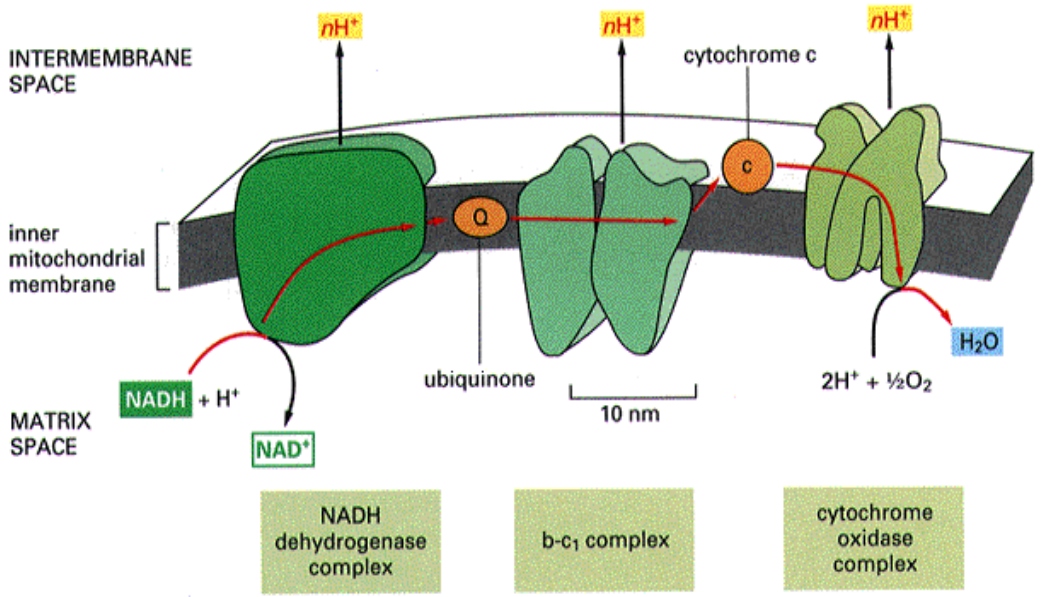
- E^o's can be determined, and ordered from stronger reducers to stronger oxidizers *in situ* using inhibitors
- Oxi/red can be followed optically from both Soret band at about 410 nm and the longer λ bands which are actually a bit more sensitive. At long λ, **oxidized cyt c (Fe^{+3})** has only one band (530 nm) but **reduced (Fe^{+2})** has an alpha (550 nm)and a beta (510 nm).
- FeS clusters can be monitored by their EPR signal, which comes from the unpaired electrons
  - Fe(III) (d5, gives either five or one unpaired electron depending on splitting)
  - Fe(II) (d6, either 4/2 or zero unpaired e- depending on splitting)

The electron transport chain

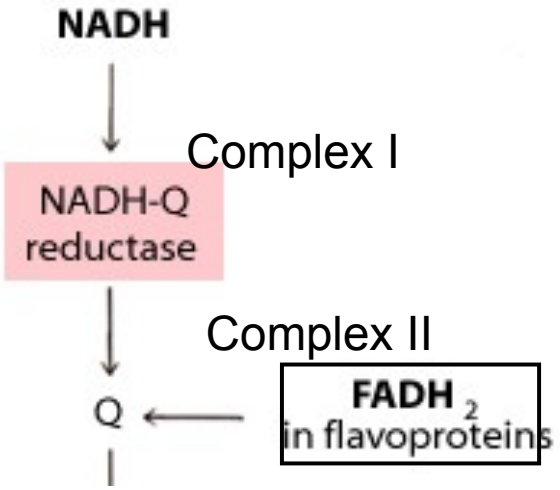
- Looks energetically



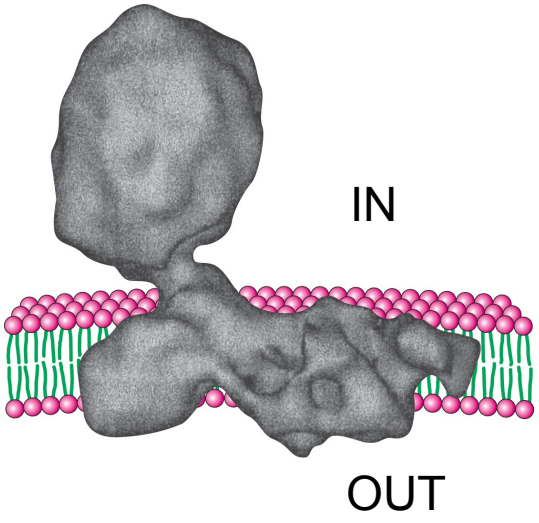
… and functionally like this (although not necessarily clustered nicely like so…)



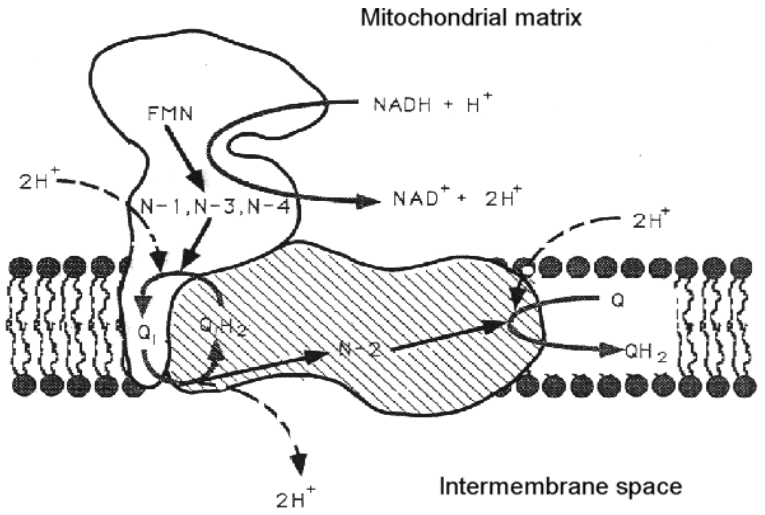
Complex I,II: Passing electrons to ubiquinone



EM reconstruction



- 2 electrons from NADH (through Complex I)
- …or 2 from succinate to $FADH_2$ (through Complex II, see TCA 5)
- Complex I
  - Electron carriers FMN, 4Fe-4S center, bound UbiQ, 2Fe-2S center
  - over 30 protein chains
- Structure unknown, except by EM
- Donates 2 electrons to ubiquinone
- Translocates 2 protons from in to out

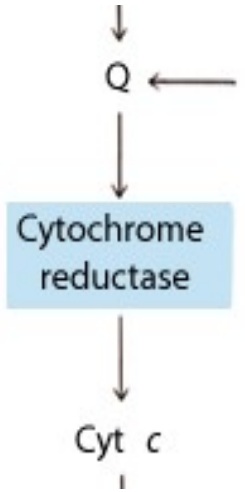


Complex I…..*What do we know about accepting electrons from NADH and donating them to ubiquinone?*

- most intricate membrane-bound enzyme known to date, 40 years since the first isolation from bovine heart mitochondria
  - 43 different subunits whose primary structures have been determined (7 are encoded by mitochondrial DNA)
  - one noncovalently-bound FMN molecule
  - 2-3 binuclear (Fe_2S_2) and 6 tetranuclear (Fe_4S_4) iron-sulfur clusters
  - three unique quinone sites are the basis of the reaction to inhibitors

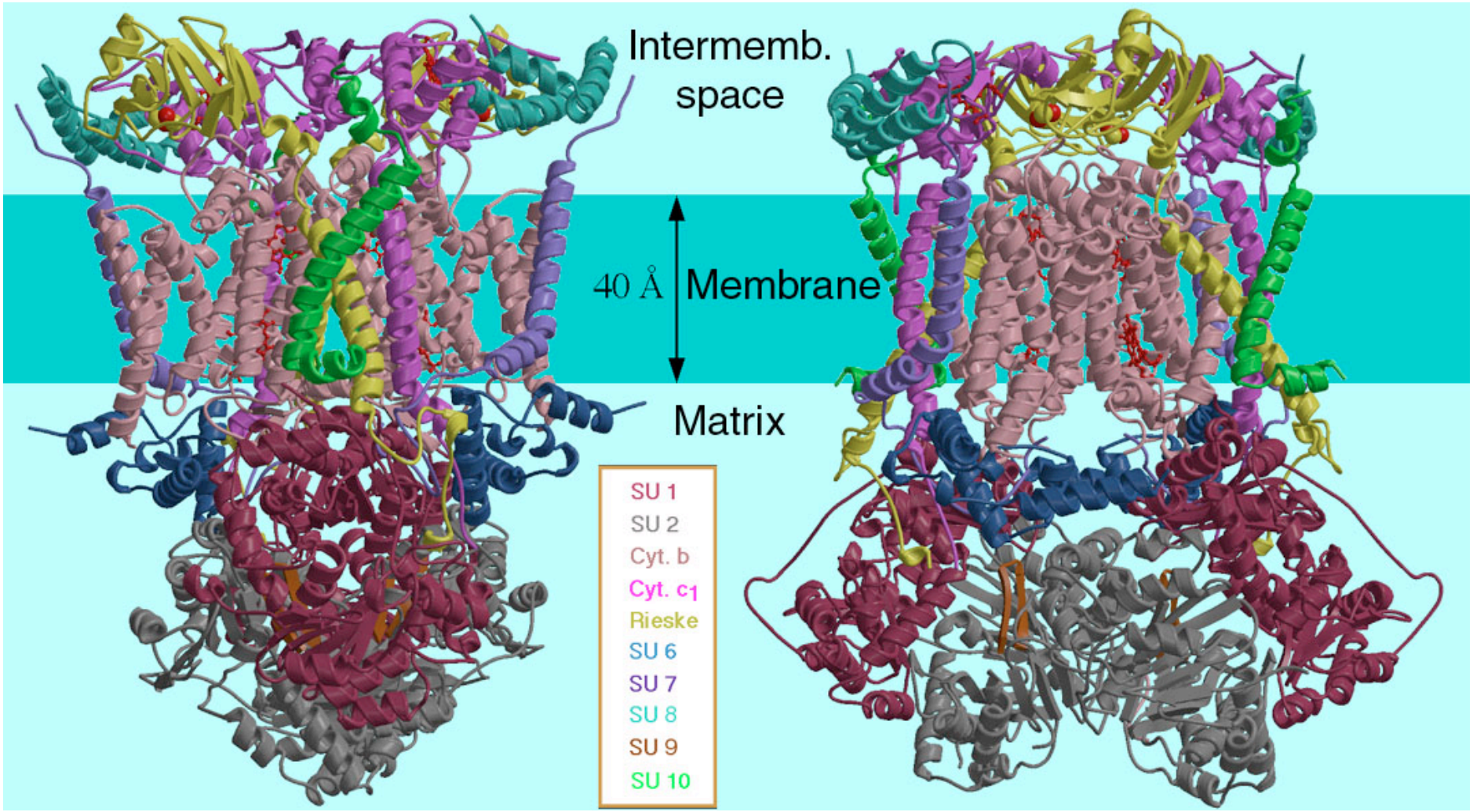
Complex III: Collecting the electrons from QH_2 - The cyt b/c_1 complex

- Cytochrome b/c_1 complex - the electron carriers
  - Carrier #e^- Eo' (V)
  - (UbiQ) 2 0.045 e donor
  - cyt b 1 0.077
  - Fe-S ? ?
  - cyt c_1 1 0.22
  - (Cyt c) 1 0.25 e acceptor



- Which suggests a sequence of transfers
- Q → cyt b → Fe-S → cyt c_1 → cyt c

Complex III: Collecting the electrons from QH_2 - The cyt bc_1 complex

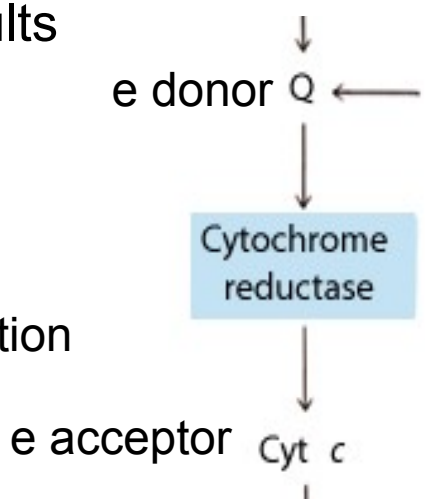


http://www.life.illinois.edu/crofts/bioph354/bc-complex_summary.html

Complex III: Collecting the electrons from QH_2 - The cyt b/c_1 complex

- Q → cyt b → Fe-S → cyt c_1 → cyt c

- Above scheme is also consistent with these results
  - In the presence of antimycin, an inhibitor of e-transport,, reduced QH_2 and cyt b accumulate, so antimycin should block between cyt b and Fe-S Protein/Cyt c_1
  - Removal of Fe-S-Protein also results in accumulation of reduced cyt b and oxidized cyt c_1



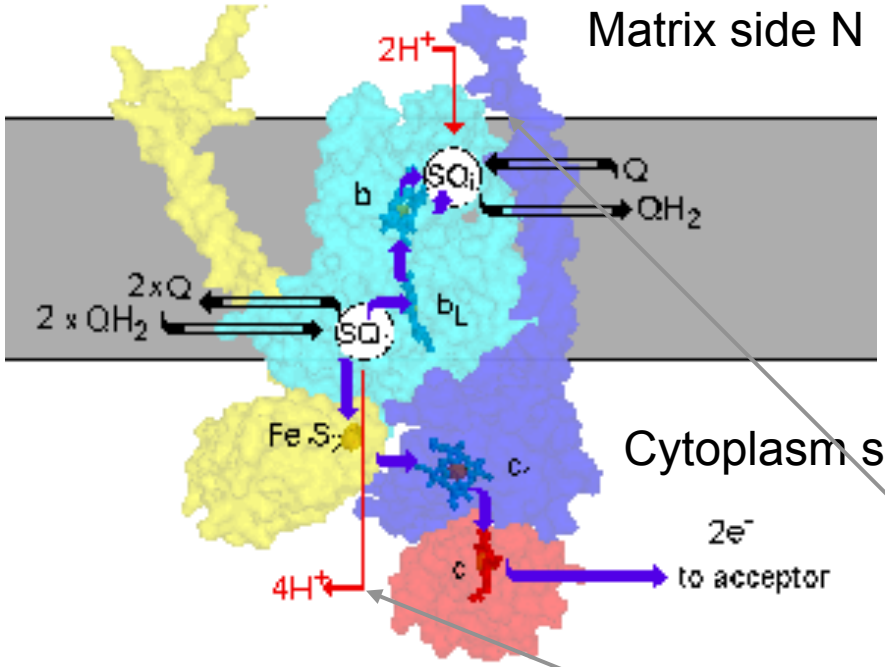
Complex III: Cytochrome b/c_1

- **The b/c_1-complex from beef heart mitochondria**

Subunit	*Redox centers*	*Mr(beef)*	*Function*
Core I	none	53.6	No catalytic, protein transport
Core II	none	46.5	No catalytic, protein transport
Cyt b (III)	heme b_H	42.6	donor to Qi-site
	heme b_L		acceptor from SQ at Qo-site
			transmembrane elec transfer
Cyt c1(IV)	heme c_1	27.3	donor to cyt c
Rieske (V)	2Fe.2S center	21.6	acceptor from Q_oH2
			donor to cyt c1
Subunit VI	none	13.3	none known
Subunit VII	none	9.5	none known
Subunit VIII	none	9.2	hinge protein (interacts w c1)
Subunit IX	none	8.0	none known
Subunit X	none	7.2	none known
Subunit XI	none	6.4	none known

Complex III: Cytochrome b/c_1 How does it work?

One subunit minus SU1 and SU2



Please note this image is inverted from previous bc1 image

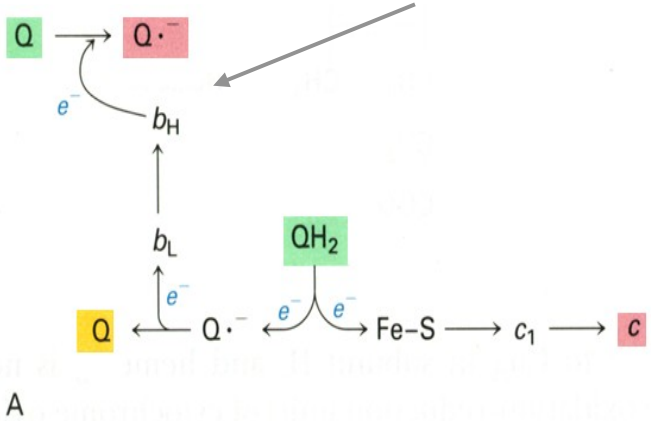
QH_2 + 2 ferricyt c^{3+} + $2H^+_N$ <==>
Q + 2 ferrocyt c^{2+} + $4H^+_P$

- Net reaction is to remove two electrons from ubiquinol to make ubiquinone in the membrane and to give 2 electrons to two molecules of cyt c (Fe(III) → Fe (II)) in the cytoplasmic/ intermembrane space (or periplasm in bacteria) **[NOTE THAT THIS DIRECT TRANSFER IS <u>NOT</u> WHAT HAPPENS]**
- (vector) 2 H+ are consumed from the matrix (N side) and end up across the membrane in the cytoplasmic (P side)
- (scalar) 2 H+ are released into the inter membrane space from every QH_2/Q oxidized for a net of 4H+ per cycle

Nothing in life is simple..

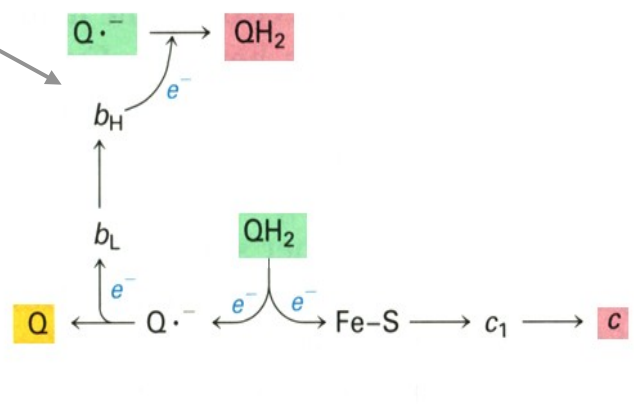
- Q → cyt b →(anti) →Fe-S → cyt c_1 → cyt c
- when antimycin is added, reduced cyt b builds up, cyt c not reduced
- when Fe-S is removed, reduced cyt b builds up, cyt c not reduced
- but when Fe-S if removed and THEN antimycin is added to the Fe-S deficient complex, reduction of cyt b is blocked???????
- Not only that, there are, in fact, two hemes in cyt b, one (b_L) with a lower electron affinity than the other (b_H)
- A proposed solution - the Q cycle:

Antimycin blocks here



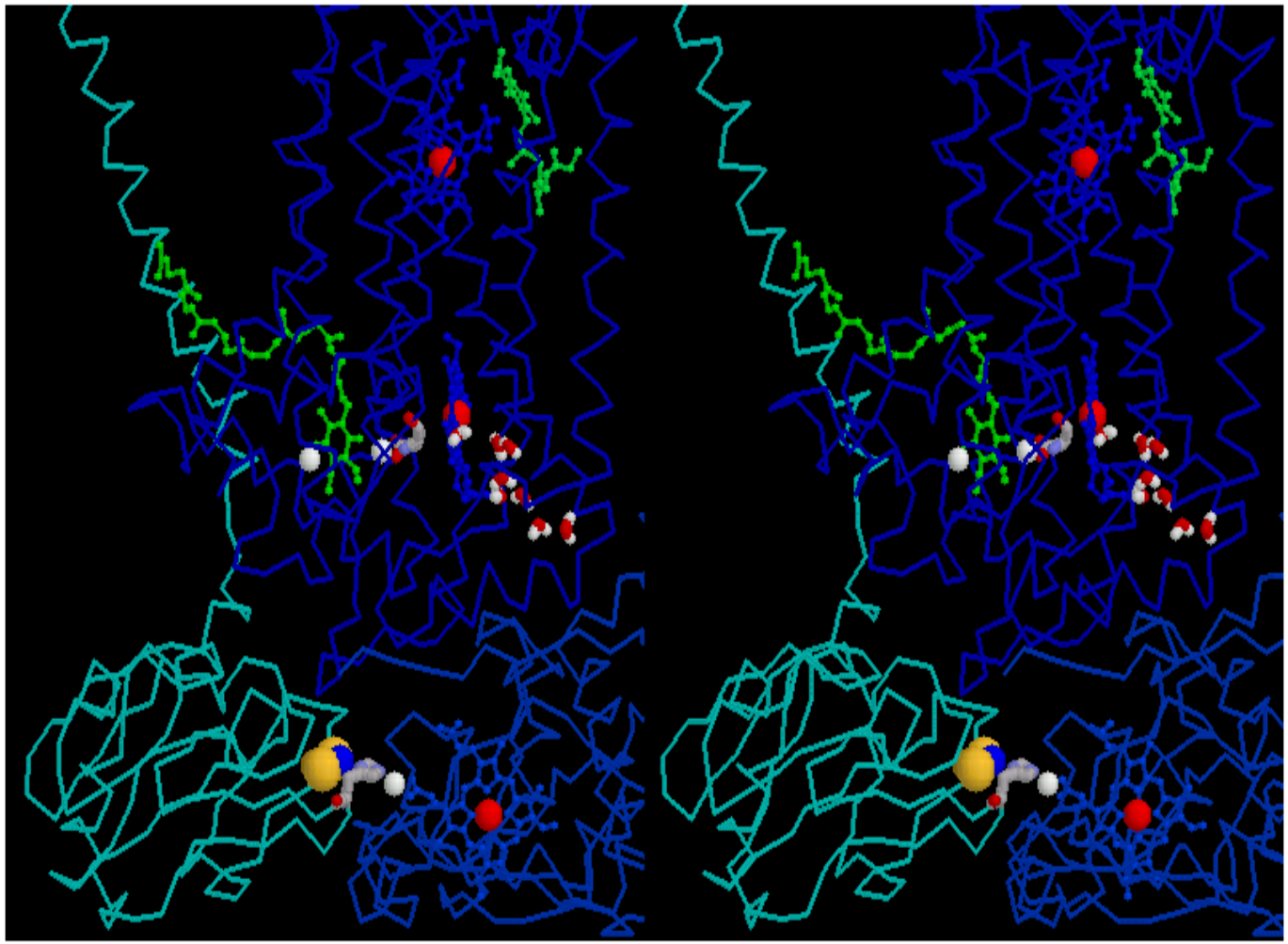
A

And then..



B

In this structure, the players and their positions can be identified...



A summary of the mechanism

http://www.life.uiuc.edu/crofts/bc-complex_site/etp-model_annotated.html

Cartoon Mechanism of Q cycle of bc1

Net chemical reaction: $QH_2 + 2\ \text{cyt c}(Fe^{+3}) \rightarrow Q + 2\ \text{cyc c}\ (Fe^{+2}) + 2\ H^+_p$ + $2\ H^+_N$ + $4\ H^+_P$

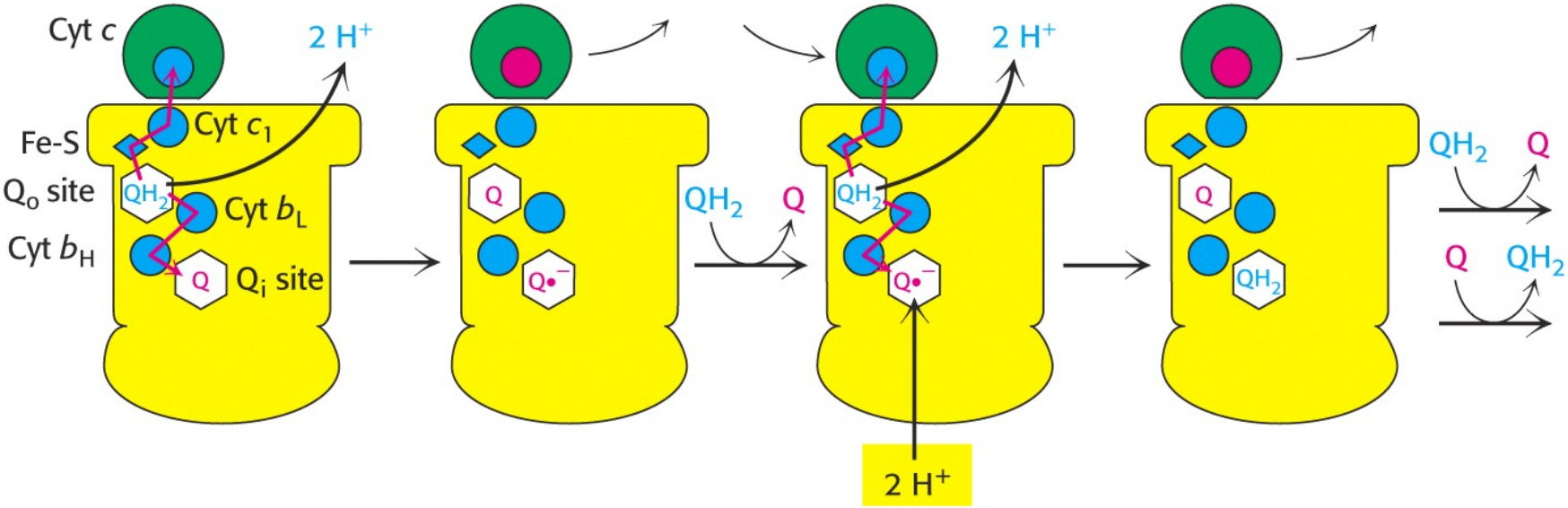
the simplicity of which hides the more complicated Q cycle below:

2 QH_2 bind to the Q_0 site and become oxidized releasing 4 e- and 4 H+

4 H+ are released to the cytoplasmic (+) side

1 Q binds to the Q_1 site and becomes reduced by 2 e- along with 2 Cyt c

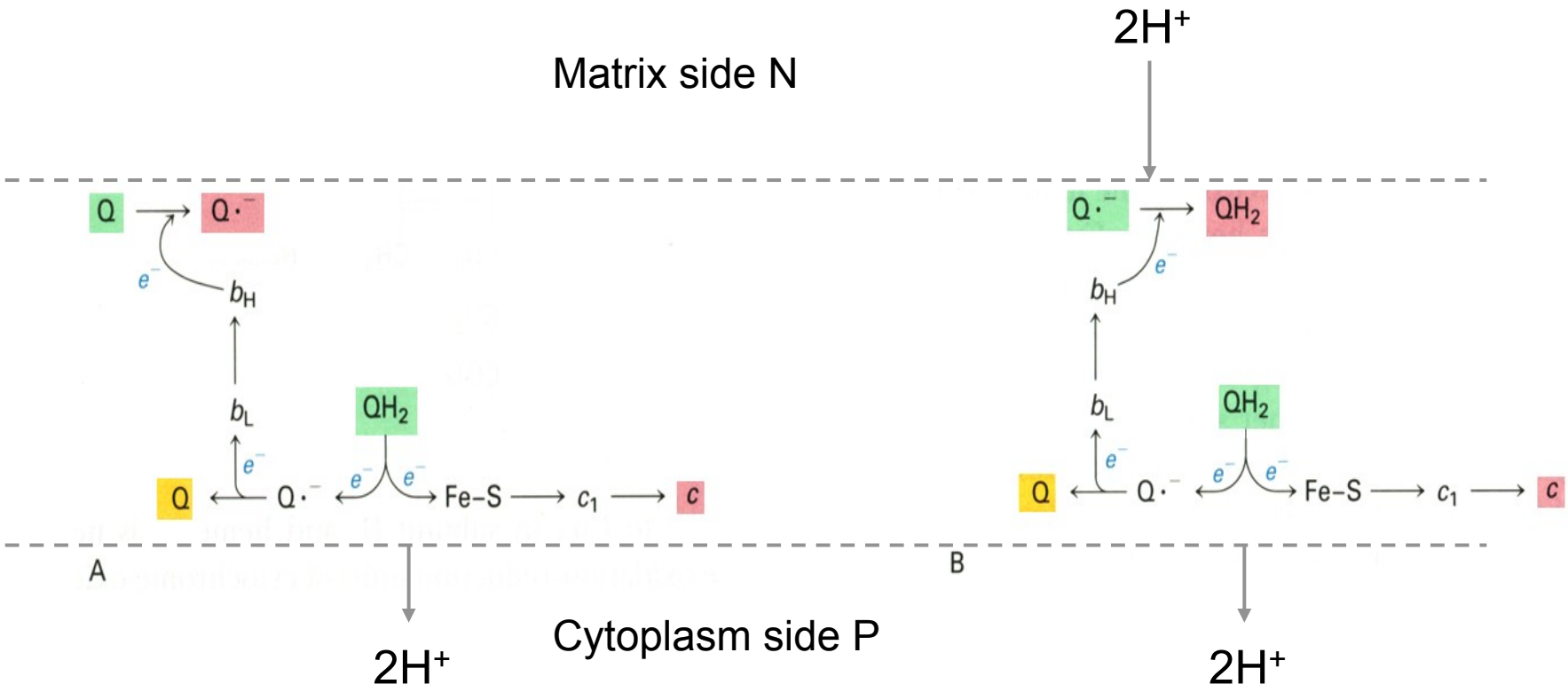
2H+ are removed from the matrix (-) side



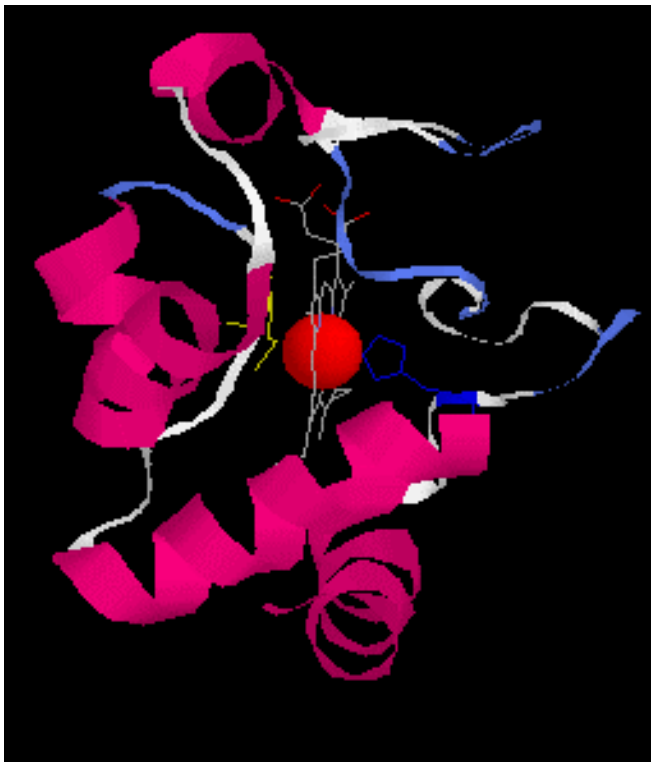
What does this complexity accomplish?

QH_2 + 2 ferricyt c^{3+} + $2H^+_N$ ➔ Q + 2 ferrocyt c^{2+} + $4H^+_P$

- Two electron → one electron step down transformer
- Proton transport results



Cytochrome C: A small mobile carrier of one electron in the intermembrane space of the mitochondrion



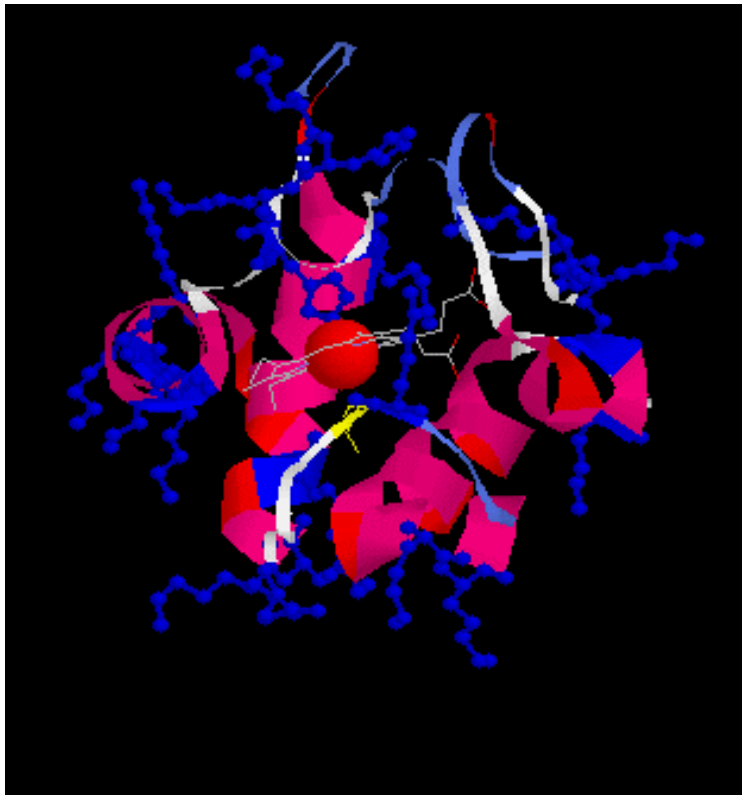
Heme group with Fe that cycles between Fe^{+2} and Fe^{+3}

Fe coordination through his18 and met80

but how does electron enter and leave?

Cyt c's throughout history have remained nearly identical in structure and react cross plants, animals and eukaryotic microorganisms !

Cytochrome C: A small mobile carrier of one electron in the intermembrane space of the mitochondrion



Cytochrome c is a protein with many, many lysines and arginines.

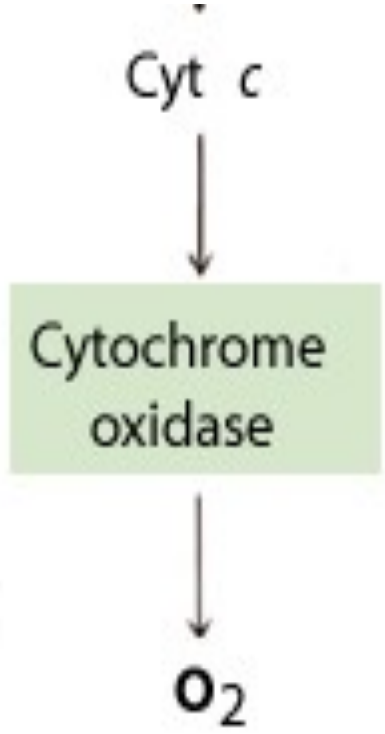
One model for both interaction with cyt b/c1 and cyt oxidase has cyt c using these positively charged residues to dock up to ite e- donor protein and e- acceptor protein and direct electron transfer directly to heme

the cluster of positive charges shown at the top of this image is called the positive “corona”

http://www-bioc.rice.edu/~graham/Bios302/InteractiveCytc.html

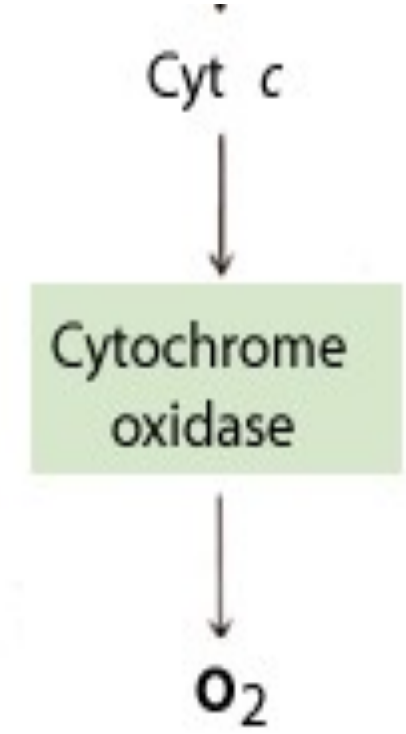
The last step: cytochrome oxidase

- Overall chemical rxn :
  - $4\ Fe^{2+} + 4\ H^{+}_{N} + O_2 \rightarrow 4\ Fe^{3+} + 2\ H_2O$
  - ⇨ 4 e^- transfer
- Energetics
  - $\Delta E_0'$ = 500 mv
  - $\Delta G^{0'} = nF(\Delta E_0') = 4x23.06x0.50 = -44$ kcal/mole
  - contribution of 4 chemical H^+ to proton gradient
    - 4 x 5.2 kcal/mole = 20.8 kcal/mole
  - contribution of 4 pumped H^+ to proton gradient
    - 4 x 5.2 kcal/mole = 20.8 kcal/mole
  - $\Delta G^{0'}$ overall = 2.4 kcal/mole
  - Highly exoergic reduction of water is coupled to moving protons across gradient, saves more than 90% of energy.



The last step: cytochrome oxidase

- Overall chemical rxn :
  - $4\ Fe^{2+} + 4\ H^{+}_{N} + O_2 \rightarrow 4\ Fe^{3+} + 2\ H_2O$
  - ⇨ 4 e^- transfer
- Internal electron carriers
- electron donor cyt c
  - Cyt a heme (E_0' = 0.29 V)
  - Cyt a_3 heme (E_0' = 0.55 V)
  - Copper ions Cu_A (two Cu's) and Cu_B
- Electron acceptor O_2
- Transfer pathway?
  - Cyt c → Cyt a → Cyt a_3 → O_2

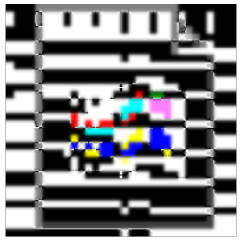


…and we have a structure

Structures of Metal Sites of Oxidized Bovine Heart Cytochrome c Oxidase at 2.8 Å

Tomitake Tsukihara, Hiroshi Aoyama, Eiki Yamashita, Takashi Tomizaki, Hiroshi Yamaguchi, Kyoko Shinzawa-Itoh, Ryosuke Nakashima, Rieko Yaono, Shinya Yoshikawa*

The high resolution three-dimensional x-ray structure of the metal sites of bovine heart cytochrome c oxidase is reported. Cytochrome c oxidase is the largest membrane protein yet crystallized and analyzed at atomic resolution. Electron density distribution of the



1OCC.pdb

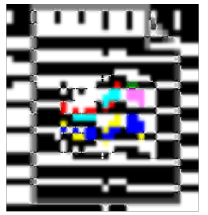
Science 269: 1069 (1995)

Structure at 2.8 Å resolution of cytochrome c oxidase from *Paracoccus denitrificans*

So Iwata, Christian Ostermeier, Bernd Ludwig* & Hartmut Michel†

Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Strasse 7, D-60528 Frankfurt/M., Germany * Johann-Wolfgang-Goethe Universität, Biozentrum, Institut für Biochemie, Molekulare Genetik, Marie-Curie-Strasse 9, D-60439 Frankfurt/M., Germany

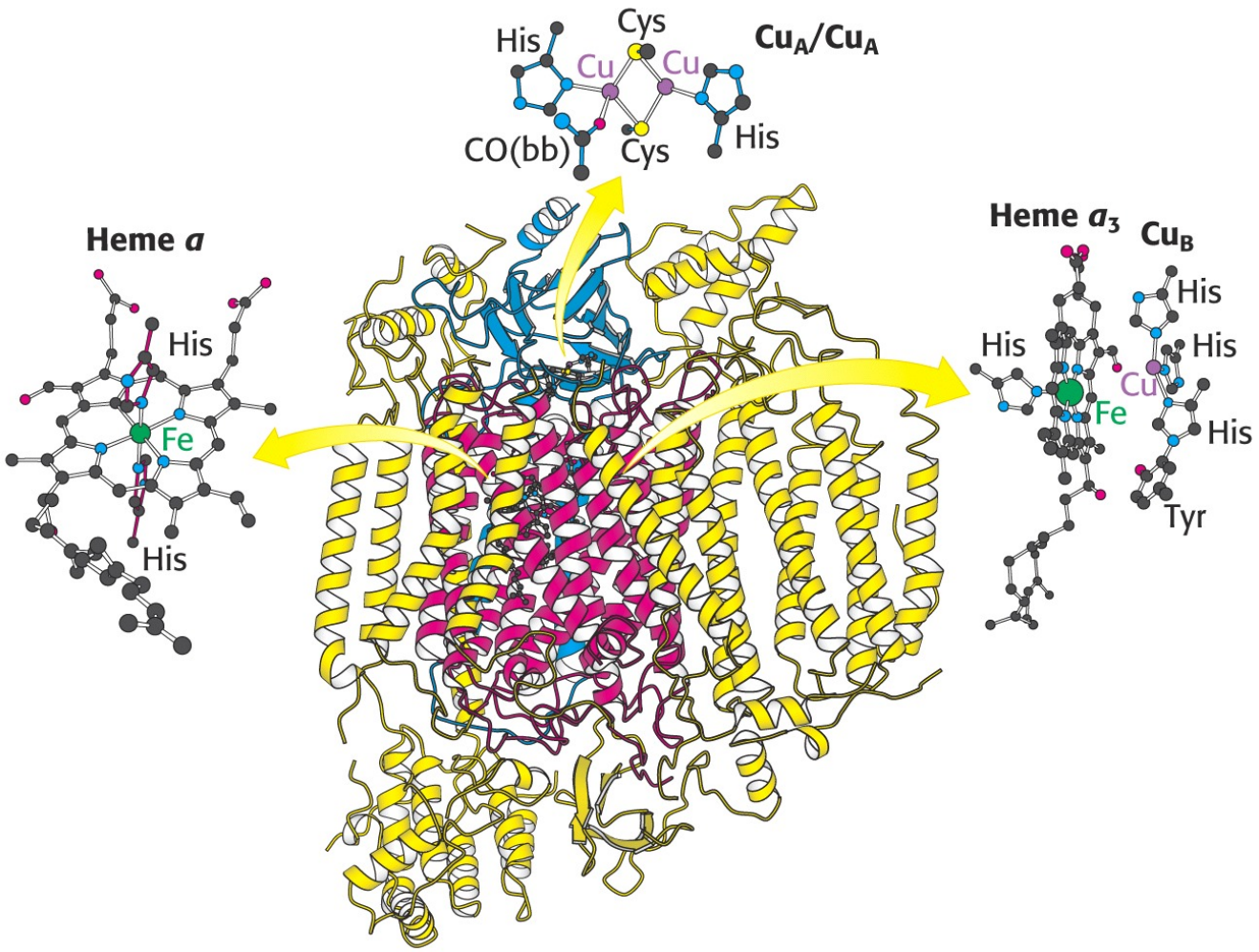
The crystal structure at 2.8 Å resolution of the four protein subunits containing cytochrome c oxidase from the soil bacterium *Paracoccus denitrificans*, complexed with an antibody F_v frag-



1AR1.pdb

Nature 376: 660 (1995)

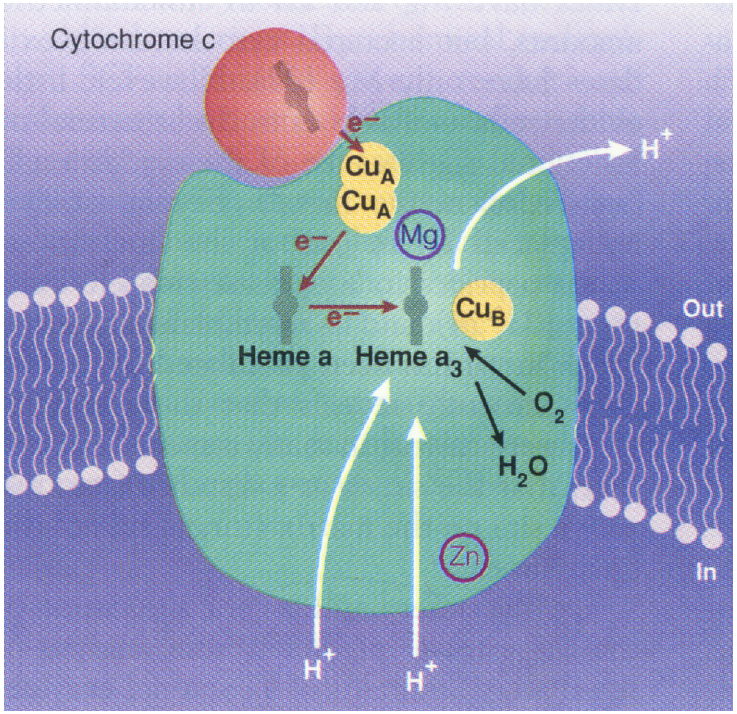
Structure of Cytochrome Oxidase



"A" differs from "C" type hemes in that:

* a formyl group replaces a methyl
* a C15 hydrocarbon chain replaces one of the vinyl groups.
* The heme is not covalently attached to the protein
* The two a and a3 differ in their spectroscopic properties because they are located in different environments

Cyt oxidase: Electron transfer reactions

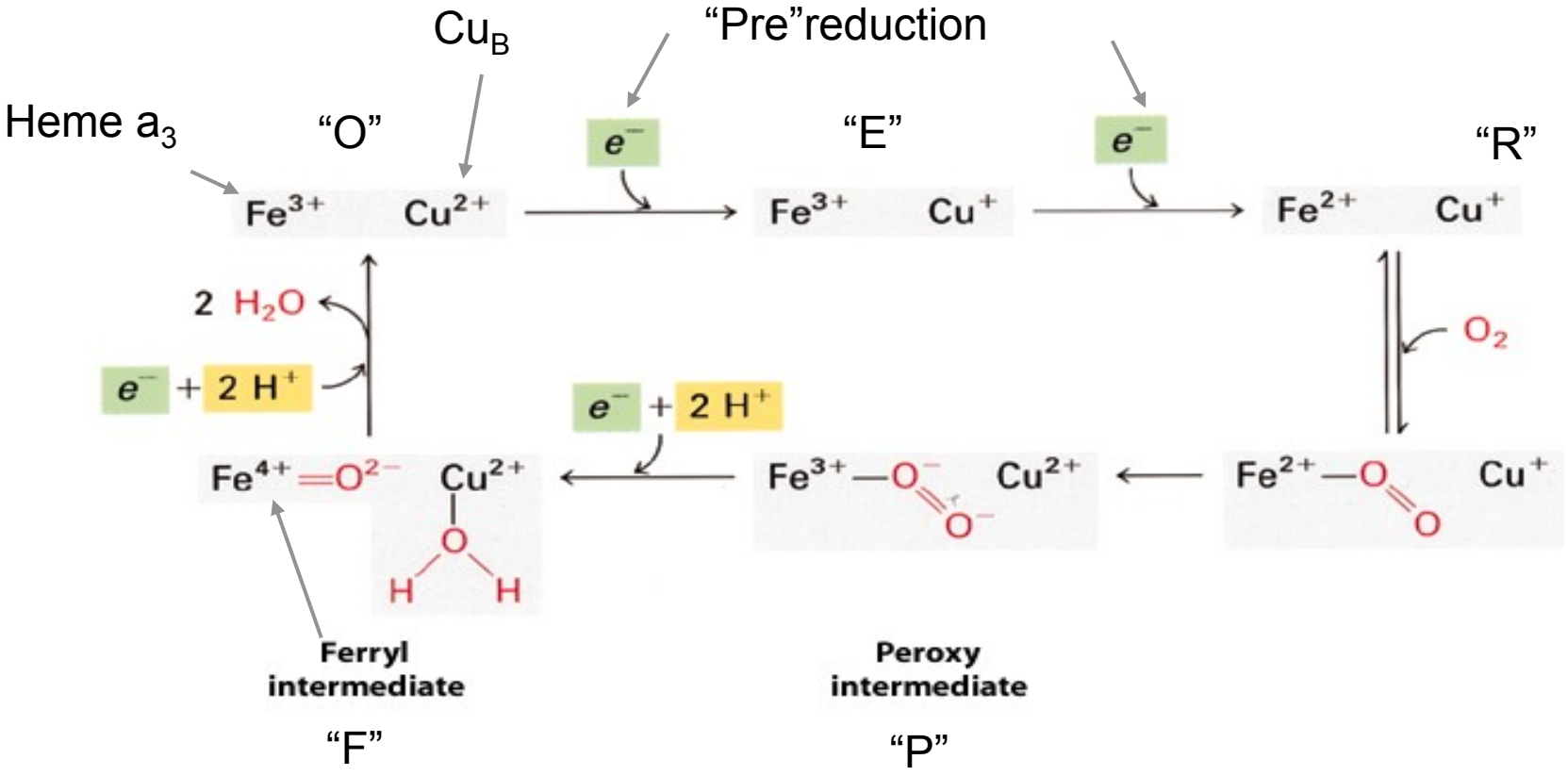


http://www-bioc.rice.edu/~graham/CcO.html

"…Electron transfer into cytochrome oxidase is initiated by binding of cytochrome c to subunit II on the external side of the membrane (see Figure). This subunit contains the CuA center…Most studies on the electron transfer indicate a linear sequence of events, with the electrons proceeding from cytochrome c to CuA, then to heme a, and on to the heme a_3-Cu_B center." (Gennis&Ferguson-Miller, 1995)

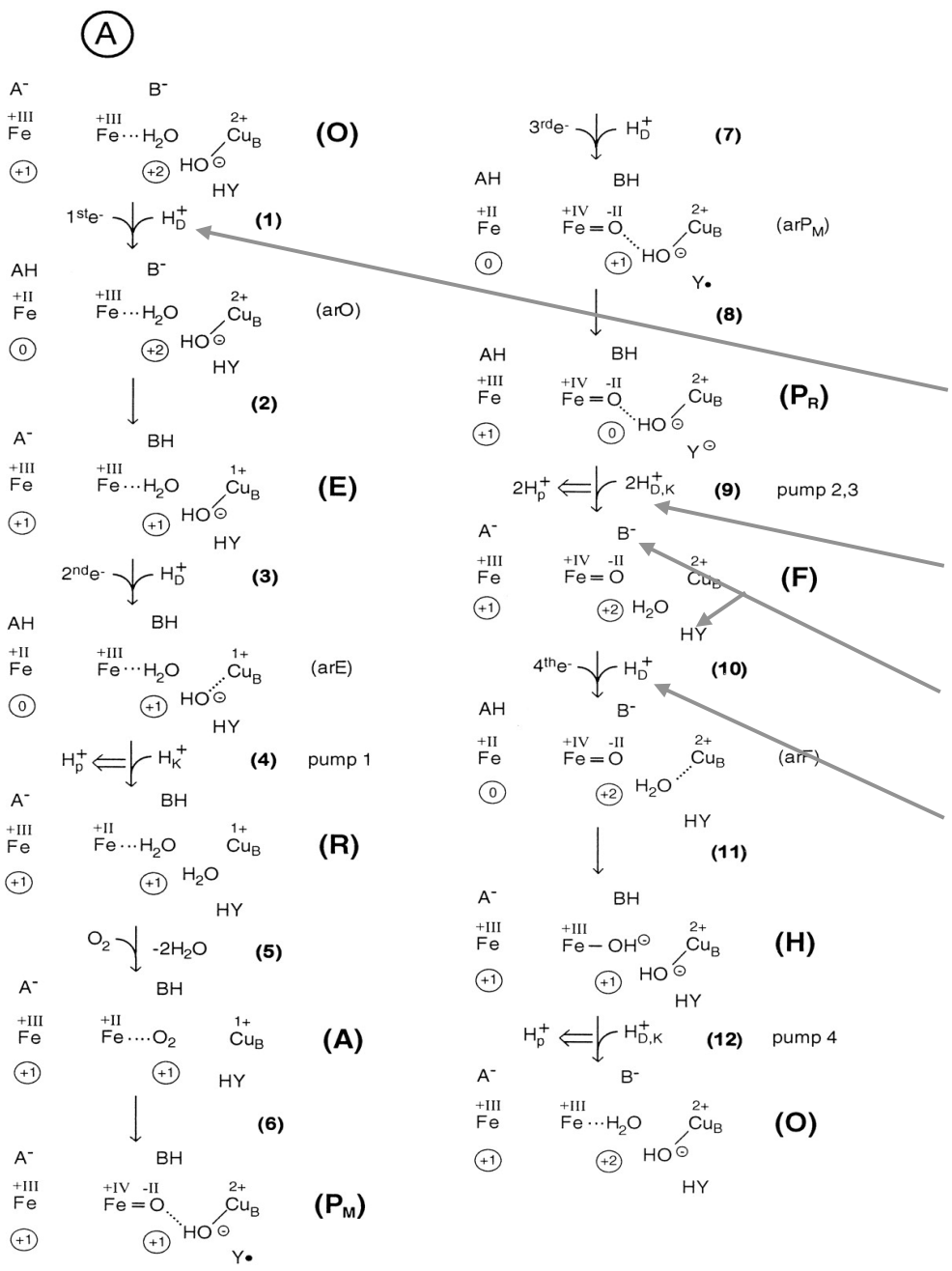
…with 4 H^+ (from inside) reacting with O_2 ("chemical" protons) AND
… with 4 H^+ pumped from inside to outside ("pumped" protons)

There's some hair-raising oxygen chemistry, Proposal I:



- …accounting for the "chemical" protons, but not their sidedness
- But how does proton pumping occur?

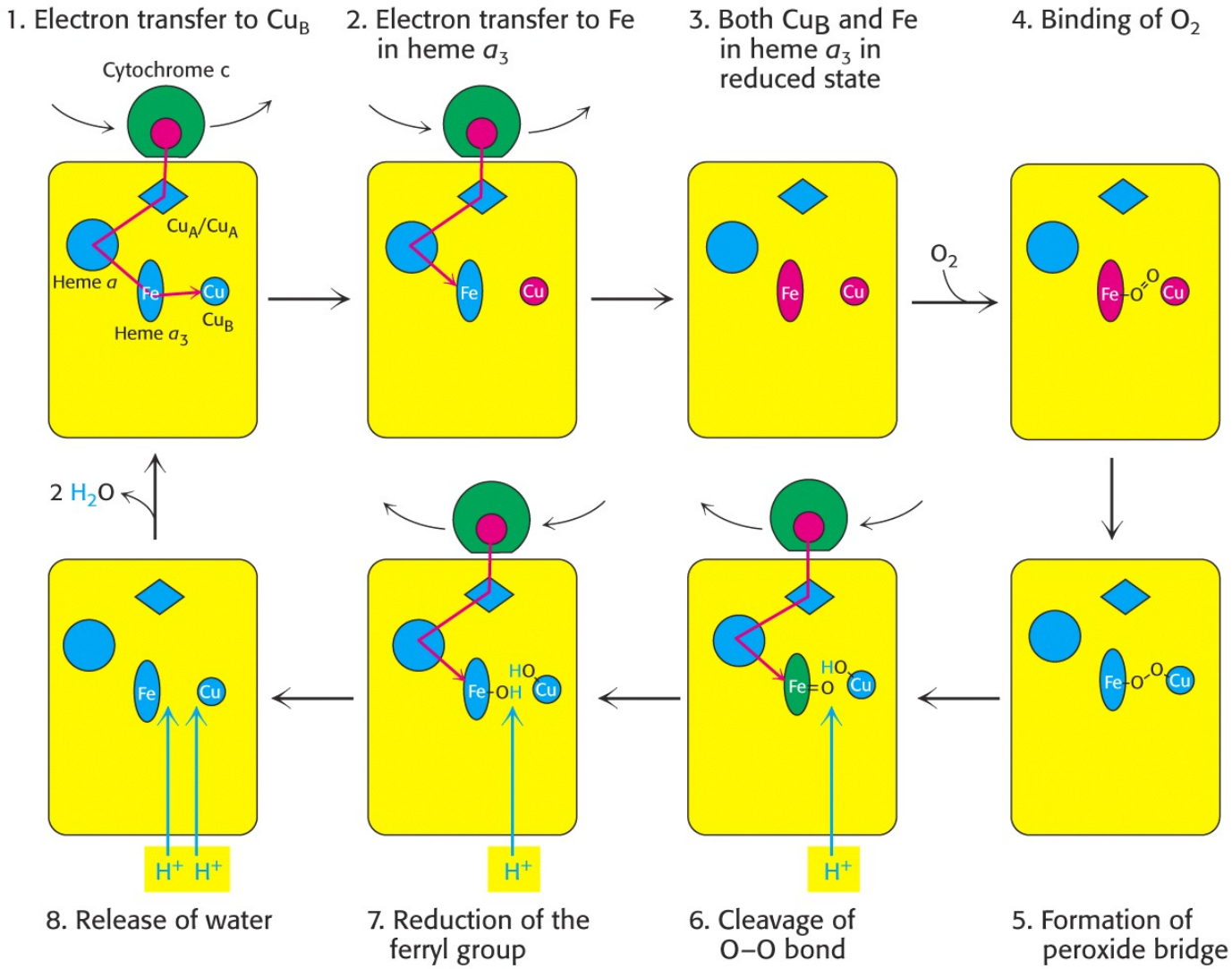
Michel mechanism Proposal 2 (if you have a structure and a Nobel prize, it doesn't have to be simple)



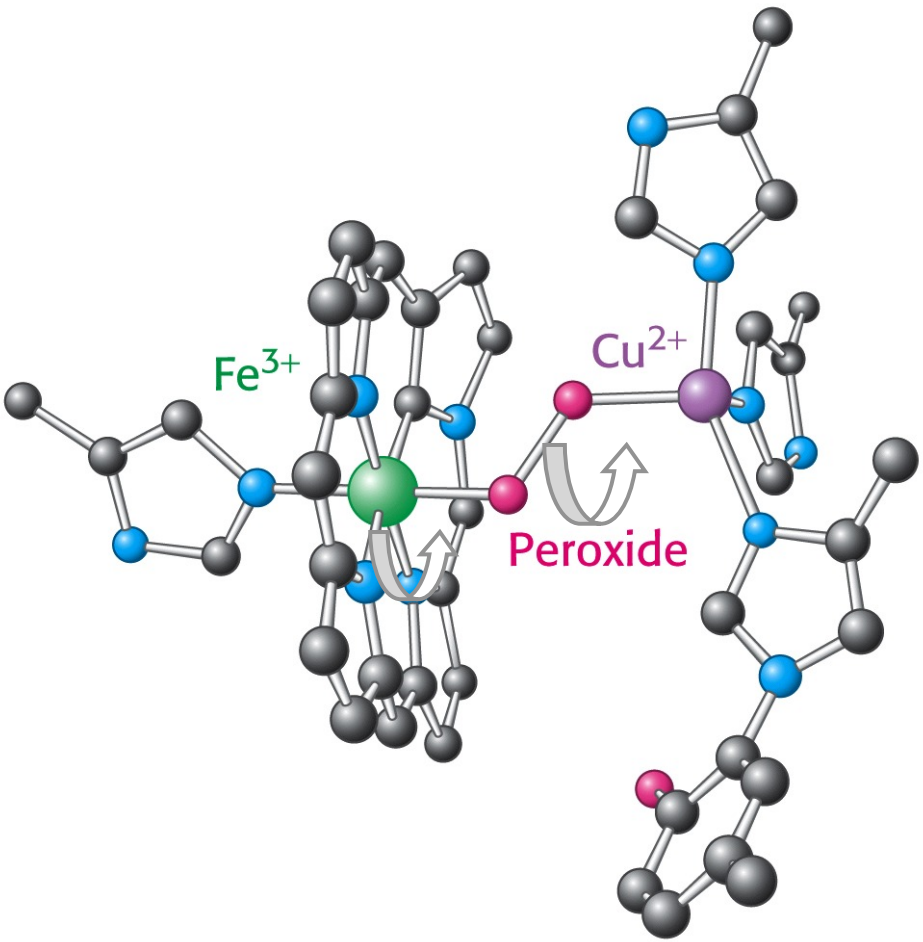
- PRINCIPLES
- H_P^+ comes in with electrons to neutralize the charge entering the membrane interior
- Entering "chemical" protons eject "pumped" protons
- Local amino acid side chains take up and give up protons
- Protons enter the core along proton "channels" or "wires", which are K,E,D,T,S sidechains extending out from α-helices in protein core (determined by mutation studies)
- E.g., "D" channel includes asp124, ser192, ser193, and glu278 in "A" chain

Cyt Oxidase Simplified Mechanism

Proposal 3



Reduction of O_2



Here's the heme a_3/Cu binculear site just prior to O-O bond breakage. At this point, two electrons have been transferred to O_2 to make it peroxide, O_2^{2-}

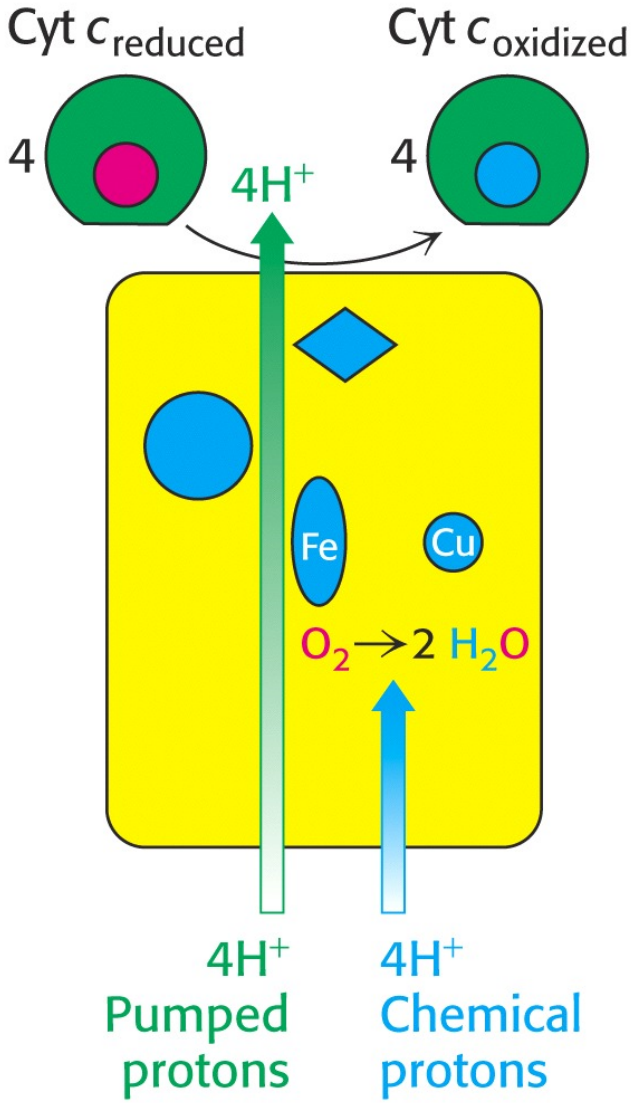
WARNING: What follows is not for the weak at heart: the 3rd electron and proton result in breakage of O-O bond and formations of Fe^{+4} =O species called the Ferryl group

O^- on the Cu gets hydrated to OH **(first chemical proton uptake)**

The ferryl goup is reduced back to Fe +3 by the fourth e-, the bound O- grabs a proton.**(2nd c. proton uptake)**

A final two protons are absorbed and two molecules of water are released. **(3rd and 4th chemical protons)**

Proton Pumping in Cyt Oxidase



Four chemical protons are removed from the matrix or N side and added to O_2

In addition, four more protons are pumped by this process through internal channels which have been identified in the crystal structure.

The pumping of protons increase the efficiency of free energy storage in the form of a proton gradient.