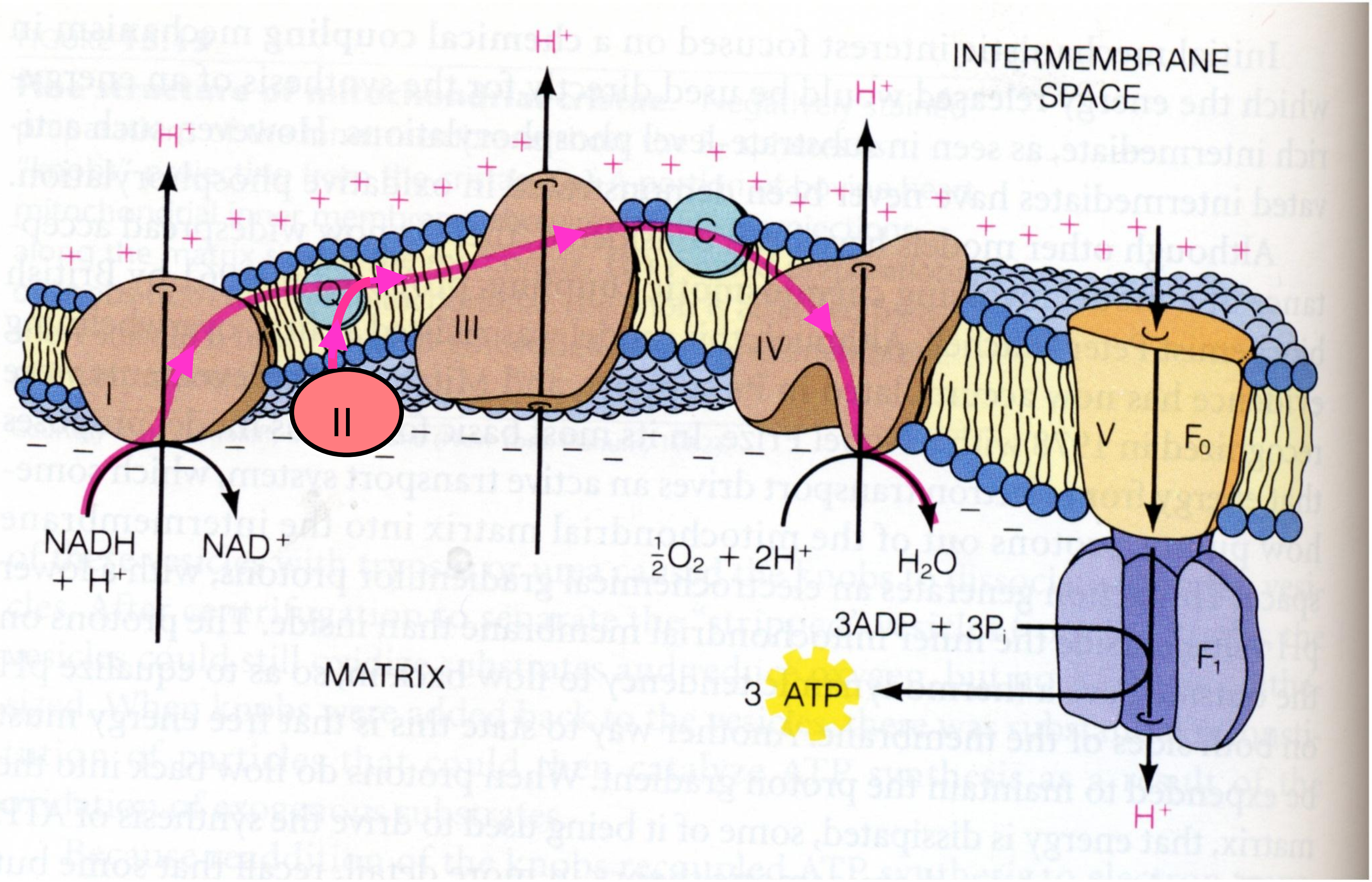
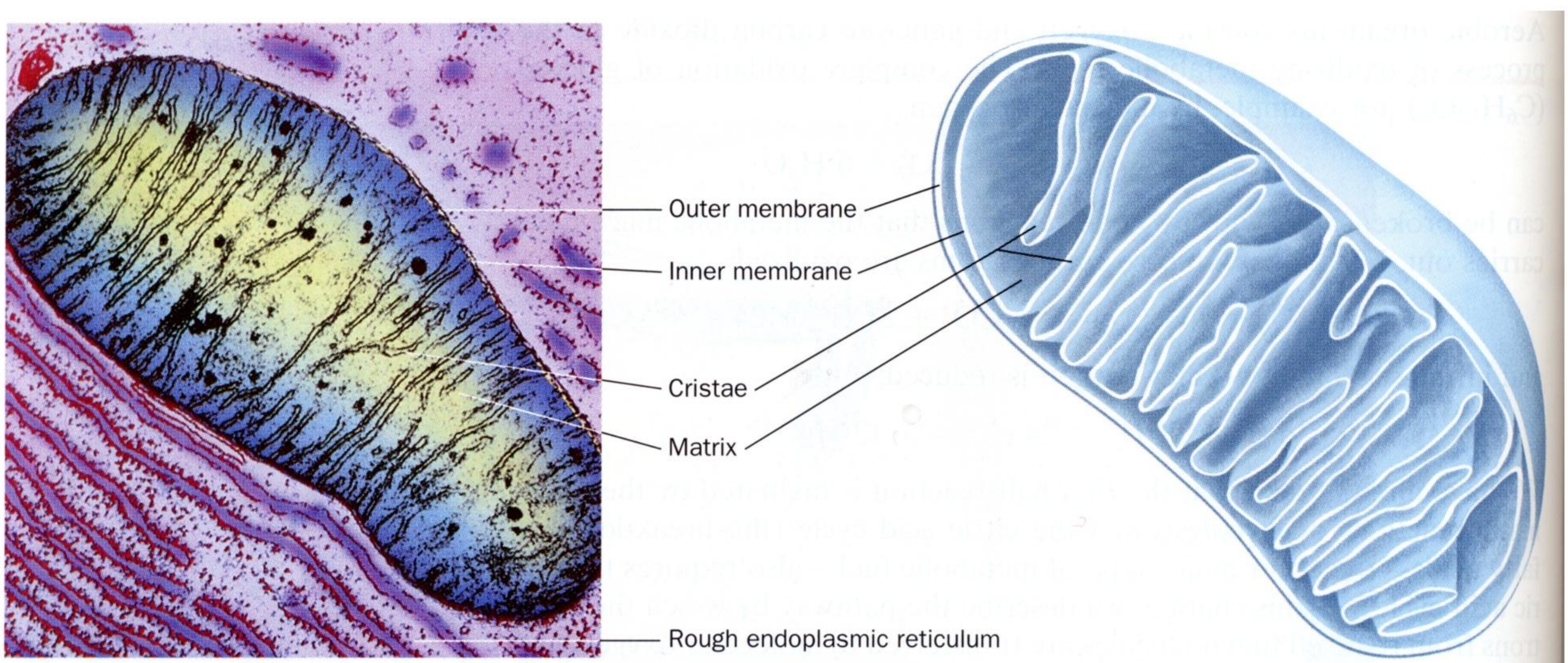
Biochemistry of Electron Transport

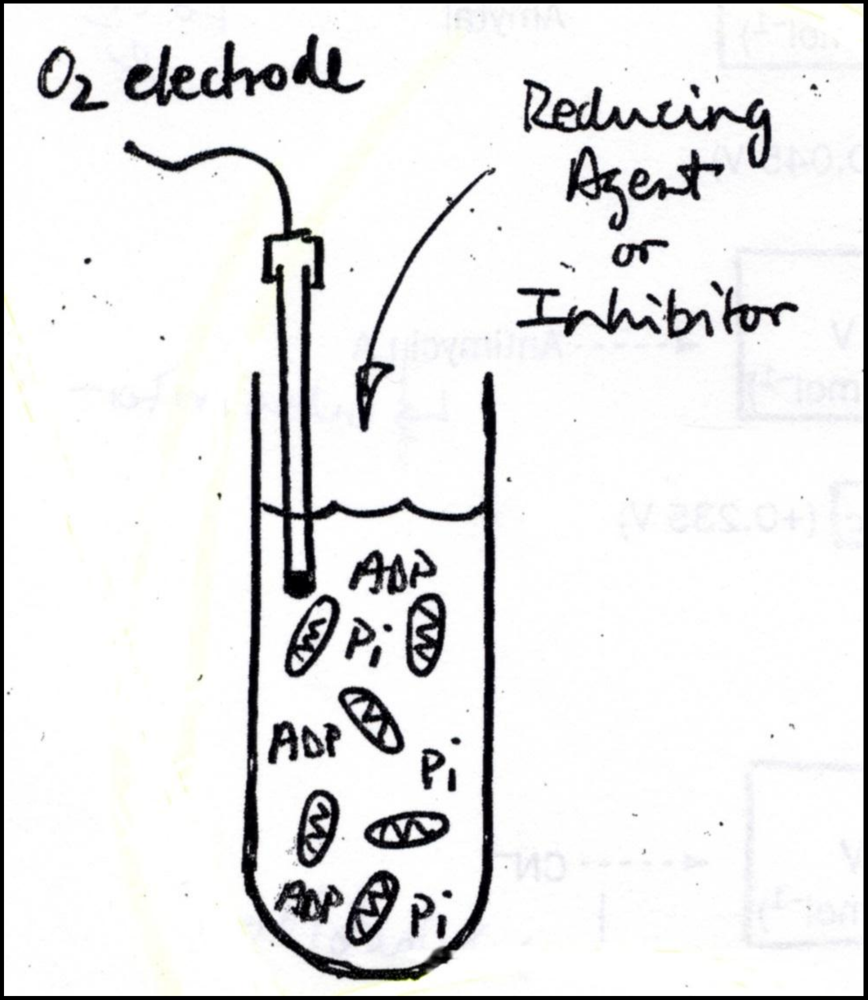
1. Pathway of the e- from NADH → O_2
2. Chemiosmotic Hypothesis
3. Synthesis of ATP


INTERMEMBRANE SPACE
H+
H+
H+
Q
C
I
II
III
IV
V
F0
F1
NADH + H+
NAD+
1/2 O2 + 2H+
H2O
3ADP + 3Pi
3 ATP
MATRIX
H+


Outer membrane
Inner membrane
Cristae
Matrix
Rough endoplasmic reticulum

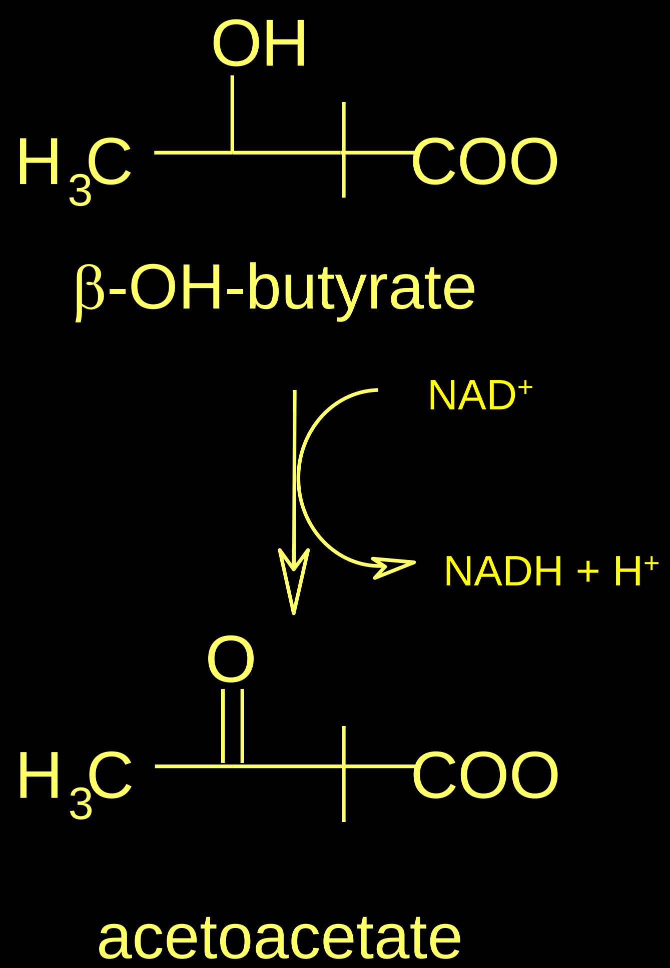
(a) (b)

"Artificial Respiration": Experiments that led to Understanding the sequence of Electron Transport Proteins



Electron Donor Systems

1



2 Succinate $\xrightarrow{}$ Fumarate

FAD → $FADH_2$

3 Ascorbic Acid

\+

TMPD(tetramethyl phenylenediamine)

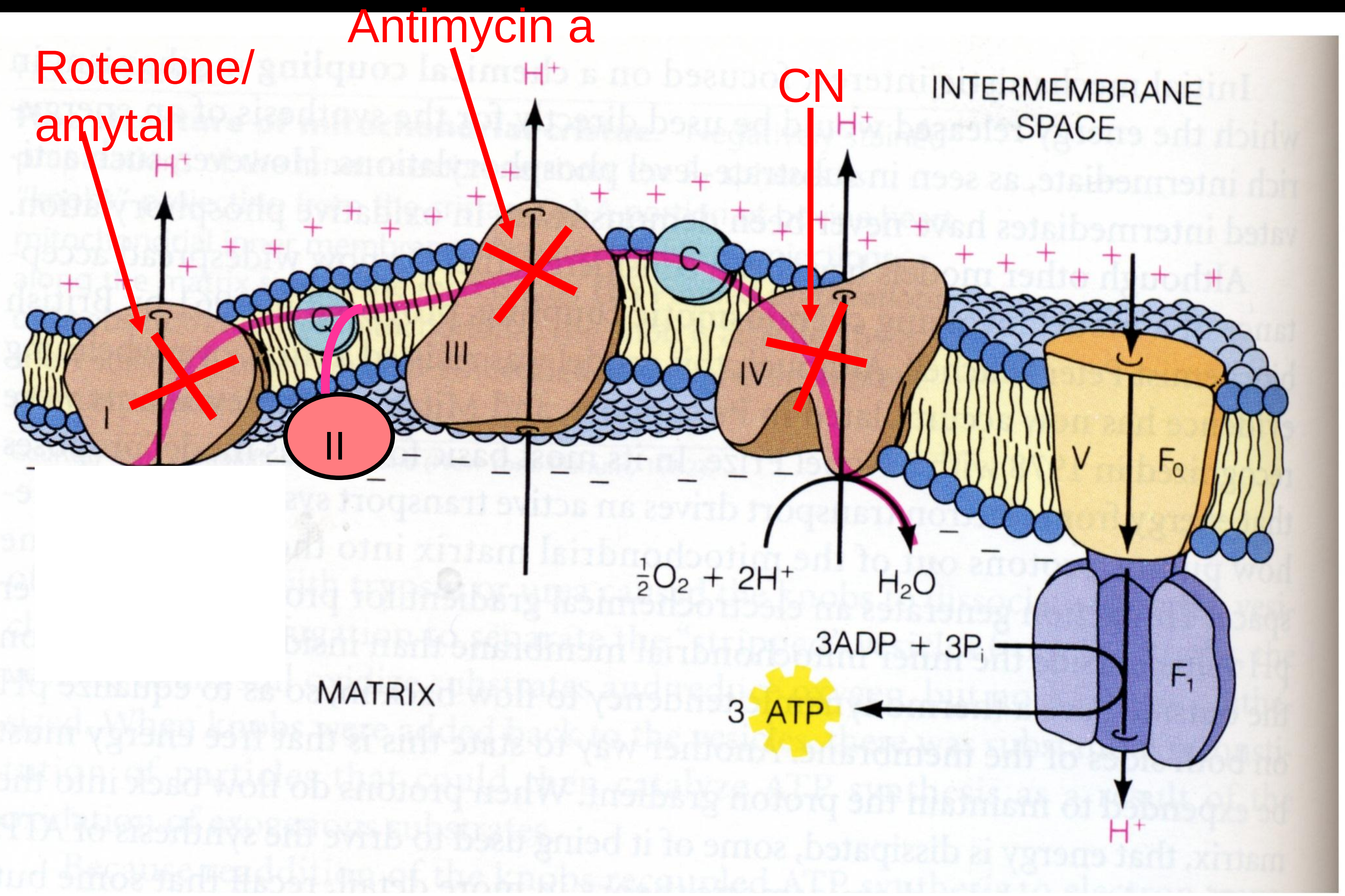
cyctochrome C

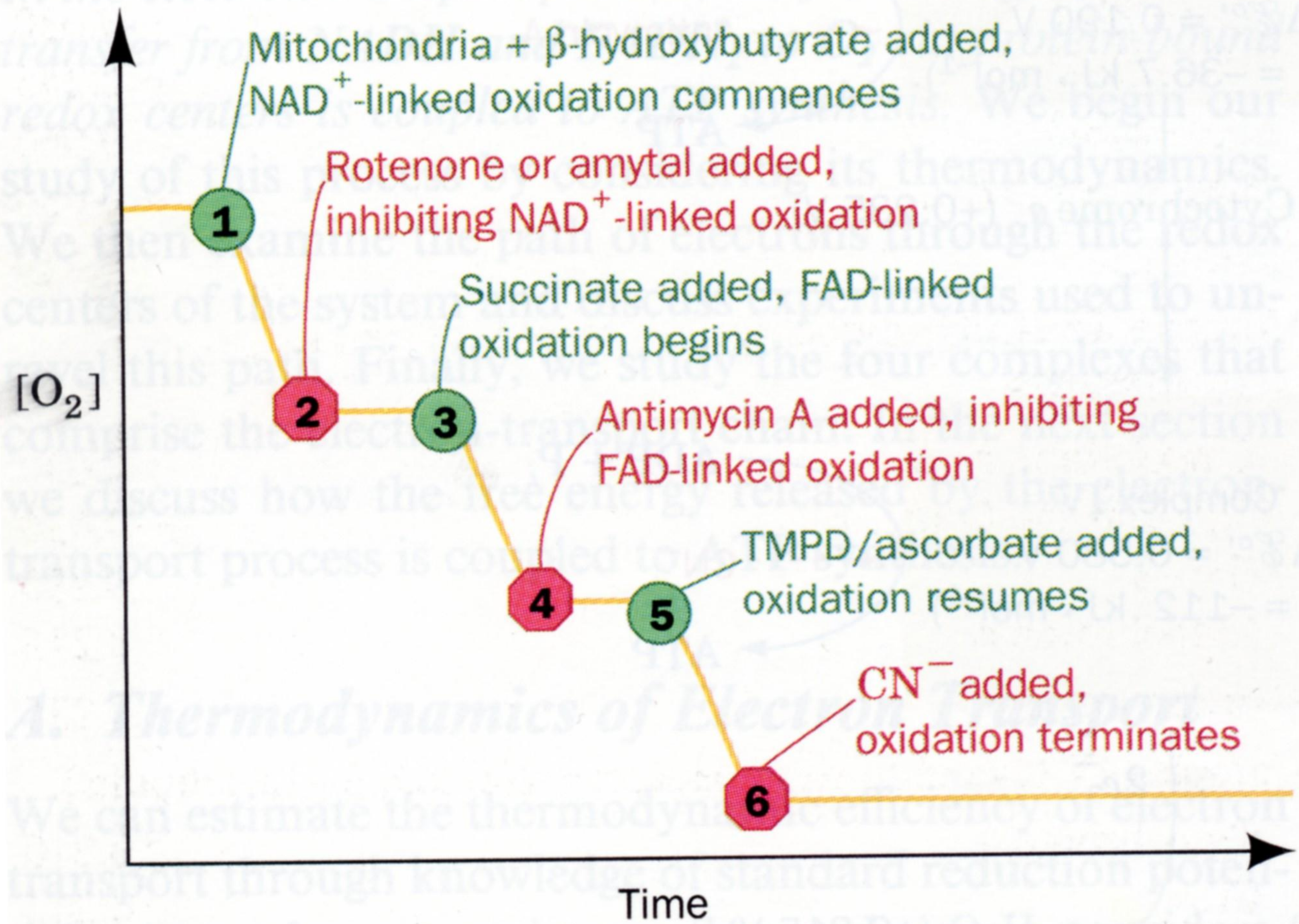
2H

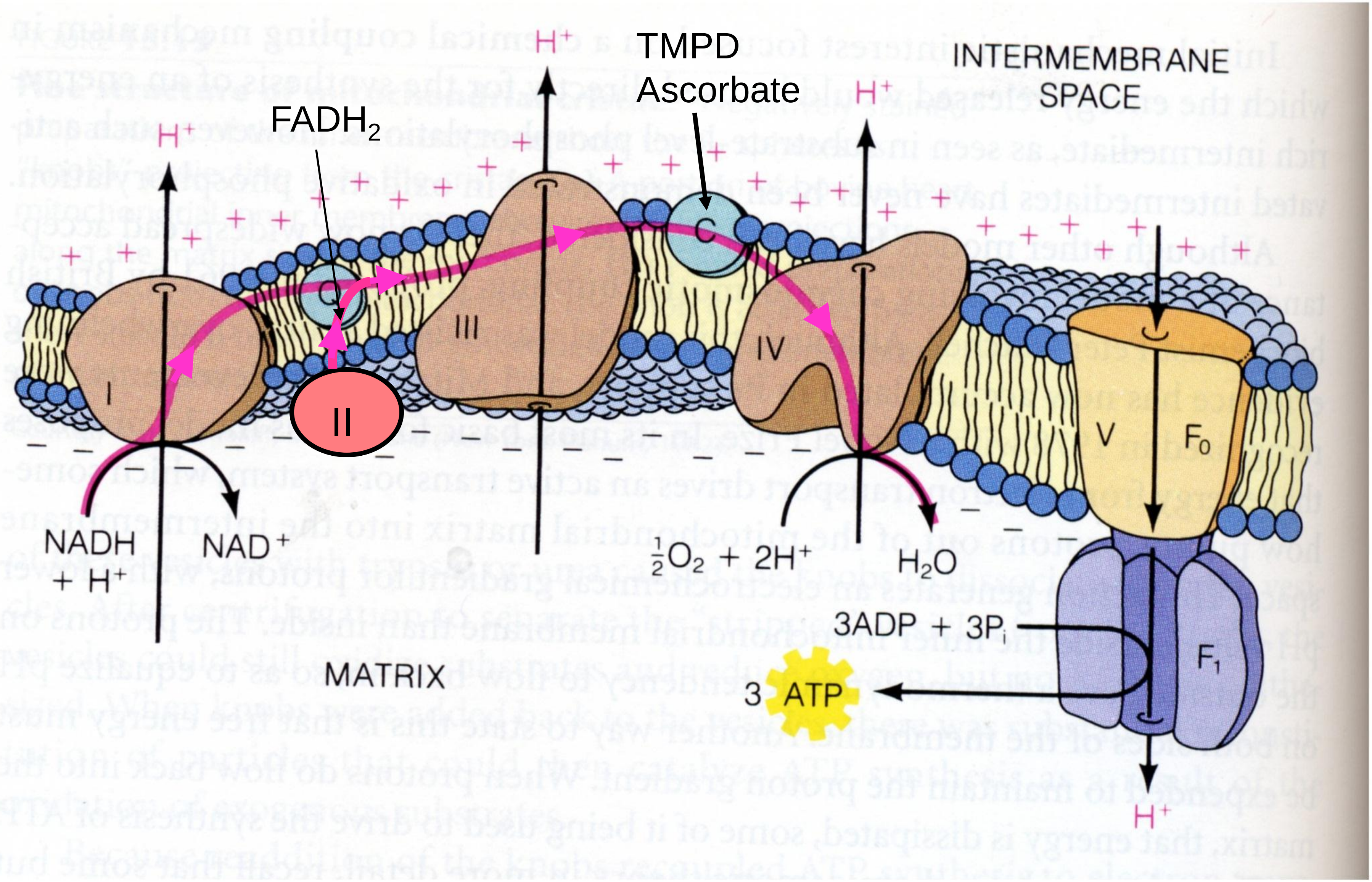
2H

Where do electrons enter the ETC?

Use: Inhibitors of electron transport

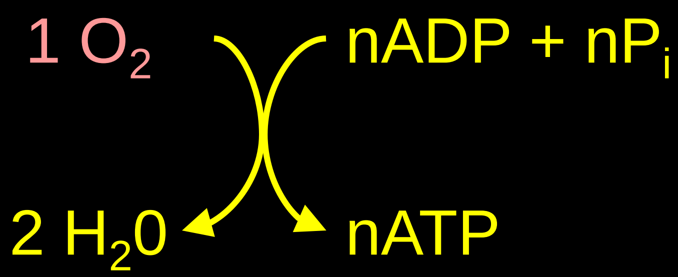

Rotenone/
amytal
Antimycin a
CN
INTERMEMBRANE
SPACE
H+
Q
C
I
II
III
IV
V
F0
F1
1/2 O2 + 2H+
H2O
3ADP + 3Pi
3 ATP
MATRIX


Mitochondria + β-hydroxybutyrate added, NAD⁺-linked oxidation commences
Rotenone or amytal added, inhibiting NAD⁺-linked oxidation
Succinate added, FAD-linked oxidation begins
Antimycin A added, inhibiting FAD-linked oxidation
TMPD/ascorbate added, oxidation resumes
CN⁻ added, oxidation terminates
1
2
3
4
5
6
[O2]
Time



H+
FADH2
TMPD
Ascorbate
INTERMEMBRANE
SPACE
Q
C
I
II
III
IV
V
F0
F1
NADH
+ H+
NAD+
1/2 O2 + 2H+
H2O
3ADP + 3Pi
3 ATP
MATRIX

How many moles of ATP are synthesized from the reduction of 1 mole O_2 ?



The Oxidation of NADH or $FADH_2$ by O_2 is Tightly Coupled to the Phosphorylation of ADP

- Measure the amount of O_2 consumed (reduced to $2H_2O$) for any given amount of ADP added.

- Experimental Conditions

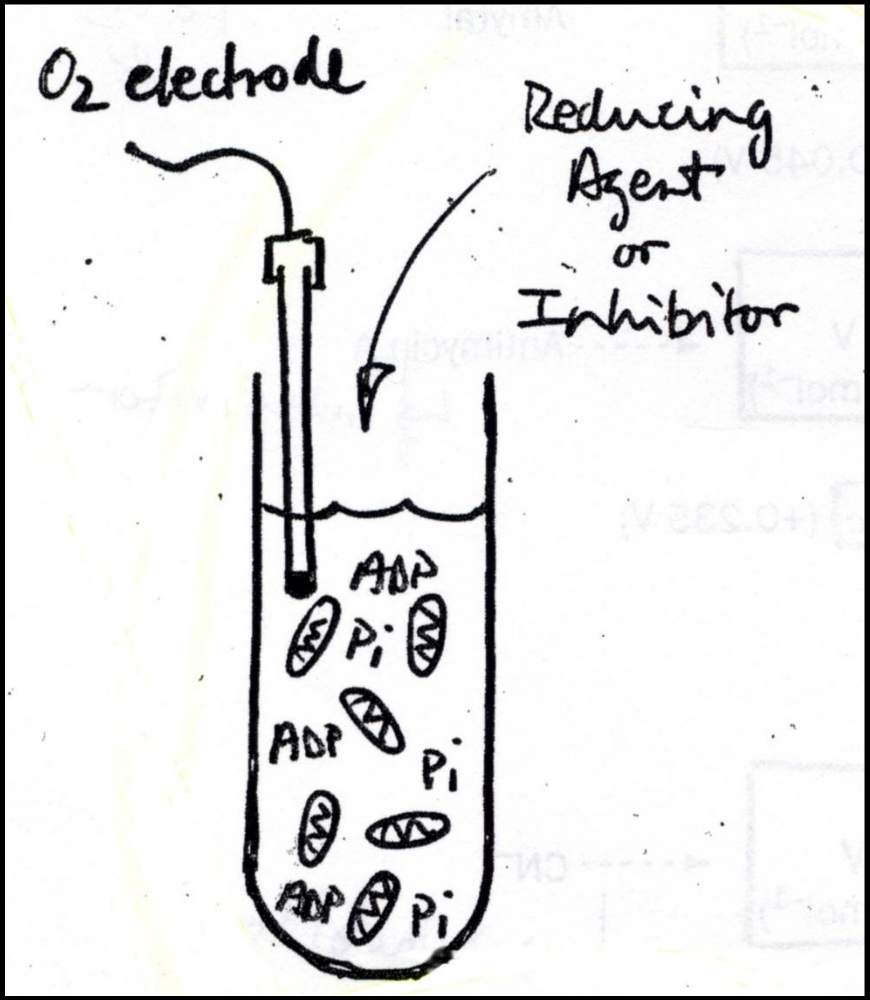
  - same as the inhibitor expt (no ADP initially, excess PO_4)

  - isolated mito's in buffer containing excess phosphate

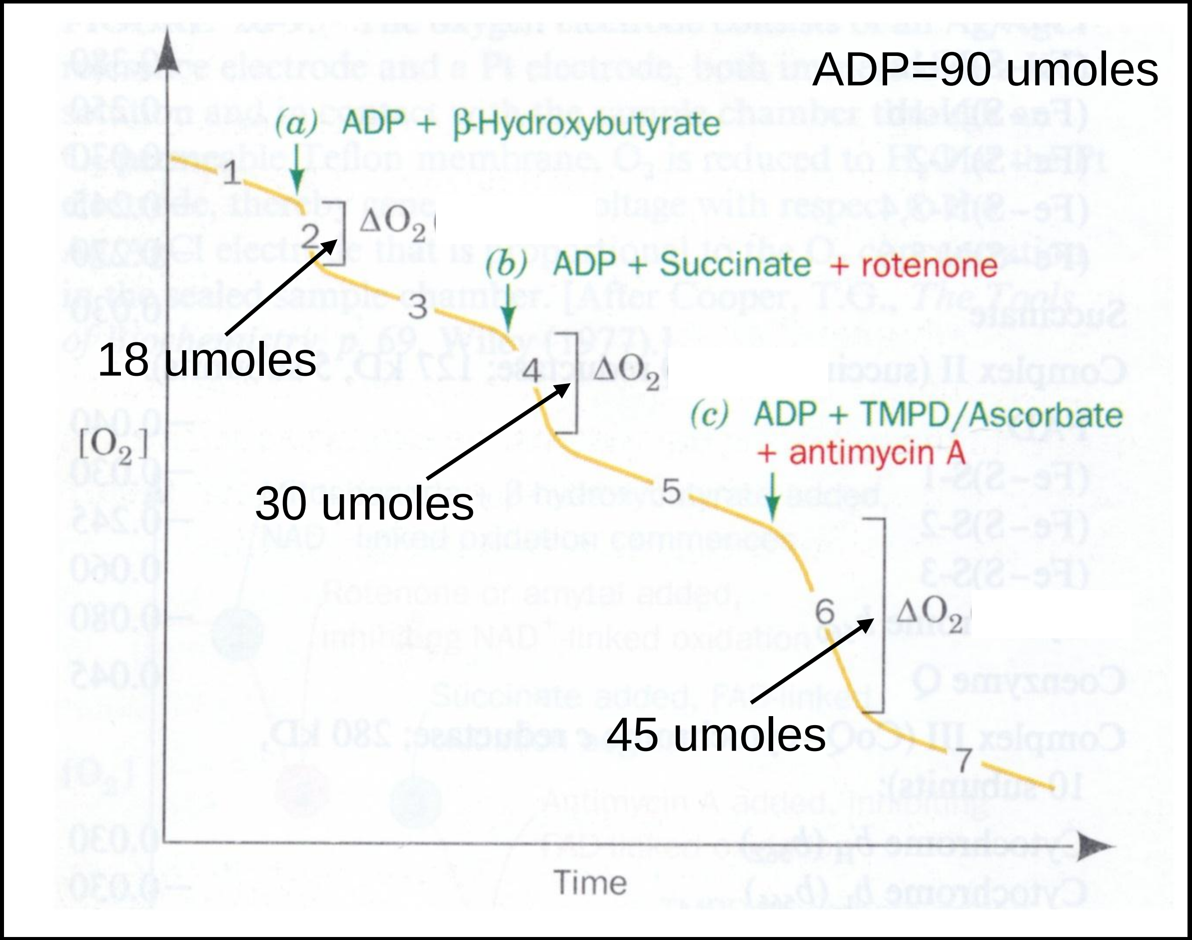
  - addition of ADP + an electron donor starts electron transport

ADP is limiting!

NADH, Pi, O_2 are in excess.



O_2 Consumption as a function of ADP P'n



Conditions: Isolated mitochondria in buffer containing excess PO_4. Reaction is initiated by addition of ADP and e- donor.

Interpretation of Results

a) β-OH-butyrate

Conversion of 90 umol ADP (or PO_4) → ATP requires 18 umol O_2 (36 umol O)

P/O = 90/36 = 2.5

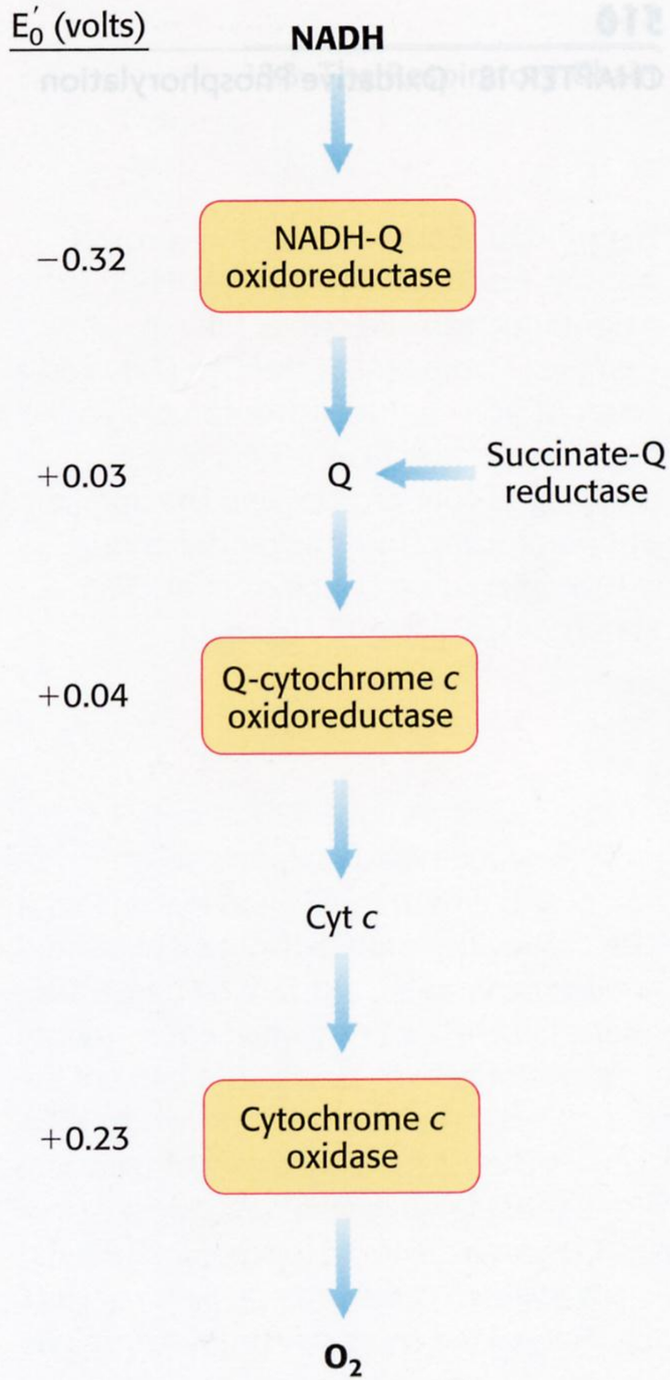
b) Succinate

Conversion of 90 umol ADP (or PO_4) → ATP requires 30 umol O_2 (60 umol O)

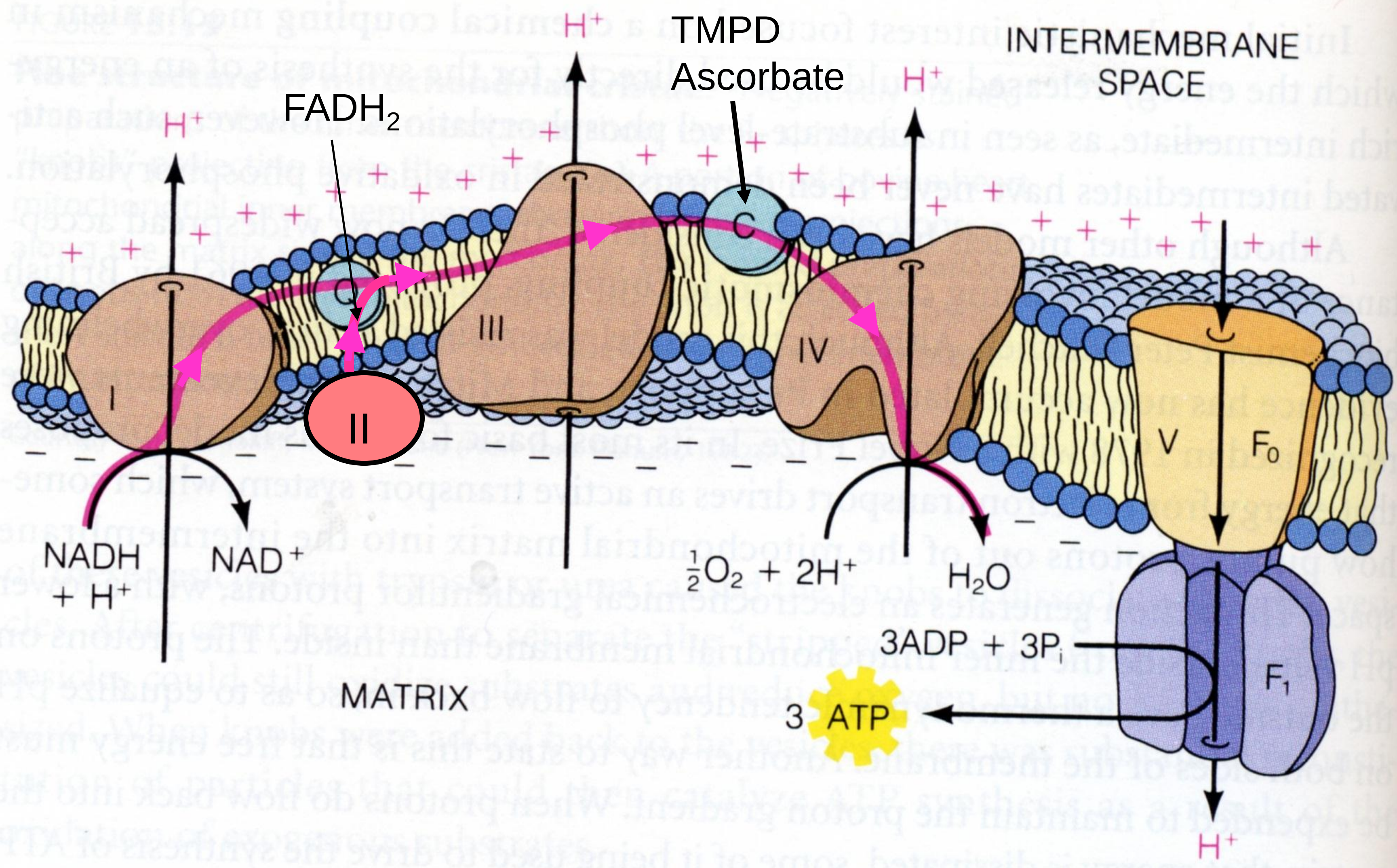
P/O = 90/60 = 1.5

c) TMPD/Ascorbate

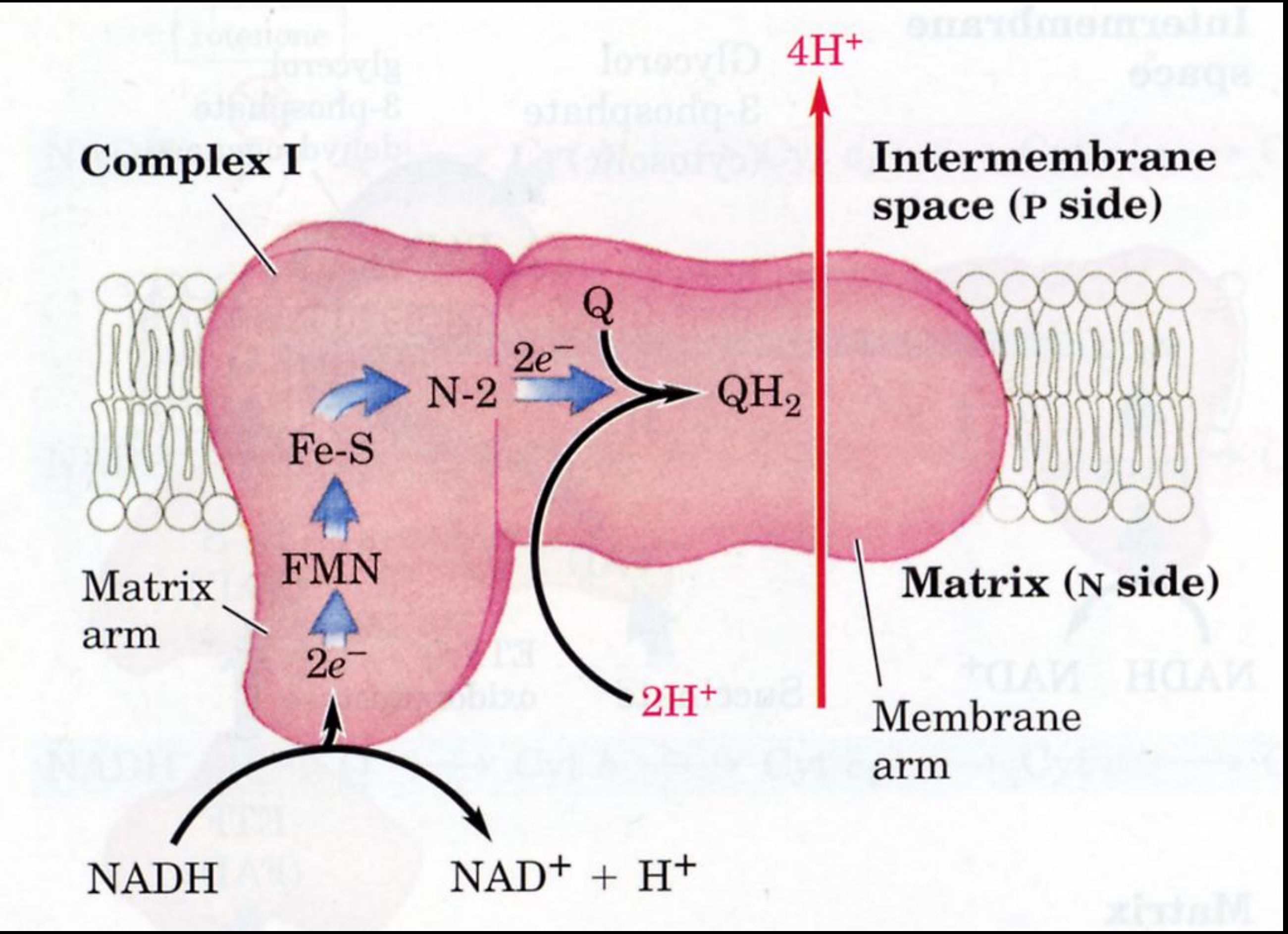
P/O = 90/90 = 1


E_0' (volts)
NADH
−0.32
NADH-Q oxidoreductase
+0.03
Q
Succinate-Q reductase
+0.04
Q-cytochrome *c* oxidoreductase
Cyt *c*
+0.23
Cytochrome *c* oxidase
O_2

How many protons are pumped for every electron pair?


INTERMEMBRANE SPACE
TMPD
Ascorbate
FADH$_2$
H$^+$
H$^+$
H$^+$
Q
C
I
II
III
IV
V
F$_0$
F$_1$
NADH + H$^+$
NAD$^+$
MATRIX
$\frac{1}{2}$O$_2$ + 2H$^+$
H$_2$O
3ADP + 3P$_i$
3 ATP
H$^+$

Complex I

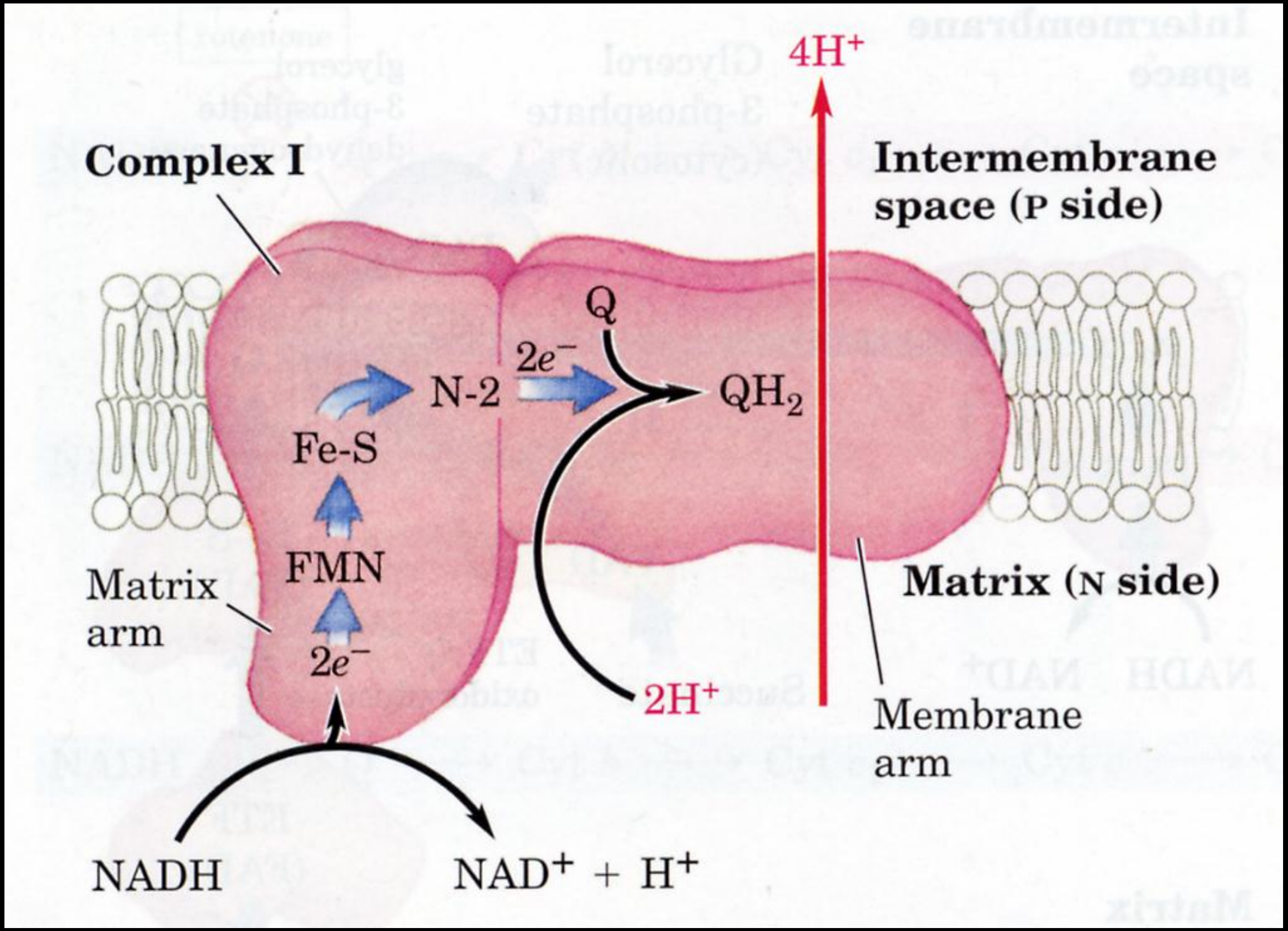


Lehninger Fig 19-9

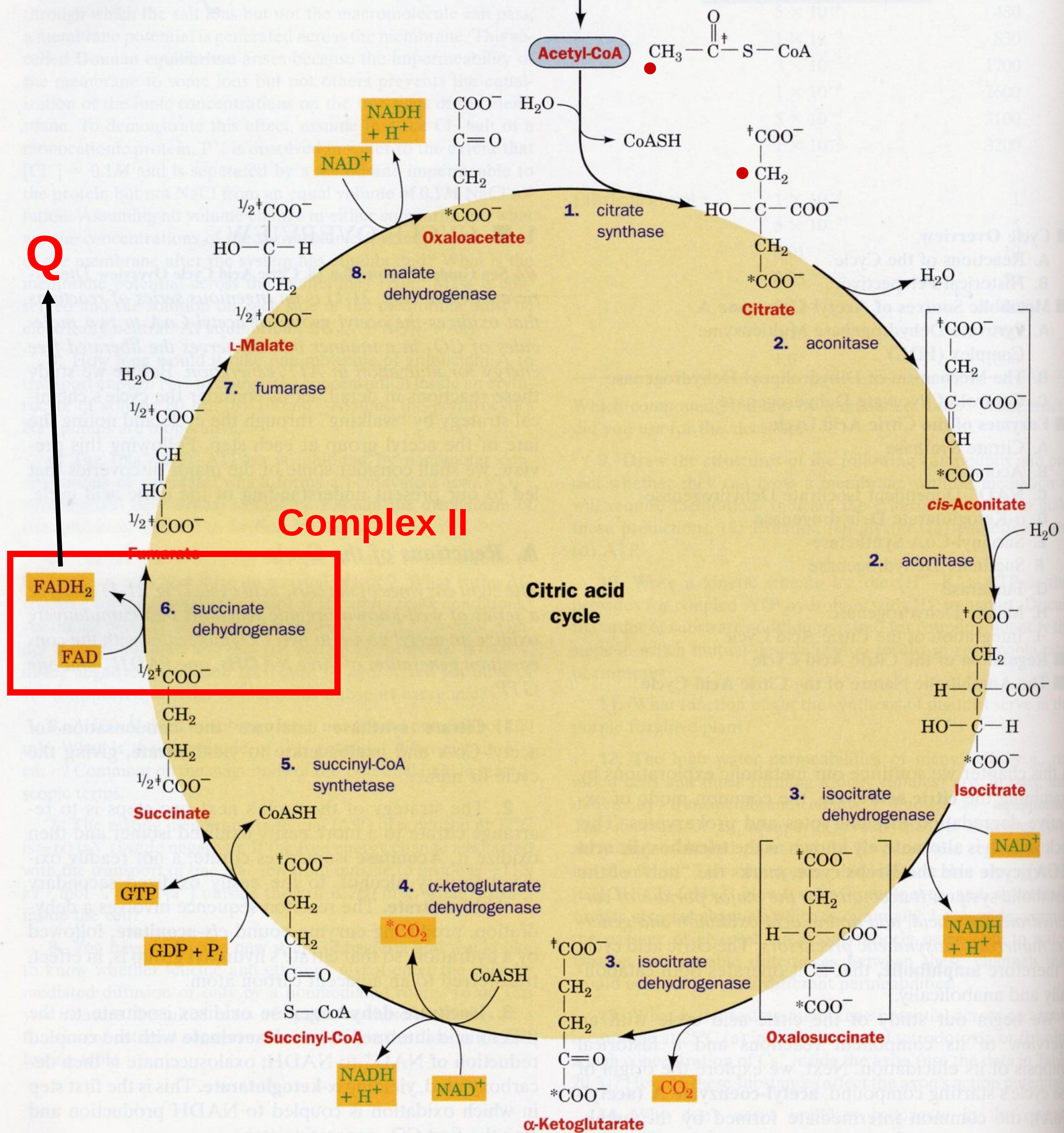
Four Types of Redox Centers in the Electron Transport Chain

1. Flavins – FMN, FAD
2. Iron – Sulfur Centers [2Fe–2S], [4Fe-4S]
3. Hemes
4. Cu Ions

Complex I

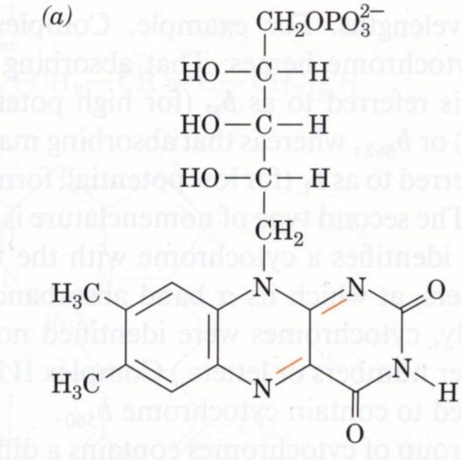


Lehninger Fig 19-9

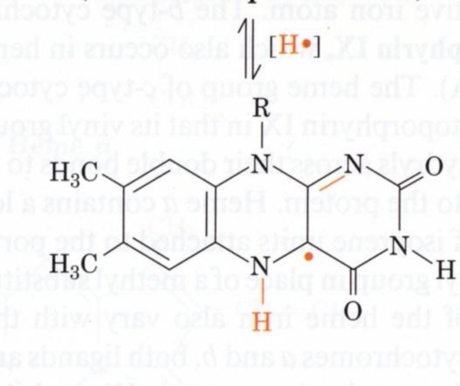


Q
Complex II
Acetyl-CoA
CoASH
1. citrate synthase
Citrate
2. aconitase
cis-Aconitate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
3. isocitrate dehydrogenase
CO2
α-Ketoglutarate
4. α-ketoglutarate dehydrogenase
CoASH
NAD+
NADH + H+
*CO2
Succinyl-CoA
5. succinyl-CoA synthetase
GDP + Pi
GTP
CoASH
Succinate
6. succinate dehydrogenase
FAD
FADH2
Fumarate
7. fumarase
H2O
L-Malate
8. malate dehydrogenase
NAD+
NADH + H+
Oxaloacetate
Citric acid cycle

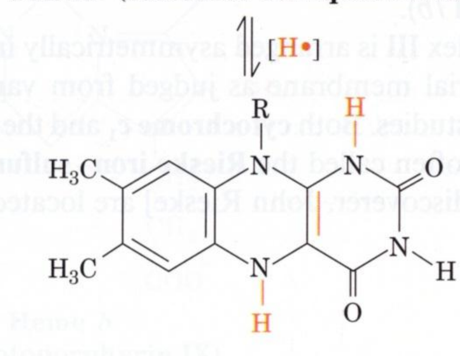
(a)



Flavin mononucleotide (FMN)
(oxidized or quinone form)

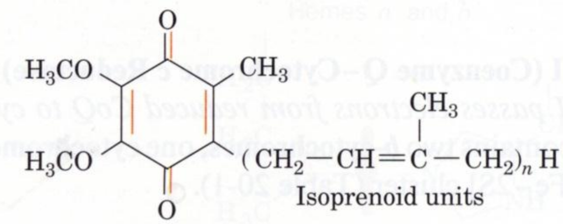


FMNH• (radical or semiquinone form)

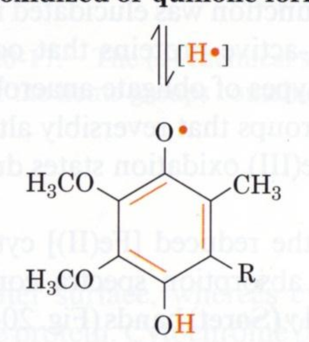


$FMNH_2$ (reduced or hydroquinone form)

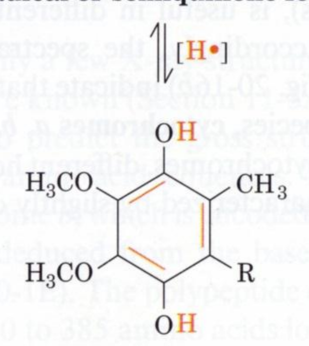
(b)



Coenzyme Q (CoQ) or Ubiquinone
(oxidized or quinone form)



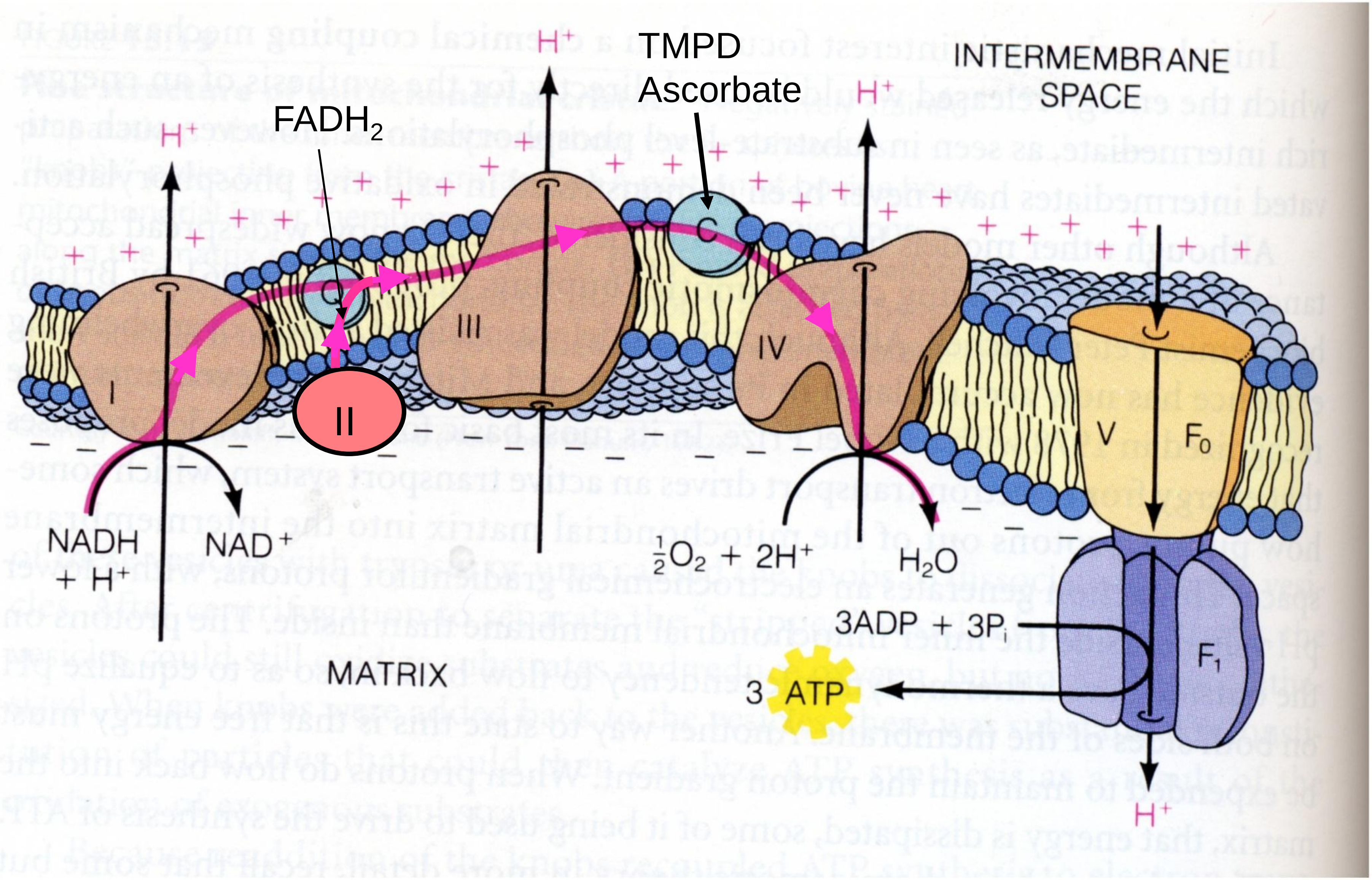
Coenzyme QH• or Ubisemiquinone
(radical or semiquinone form)



Coenzyme QH_2 or Ubiquinol
(reduced or hydroquinone form)

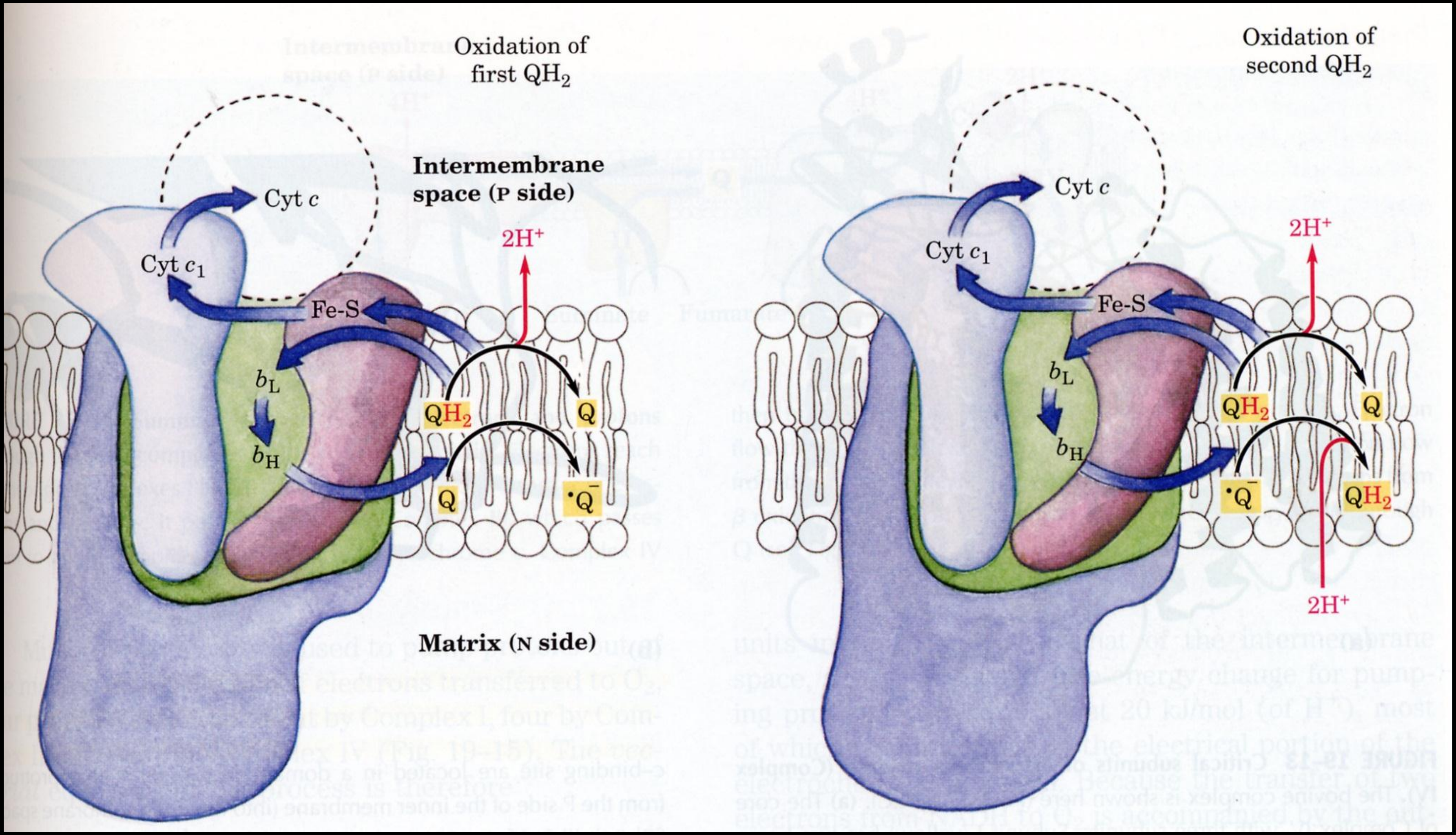
- Oxygen is a bi-radical
- Can accept e-s only 1 at a time
- Problem: ETC starts with e- pairs….

$$\bullet O_2 \bullet$$



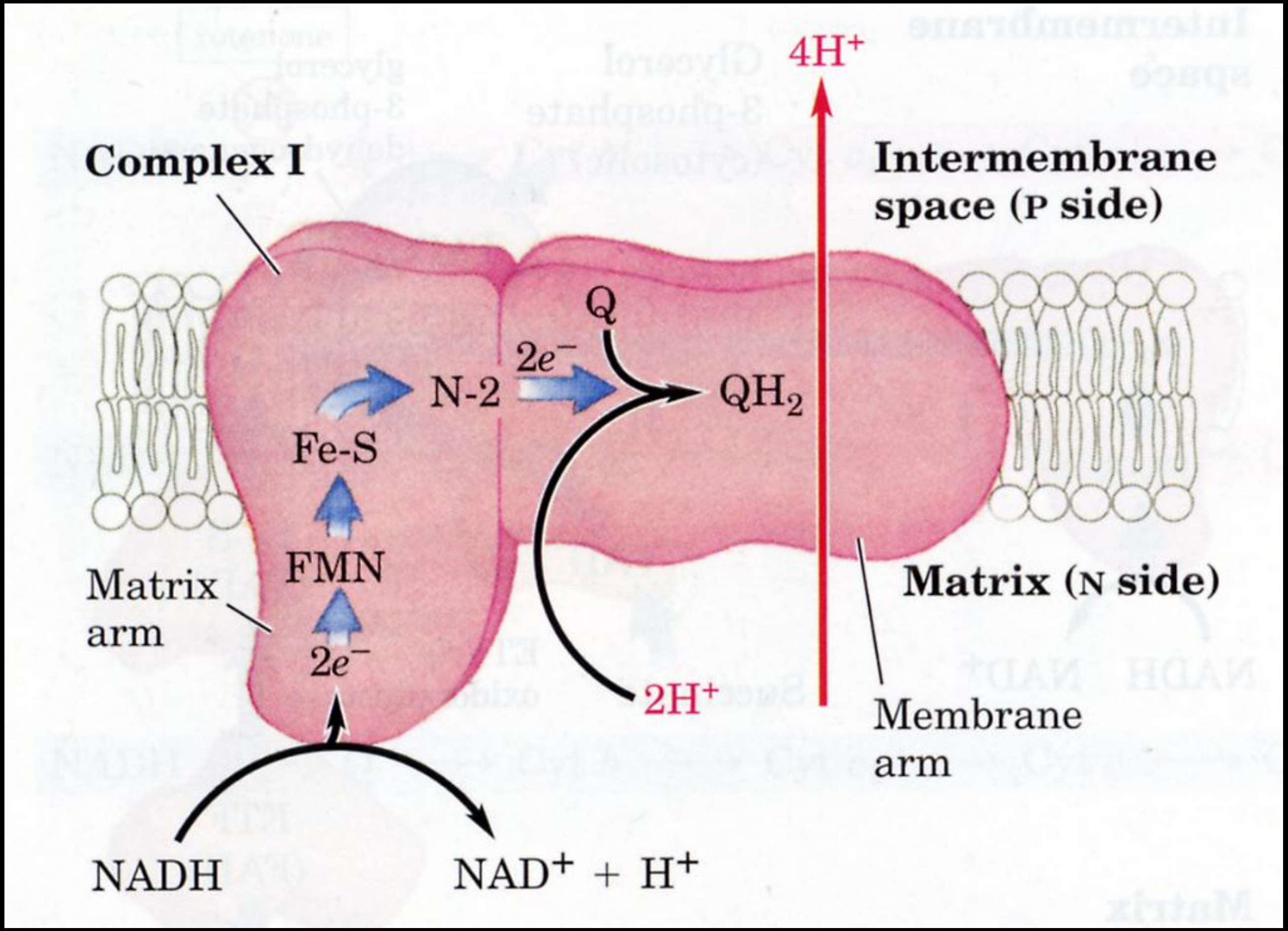
TMPD
Ascorbate
INTERMEMBRANE
SPACE
FADH2
H+
Q
C
I
II
III
IV
V
F0
F1
NADH
+ H+
NAD+
MATRIX
1/2 O2 + 2H+
H2O
3ADP + 3Pi
3 ATP

Complex III

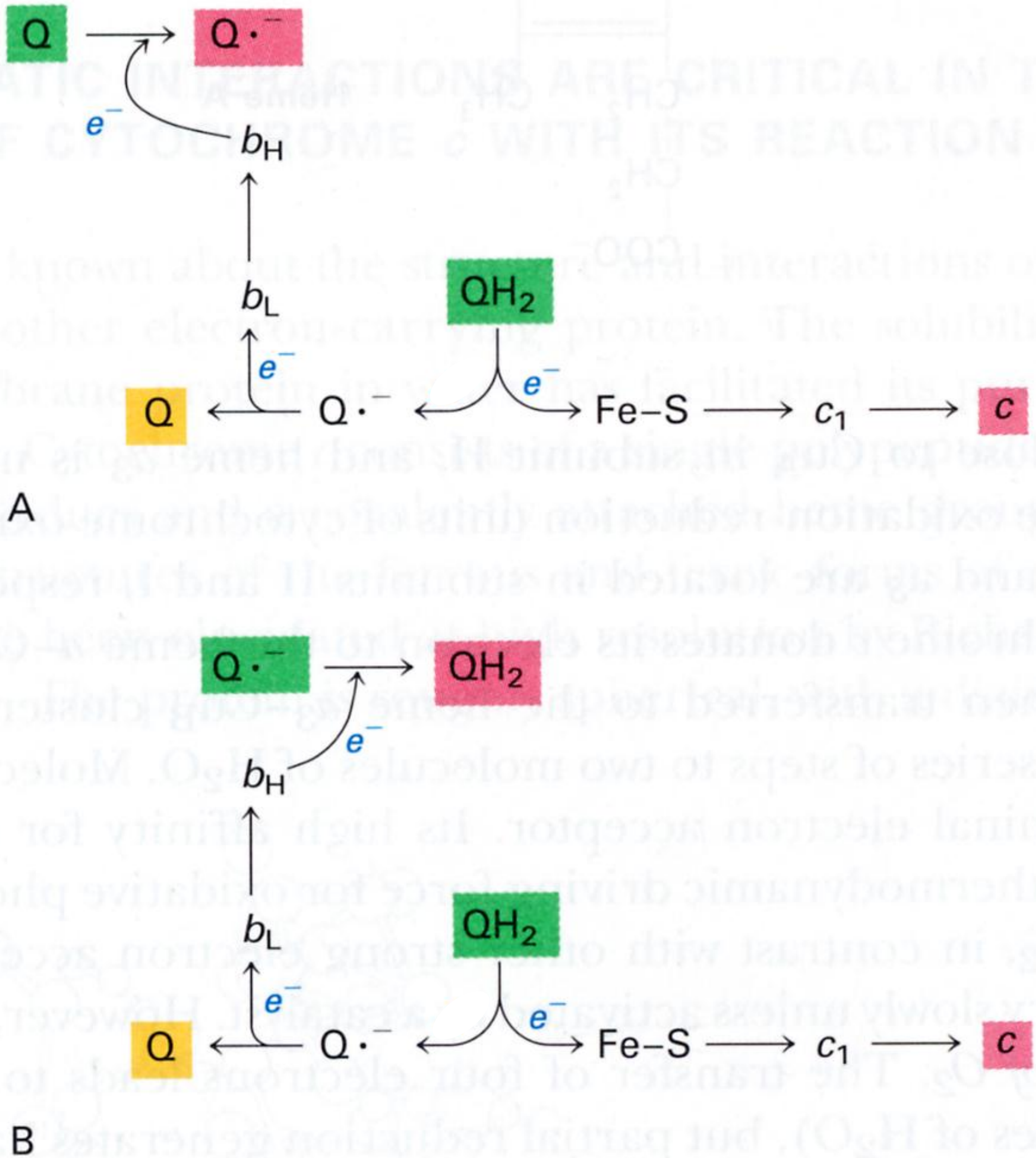


Lehninger Fig 19-12

Complex I

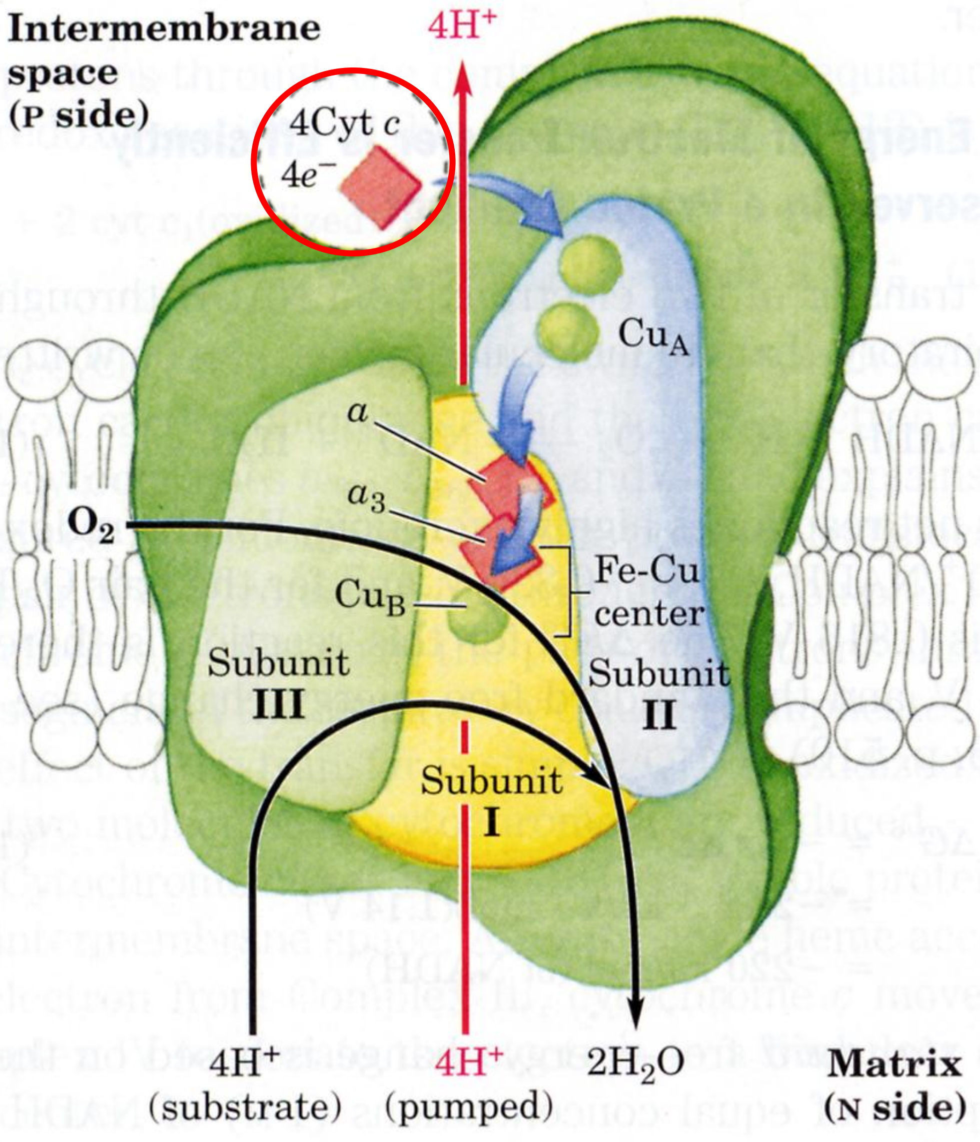


Lehninger Fig 19-9



Q
Q·⁻
e⁻
b_H
b_L
QH₂
e⁻
e⁻
Q
Q·⁻
Fe–S
c₁
c
A
Q·⁻
QH₂
e⁻
b_H
b_L
QH₂
e⁻
e⁻
Q
Q·⁻
Fe–S
c₁
c
B

Complex IV (cytochrome oxidase)



Oxygen free radicals

$\cdot O_2 \cdot \; + \; e^- \longrightarrow \cdot O_2 :^- \quad (HO_2 \cdot)$ Superoxide

$\cdot O_2 \cdot \; + \; 2e^- \longrightarrow :O_2:^{2-} \quad (H_2O_2)$ Peroxide

$\underset{\cdot\cdot}{\ddot{O}}::\underset{\cdot\cdot}{\ddot{O}} \; + \; 3e^- \longrightarrow :\underset{\cdot\cdot}{\ddot{O}}:^{2-} \; + \; :\underset{\cdot\cdot}{\ddot{O}}\cdot^-$

(H_2O) Hydroxy radical

$O_2 \; + \; 4e^- \longrightarrow 2H_2O$

$\cdot O_2^- \; + \; H_2O_2 \longrightarrow O_2 \; + \; \underline{HO}\cdot$

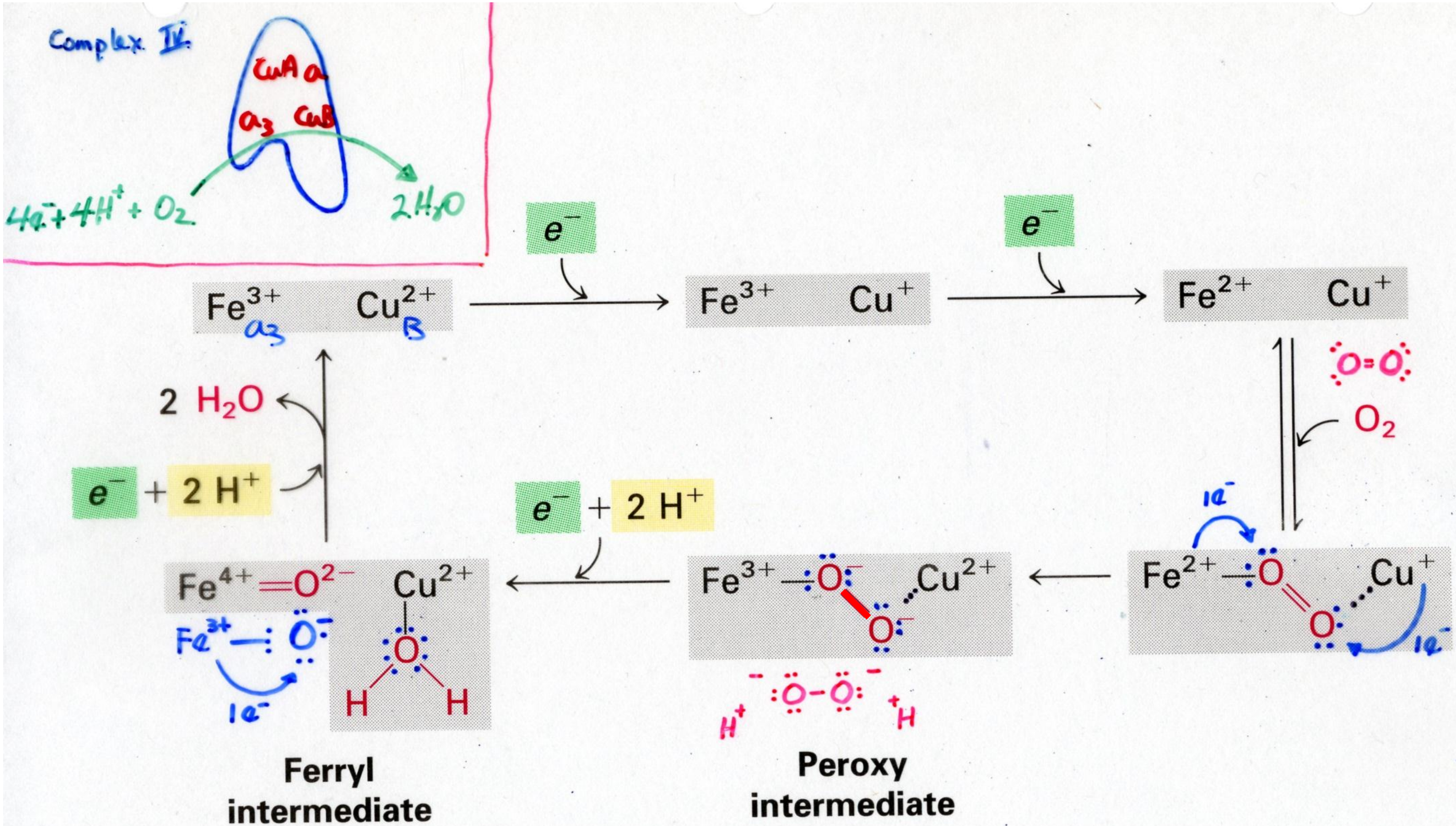
O_2 free radical scavaging systems

$$\bullet O_2^- + \bullet O_2^- \xrightarrow{+2H^+} H_2O_2 + O_2$$

Superoxide Dismutase

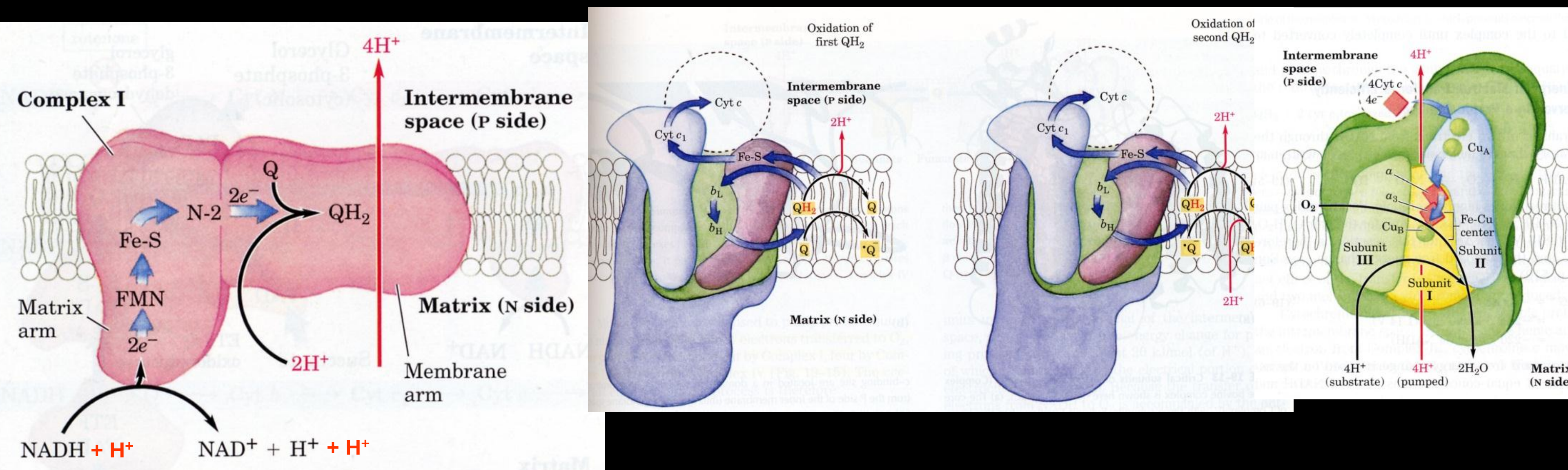
$$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase



Complex IV
CuA a
a3 CuB
4e⁻ + 4H⁺ + O2
2H2O
e⁻
e⁻
Fe3+ Cu2+
a3 B
Fe3+ Cu+
Fe2+ Cu+
O=O
O2
2 H2O
e⁻ + 2 H+
e⁻ + 2 H+
1e⁻
1e⁻
1e⁻
Fe4+=O2− Cu2+
Fe3+—O⁻
Fe3+—O⁻ O⁻ Cu2+
Fe2+—O=O Cu+
O–O
H+ H+
H H
Ferryl intermediate
Peroxy intermediate

10 H^+'s are pumped per $2e^-$s transported from NADH to ½ O_2



Look at Fig. 19-19 Lehninger