Oxidative Phosphorylation

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- We have seen that the proton motive force conserves about 200 kJ of free energy per ‘mole’ of electron pairs, more than enough to drive the synthesis of ATP (~50 kJ/mol under cellular conditions).
- But to do so, there must be a coupling between the gradient and the synthesis of ATP from ADP and Pi.
- That coupling mechanism is provided by the ATP synthase enzyme.

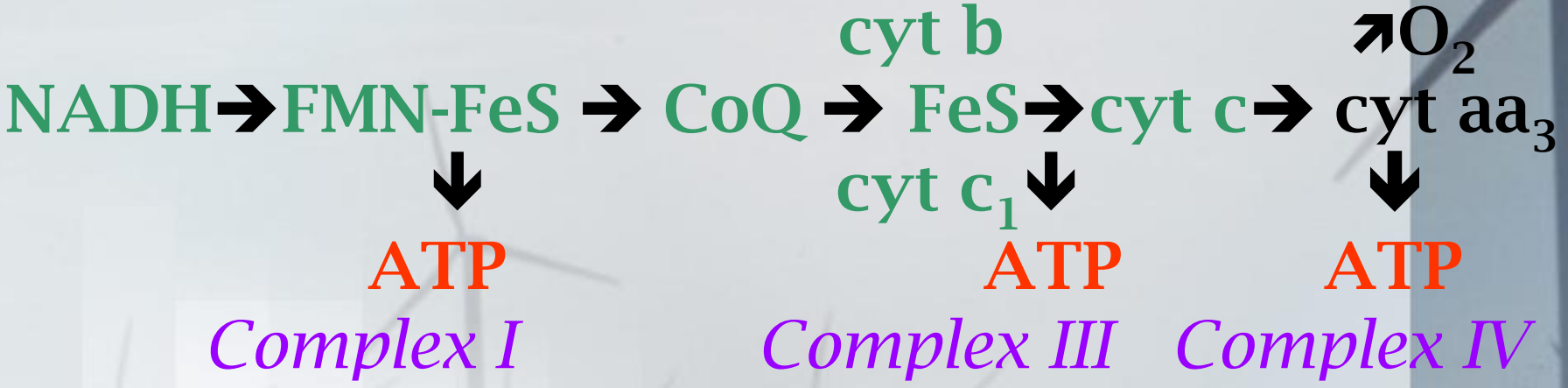
Oxidative Phosphorylation

-- (ox-phos)

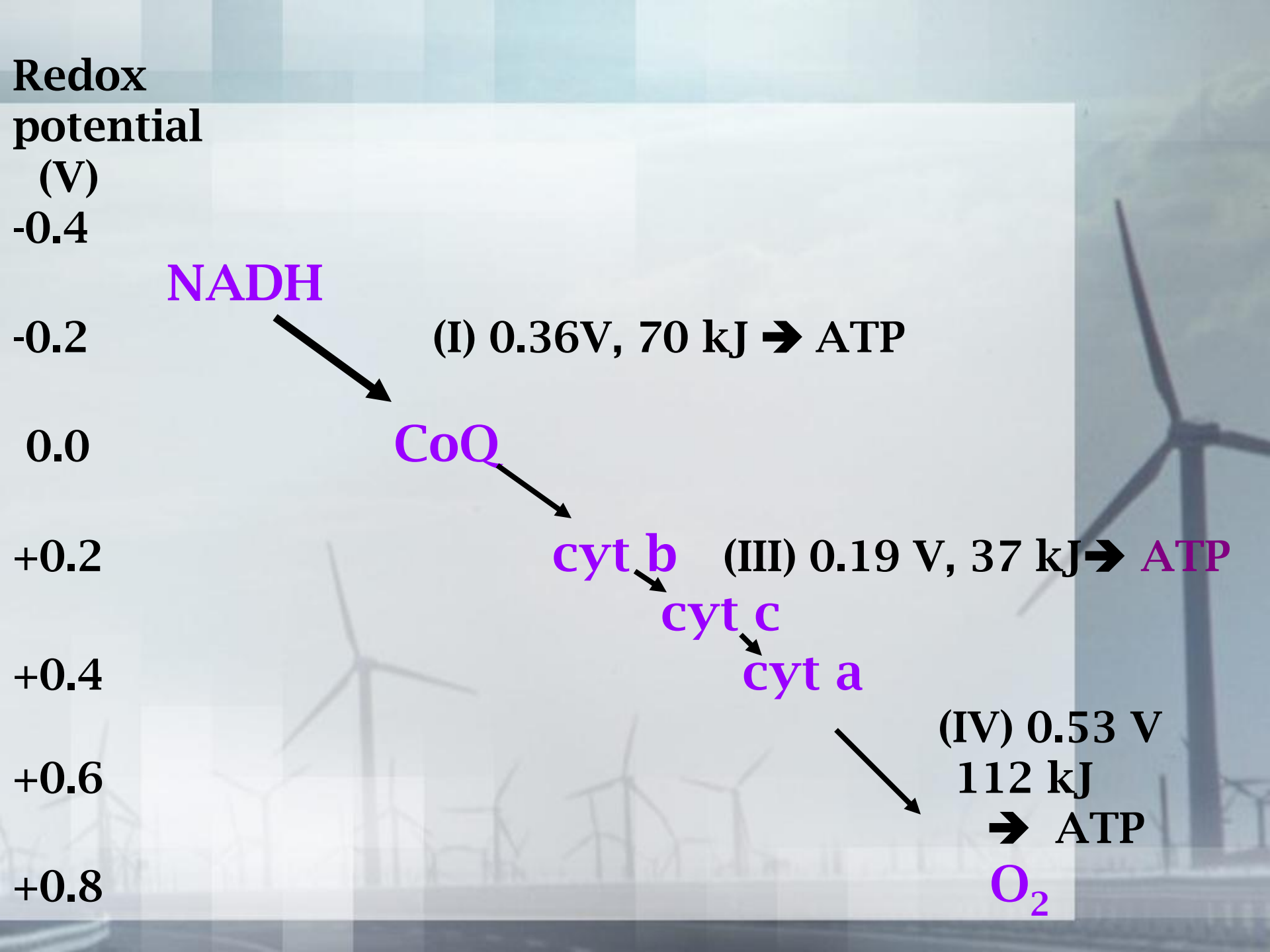
Definition: Production of ATP using transfer of electrons for energy

= coupled

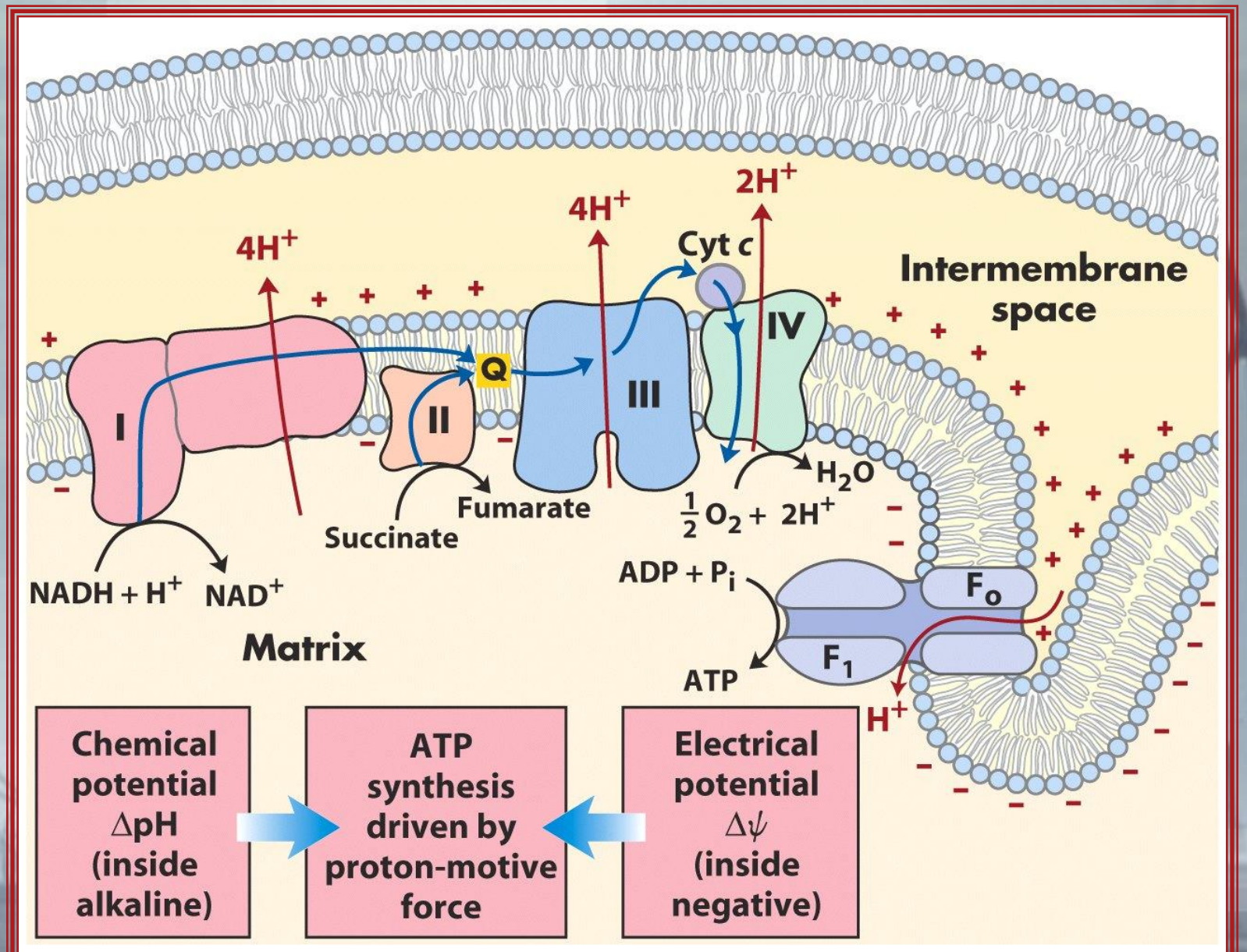
--for NADH, we know



Note: Several small energy steps


Redox potential (V)
-0.4
-0.2
0.0
+0.2
+0.4
+0.6
+0.8
NADH
(I) 0.36V, 70 kJ ➔ ATP
CoQ
cyt b
(III) 0.19 V, 37 kJ➔ ATP
cyt c
cyt a
(IV) 0.53 V
112 kJ
➔ ATP
O_2

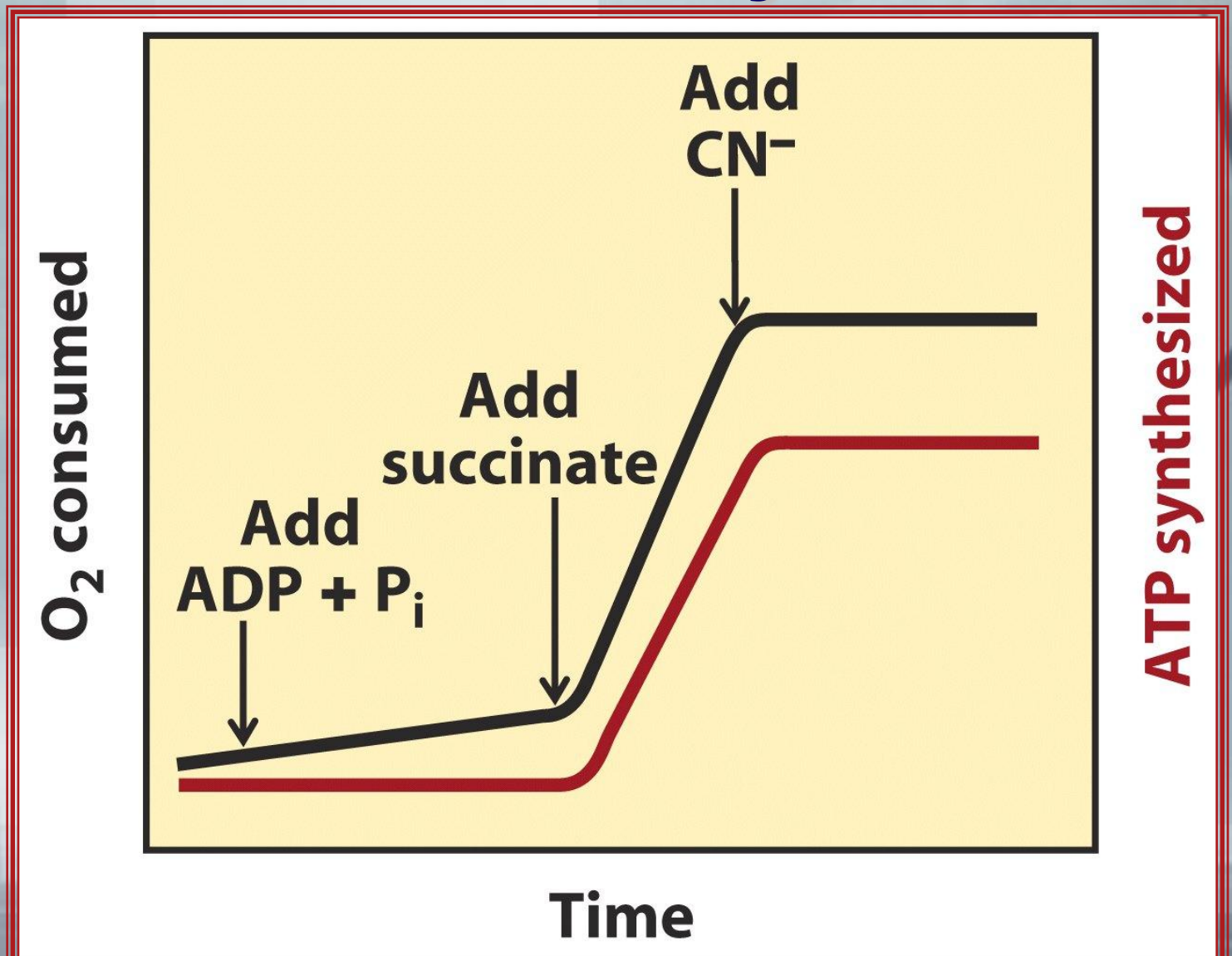
Chemiosmotic Model of ATP Synthesis



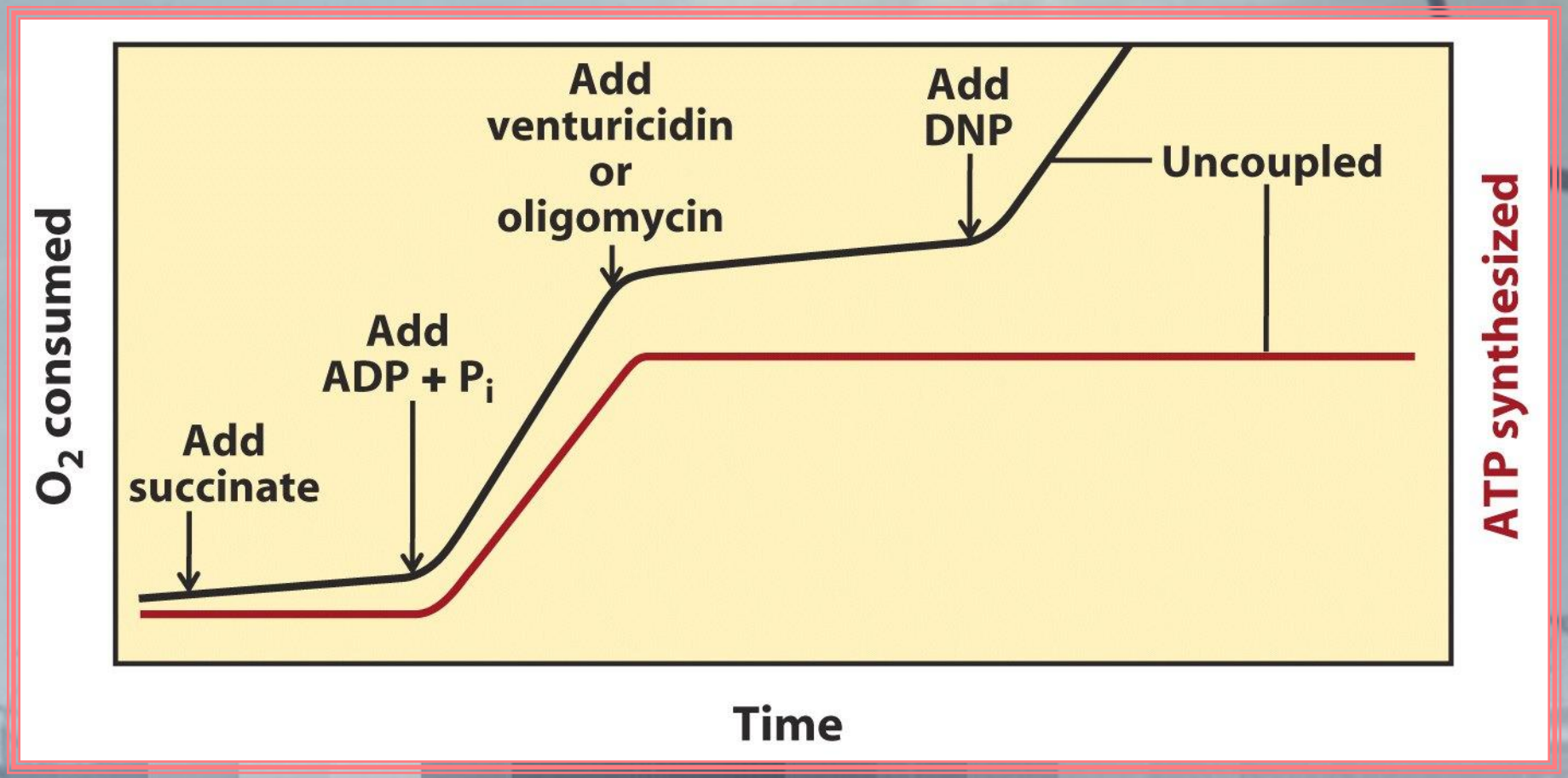
- The reaction carried out by ATP synthase can be written as:
- ADP + Pi + *n*H+out → ATP + H2O + *n*H+in
- Because energy from substrate oxidation is required for ATP synthesis, it's not surprising that inhibitors of electron transport (*e.g.* cyanide, carbon monoxide) block ATP synthesis by mitochondria.
- But the converse is also true: inhibition of ATP synthesis blocks electron transport .

- This is because there is obligate coupling between electron transport and ATP synthesis.
- If ATP synthesis is blocked, a proton gradient is established until the cost in free energy of pumping protons out is no longer less than the energy obtained from electron transfer.
- The idea that electron transport exists solely to generate a proton gradient for ATP synthesis can be tested by artificially inducing a proton gradient in the absence of electron transfer .

Blocking electron transfer blocks ATP synthesis

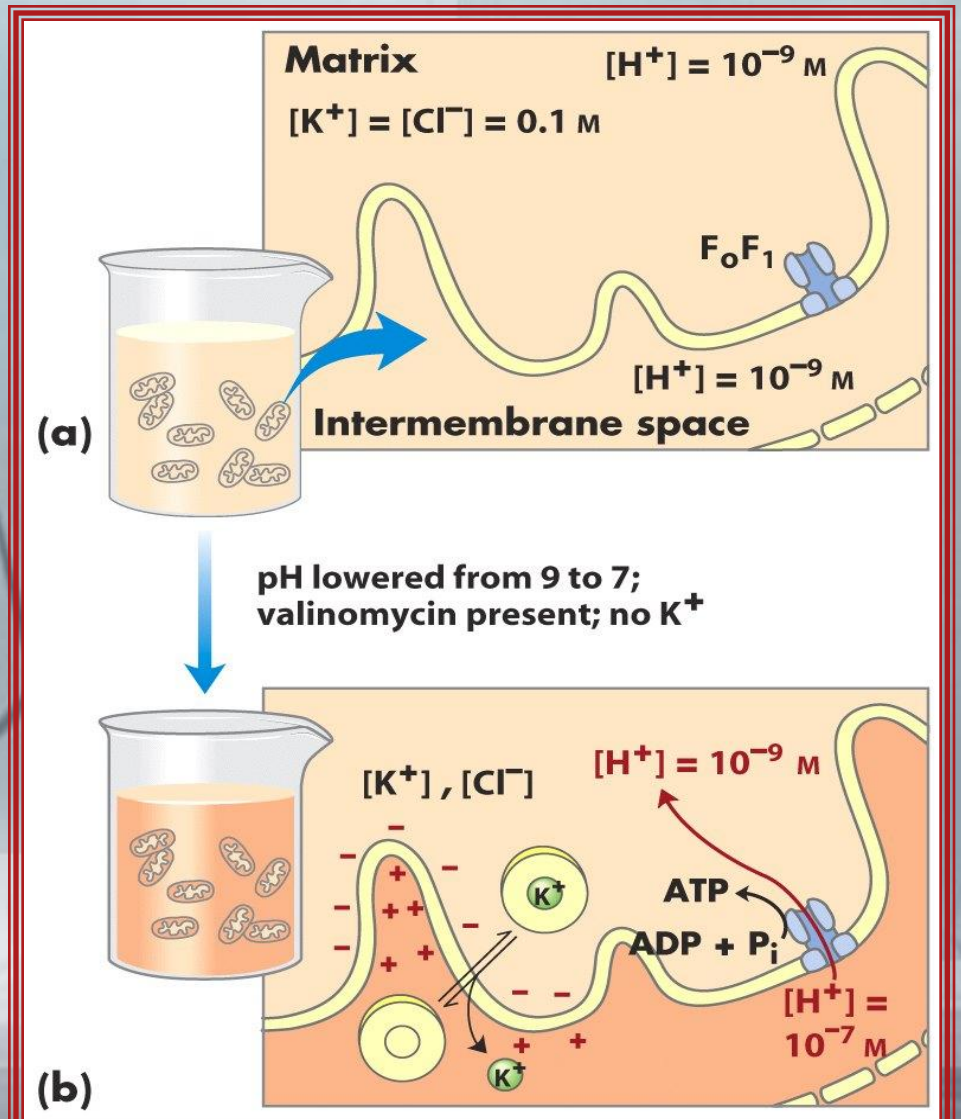


And blocking ATP synthesis blocks electron transfer to O_2

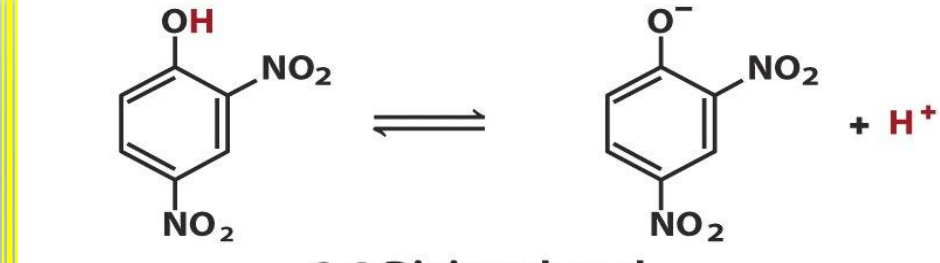


- Such experiments yield ATP, demonstrating that the electron transfer is only required because it produces a proton gradient.
- Certain chemicals can uncouple electron transfer from ATP synthesis.
- 2,4-dinitrophenol (DNP) and carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) are weak acids that are also hydrophobic.
- Because of this combination of properties they can diffuse across membranes and can also carry protons across, releasing them inside the mitochondria and dissipating the proton gradient .

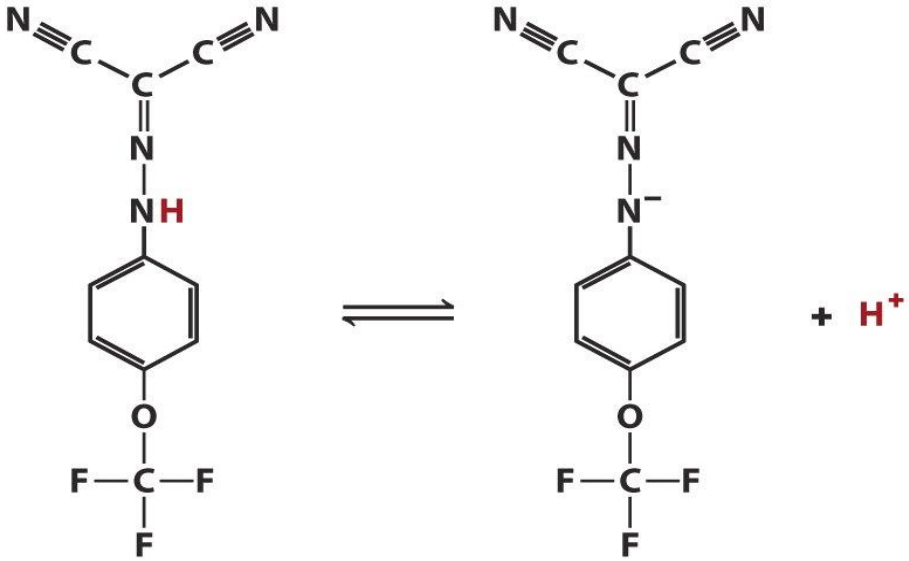
Role of the proton gradient in ATP synthesis



Chemical uncouplers of oxidative phosphorylation



2,4-Dinitrophenol
(DNP)



Carbonylcyanide-*p*-
trifluoromethoxyphenylhydrazone
(FCCP)

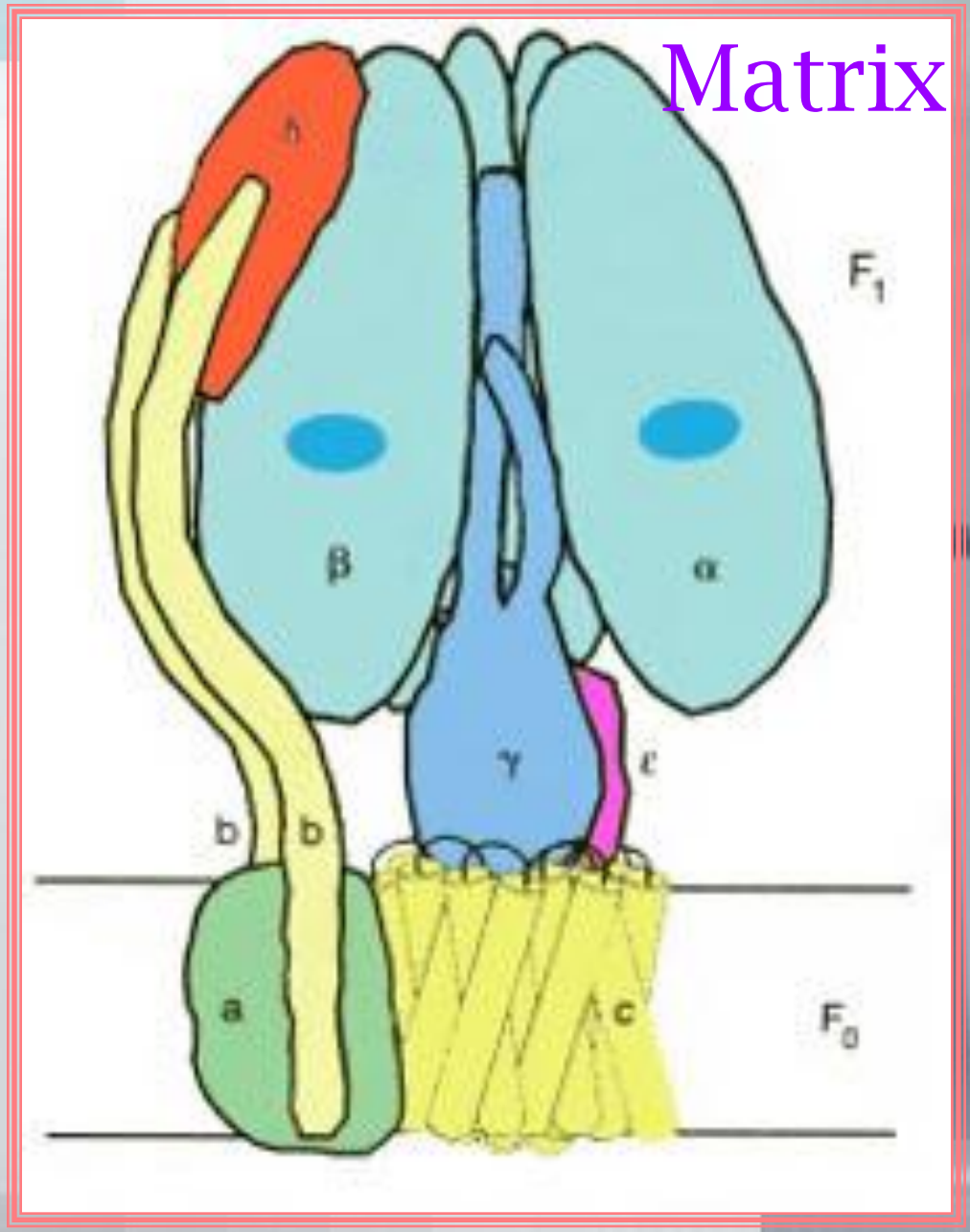
The ATP synthase enzyme

- We first encountered ATP synthase, or the FoF1 ATPase as it has been called historically.
- Now we'll examine the mechanism by which it performs active transport in reverse, allowing protons to move down their electrochemical gradient into the mitochondria while coupling this movement to the synthesis of ATP.

- ATP synthase is composed of two domains, the Fo domain and the F1 domain. (The letter ‘o’ in the Fo stands for oligomycin-sensitive).
- The Fo domain is the ion channel and the F1 domain is the ATPase domain.
- These domains are, of course, closely linked together so that proton movement can be coupled to ATP synthesis.

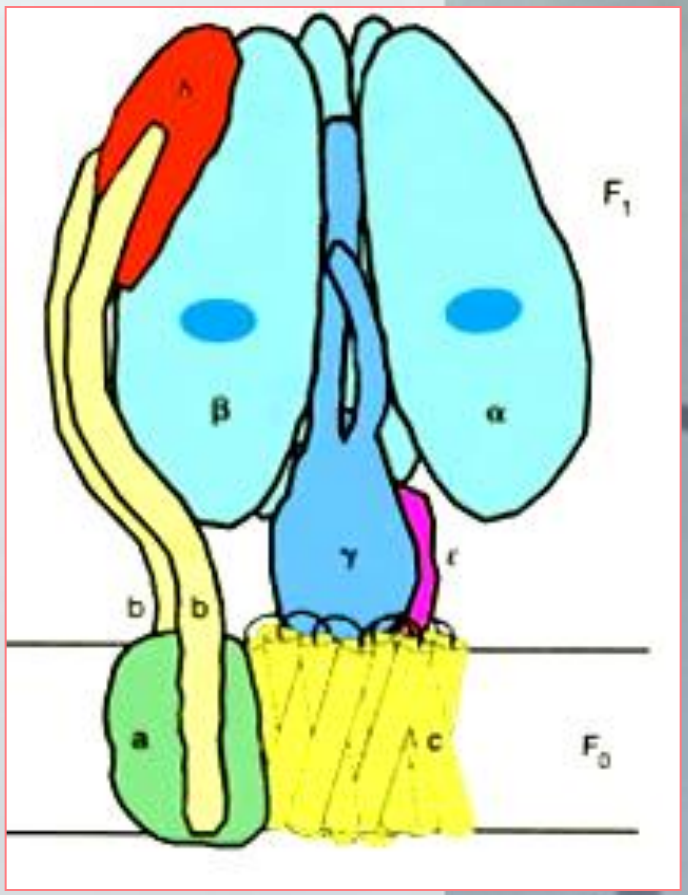
ATP Synthetic Machinery

= F_oF_1 ATP synthase Complex
-- in inner mitochondrial membrane

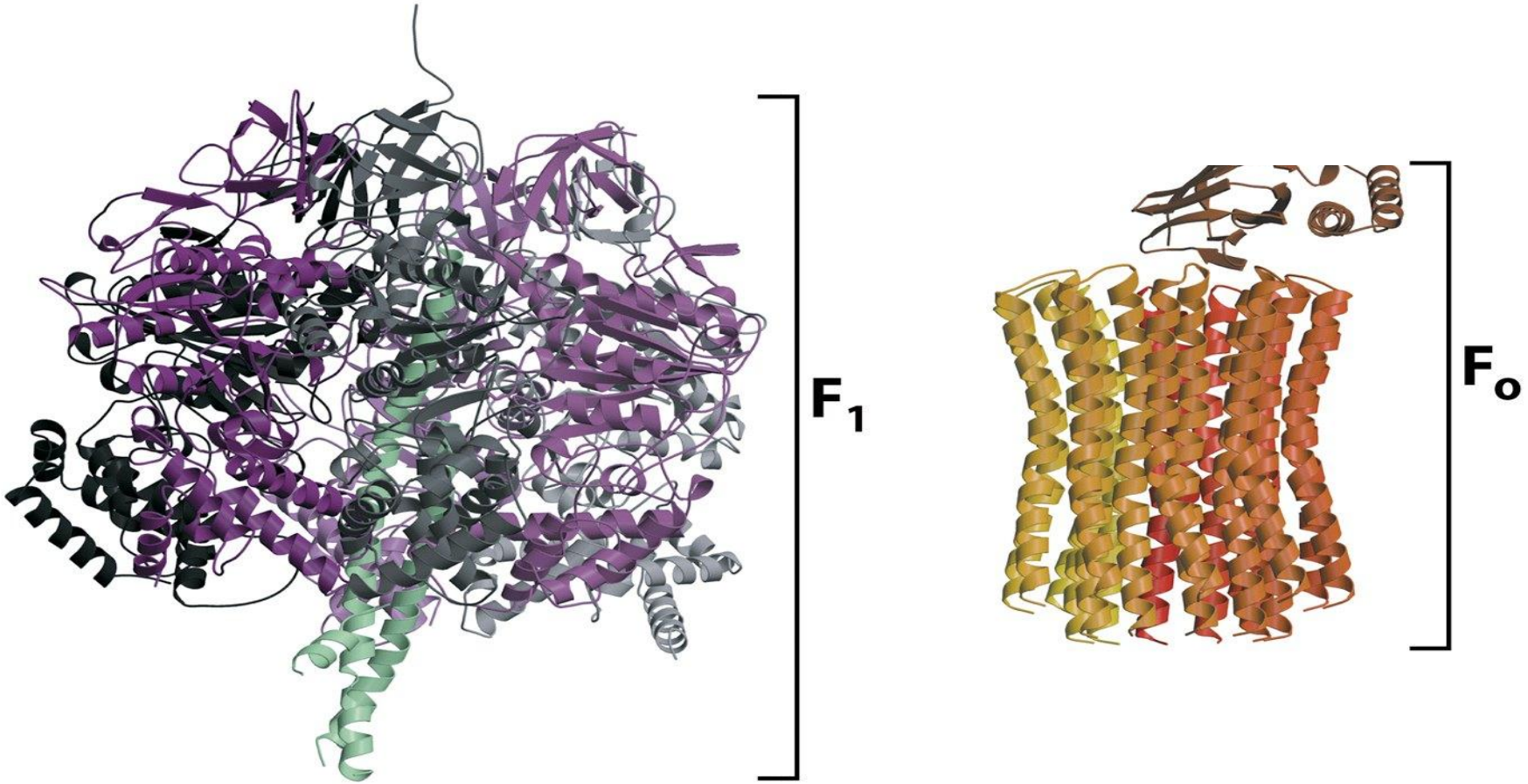


-- knob-like projections on the matrix side called F_1 spheres.

-- responsible for ATP production since when removed by trypsin treatment, the resulting membranes still transport electrons but do not make ATP.



Structures of the F_o and F_1 domains

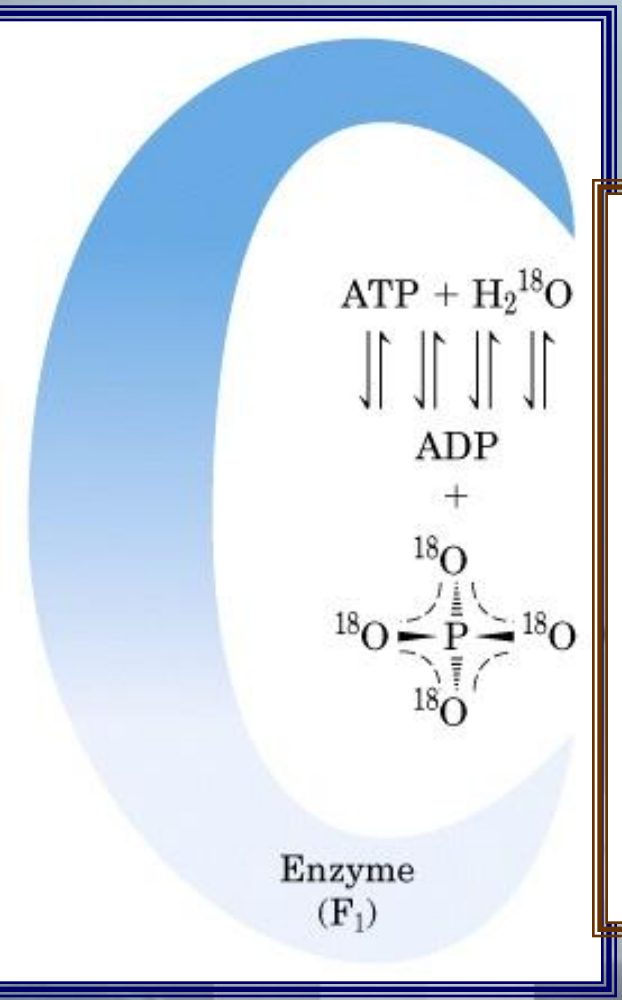


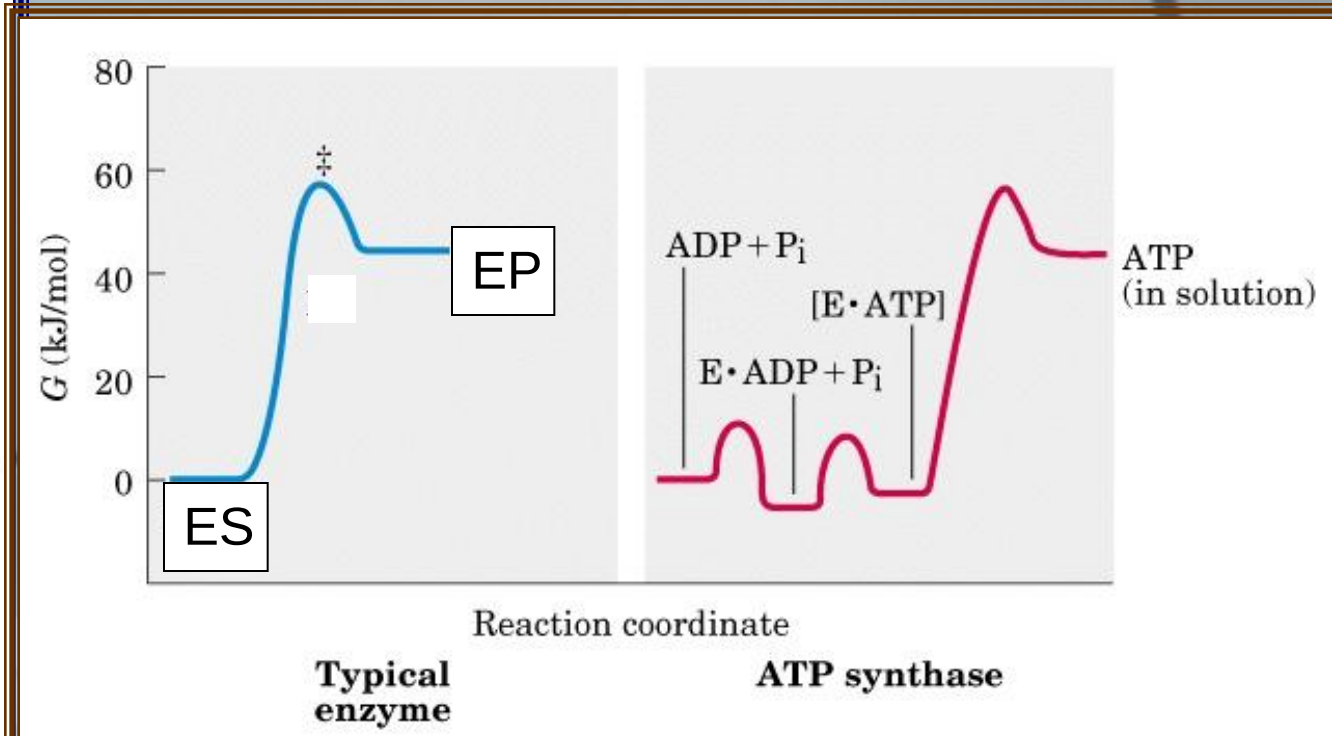
a, b, and c subunits
only c subunits shown here

The Fo domain

- Contains three subunits, a, b, and c in proportion ab2c10-12.
- The Fo domain forms a channel though the membrane that regulates the passage of protons.
- The b subunits form a stalk that holds the F1 ATPase relative to the b domain and the membrane.

Hydrolysis Reaction is Energetically Neutral on F_0 ATPase



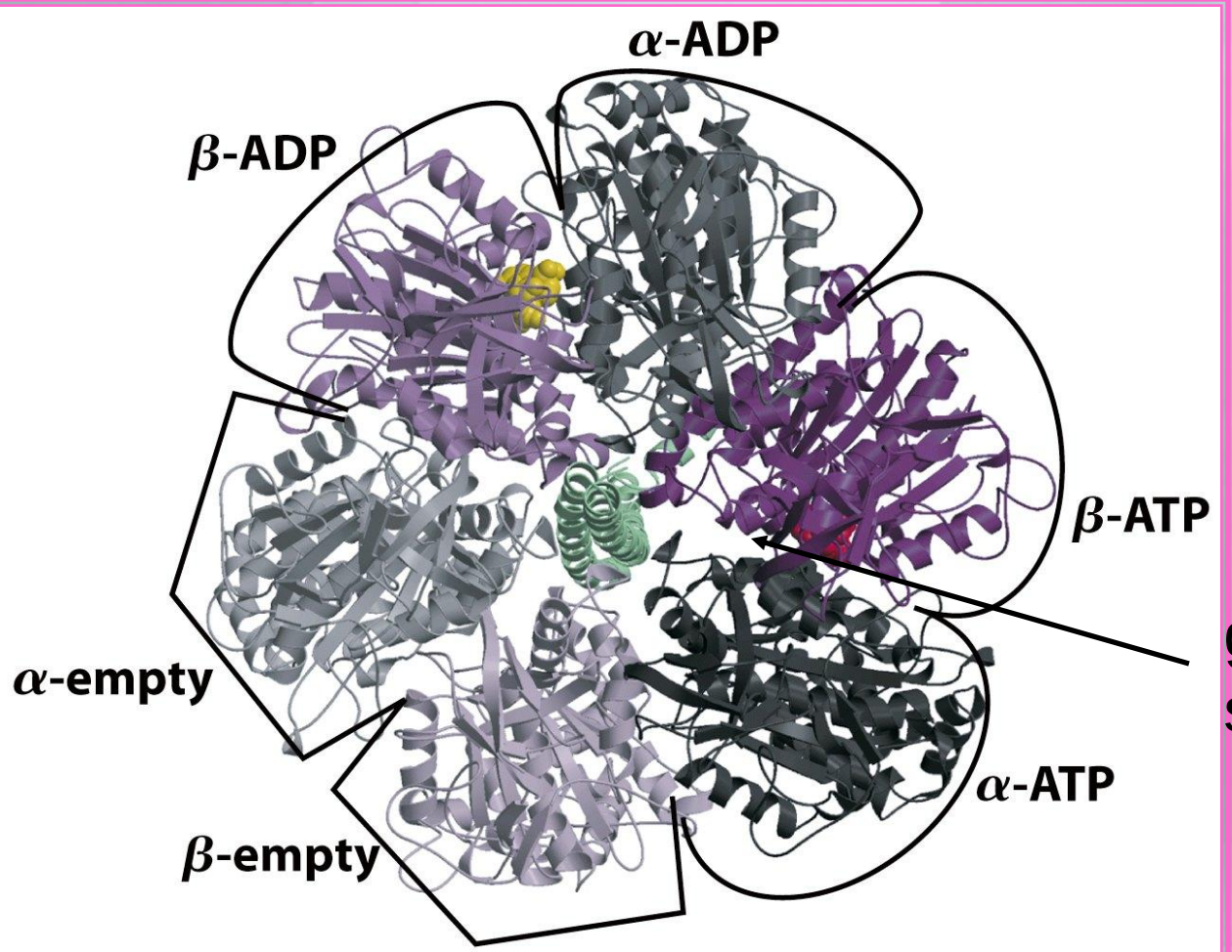


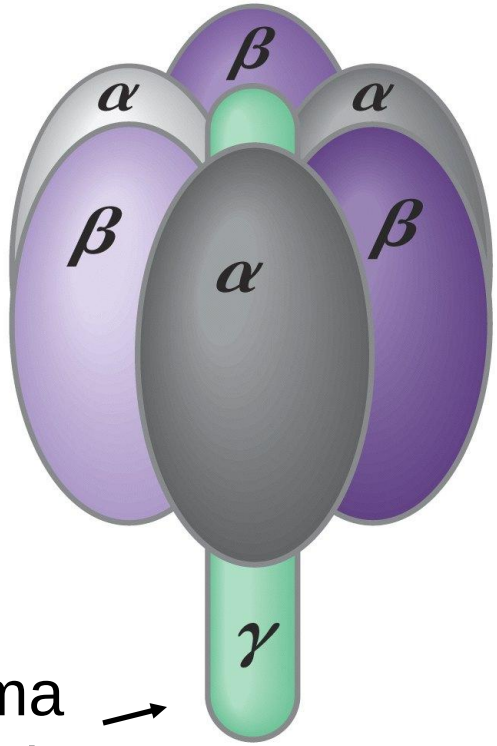
The F1 ATPase domain

- Consists of nine subunits of five different types: a3b3gse.
- Each of the b sites subunits has an active site for ATP synthesis.
- The recent crystal structure showed that each of the b subunits is in a different conformation, and these differences affect their nucleotide binding sites.
- These different conformations differ in their affinities for ATP and ADP, and these differences are critical to the mechanism of ATP synthesis.

- In at least one of the conformations, the reaction ATP ←→ ADP + Pi is readily reversible.
- In fact, it has a free energy change near zero, oscillating back and forth.
- (Remember, although enzymes cannot change the equilibrium between species *free in solution*, the same prohibition does not apply when considering the equilibrium in an enzyme's active site).

Subunits of the F_1 ATPase Domain





gamma subunit

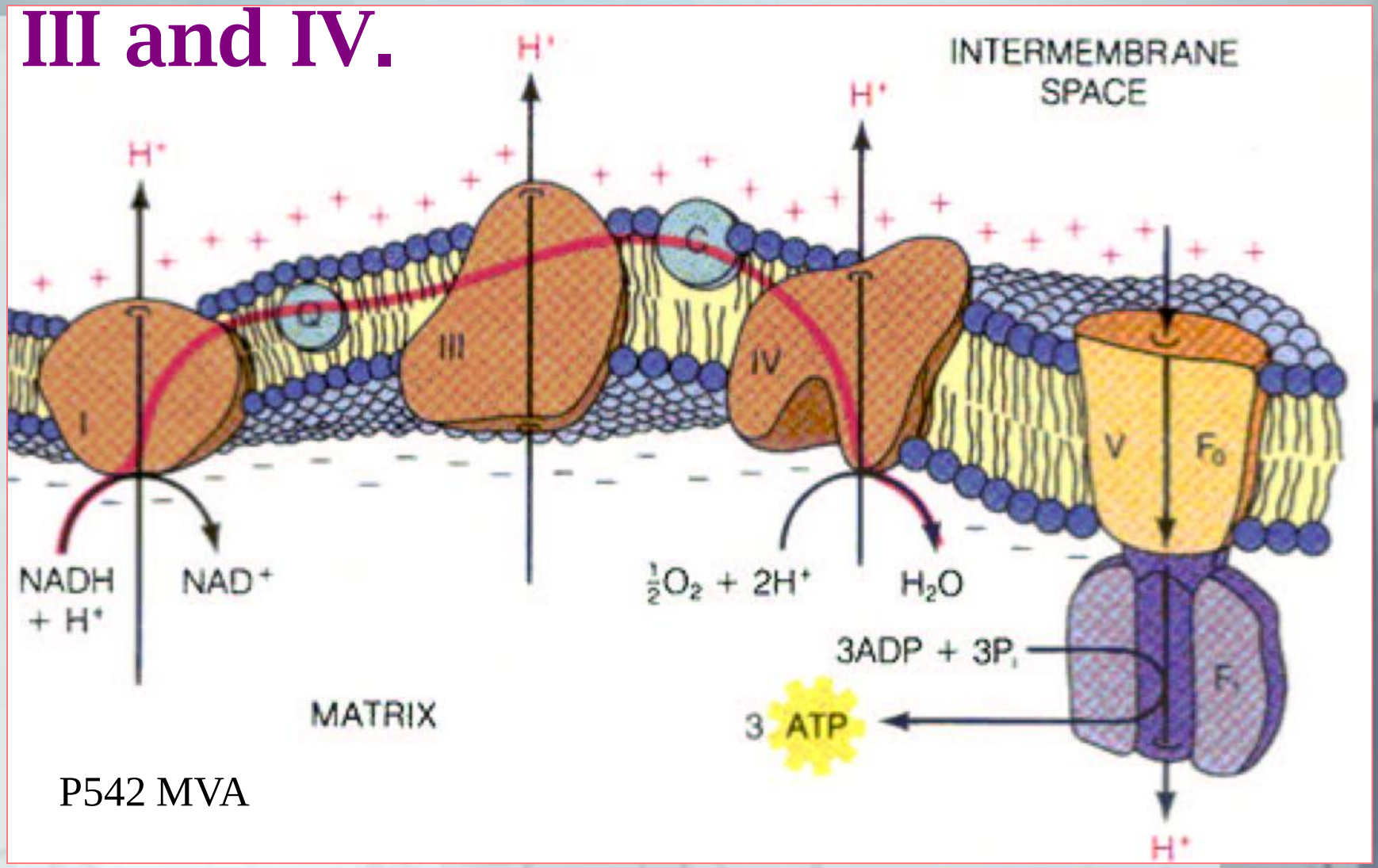
Mechanism of ATP synthesis

- Although the exact mechanism is not yet known, features of it are now clear.
- When protons pass through the Fo domain, it rotates within the membrane.
- The b subunits of the F1 domain does not rotate because they are held in place by the b stalk of the Fo domain.
- However, the g subunit, which protrudes between the b subunits, does rotate along with the Fo domain.
- The rotation of the g subunit causes the b subunits to change between their conformational states .

- The conformations are attained in a directed order:
- the first state favors ADP + Pi, then the conformation that favors ATP, and then the conformation that binds nucleotides weakly.
- Thus, ADP and Pi bind in the first step, they are converted to ATP in the second step, and are released as ATP in the third step.

- A critical feature is that the rotation only occurs one way! Proton passage through the Fo channel dictates that the g subunit rotate in the way that changes the b subunits through their conformational states in the right order for ATP synthesis.
- Because protons are flowing down their gradient, this rotational movement can be maintained.

The proton pumps are *Complexes* I, III and IV.

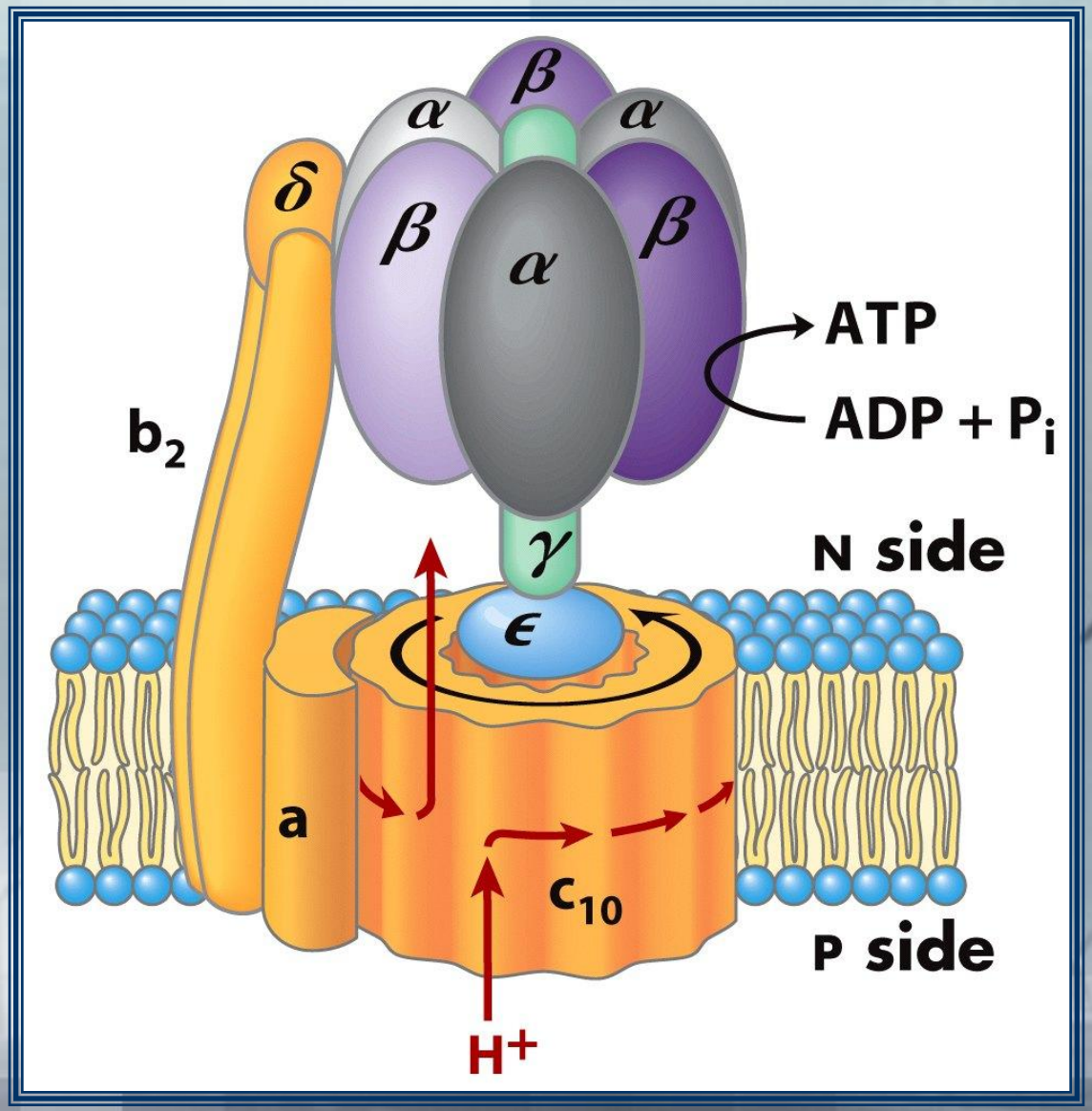


P542 MVA

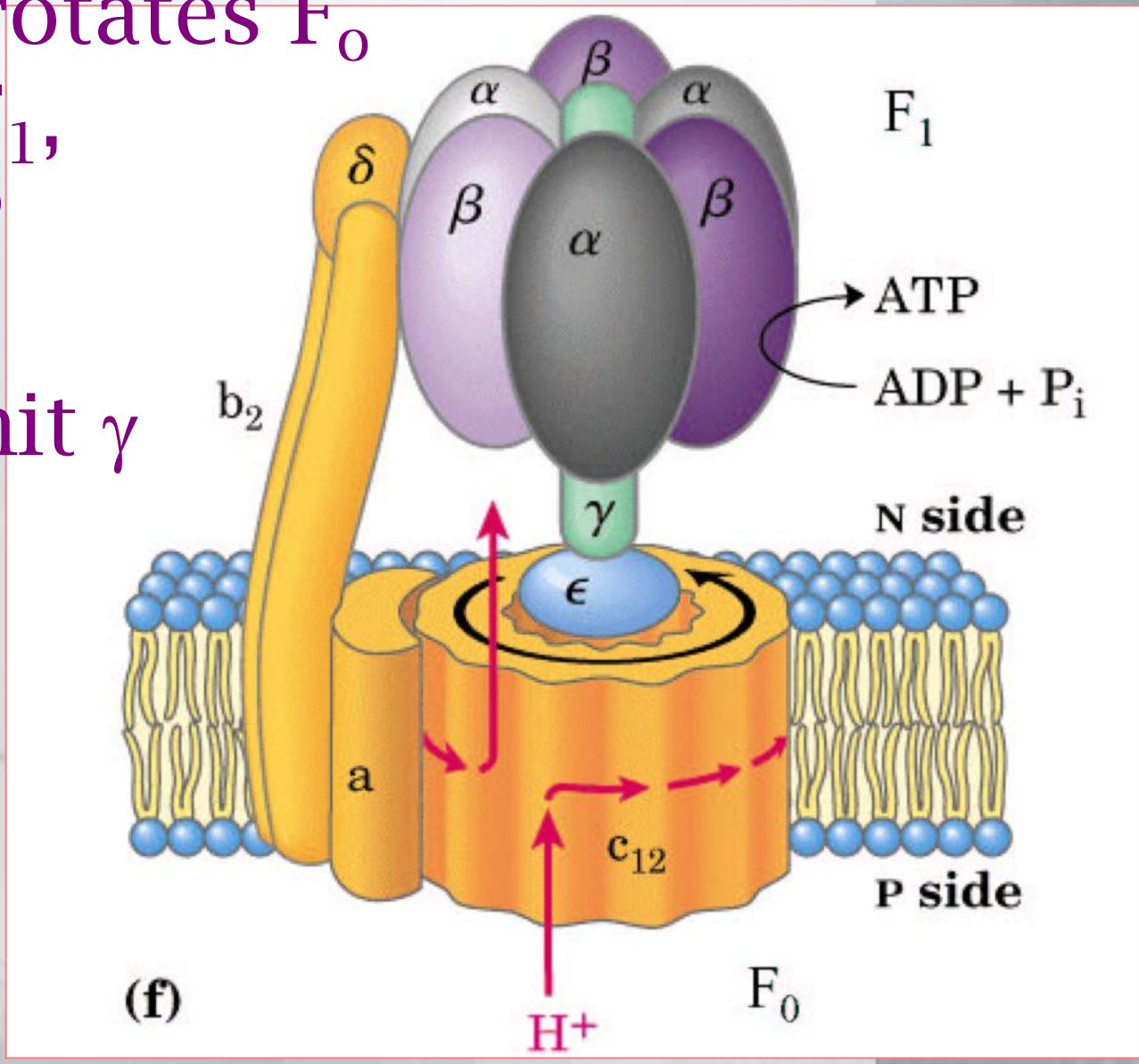
Protons return thru ATP synthase

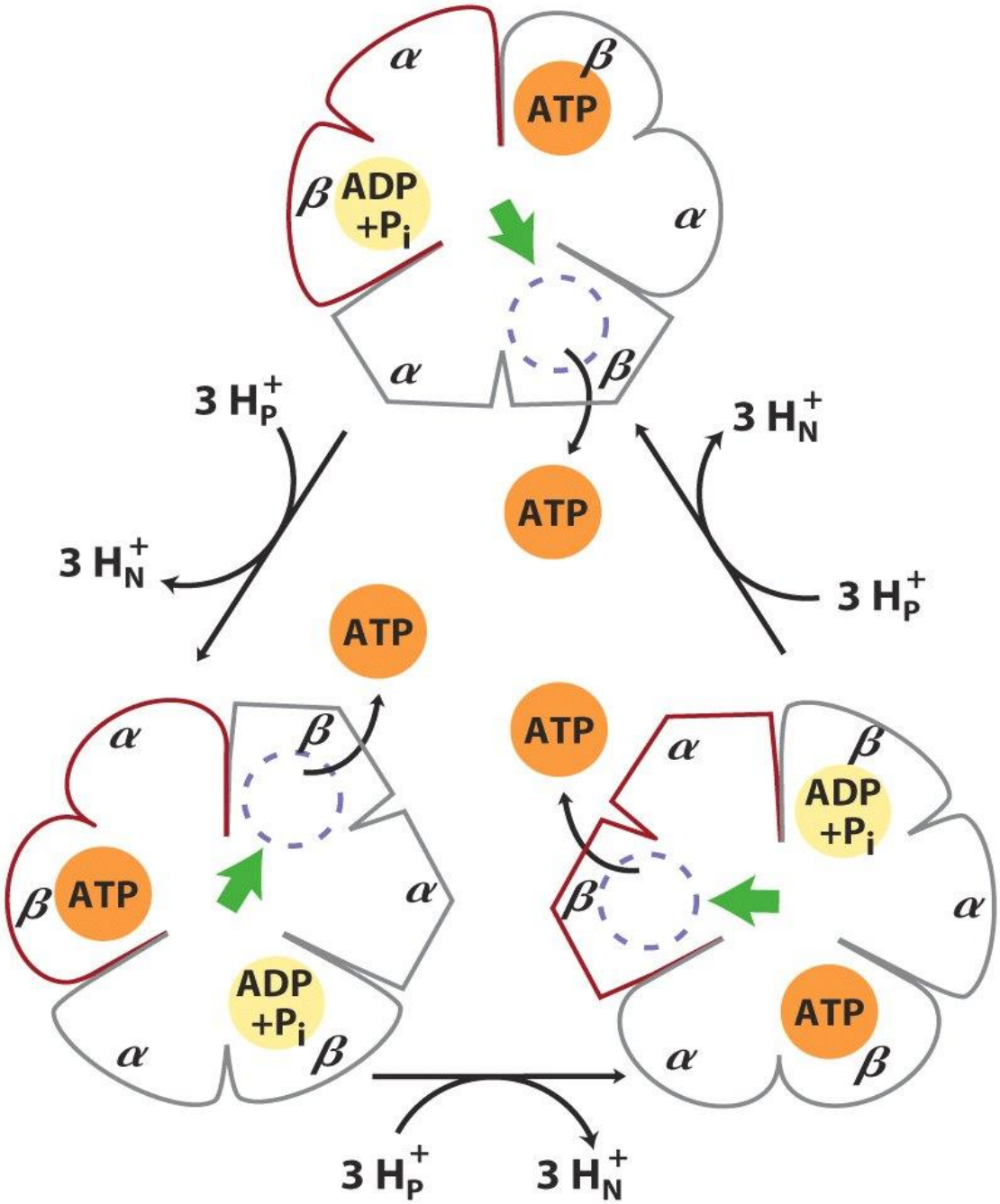
- If it were not for the protons flowing down their gradient, the rotation could operate in reverse and the F1 domain would hydrolyze ATP instead of synthesizing it (as it does if the F1 domain is removed from the Fo domain.
- The rotation of the g subunit has been verified in single molecule fluorescence experiments in which an actin filament was attached to the subunit and directly observed to rotate.

A Rotational Enzyme

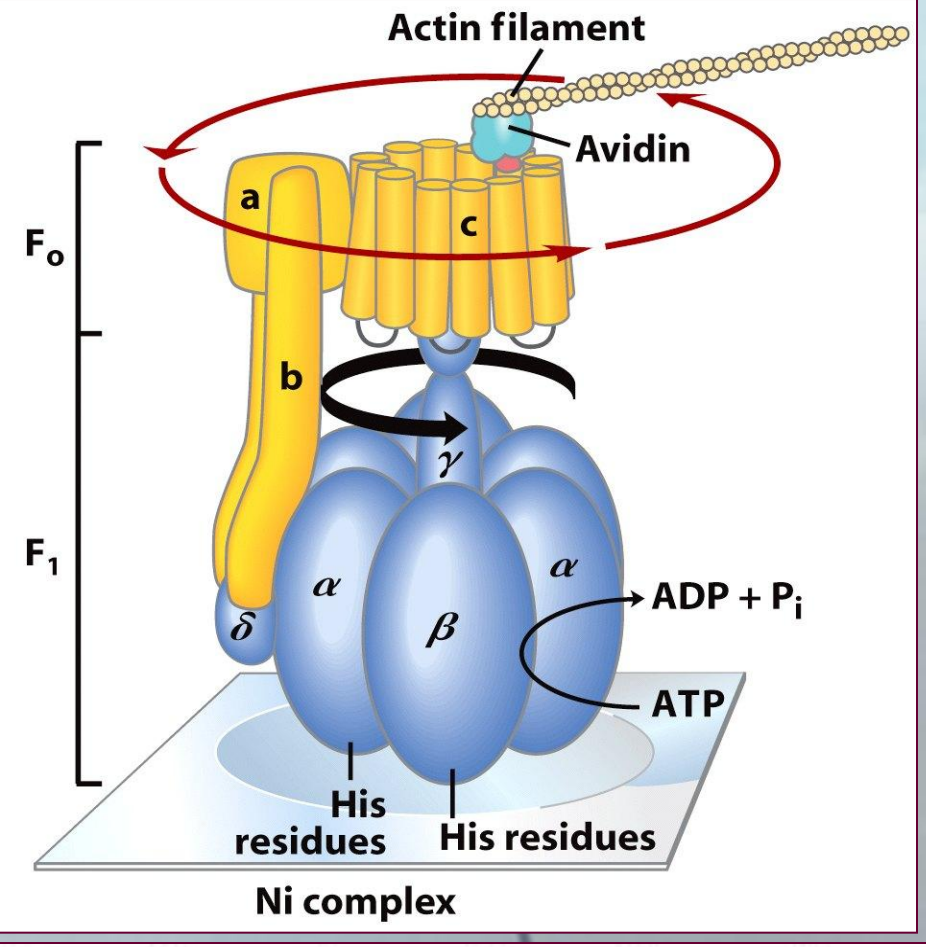


The return of protons "downhill" through F_o rotates F_o relative to F_1, driving ATP synthesis.
Note: Subunit γ rotates through F_1.

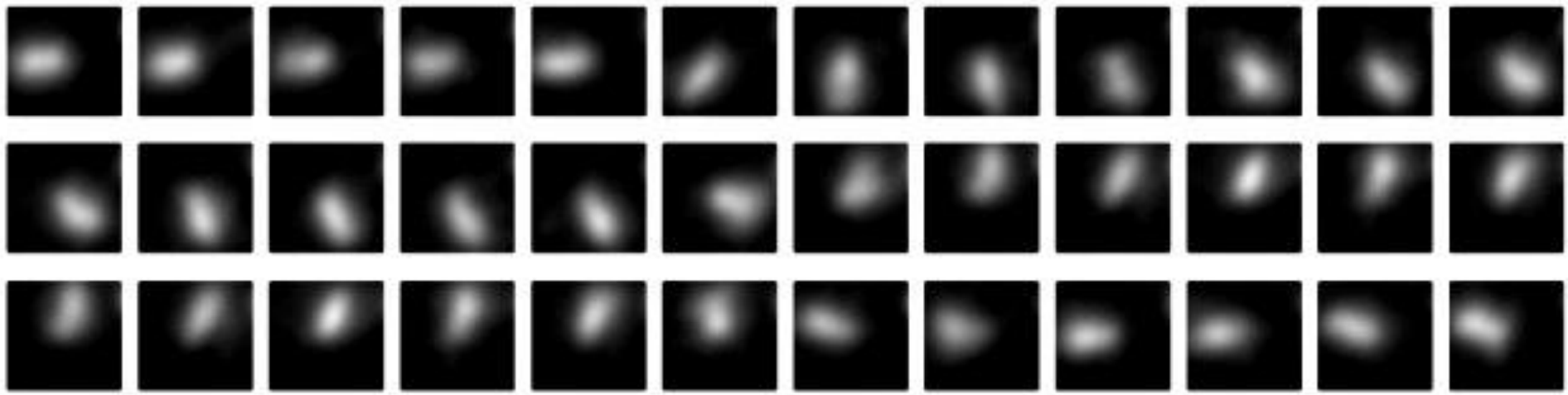




Changes In Nucleotide Affinity Are Linked to Rotation



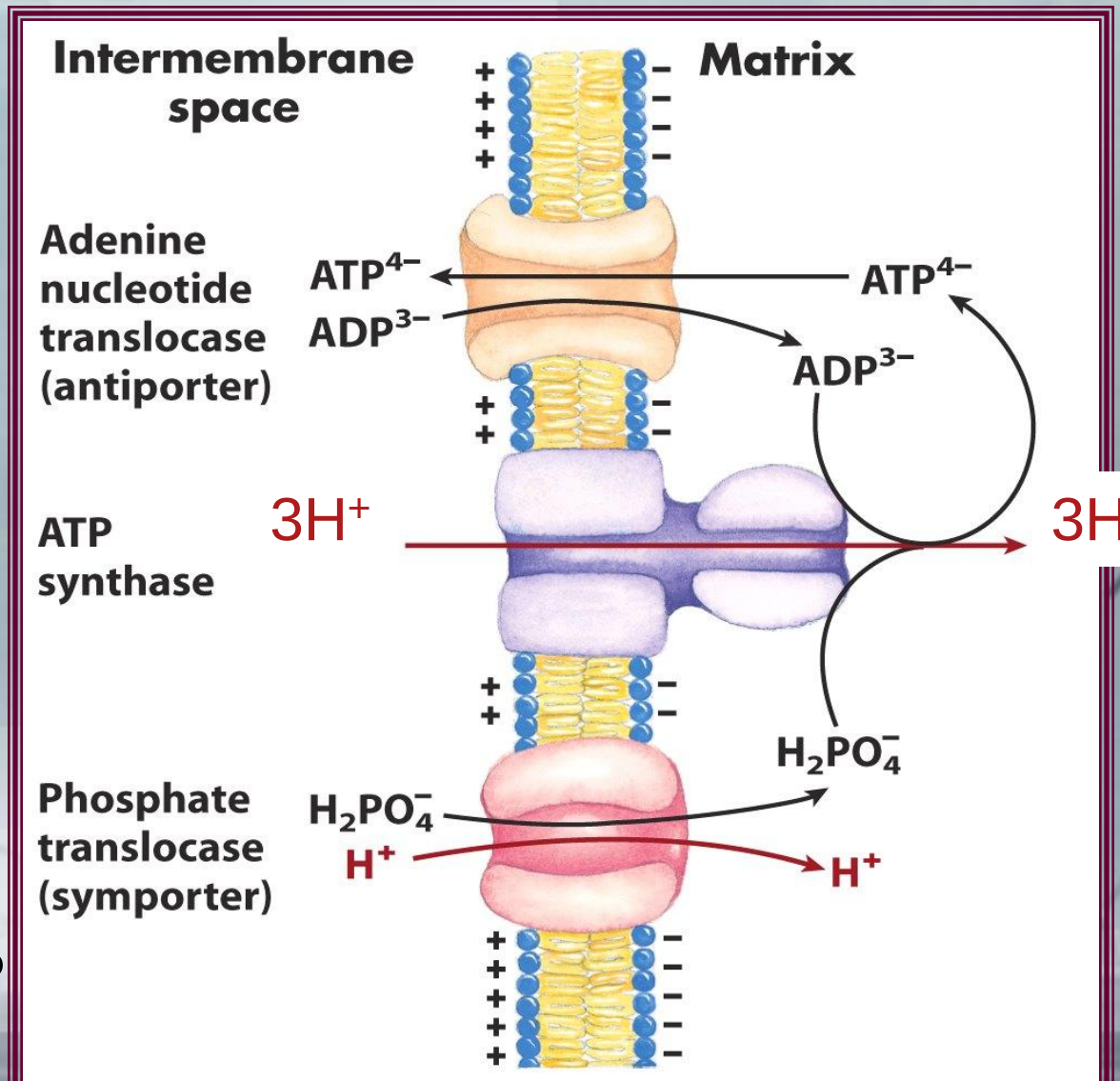
Single Molecule Demonstration of Rotation



Stoichiometry of oxygen consumption and ATP synthesis

- Before the chemiosmotic model for oxidative phosphorylation, it was thought that there would be an integral number of ATP molecules synthesized for each transfer of a pair of electrons from NADH.
- However, with the hypothesis of the proton gradient this assumption no longer held: each electron could give some number of protons pumped, and synthesis of ATP could require any number of protons flowing back in.

Phosphate Transport Is Coupled to Proton Gradient



About 4 H^+ per ATP

- Now the consensus values are: ~10 protons pumped out per pair of electrons, and about 4 protons required per ATP.
- (Actually only 3 are thought to be required to flow through Fo for synthesis of an ATP, one for each conformational change, but a 4th is thought to be required for import of ATP and Pi into the mitochondria and ATP out.) .
- These values make the ratio 2.5 ATPs synthesized for each pair of electrons from NADH.

TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	*Direct product*	*Final ATP*
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

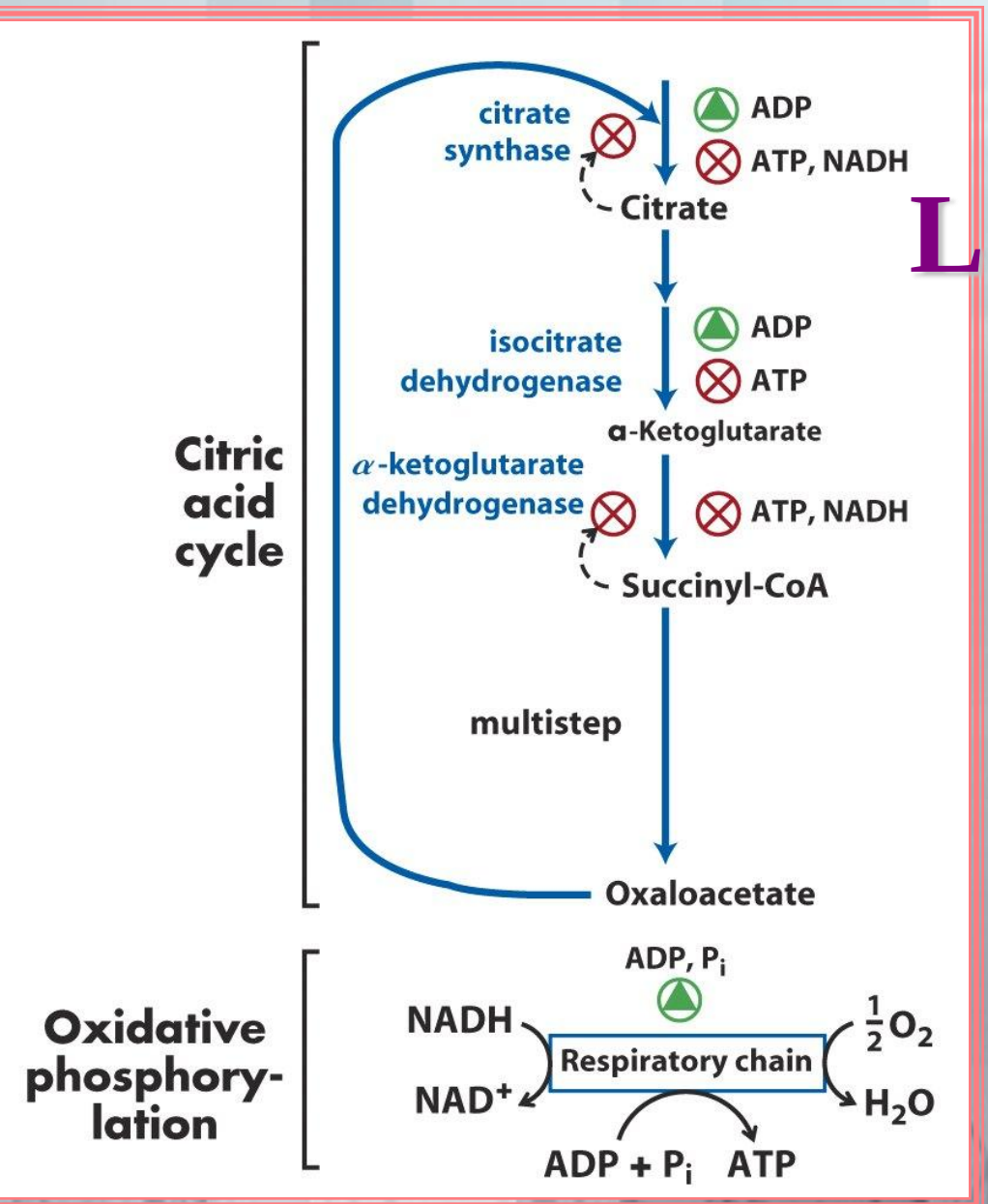
*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

Regulation of oxidative phosphorylation

- Complete oxidation of a glucose molecule yields 30-32 molecules of ATP .
- Glyolysis only yields 2! Aerobic metabolism accounts for the vast majority of ATP molecules synthesized and therefore the regulation of ATP synthesis by oxidative phosphorylation is critical.
- Rate of respiration is generally limited by the availability of ADP.
- In some animal tissues the change in respiration rate due to changes in ADP concentration can be as much as a factor of 10.

- Another parameter that affects the respiration rate and provides feedback on the energy status of cells is the mass-action ratio of the ATP-ADP system: [ATP]/([ADP][Pi]).
- Normally the concentration of ATP is much larger than that of ADP; nucleotides are almost fully phosphorylated.
- When this ratio drops, respiration increases until the ratio returns to its normal value.
- ATP and ADP levels do not only regulate oxidative phosphorylation.
- As we have seen, they are involved in regulation of carbohydrate metabolism all the way through glycolysis and the citric acid cycle also.

ATP and ADP Levels Are Critical For Regulation

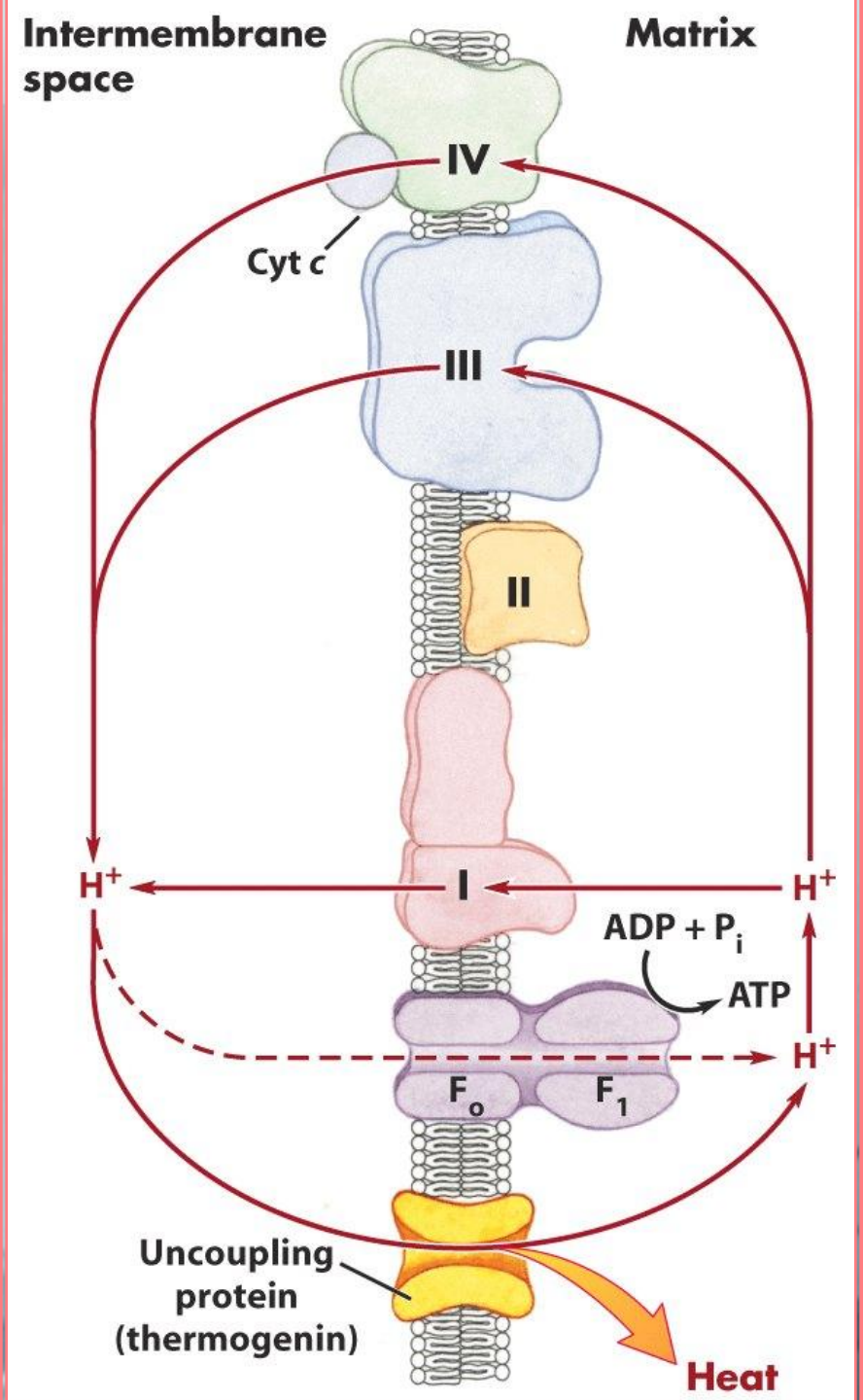


Catabolism is regulated at multiple stages by ATP and ADP concentrations

Uncoupled mitochondria in brown fat

- Newborn mammals have a type of tissue called brown fat, which oxidizes fuel to produce heat instead of ATP.
- The mitochondria in these cells have a protein called thermogenin, which provides a path for protons to return to the matrix without passing through the FoF1 ATPase.

- Thus, energy of oxidation is not conserved by ATP formation but is instead dissipated as heat.
- Tissue is called brown fat because it has a high concentration of mitochondria and thus large amounts of cytochromes, which give the tissue a brown color.
- Hibernating animals also use brown fat to generate heat during dormancy.



Brown Fat: Electron Transport Produces Heat Instead of ATP

Summary

1. Most of the ATP from oxidation of glucose is synthesized by coupling electron transport to the generation of a proton gradient. As electrons are transported to oxygen, protons are pumped out.

2. ATP synthase allows protons to re-enter the mitochondrial matrix and couples this favorable process to the unfavorable process of ATP synthesis. ATP synthesis involves rotation of part of this enzyme.

3. Oxidative metabolism is heavily regulated by concentrations of ATP, ADP, and P_i.