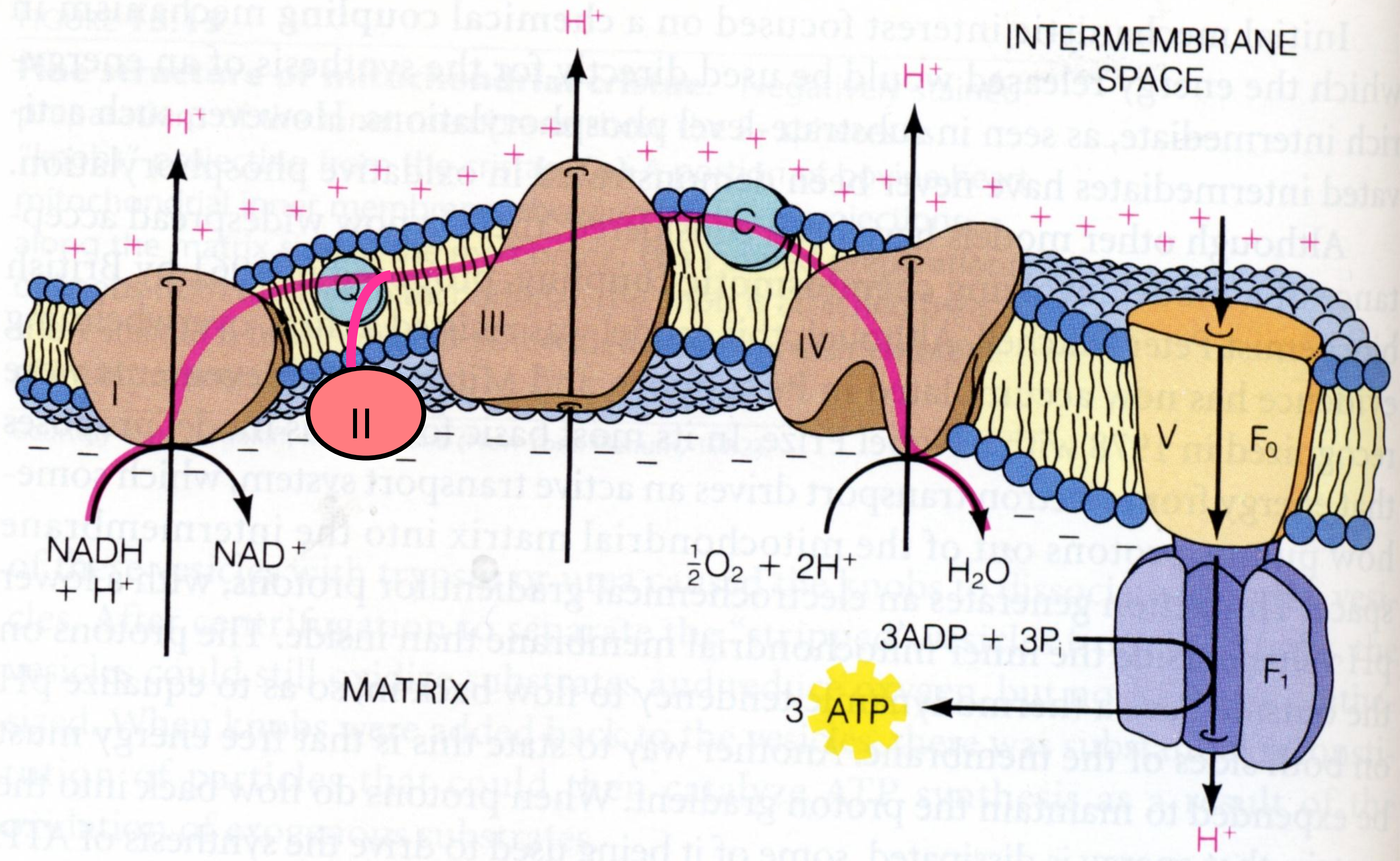
Biochemistry of Oxidative P’n

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

The Chemiosmotic Hypothesis

- The accepted model for coupling oxidation to P'n.

- Free energy of electron transport is harnessed by pumping H^+ from the matrix to the intermembranal space (cytosol) to create a proton gradient.

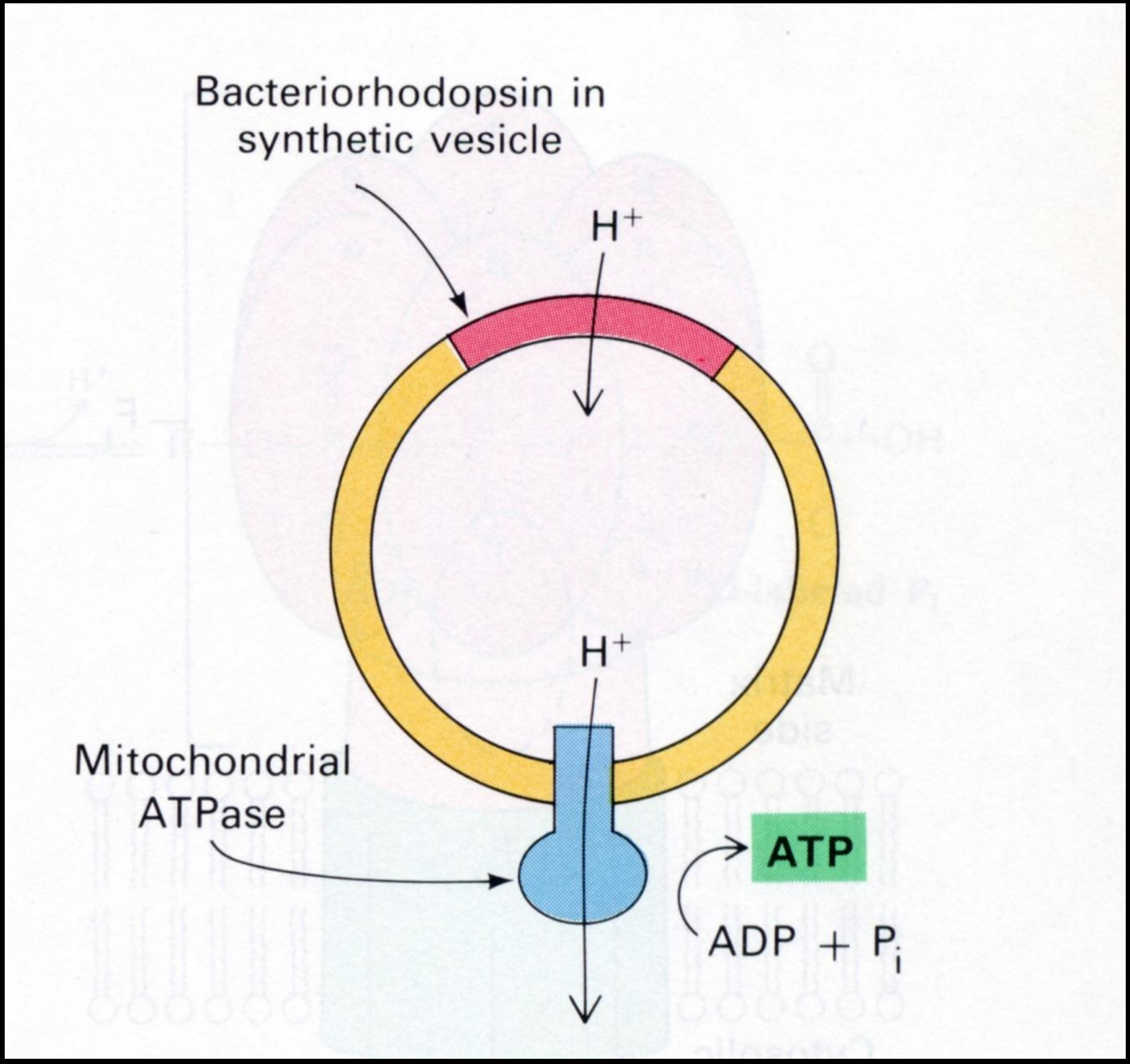

INTERMEMBRANE SPACE
H+
H+
H+
Q
C
I
II
III
IV
V
F0
F1
NADH + H+
NAD+
1/2 O2 + 2H+
H2O
3ADP + 3Pi
3 ATP
MATRIX
H+

Evidence that supports the chemiosmotic hypothesis:

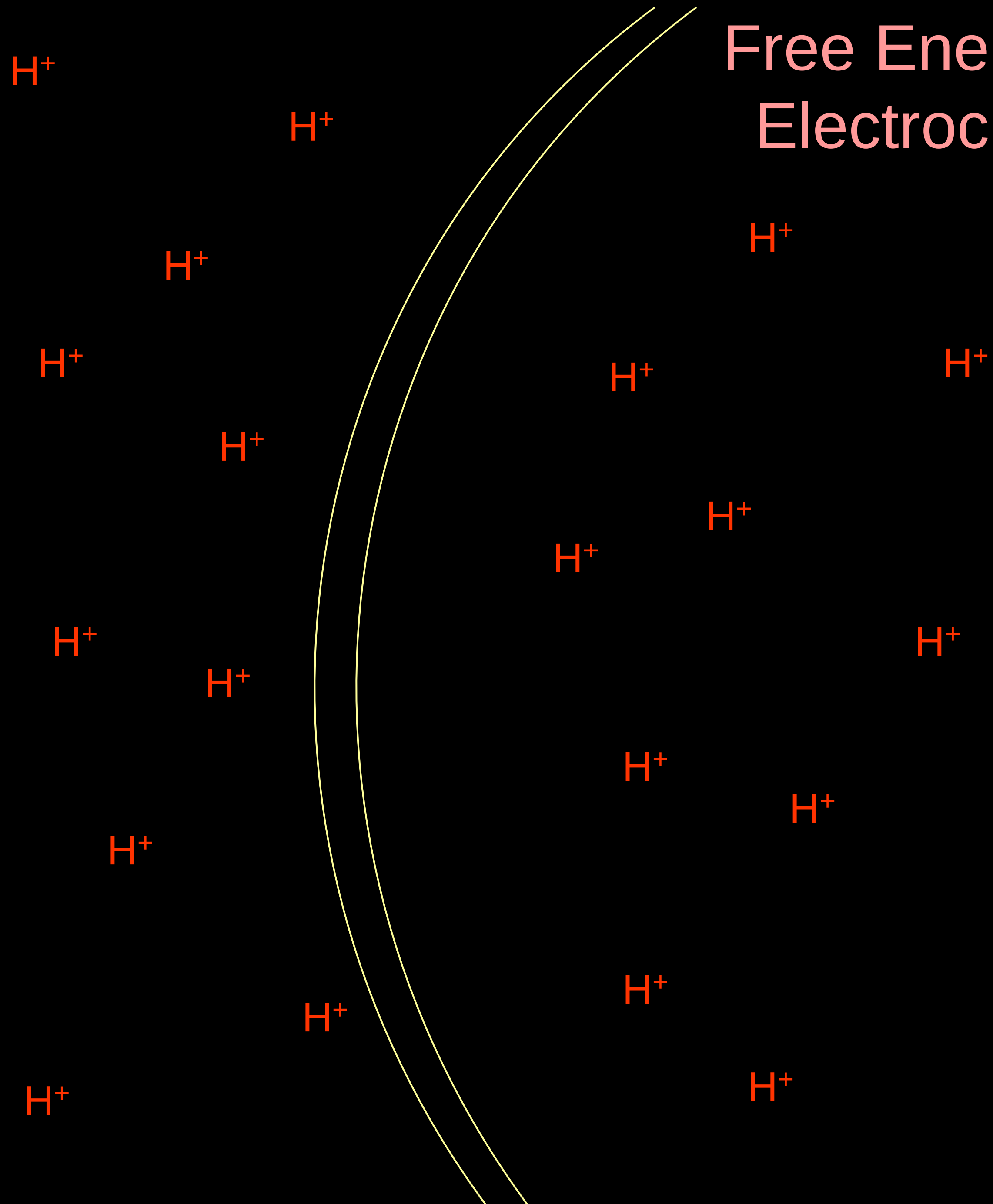
1. e- transport correlates with generation of a proton gradient
2. An artificial pH gradient leads to ATP synthesis in intact mitochondria
3. Complex I,III, and IV are proton pumps
4. A closed compartment is essential
5. Proton carriers (across IMM) “uncouple” oxidation from P’n.

Con’t

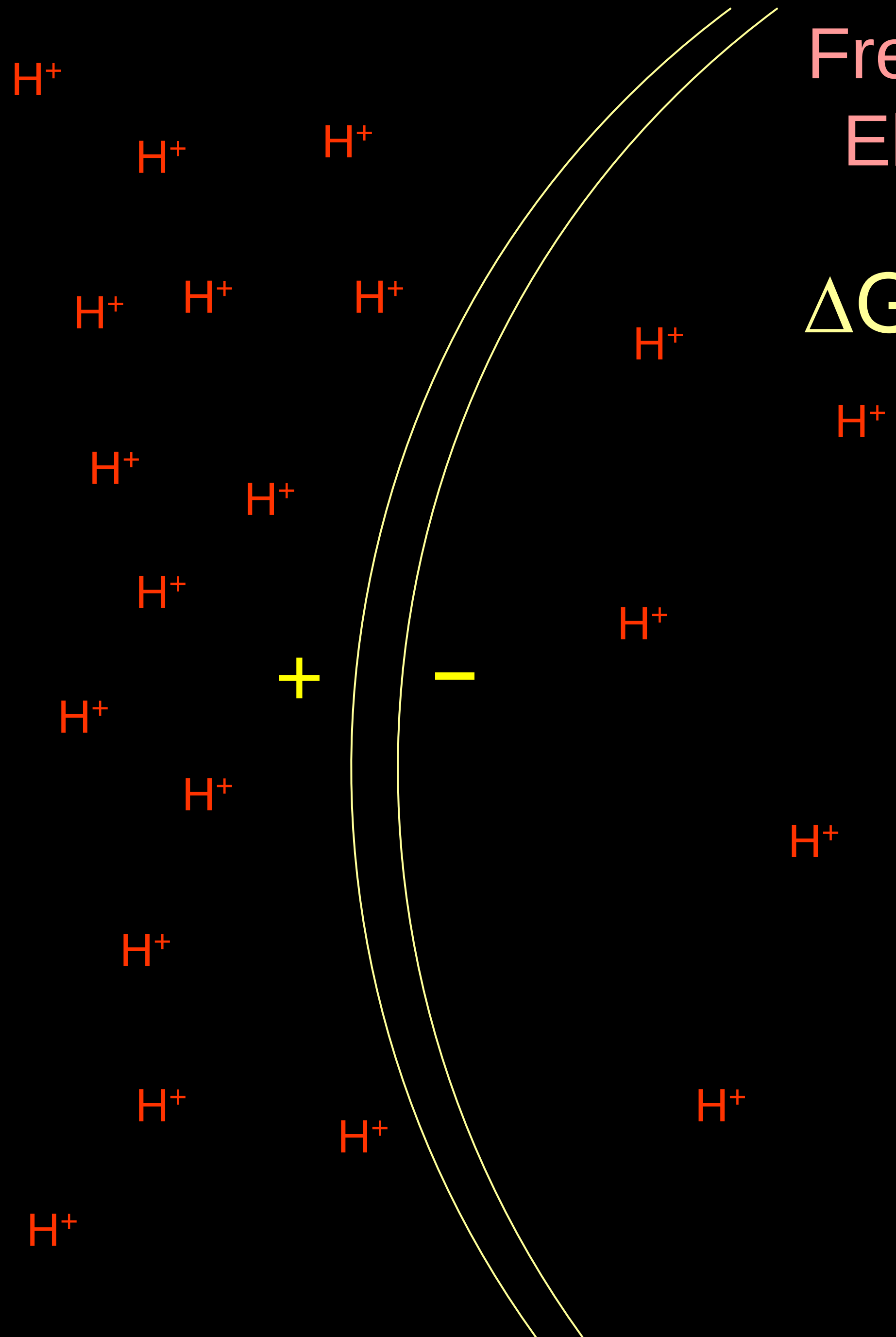
6. ATP Synthesis occurs in artificial liposomes in response to light, if the ATP sythase is reconstituted with bacterial rhodopsin



Free Energy Associated with Electrochemical Gradients

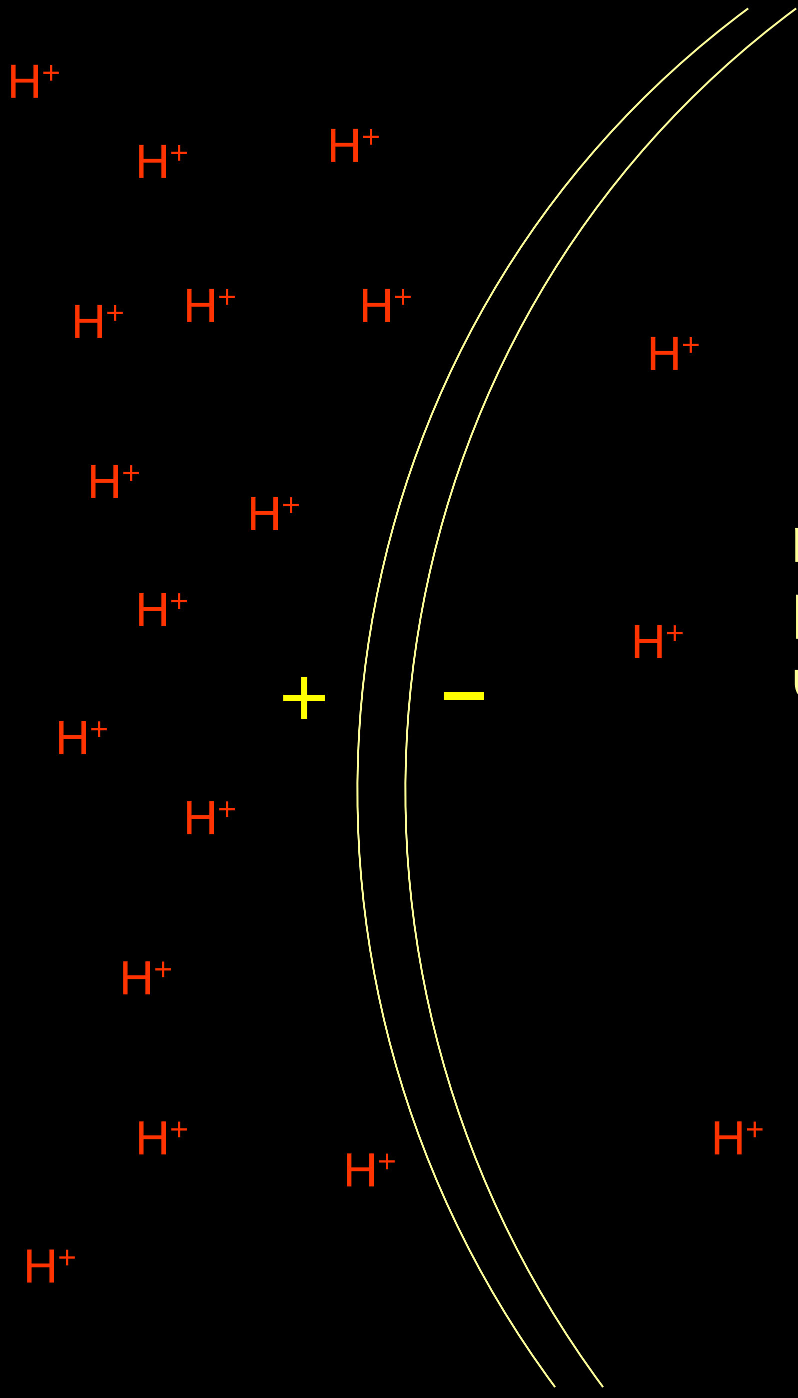


Free Energy Associated with Electrochemical Gradients



$$\Delta G = \qquad -2.3RT\Delta pH$$

Free Energy Associated with Electrochemical Gradients



$$\Delta G = -mF\Delta\Psi - 2.3RT\Delta pH$$

H^+

m = ion charge

F = Faraday constant = 23 kcal/mol/V

Ψ = membrane potential (V)

H^+

$$\Delta G = -mF\Delta\Psi - 2.3RT\Delta pH$$

In respiring mitochondria:

$\Delta\Psi$ = ~0.18 V

Δ pH = ~1 pH unit

So: ΔG = -(1)(23 kcal/mol/V)(0.18 V) – (1.4 kcal/mol)(1)
= -5.5 kcal/mol (23.3 kJ/mol)

Therefore: pumping 1 mole of H^+ requires 5.5 kcal

Assuming that oxidation of 1 mol NADH results in 10 mol H^+ being pumped, 55 kcal is required.

Synthesis of ATP requires 12 kcal/mol. Therefore 2.5 mol ATP's can easily be made from 1 mol NADH.

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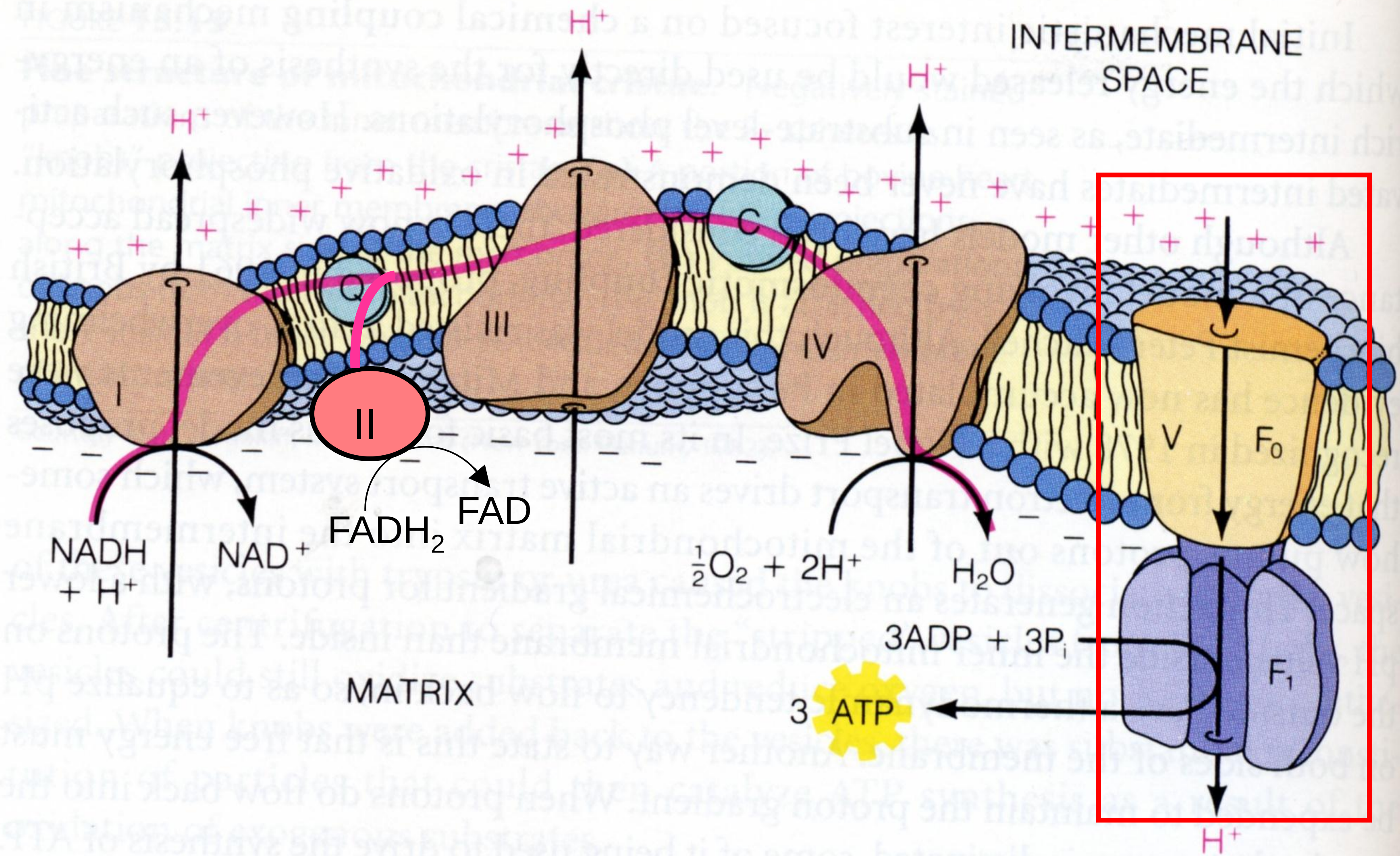
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H+
H+
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NADH + H+
NAD+
FADH2
FAD
1/2 O2 + 2H+
H2O
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3 ATP
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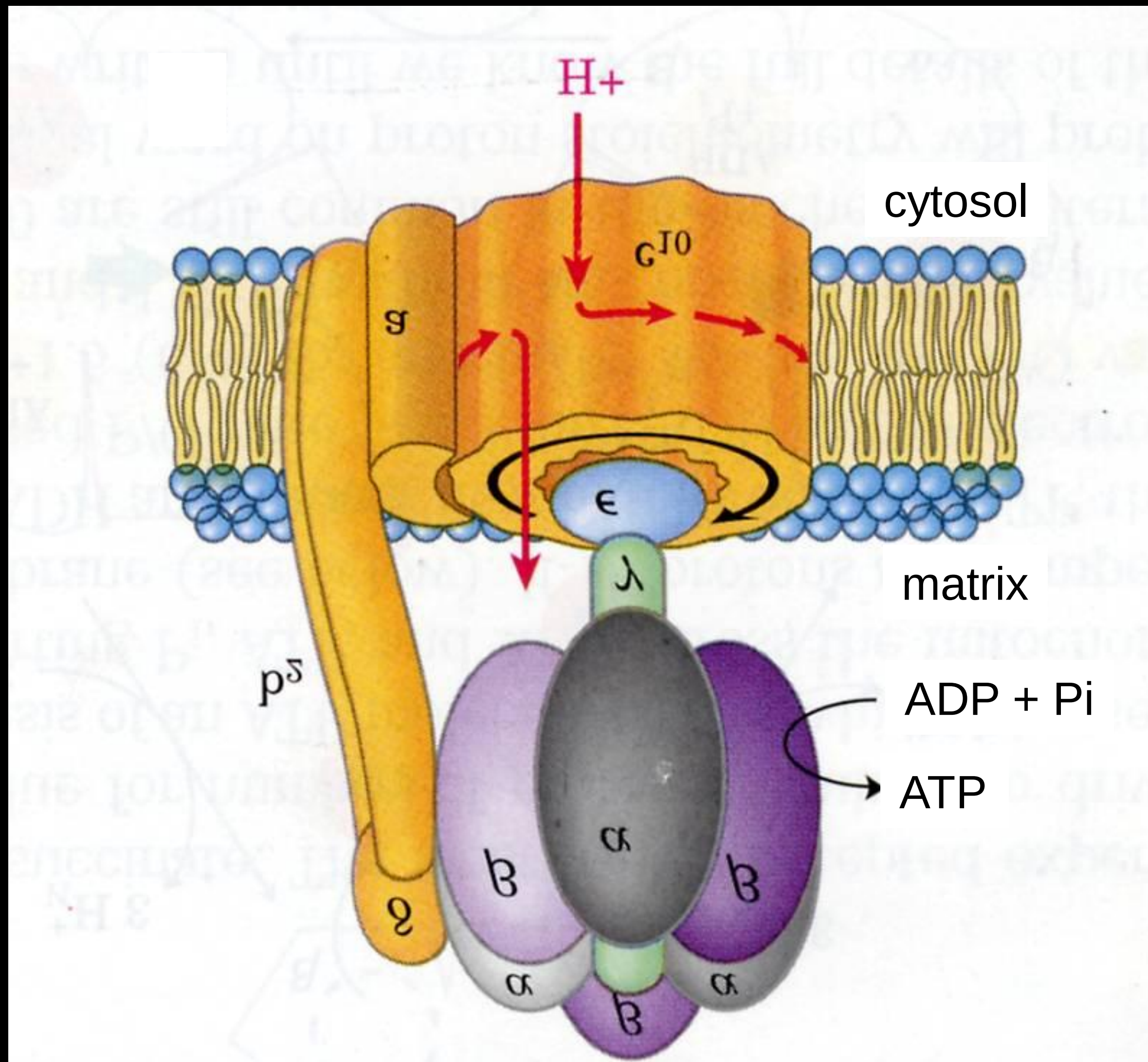
MATRIX

ATP Synthase



Science

14 January 2005

Vol. 307 No. 5707
Pages 165–300 $10

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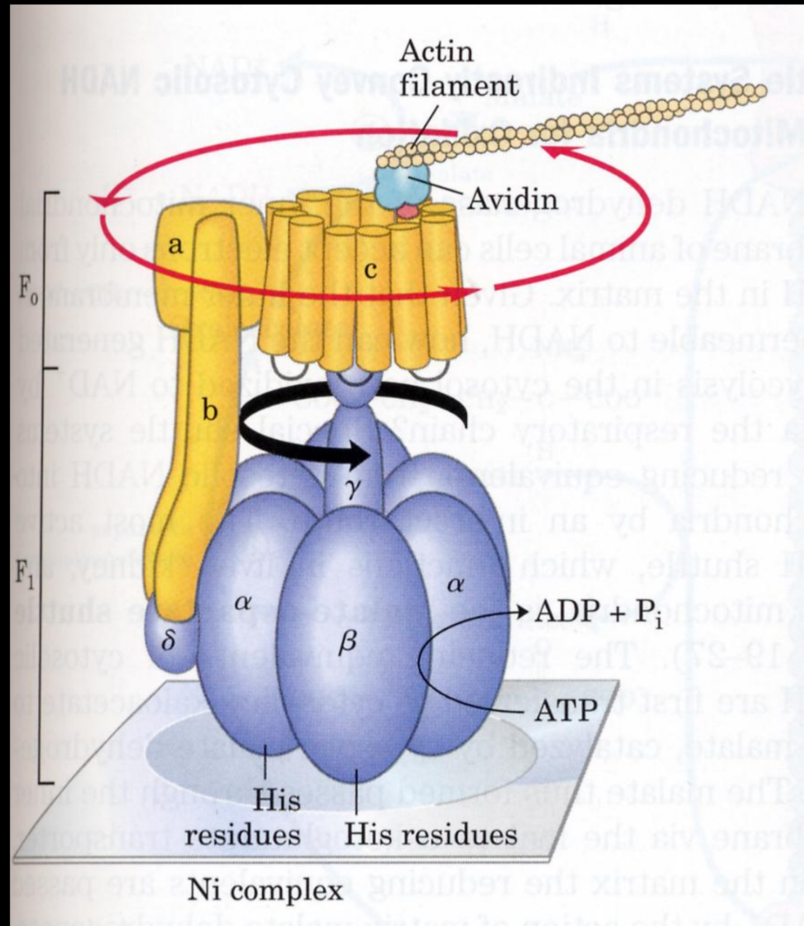
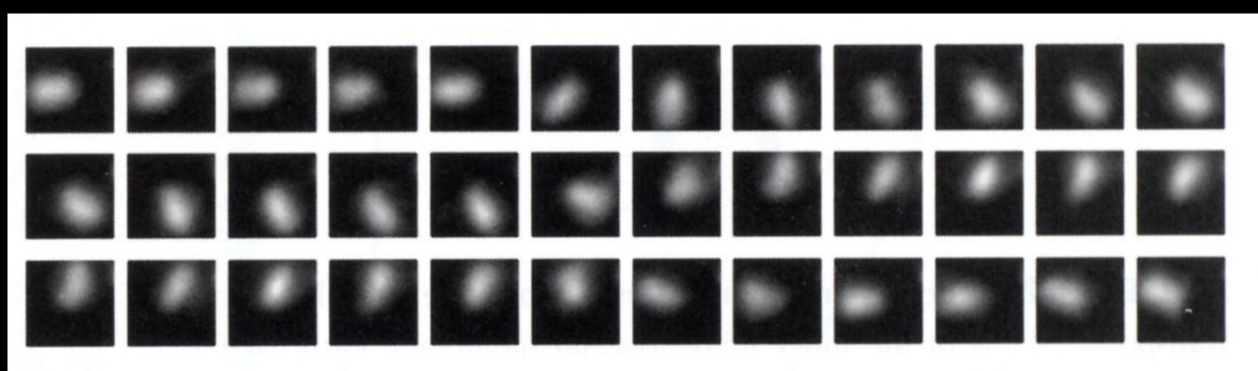
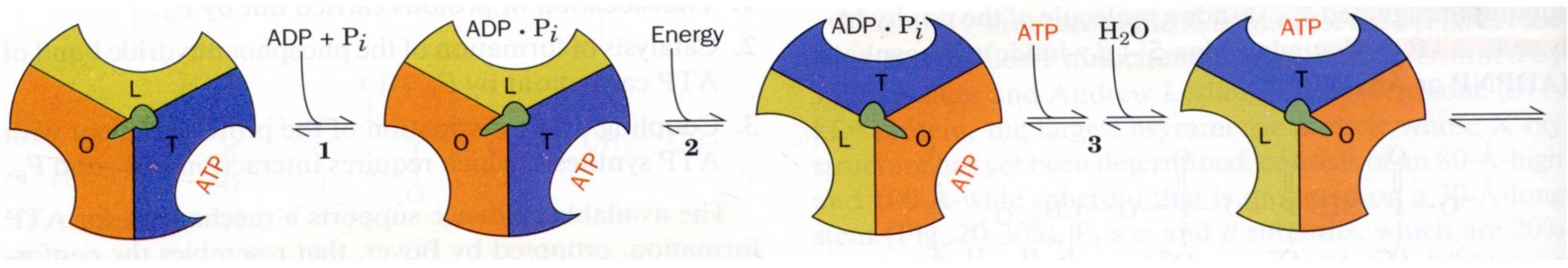

Actin filament
Avidin
a
c
F0
b
γ
F1
α
α
ADP + Pi
δ
β
ATP
His residues
His residues
Ni complex



Coupling H^+ translocation through ATP Synthase with ATP synthesis

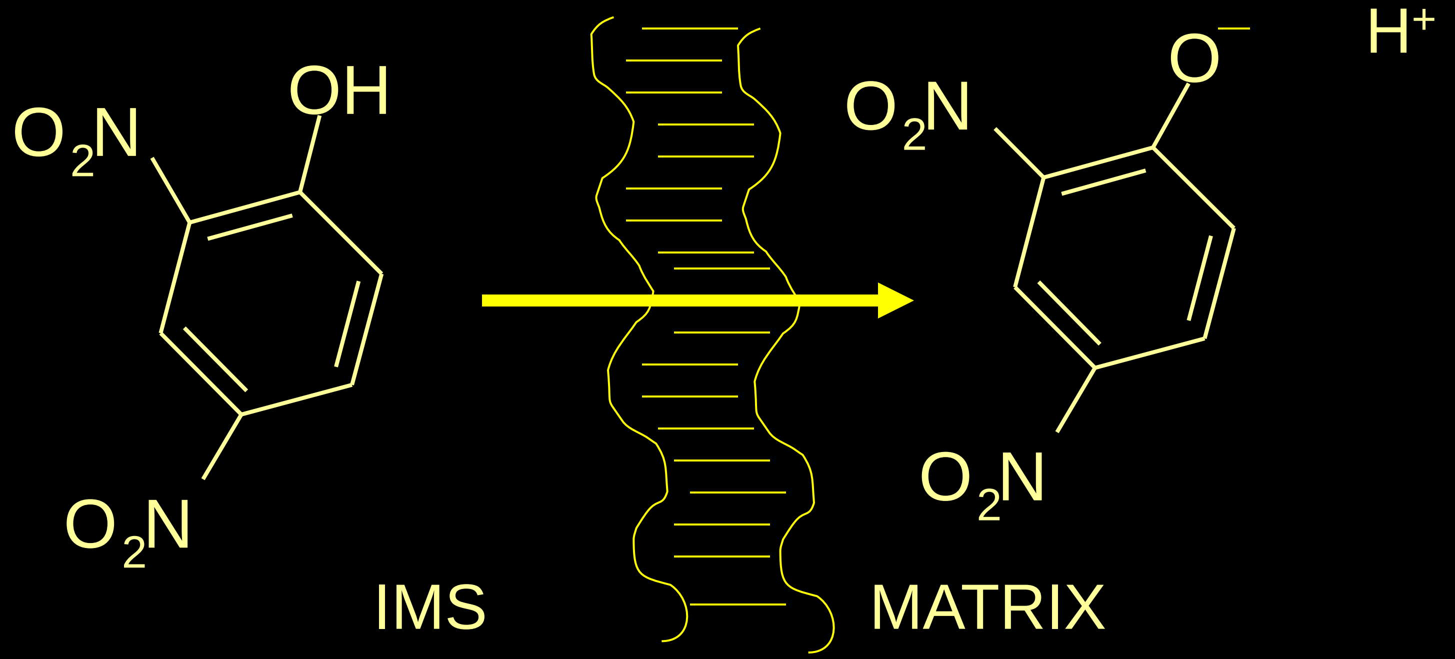


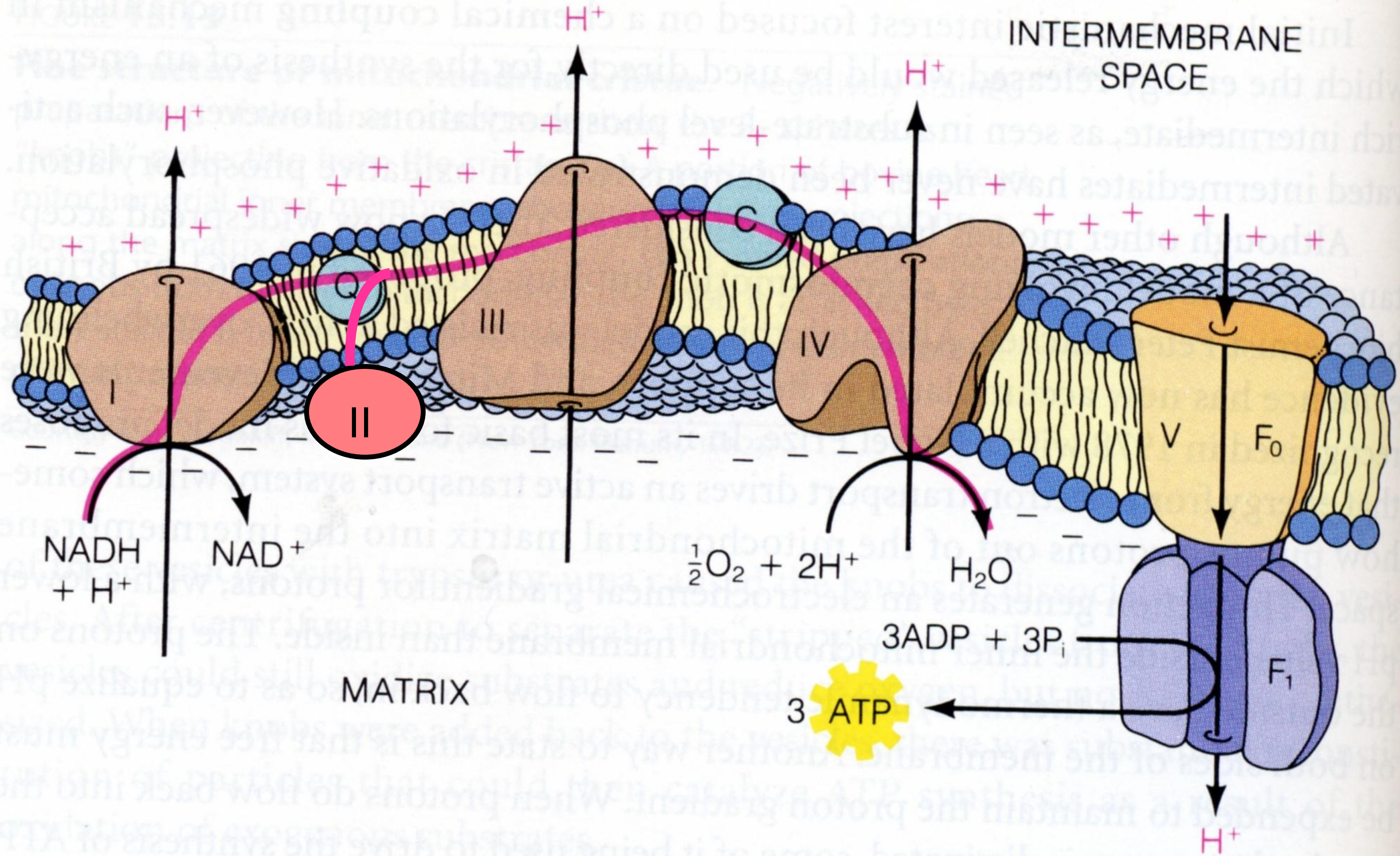
Free energy of H^+ translocation "forces" an internal cam shaft to rotate, which changes the conformation of each subunit during one complete turn.

Inhibitors of Oxidative Phosphorylation

1. Inhibitors of Complexes I, III, & IV.
2. Oligomycin – antibiotic which binds to ATP synthase and blocks H^+ translocation.
3. Uncouplers:

   a) Dinitrophenol (DNP).

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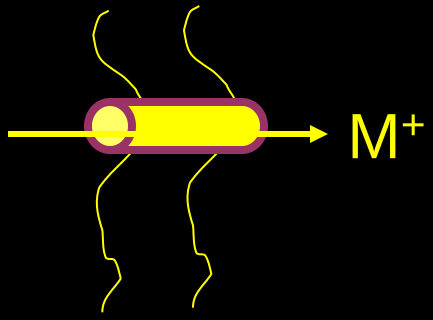
b) Ionophores

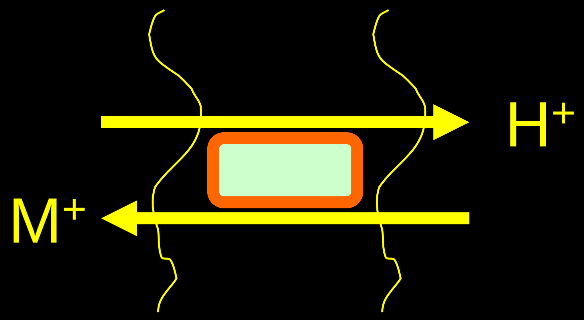
i) Valinomycin – carries charge but not H^+'s.

- Dissipates electrical gradient.

ii) Nigericin – carries protons but not charge.

- Dissipates chemical gradient. (due to H^+)



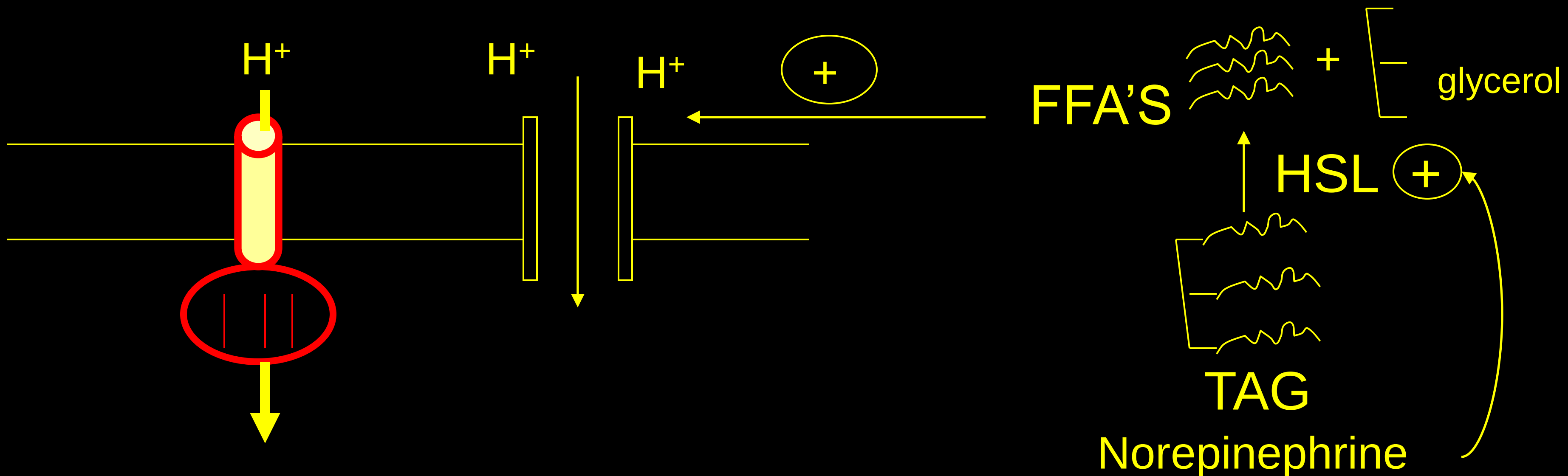


$M^+ = K^+ >> Na^+$ $M^+ = K^+ >> Na^+$

c) Thermogenin – active component of brown fat.

- acts as a H^+ channel in the IMM of brown fat mitochon.
- effect: P/O << 1.

Regulation of Thermogenin Conductance



**Uncoupling (and heat generation) occur only if plenty of FFA substrate is available. If not, ATP synthesis prevails.

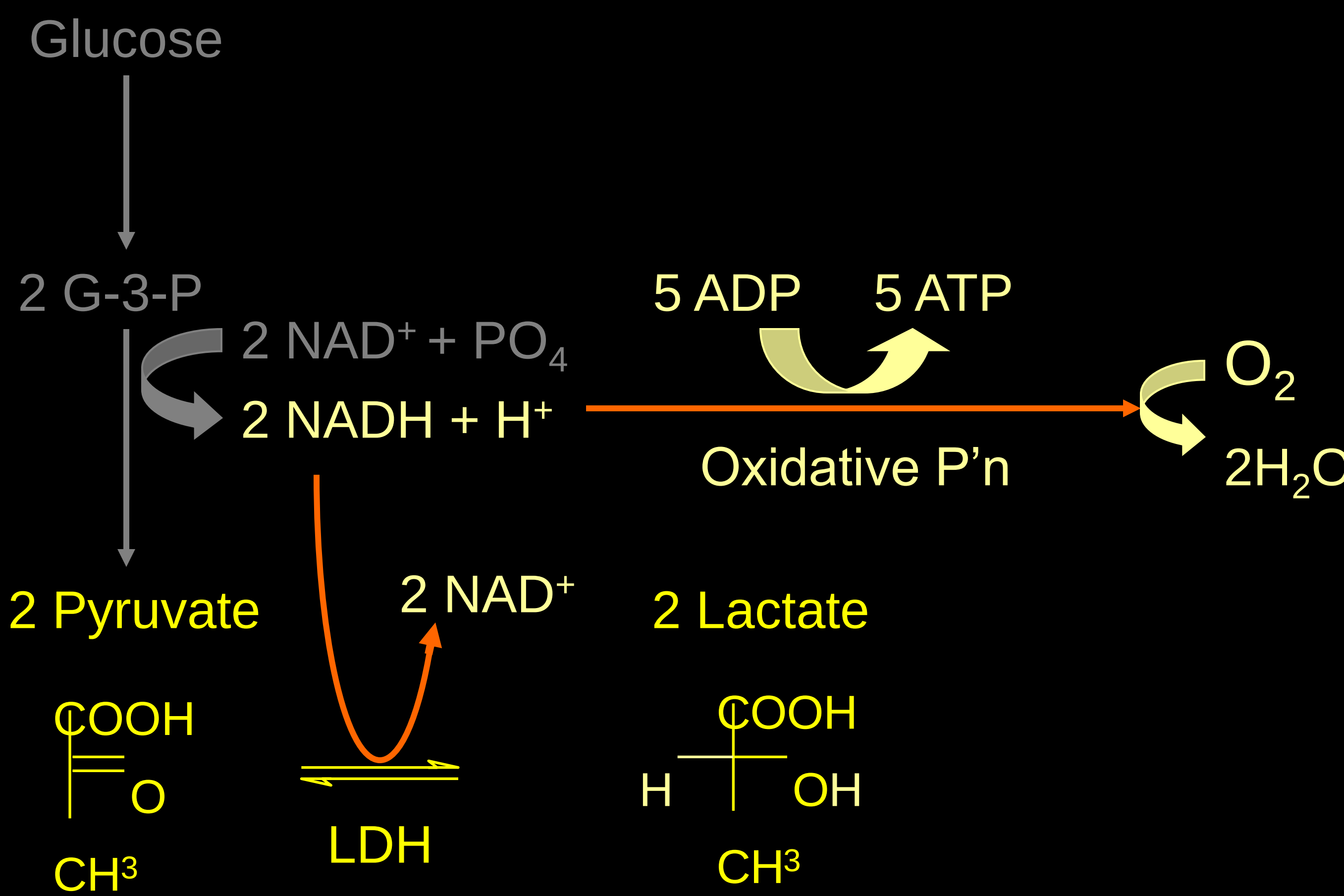
ATP Inventory

For homework: Rationalize the following:

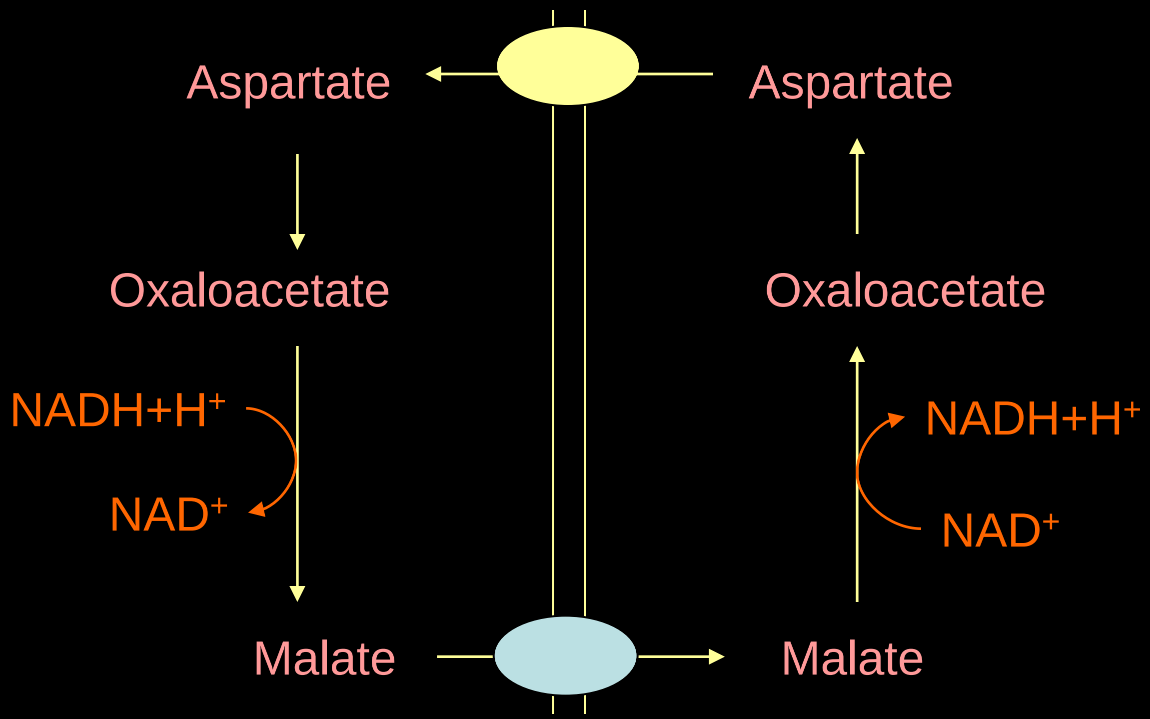
<u>From 1 Glucose</u>

10 NADH	25 ATP
2 $FADH_2$	3 ATP
2 GTP	2 ATP
	2 ATP
	32 ATP

Fates of cytosolic NADH



Cytosolic NADH enters the mitochondria via the malate-aspartate shuttle



End of Oxidative Phosphorylation

Glycogen Breakdown and Synthesis

Chapter 15 Lehninger

Please read on your own:
Figures 25, 27, 29, 30, 34, 37