ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

OXIDATIVE PHOSPHORYLATION 2

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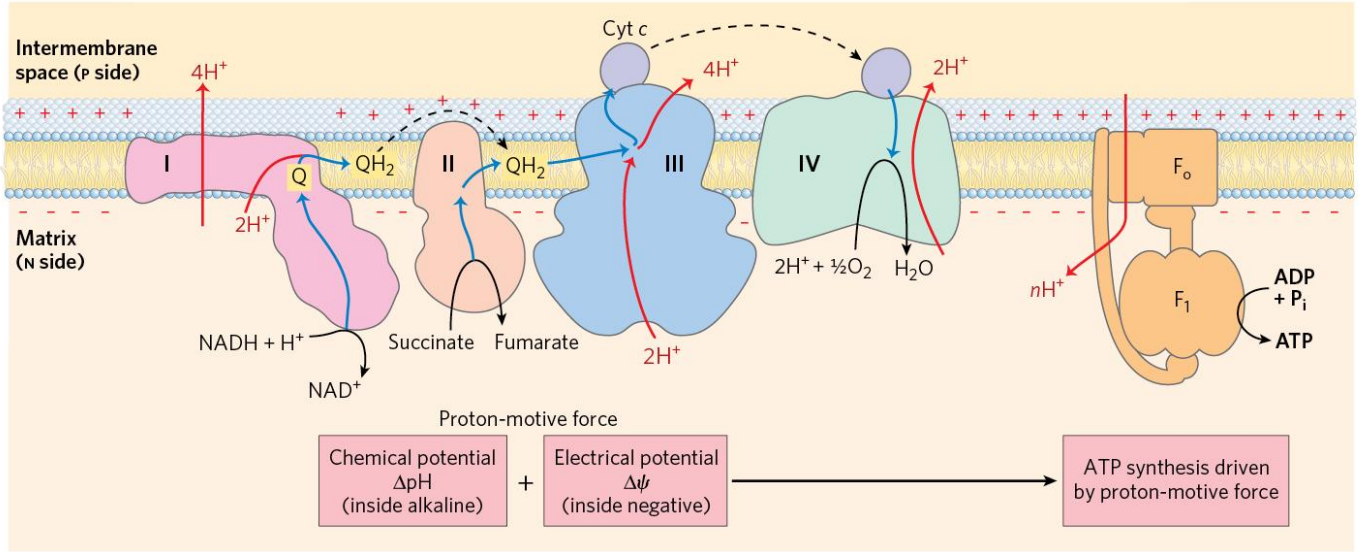
CHEMIO-OSMOTIC COUPLING

Chemiosmotic model describes the coupling of ATP synthesis to an electrochemical proton gradient (the proton-motive force).

1) The respiratory chain pumps protons from the matrix to the IMS exploiting the negative free energy change of electron transfer.

2) The proton gradient thus created is a form of energy.

3) The protons passively return to the matrix through ATP synthase.



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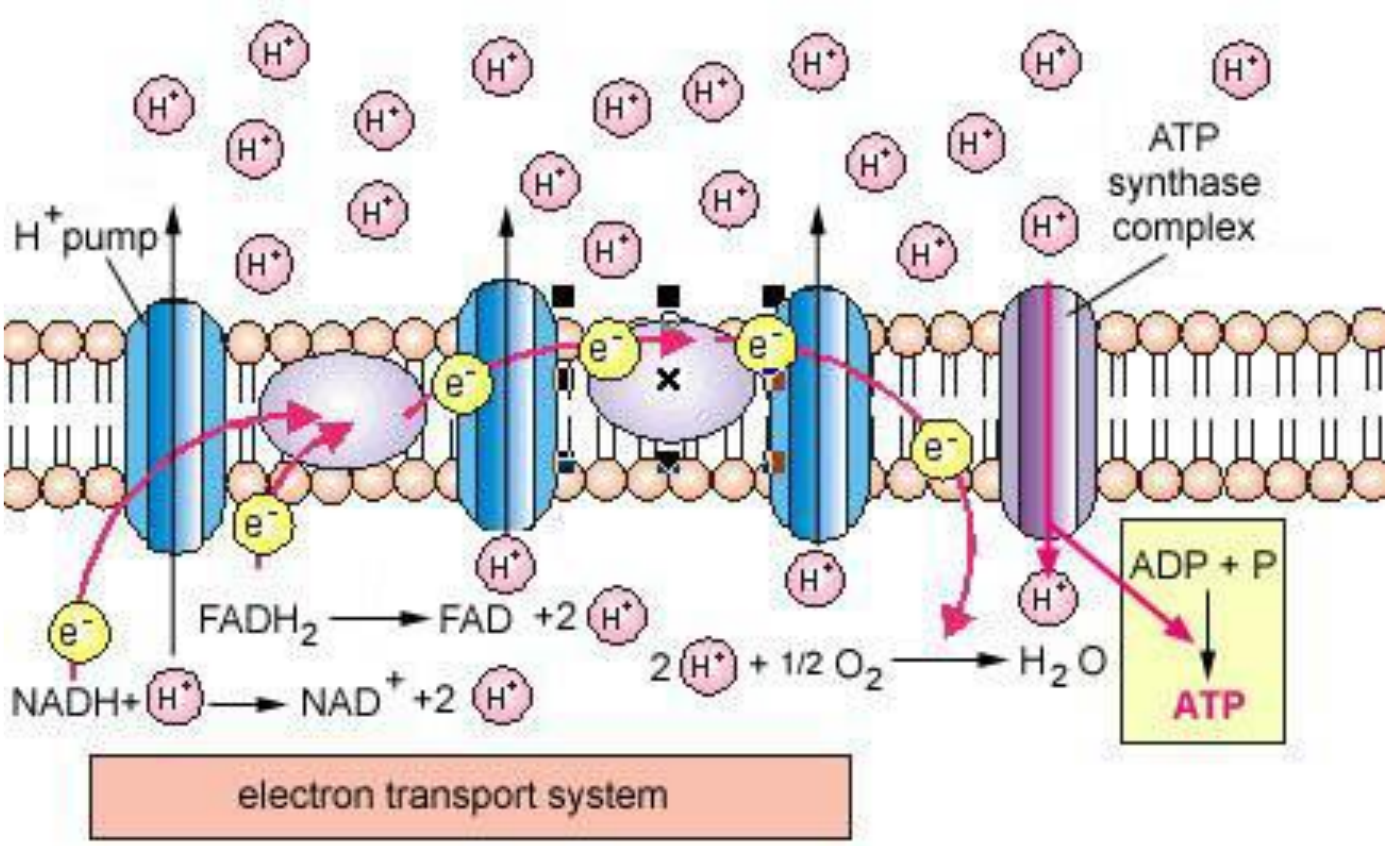
CHEMIO-OSMOTIC COUPLING

Chemio-osmotic mechanism of oxidative phosphorylation.

Equation for ATP synthesis is:

$ADP + P_i + nH^+_{out} \rightarrow ATP + H_2O + nH^+_{in}$



CHEMIO-OSMOTIC COUPLING

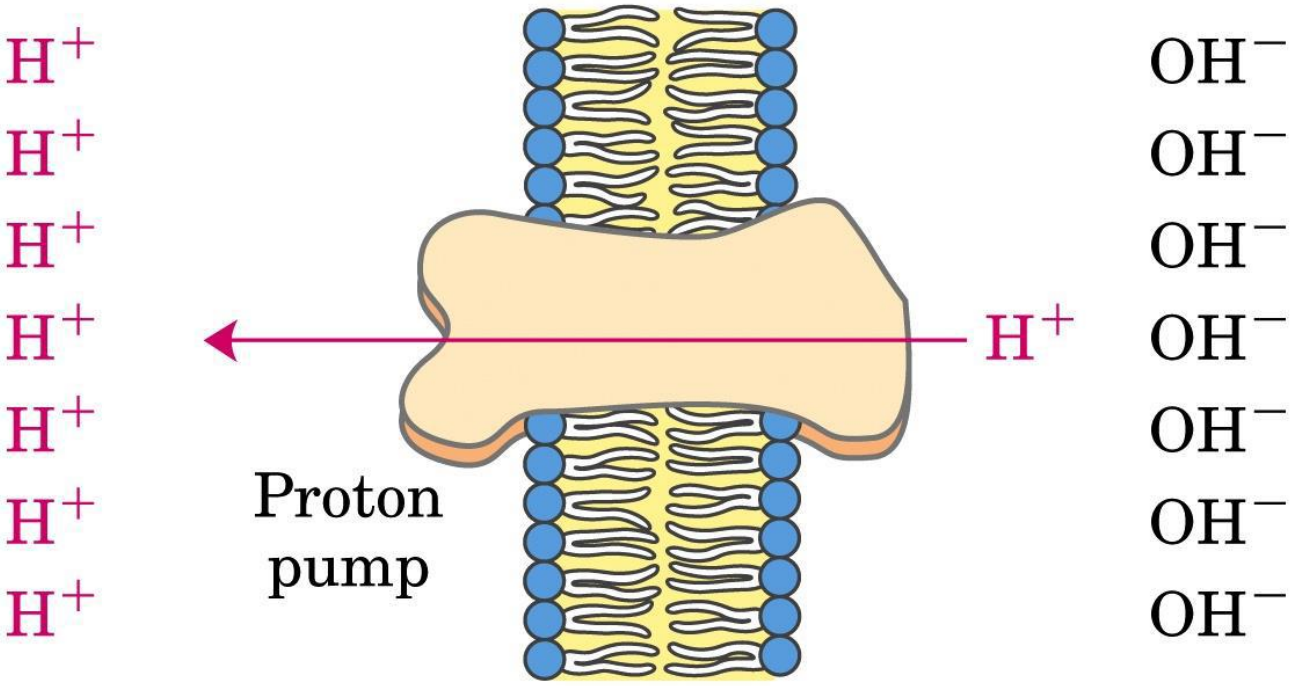
An endoergonic reaction may occur if coupled to an exoergonic reaction via a common intermediate:

- the dogma assumed that such a common intermediate *may only be a chemical species;*
- for the chemio-osmotic hypothesis the intermediate *is a physical state* (proton gradient).

CHEMIO-OSMOTIC COUPLING

P side $[H^+]_P = C_2$

N side $[H^+]_N = C_1$



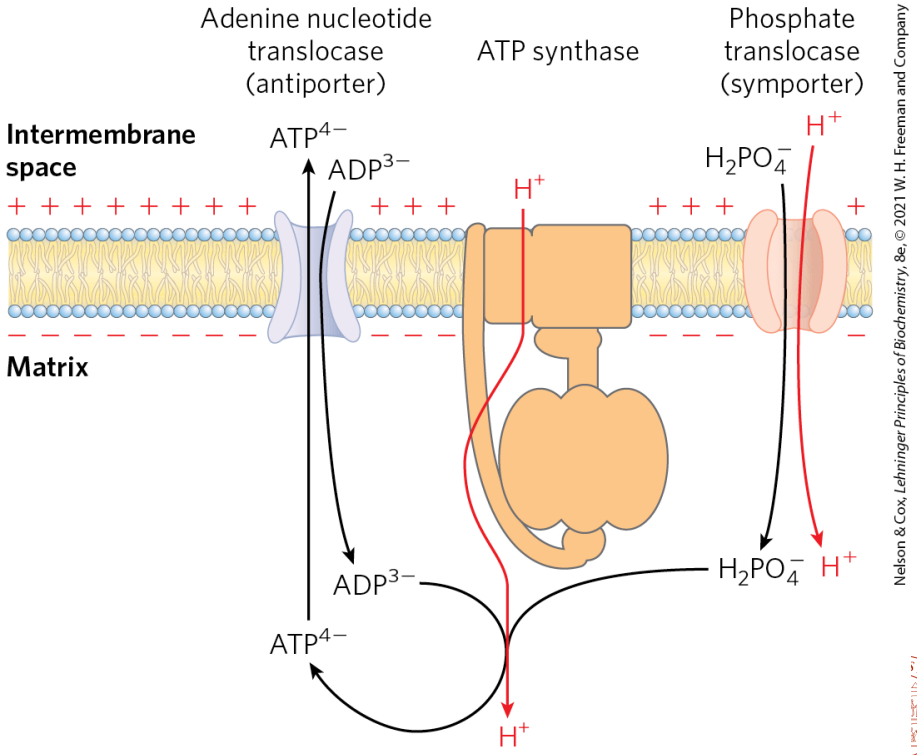
$$\Delta G = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi$$

$$= 2.3RT\,\Delta\text{pH} + \mathcal{F}\Delta\psi$$

CHEMIO-OSMOTIC COUPLING

Protons passively flow back into the matrix through the ATP synthase and the energy is used to synthesize ATP from ADP and phosphate ($3H^+$/ATP generally assumed). ADP and P_i must be recovered from the cytosol to the matrix, consuming an additional H^+.

Total: 4 H^+/ATP synthesized.



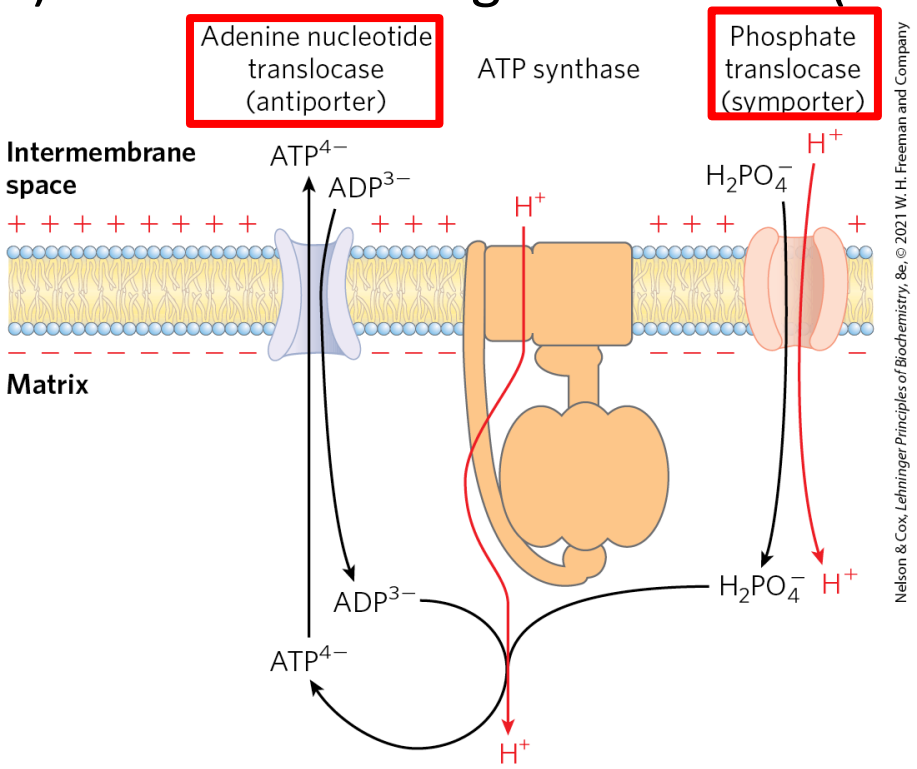
CHEMIO-OSMOTIC COUPLING

The entry of phosphate together with ATP/ADP exchange consume one H^+.

Phosphate enters as a symport with H^+; since phosphate has a negative charge, the transport is electroneutral.

ATP (4 negative charges) exits in exchange with ADP (3 negative charges).

Total: entry of one H^+



STOICHIOMETRY OF OXIDATIVE PHOSPHORYLATION

$NADH + H^+ + ½\ O_2 \rightarrow NAD^+ + H_2O$

$10\ H^+ \rightarrow$ out

$ADP + P_i \rightarrow ATP$

$4\ H^+ \rightarrow$ in

Stoichiometry

$10\ H^+ (½\ O_2)/4\ H^+$ (ADP) = 2.5 **(P/O ratio)**

Consuming ½ O_2 drives the synthesis of 2.5 ATP (or rather 1 O_2 drives the synthesis of 5 ATP)

STOICHIOMETRY OF OXIDATIVE PHOSPHORYLATION

Succinate + ½ O_2 → Fumarate + H_2O

6 H^+ → out

ADP + P_i → ATP

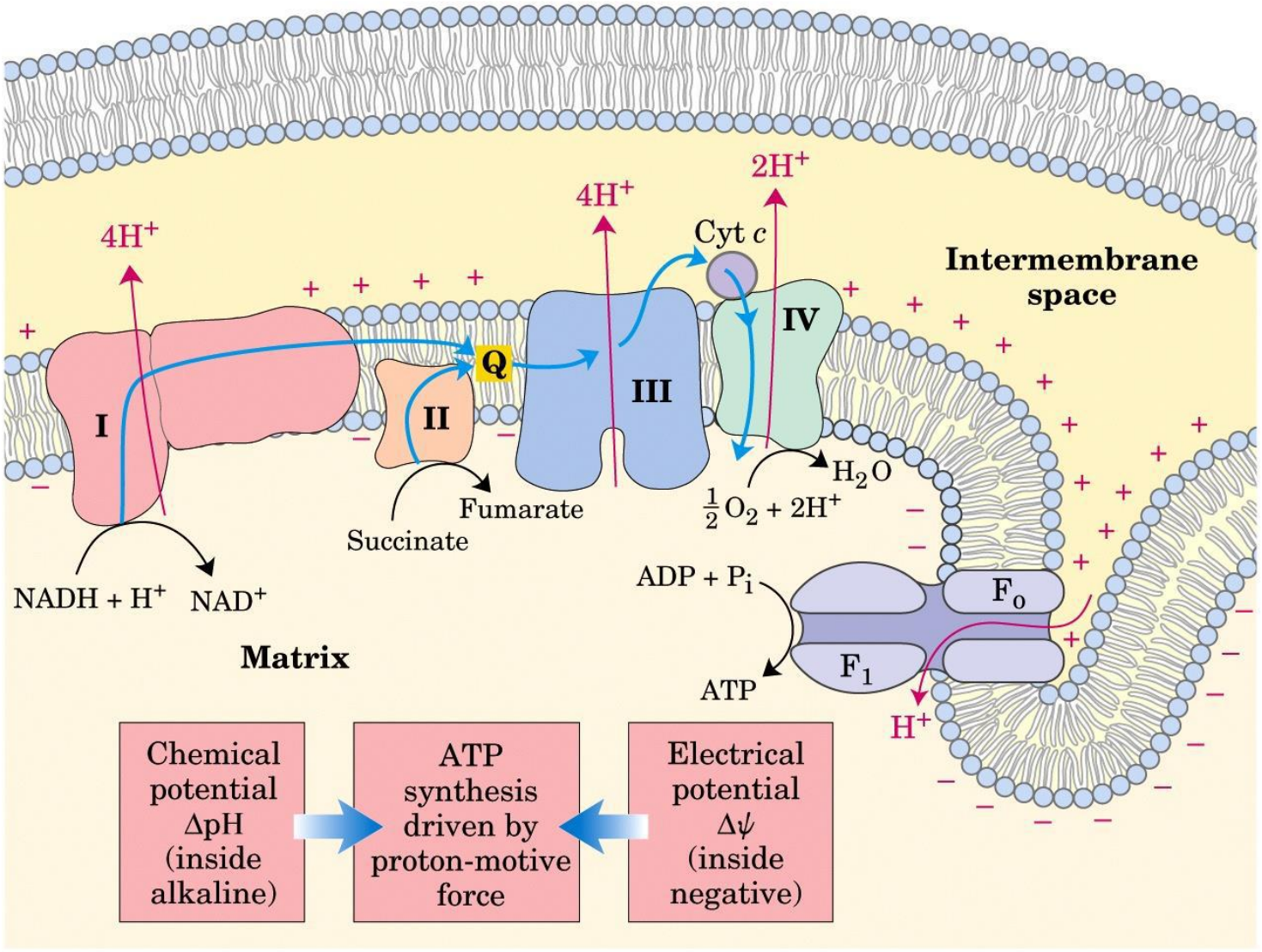
4 H^+ → in

Stoichiometry

6 H^+ (½ O_2)/4 H^+ (ADP) = 1.5 **(P/O ratio)**

Consuming ½ O_2 drives the synthesis of 1.5 ATP (or 1 O_2 drives 3 ATP).

CHEMIO-OSMOTIC COUPLING

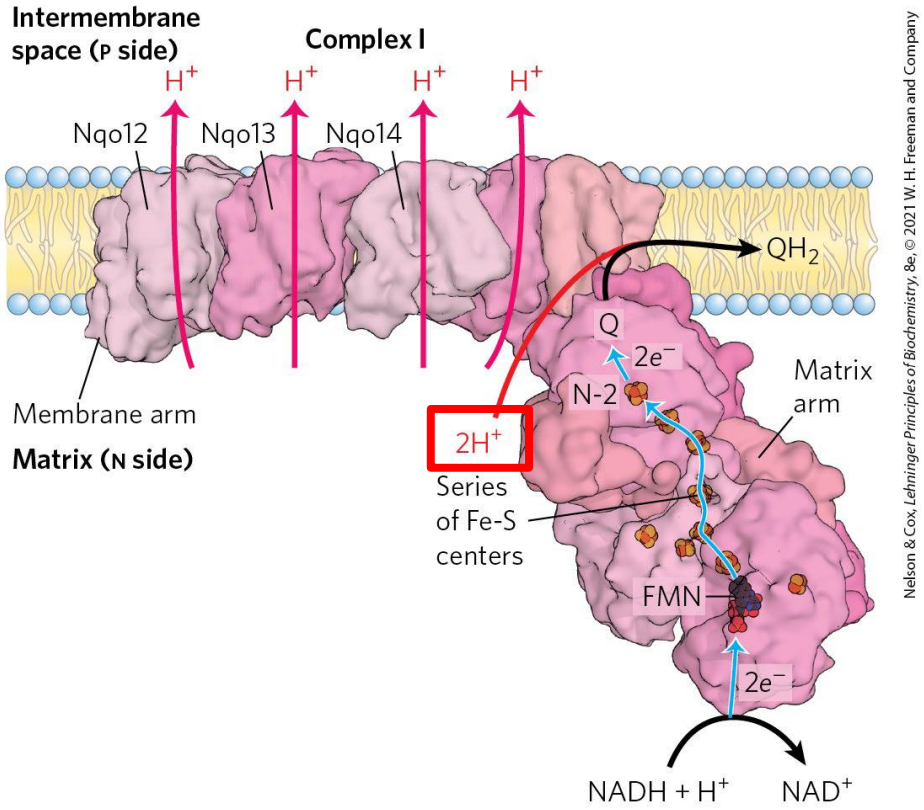


CHEMIO-OSMOTIC COUPLING

In Complex I at $FMNH_2$ → FeS there is loss of 2 H^+ in the matrix.

Does this affect the stoichiometry of H^+ pumping?

No, these are "scalar" (not "vectorial") H^+ that are recovered by reduction of CoQ to $CoQH_2$ still at the matrix side.



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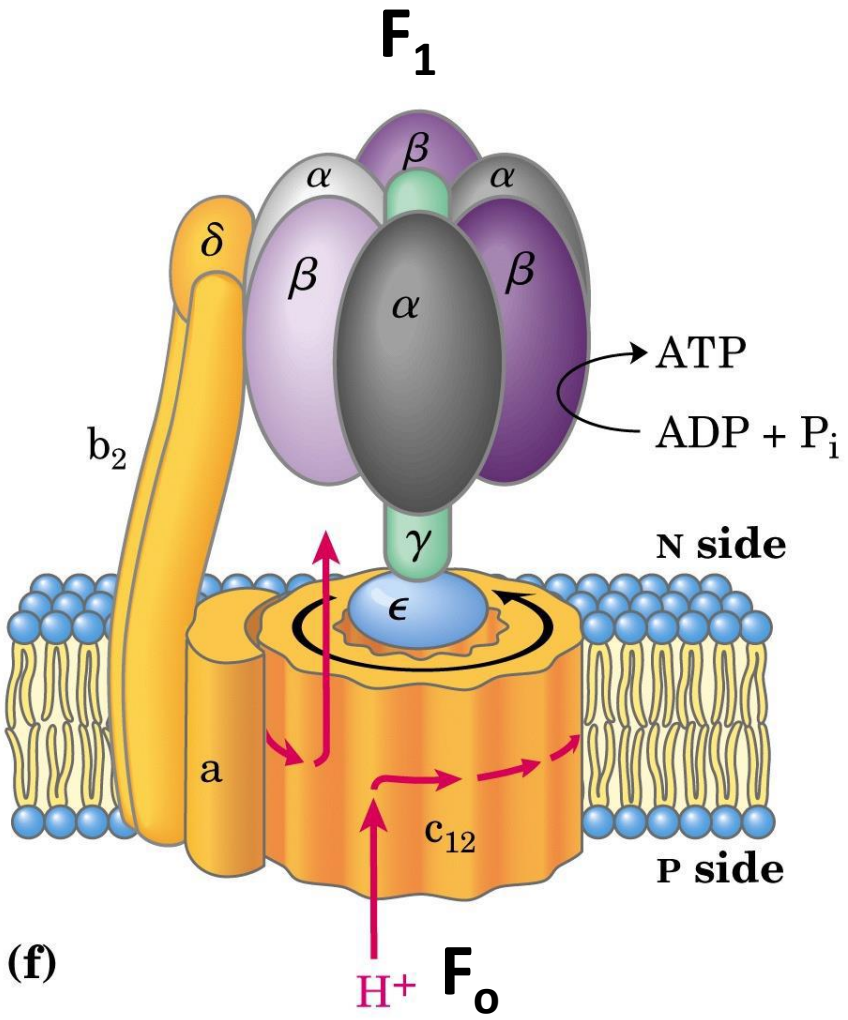
ATPase/ATP synthase

ATPase enzyme contains two distinct components:

- **F_o**: an integral membrane protein with a proton pore.
- **F_1** (originally **F_1 ATPase**): a peripheral membrane protein that catalyzes hydrolysis of ATP when isolated.



ATPase/ATP synthase

F_1: hydrophilic, protruding into the matrix.

Subunit composition: $\alpha_3\beta_3\gamma\delta\varepsilon$. Active site is in subunits β (3 active sites for ATP synthesis).

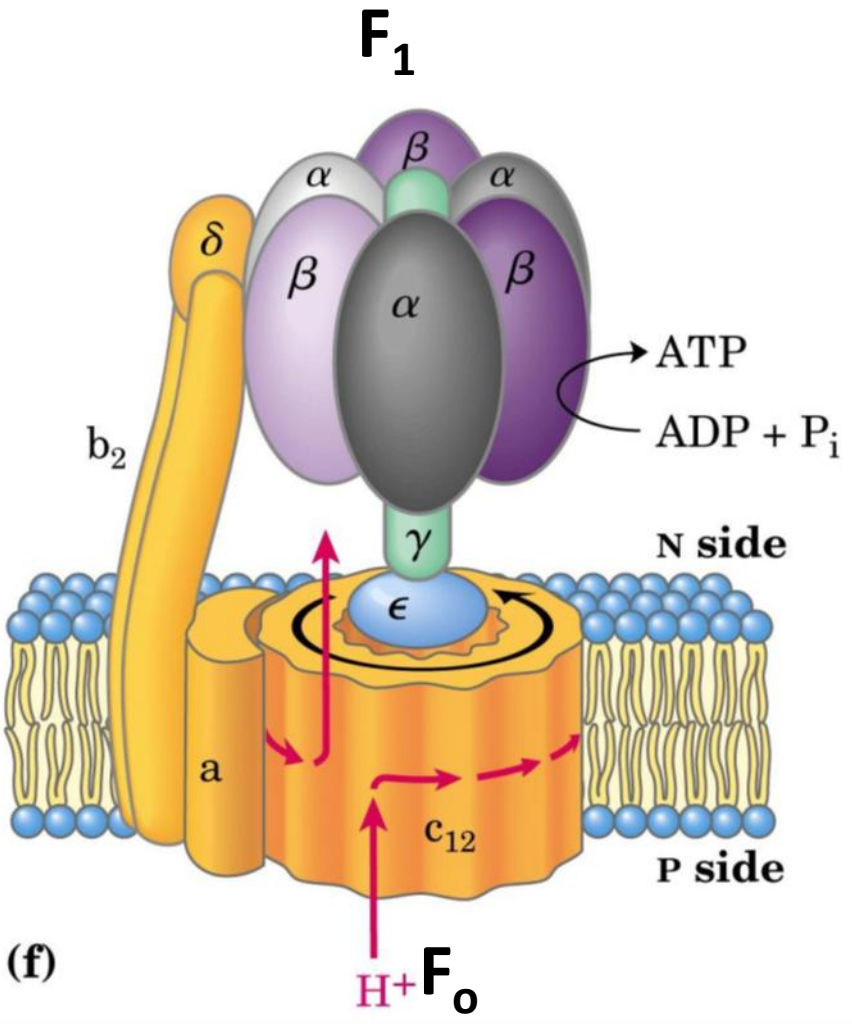
Isolated F_1 catalyzes ATP hydrolysis.

F_o: hydrophobic, trans-membrane.

Subunit composition: $abc_{8\text{-}12}$.

Interface a-c forms a H^+ channel.

When coupled to a proton gradient $F_o + F_1$ synthesizes ATP.



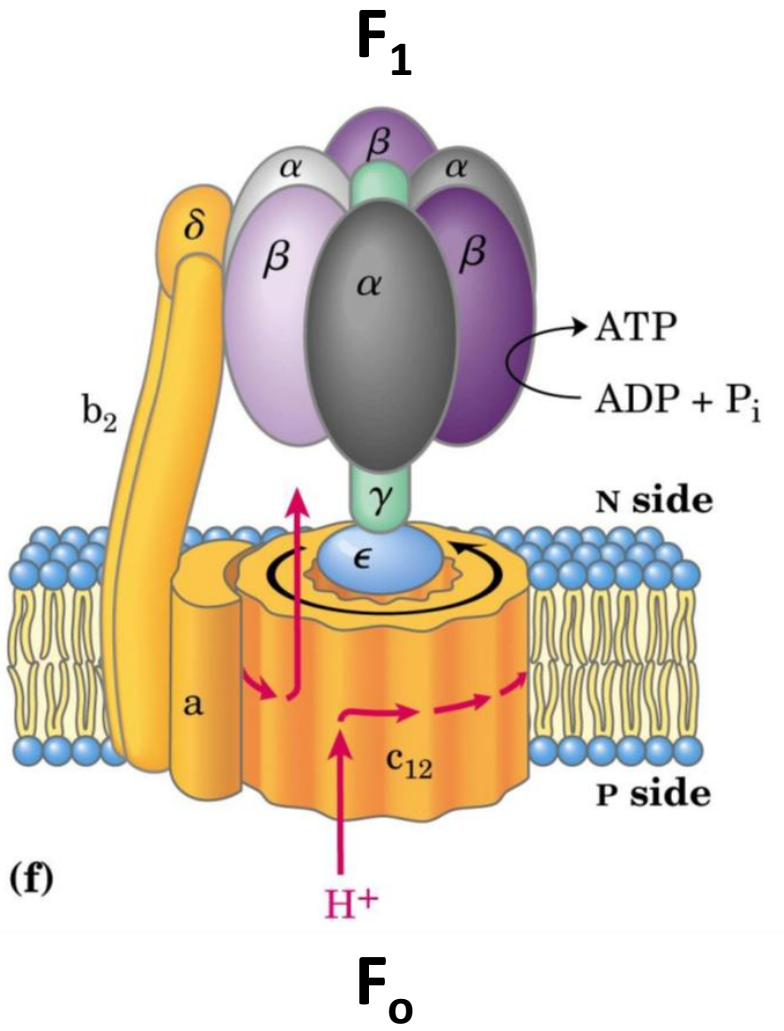
ATPase/ATP synthase

Subunit **a** (also called **ATPase-6)** is immobilized by contact with subunit **b** that also fixes F_1.

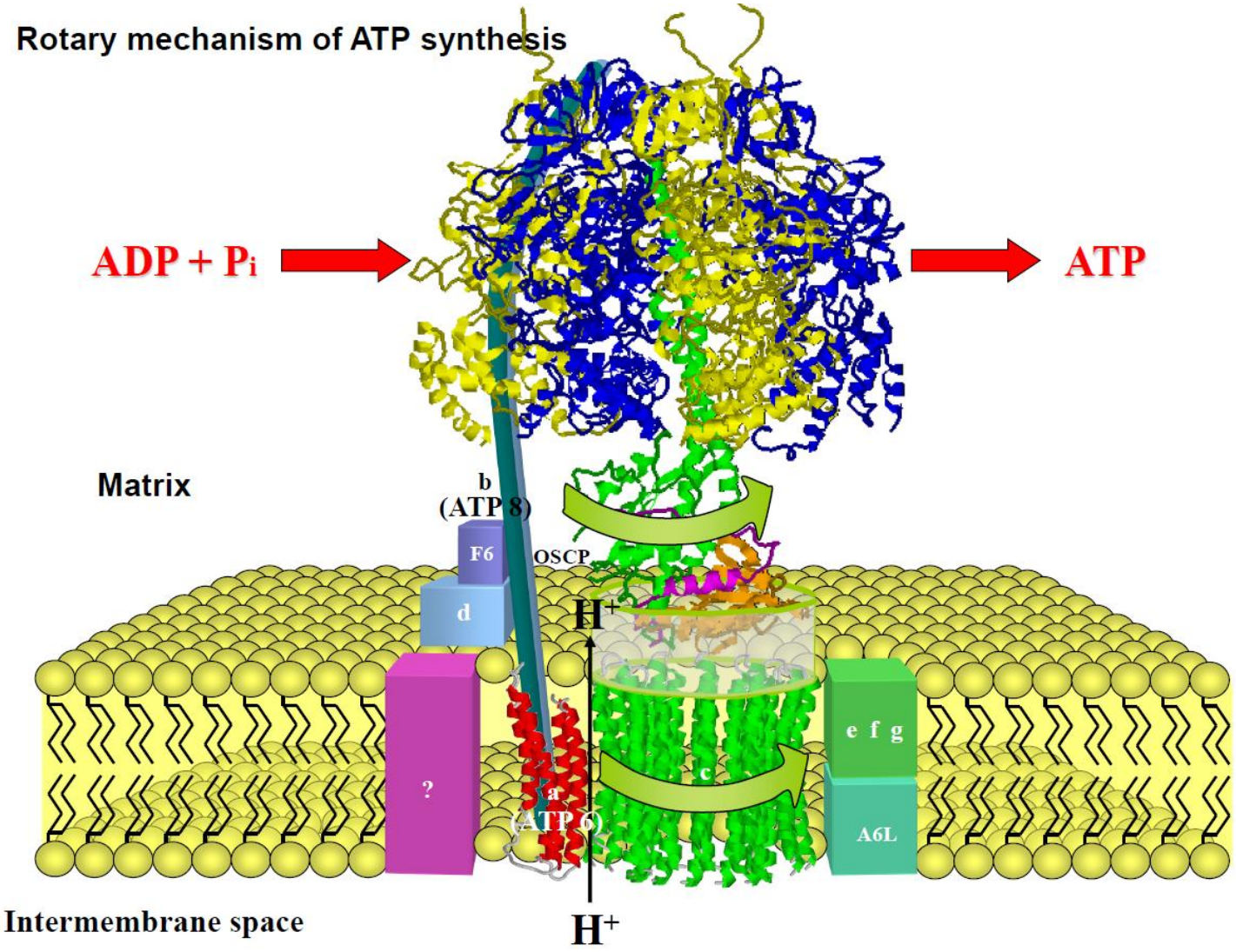
Protons enter passively from IMS and induce rotation of **c** polymer in such way that consecutive **c** subunits contact subunit **a** during rotation.

Rotation of **c** subunits induces rotation of **γ** subunit within F_1, exposing to H^+ alternative active sites.

In the active site ATP is synthesized without energy expense, $3H^+$ are required to open the active site and deliver ATP in the water phase.



ATPase/ATP synthase



ATPase/ATP synthase

Since metabolic reactions take place in water, the aqueous medium will affect the equilibrium, thus the ΔG values include the concentration of pure water (ca 56 M).

For the synthesis of ATP

$ADP + P_i \rightarrow ATP + H_2O$

Under standard conditions the concentrations are, respectively

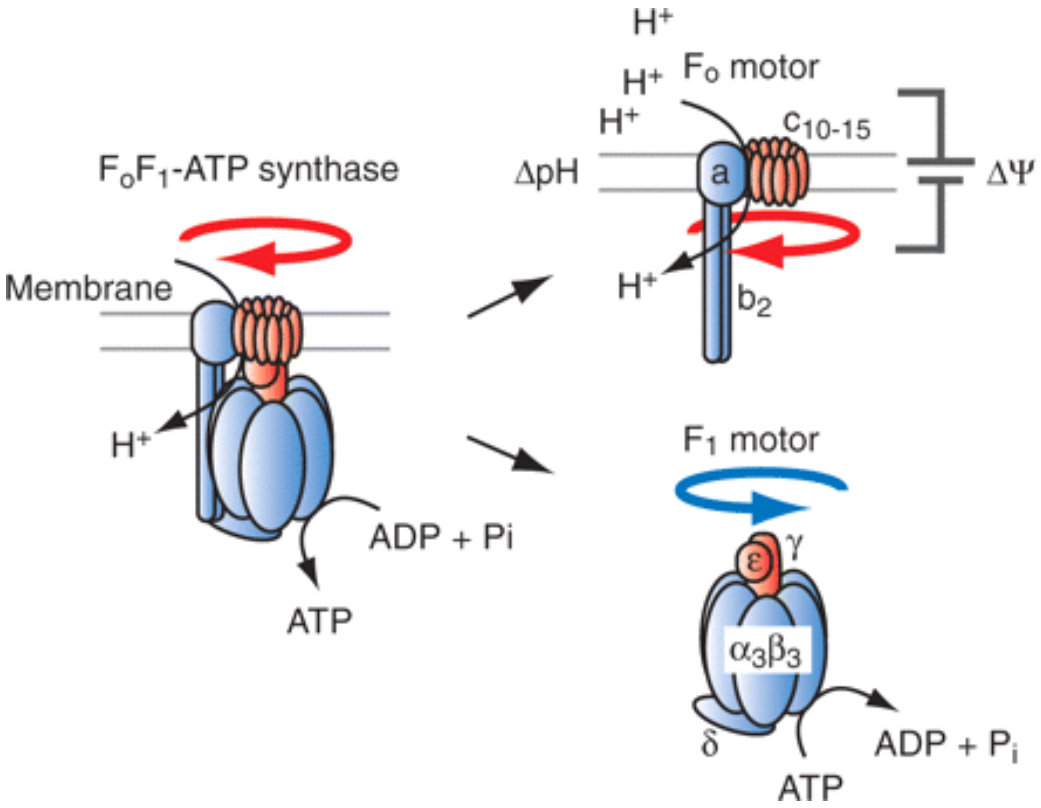
1M 1M 1M 56 M and the ΔG° is + 30.5 kJ/mol.

In the active site of ATP synthase there is no water, so the ΔG° would be proportionally: + 30.5/56 = 0.5 kJ/mol

Therefore, in a hydrophobic environment, ATP synthesis does not require energy. However, energy is required to push ATP in the water medium by a protein conformational change induced by H^+.

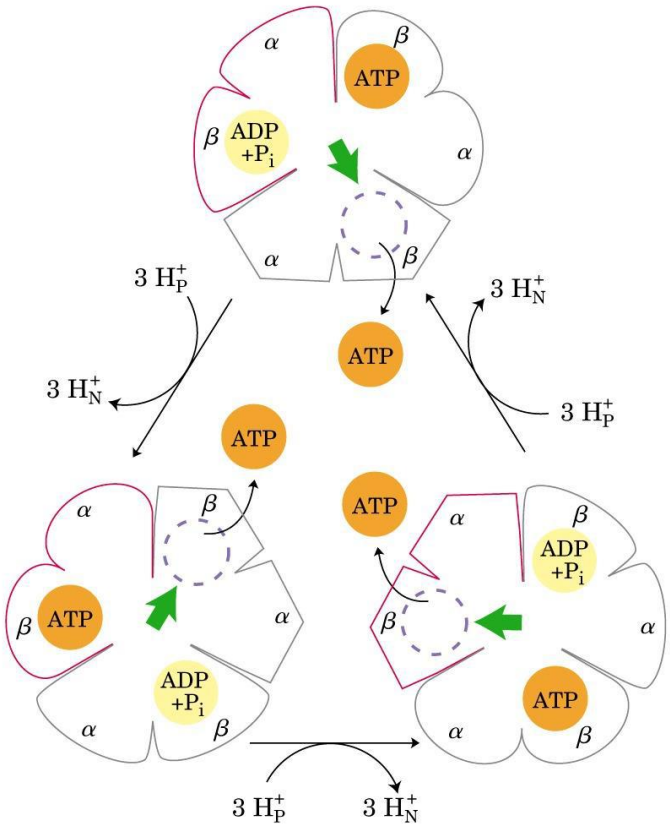
ATPase/ATP synthase

When F_1F_o is membrane-bound without a H^+ gradient (e.g. uncoupled conditions), ATP hydrolysis, as in the figure, will induce a rotation and a H^+ translocation towards the IMS: ATPase creates a H^+ gradient.



ATPase/ATP synthase

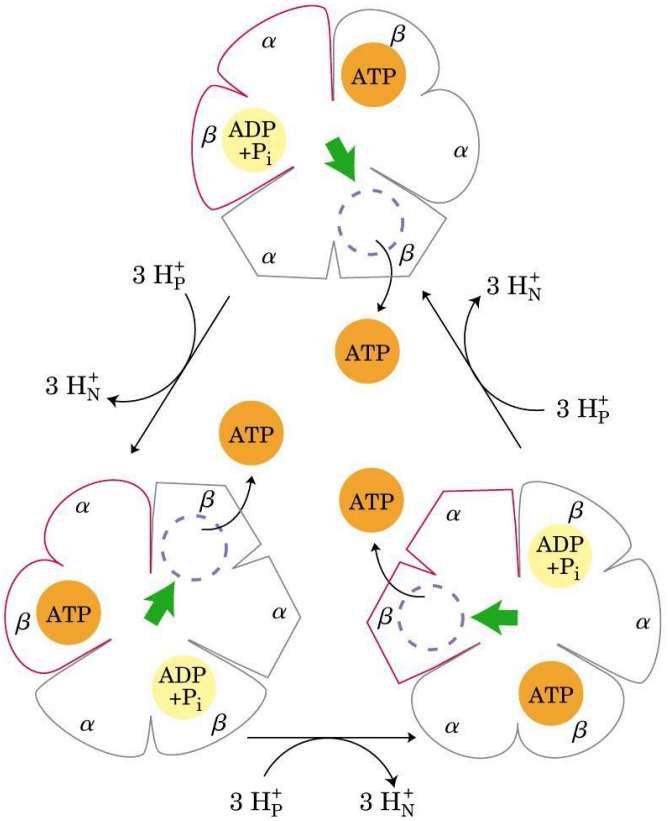
Rotational catalysis is the mechanism by which the flow of protons through F_o causes the c ring to rotate and, in turn, trigger the subunit conformational changes in F_1.



ATPase/ATP synthase

Binding-change model: the active site of each β subunit cycles is through the three conformations:

- β-ATP – tight binding
- β-ADP – loose binding
- β-empty – very loose binding

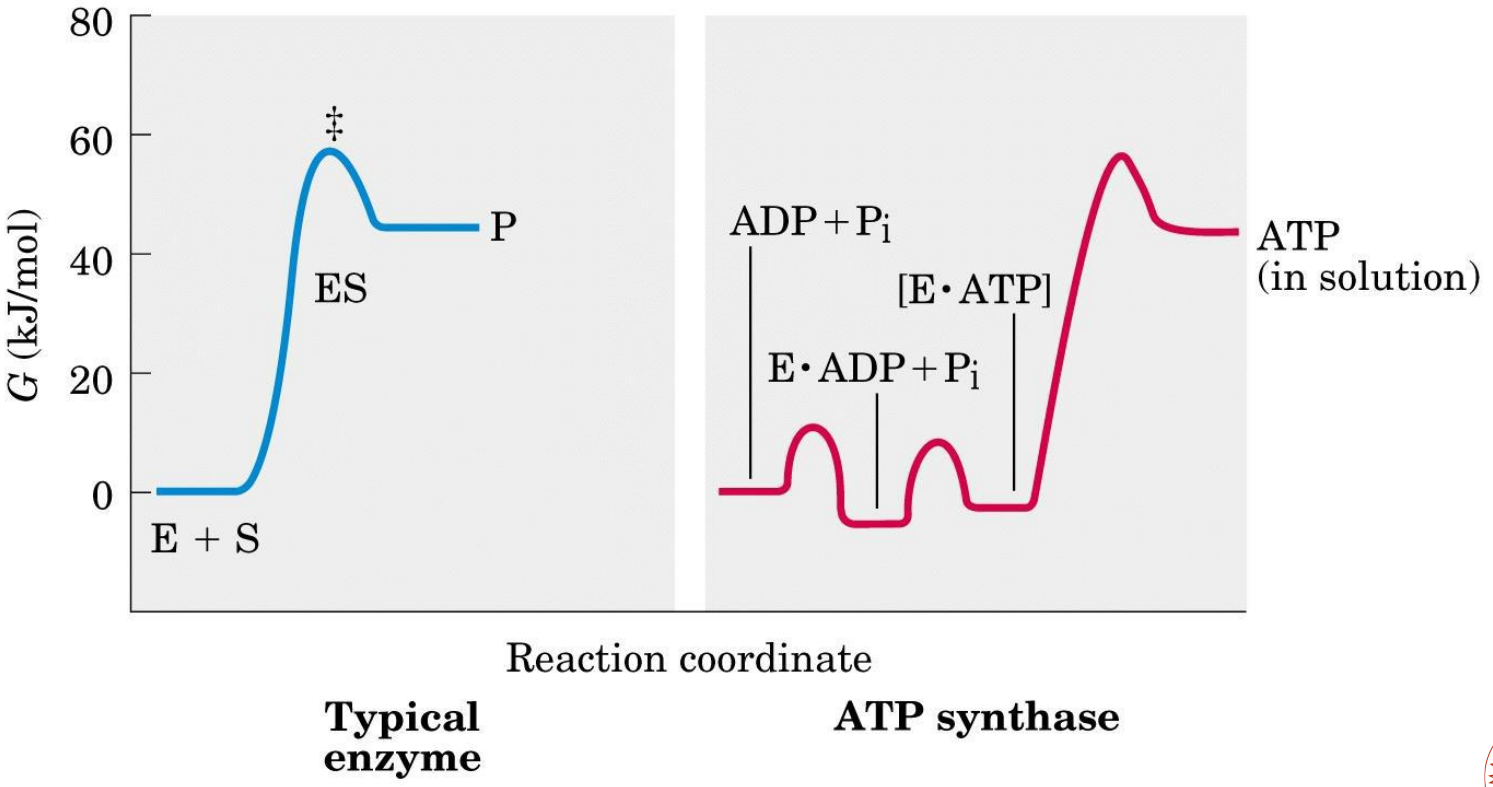


- translocation of three protons fuels synthesis of one ATP
- each complete rotation synthesizes three ATP

ATPase/ATP synthase

$ADP + P_i \rightarrow ATP + H_2O$

The free energy required for the release of ATP is provided by the proton-motive force.



RESPIRATORY CONTROL

1. $NADH + H^+ + ½\ O_2 + 10\ H^+in \rightarrow NAD^+ + H_2O + 10\ H^+_{out}$

2. $10\ H^+_{out} + 2.5\ ADP + 2.5\ P_i \rightarrow 2.5\ ATP + 10H^+_{in}$

Protons are transported through ATP synthase obligatorily coupled to ATP synthesis.

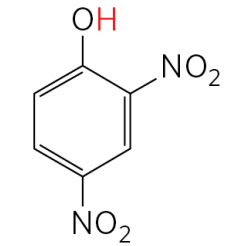
When ADP is lacking or ATP synthase is inhibited (oligomycin) protons cannot flow through F_o.

When reaction 1 reaches equilibrium, respiration stops. The rate is not zero because the membrane has some permeability to H^+. Membrane damage increases H^+ leak.

UNCOUPLERS

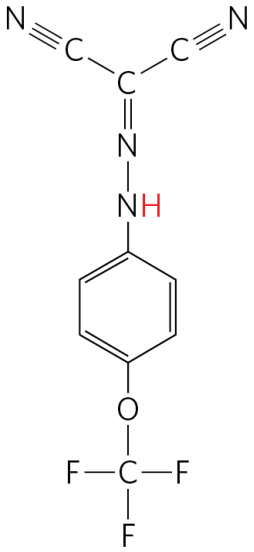
Uncouplers are membrane-permeable H^+ carriers which dissipate H^+ gradient (weak acids that carry protons across the membrane).

Uncouplers reduce or prevent ATP synthesis, but do not prevent e^- transport.

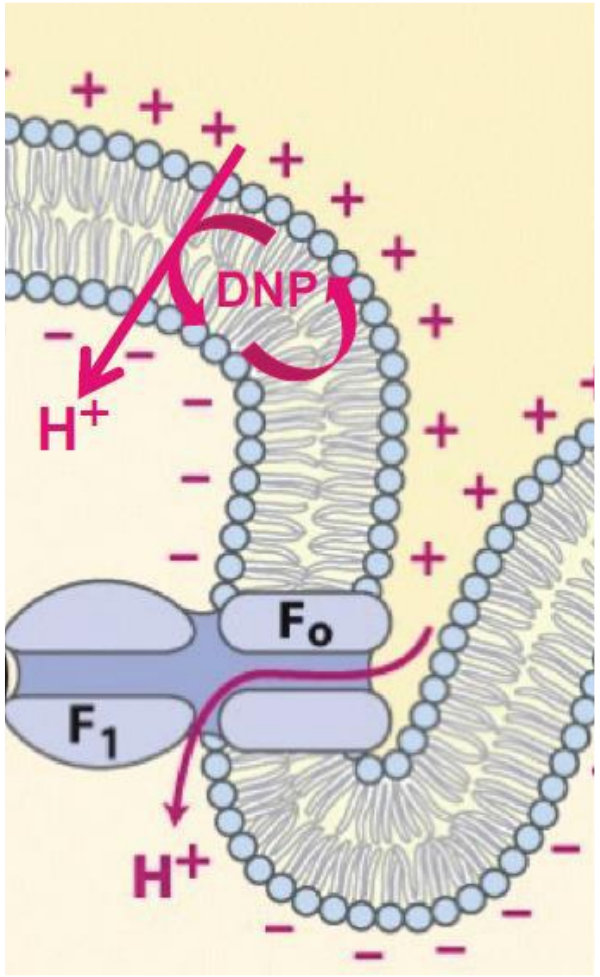


2,4-Dinitrophenol
(DNP)

Weak acids



Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone
(FCCP)



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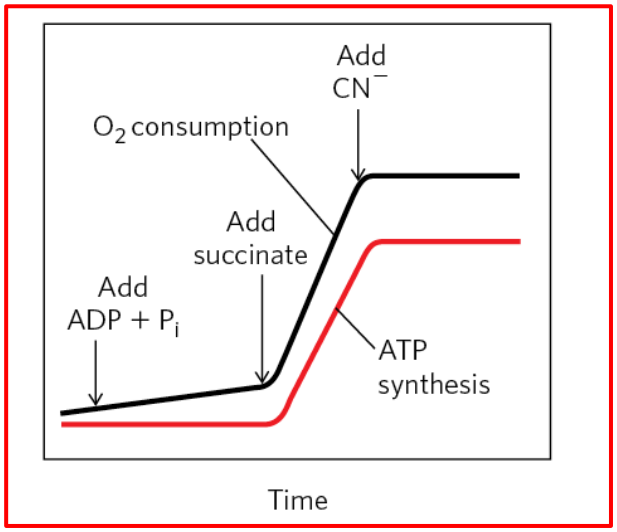
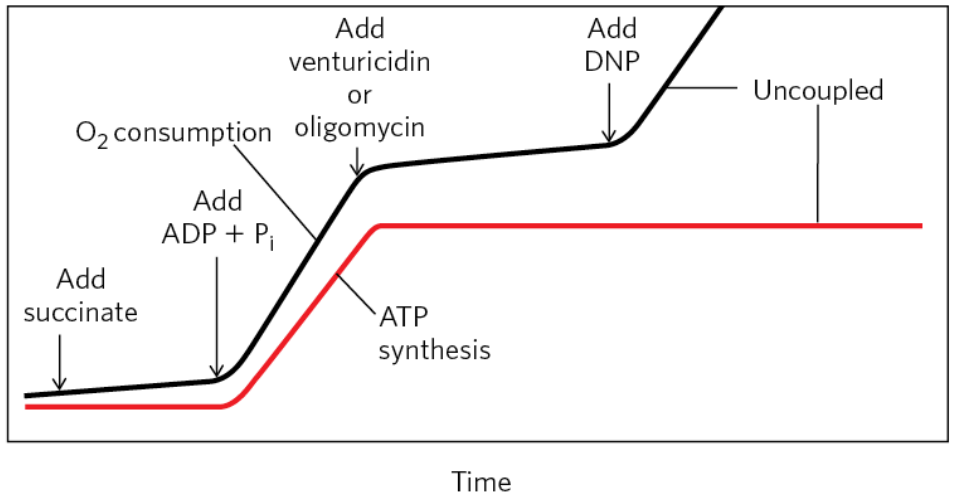
UNCOUPLERS

Protons will not flow through ATP synthase unless other substrates (ADP and P_i) are present (enzymes cannot catalyze a part of the overall reaction). Thus, in coupled mitochondria e^- transport/H^+ pumping does not occur without ATP production.



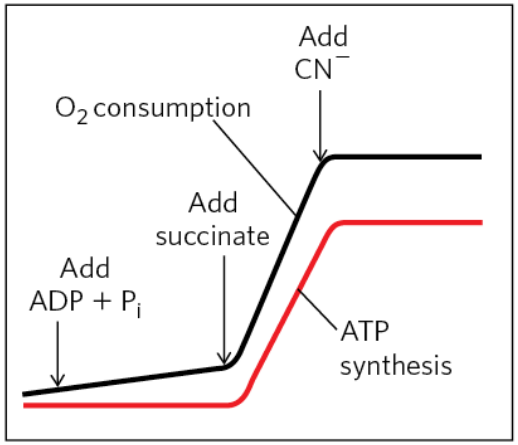
(a)

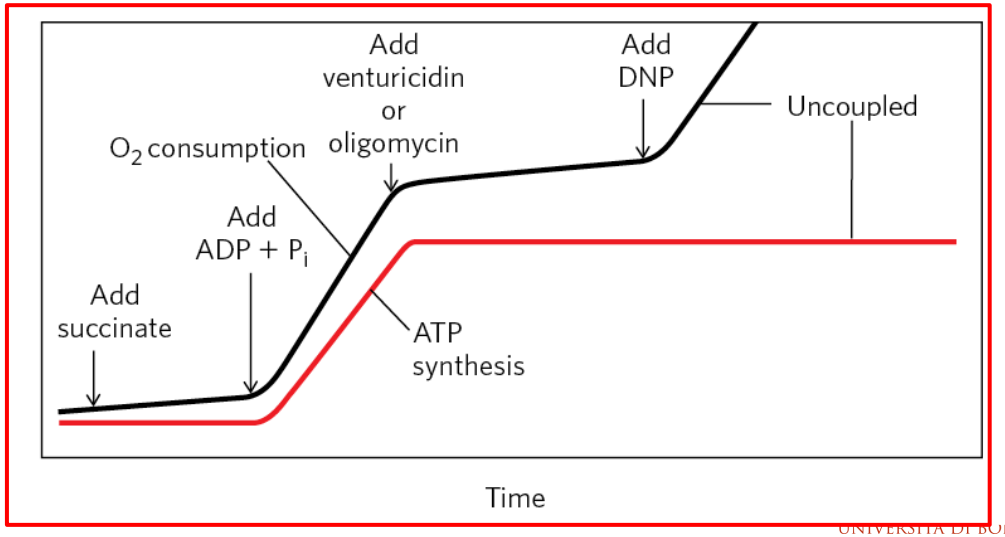


(b)

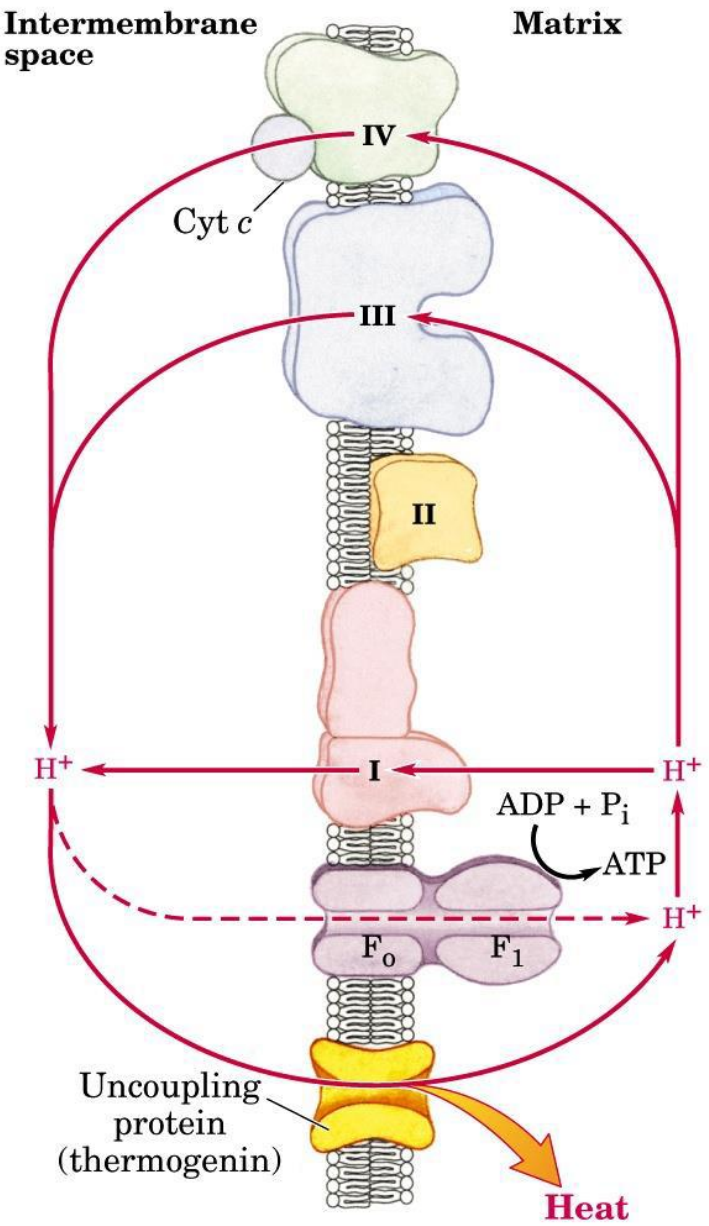
UNCOUPLERS

1) With succinate alone, Electron Transport (ET) builds up the proton gradient until an equilibrium is reached.
2) With ADP + P_i, protons flow through ATP synthase, ET proceeds and O_2 is consumed.
3) Oligomycin blocks ATP formation by inhibiting ATP synthase. No proton flow and ET again creates a proton gradient.
4) Uncoupler (DNP) dissipates the proton gradient and permits ET in absence of ATP synthesis.





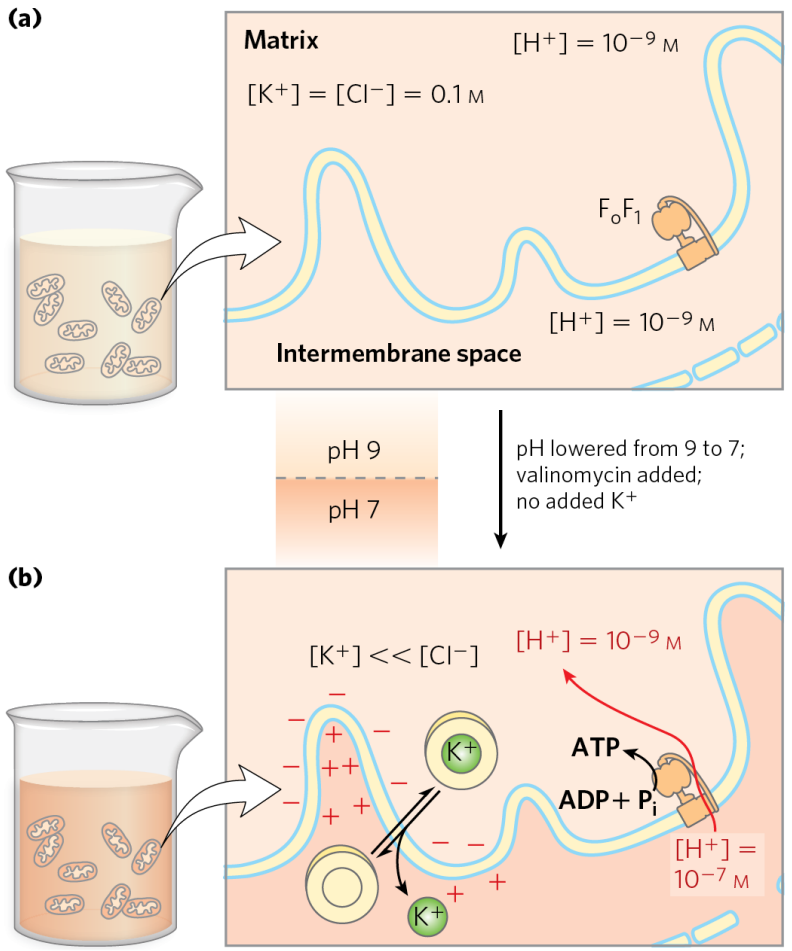
UNCOUPLERS



UNCOUPLERS

In the absence of an oxidizable substrate, the proton-motive force alone drives ATP synthesis without electron transport.

Valinomycin is a polypeptide used in K^+ transport.



OXPHOS INHIBITORS

Respiratory Inhibitors (rotenone, Antimycin A, KCN…..) block electron transfer → ATP synthesis.

ATP synthase Inhibitors (oligomycin) block ATP synthesis → respiration slow.

Uncouplers (DNP, FCCP) Proton carriers, dissipate membrane potential → increase electron transfer and block ATP synthesis.

OXPHOS

Membrane potential may be used to transport molecules against gradient

- Calcium influx (Ca^{2+} uniport)
- Cotransport phosphate/H^{+}
- ADP^{3-}/ATP^{4-} exchange (exit of a negative charge)
- Pyruvate/OH^{-} exchange (exit of a negative charge)

BIOCHEMISTRY OF MITOCHONDRIAL DISEASES

In the ’80s it was discovered that mtDNA mutations cause hereditary diseases: since then, mitochondrial cytopathies have become a hot subject of investigation.

Respiration and Oxidative Phosphorylation defects increased glycolysis with lactic acidosis.

Often increased production of Reactive Oxygen Species (ROS).

But a dramatic breakthrough has been understanding that mtDNA mutations may be at the basis of aging, degenerative diseases, and cancer.

REACTIVE OXYGEN SPECIES (ROS)

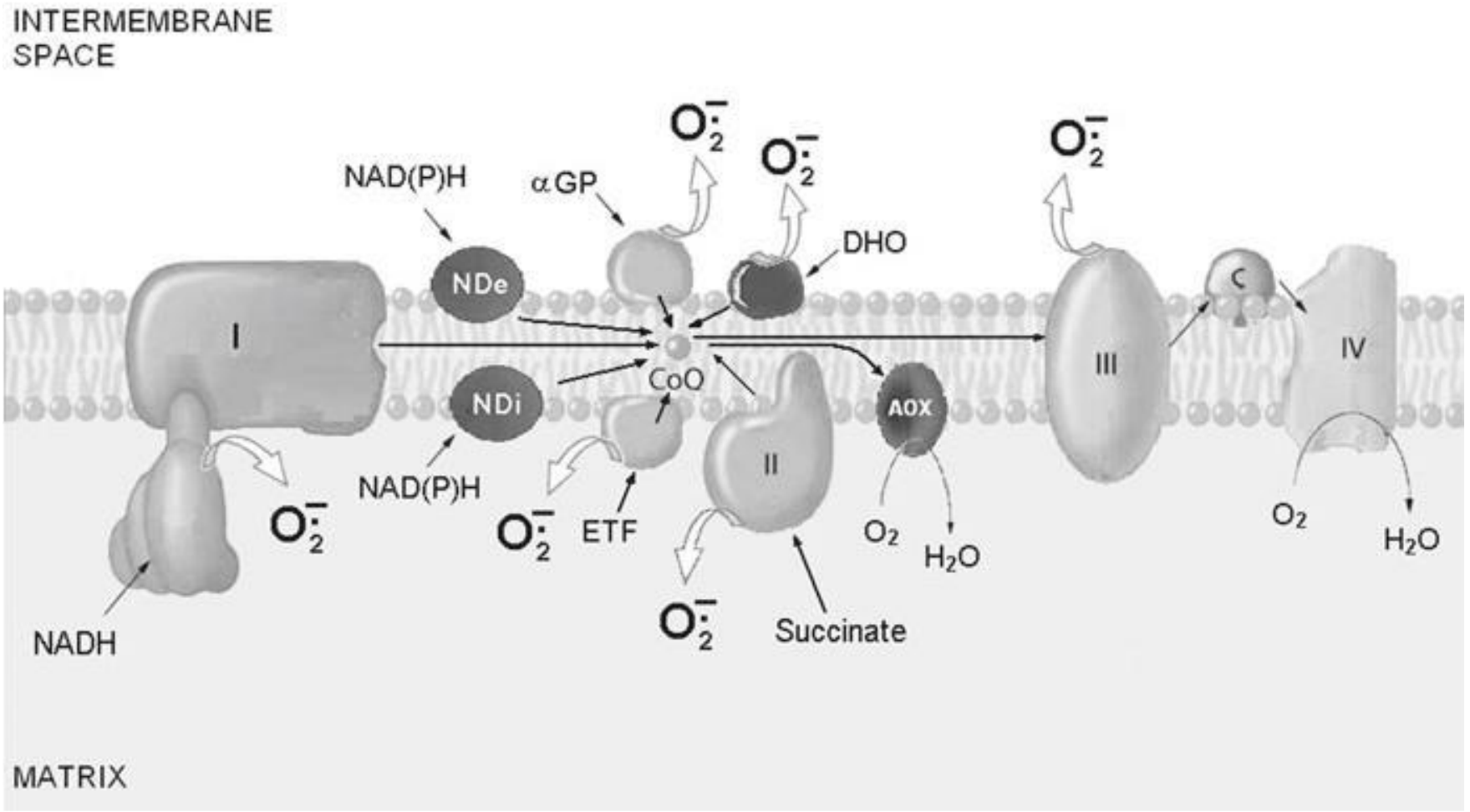
$O_2 + 1\ e^- \rightarrow \mathbf{O_2^{\bullet -}}$	Superoxide
$O_2^{\bullet -} + 1\ e^- + 2\ H^+ \rightarrow \mathbf{H_2O_2}$	Hydrogen peroxide
$H_2O_2 + 1\ e^- \rightarrow \mathbf{OH^\bullet} + OH^-$	Hydroxyl radical
$OH^- + 1\ e^- + H^+ \rightarrow H_2O$	

REACTIVE OXYGEN SPECIES (ROS)

Sources of ROS:

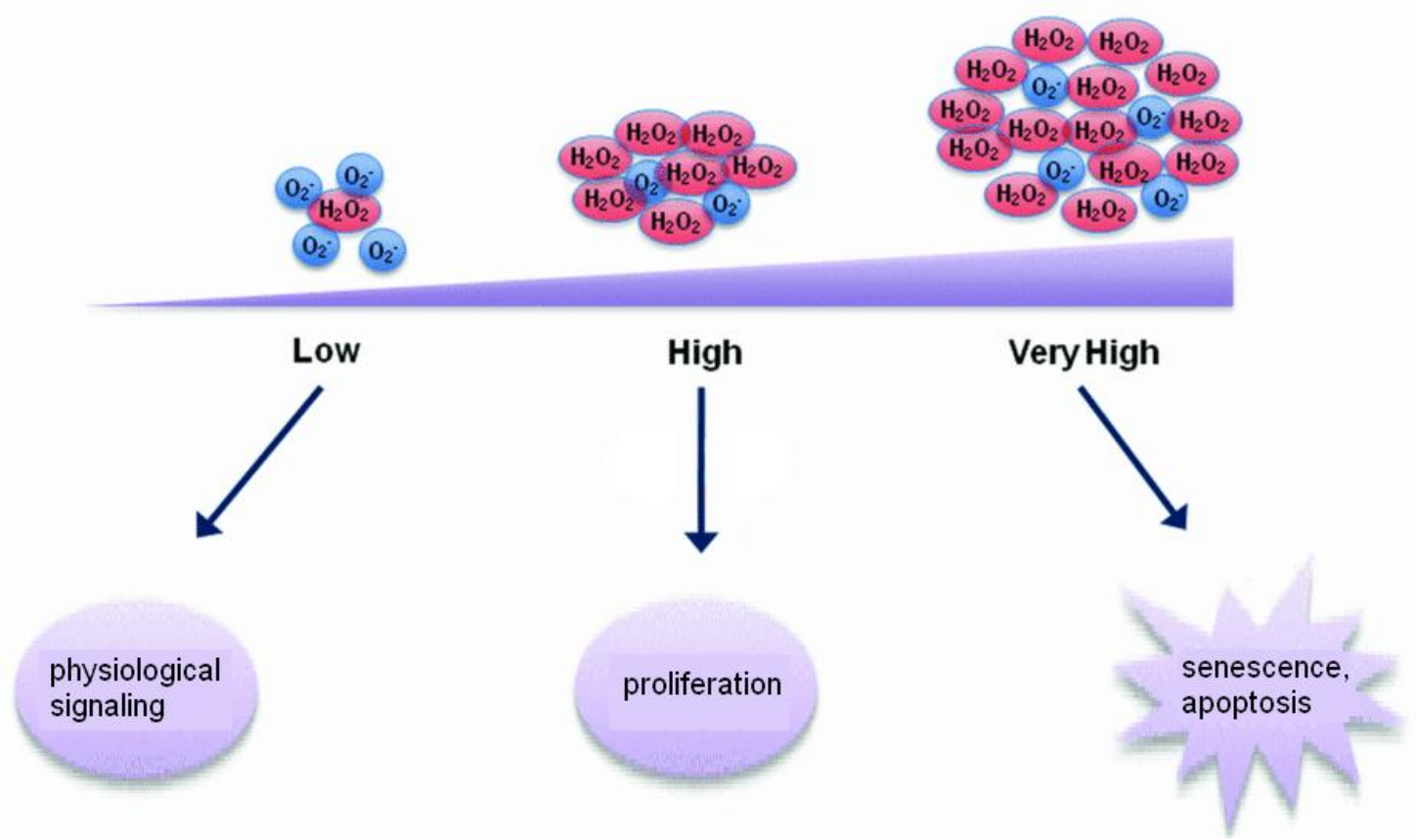
- Ionizing and UV radiation
- Xenobiotics (non natural compounds)
- **Smoking**
- Transition and heavy metal ions
- NADPH oxidase (phagocytes and other cells)
- Microsomal oxidations (cytochrome P-450, catalysing hydroxylation reactions)
- Other metabolic oxidations (amine oxidases, xanthine oxidase etc,)
- **Mitochondrial respiratory chain**

REACTIVE OXYGEN SPECIES (ROS)



REACTIVE OXYGEN SPECIES (ROS)

ROS promote intracellular signalling, in part, by the oxidative inactivation of phosphatases.



REACTIVE OXYGEN SPECIES (ROS)

Mechanisms of protection from ROS:

- Induction of protective factors: *nFkB, HSP*
- Repair mechanisms
- Removal mechanisms (*proteolysis, autophagy, apoptosis*)
- Antioxidant Enzymes (*SOD, GPx, catalase*)
- Chemical Antioxidants (water- and lipid-soluble)

MITOCHONDRIA AND PATOLOGY

Current consensus that most human pathologies have common etiologic and/or pathogenic mechanism in mitochondria, in connection with their major involvement in ROS generation and of the accumulation of mtDNA somatic mutations. The consequent loss of energy production by OXPHOS induces cellular degeneration and cell death.

MITOCHONDRIA AND PATOLOGY

Aging is the major predisposing cause of many pathologies:

- Ischemic diseases (myocardial infarct, stroke…)
- Degenerative diseases of the skeletrical system
- Neurodegenerative diseases (Parkinson d., Alzheimer d.)
- Type 2 diabetes
- Cancer

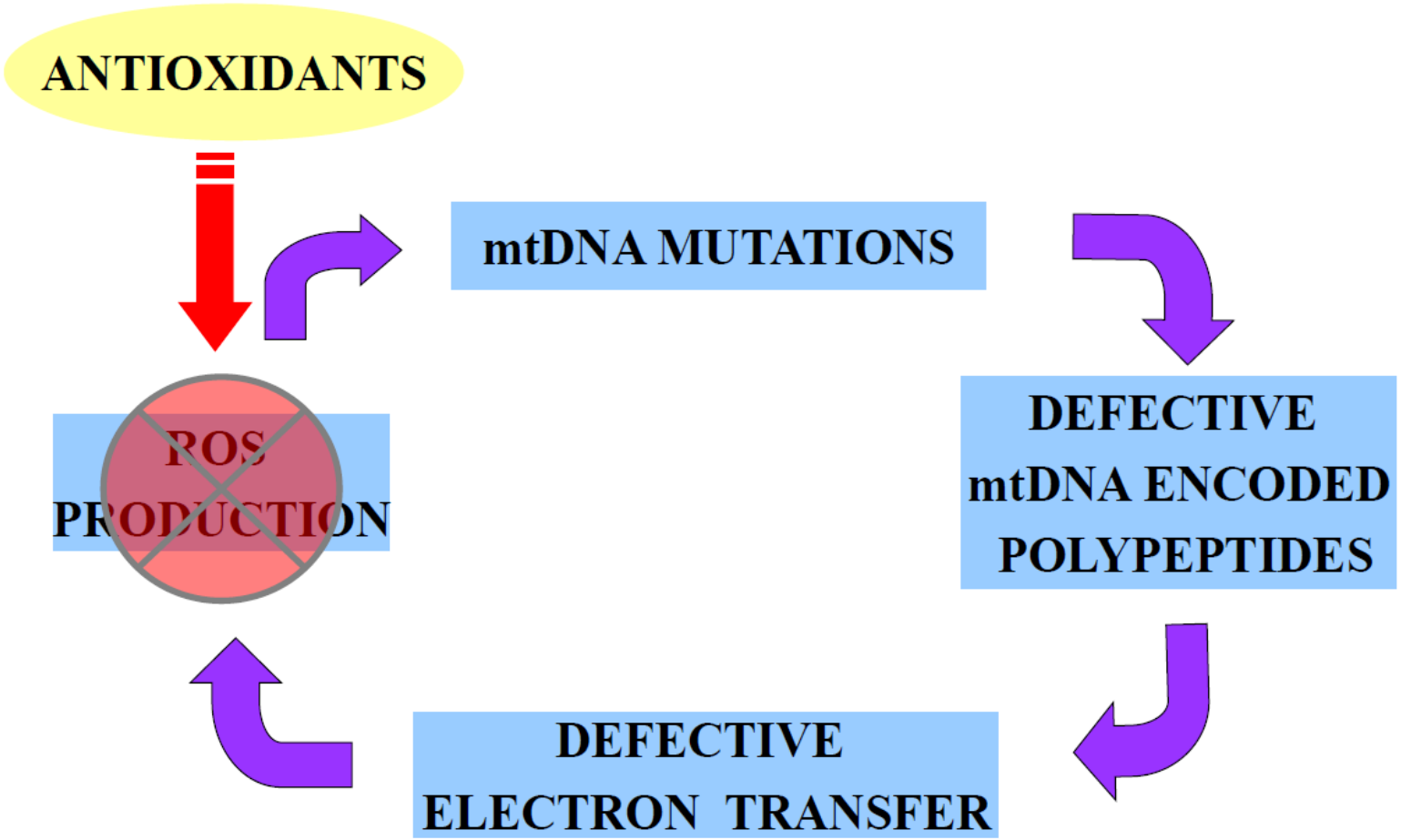
MITOCHONDRIA AND PATOLOGY

Molecular bases of the mitochondrial theory of aging:

- Mitochondria are major ROS producers
- Mitochondrial DNA (mtDNA) not protected
- Mutations and deletions of mtDNA
- Damage to respiratory chain and ATP synthase
- Energetic deficiency and cell death

MITOCHONDRIA AND PATOLOGY

Vicious circle of oxidative stress and aging



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