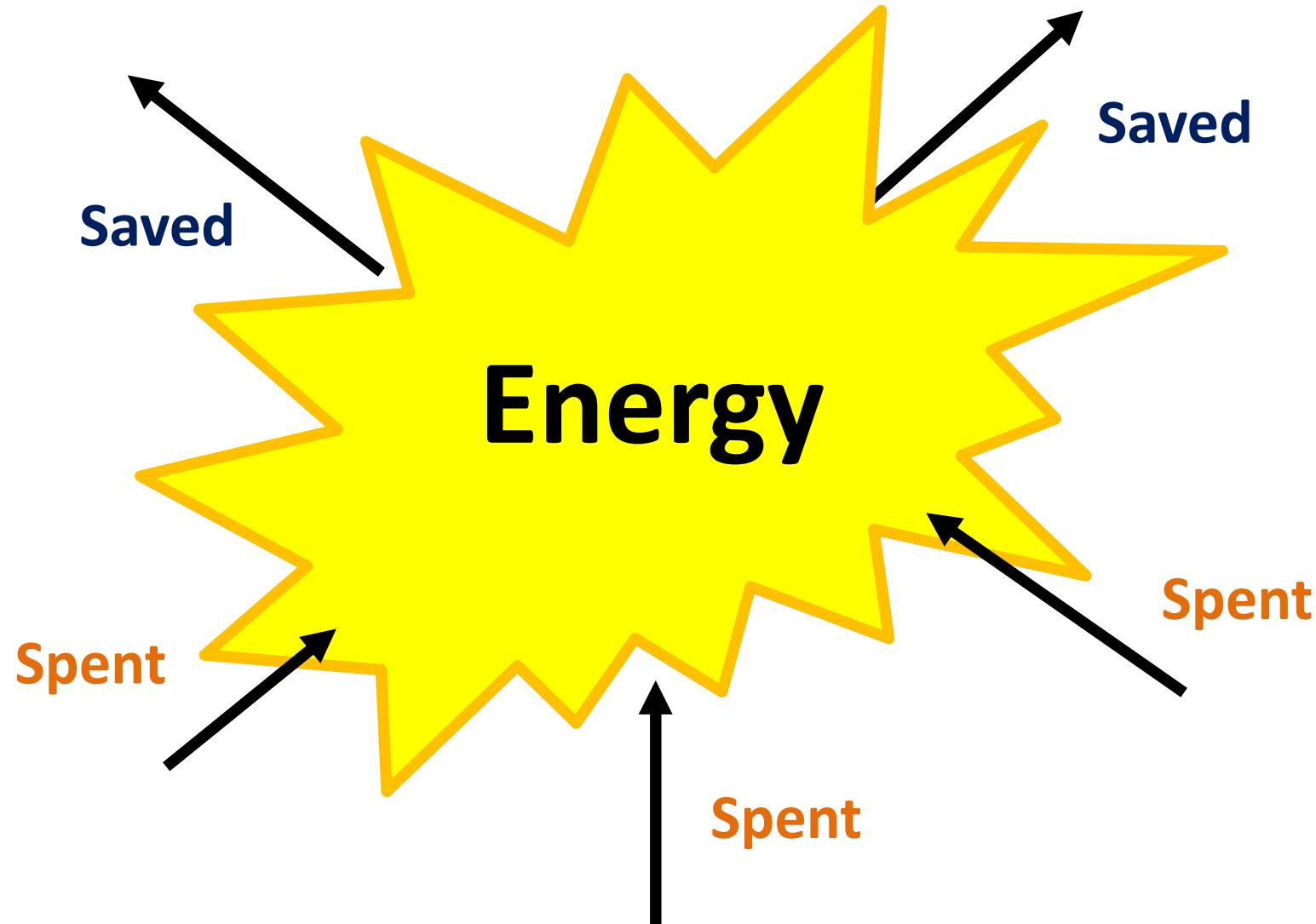
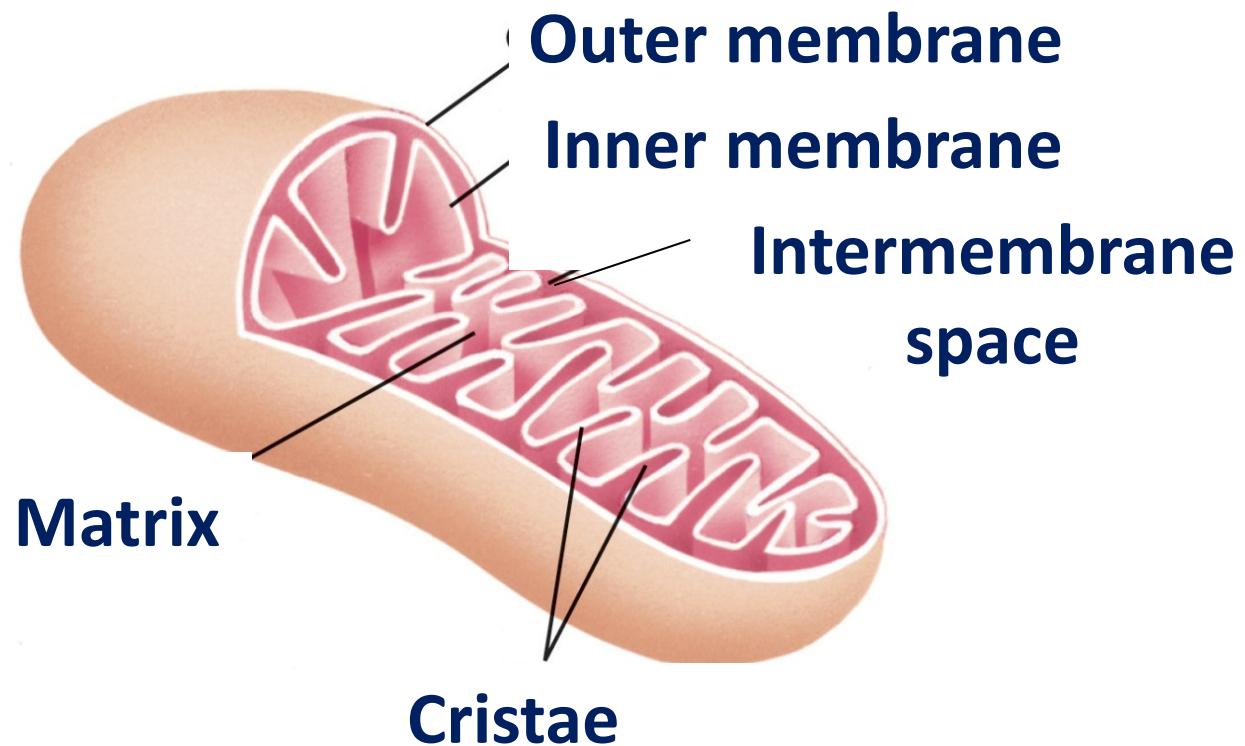


Electron Transport and Oxidative Phosphorylation

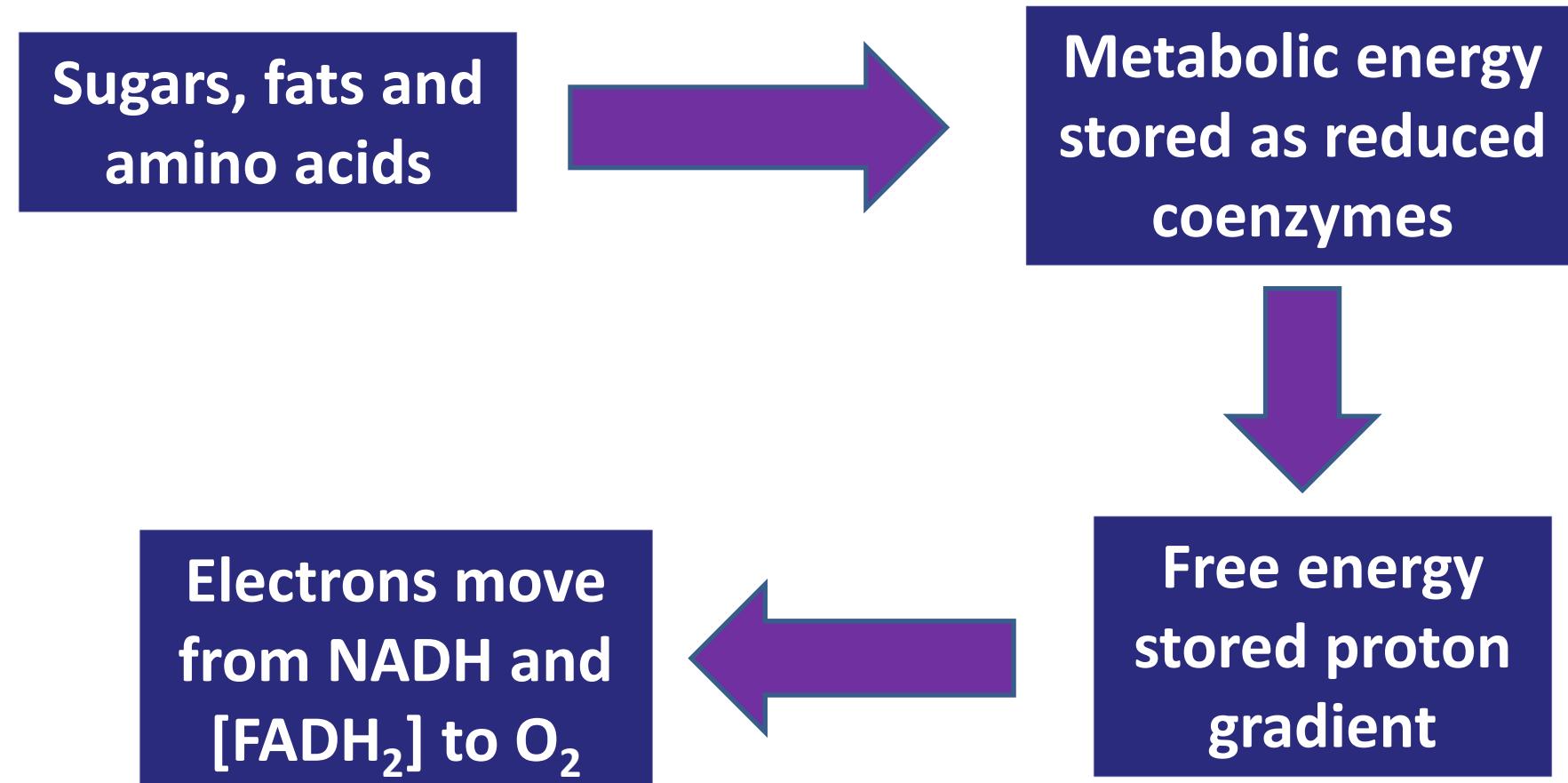
Chapter 20



Mitochondrial compartments



Organisation of the ETC



Molecular species in the ETC

- **Flavoproteins**: Tightly bound FMN and FAD
- **Coenzyme Q** (ubiquinone) abbreviated CoQ or UQ
- **Cytochromes** (*b*, *c*, *c1*, *a* & *a3*): Haeme group
- **Iron-sulphur proteins**: Fe²⁺ and Fe³⁺ states
- **Protein-bound copper**: Cu⁺ and Cu²⁺

4 protein complexes of the ETC

- Large, multisubunit complexes embedded in the mitochondrial membrane

COMPLEX I:

Accepts e⁻ from: NADH
Product: Reduced CoQ

COMPLEX II:

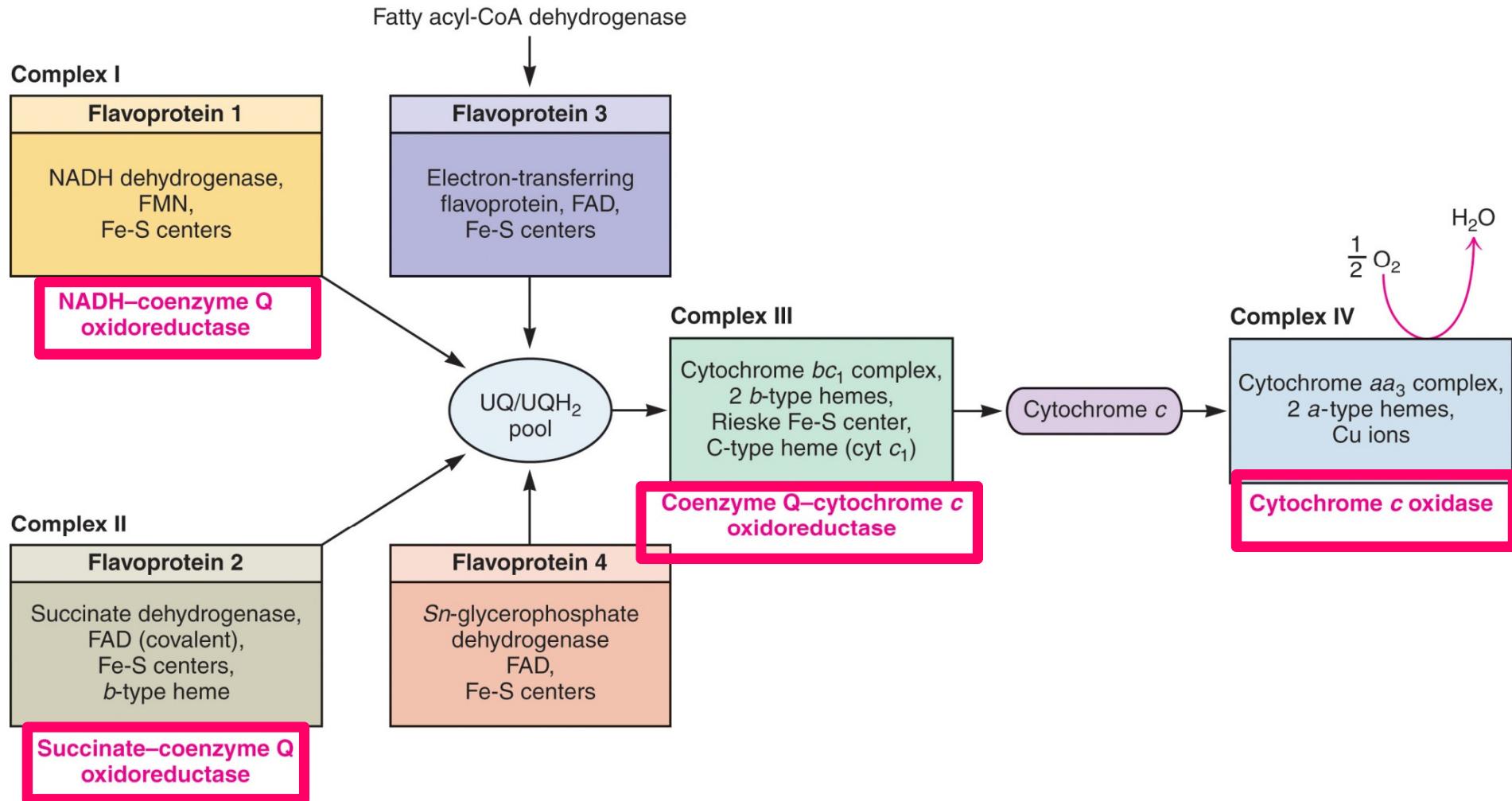
Accepts e⁻ from: Succinate
Product: Reduced CoQ (UQH₂)

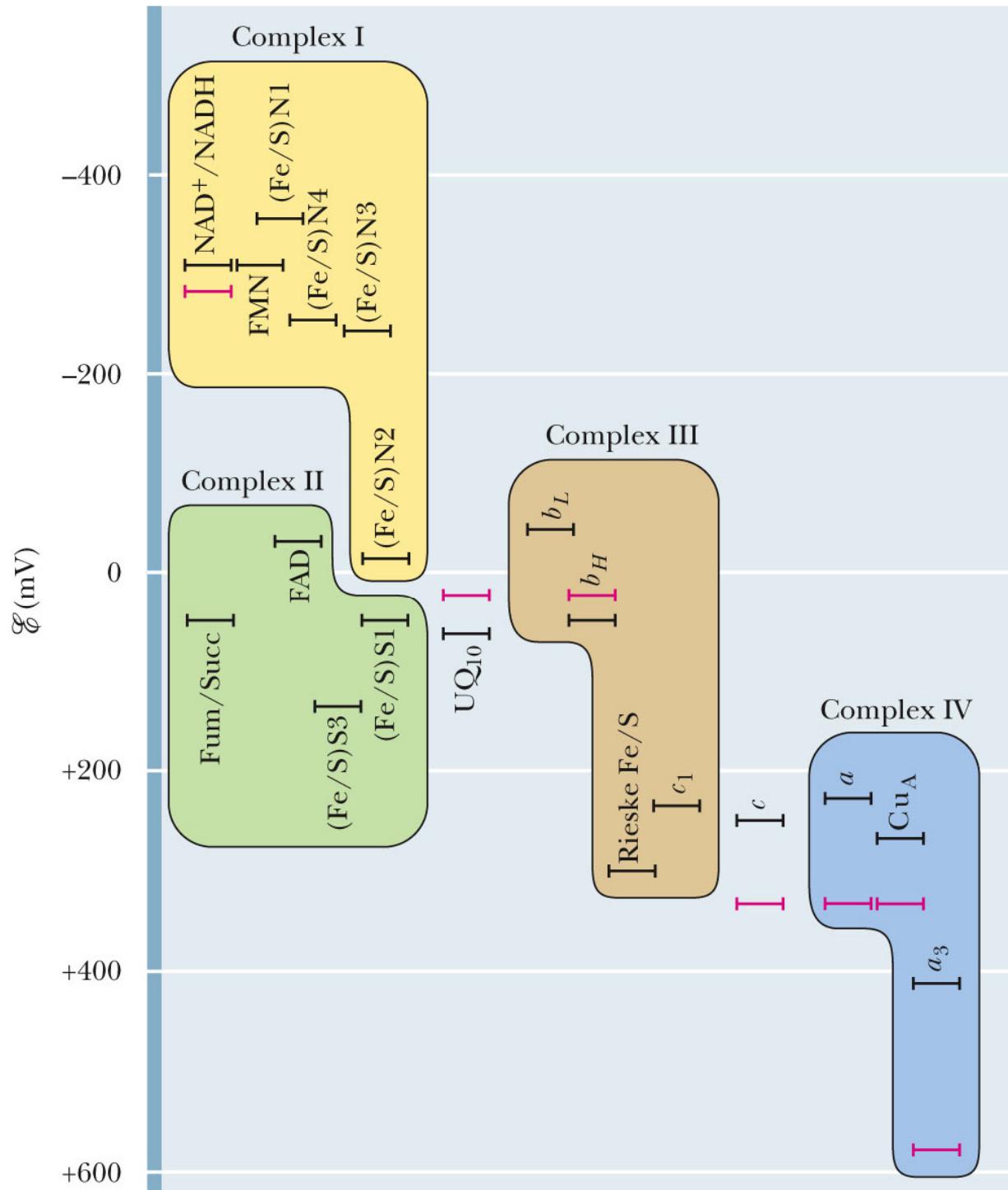
COMPLEX III:

Accepts e⁻ from: UQH₂
Product: Cytochrome c

COMPLEX IV:

Accepts e⁻ from: Cytochrome c
Product: Molecular oxygen





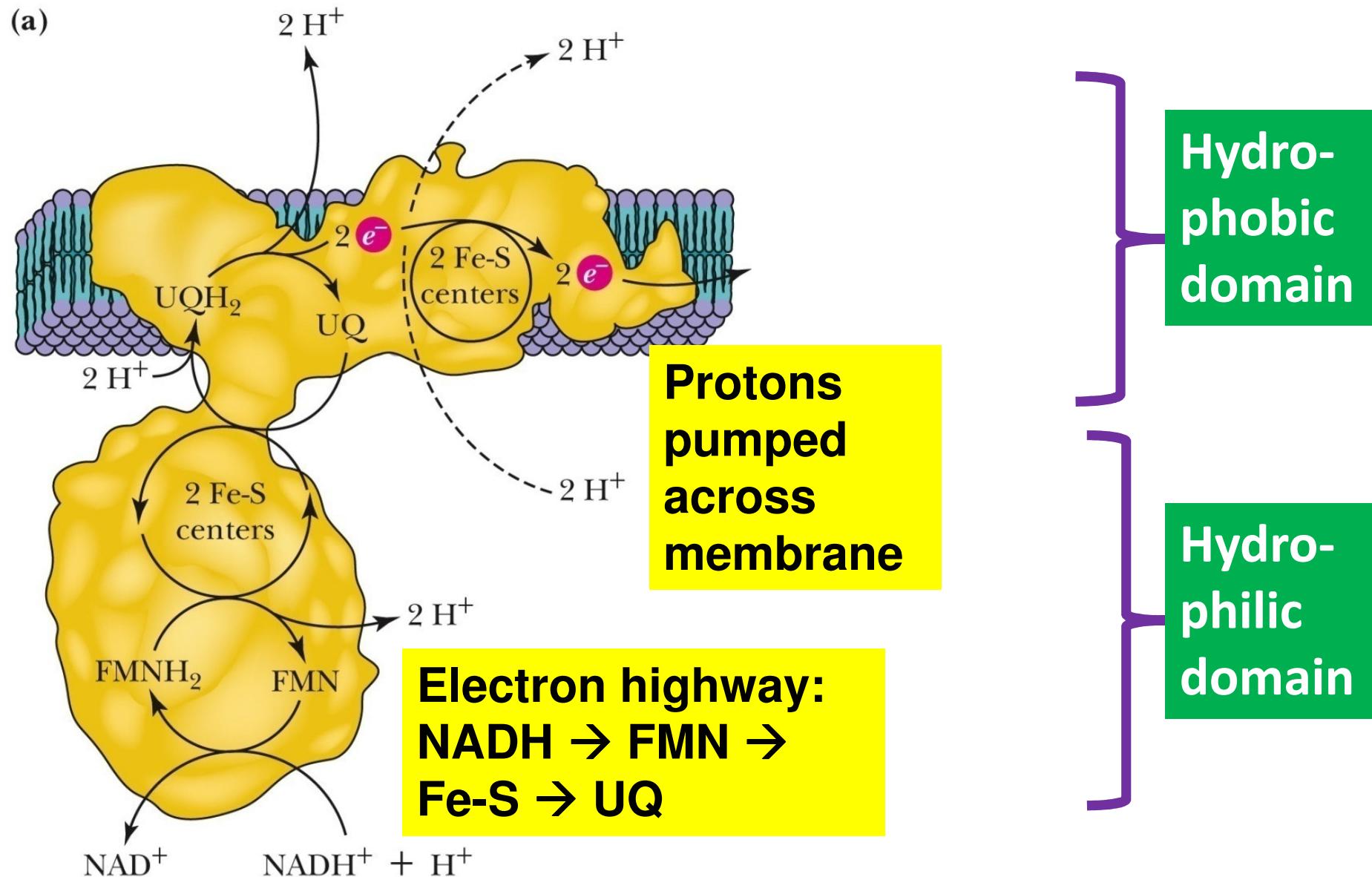
The
complexes
are not
arranged in
a linear
sequence

Complex I oxidises NADH and reduces Coenzyme Q

- NADH-UQ oxidoreductase
- Transfers electrons from **NADH** → **coenzyme Q**
- 45 polypeptide chains
- One molecule of FMN
- 8 or 9 Fe-S clusters

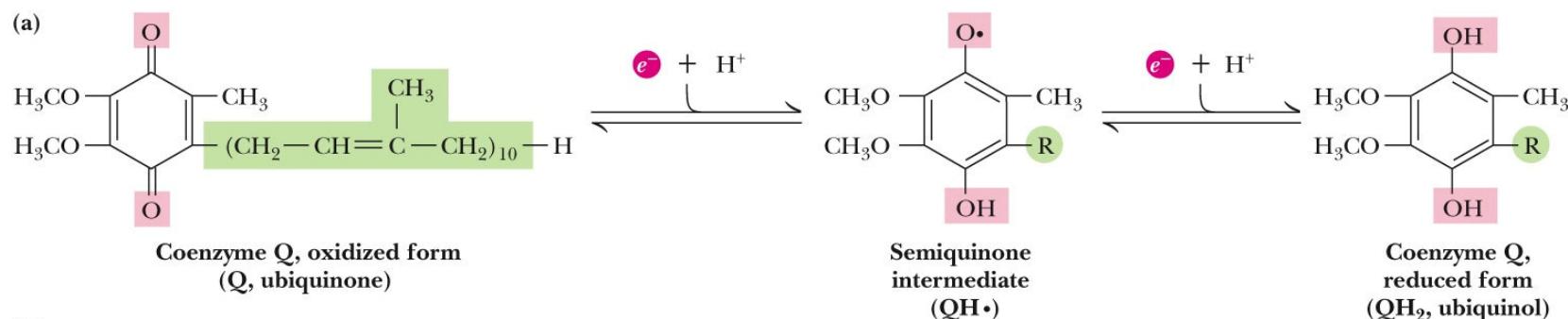
Protons transferred from matrix side (N) to cytosolic side (P) of inner mitochondrial membrane

Complex I: structure suited to function



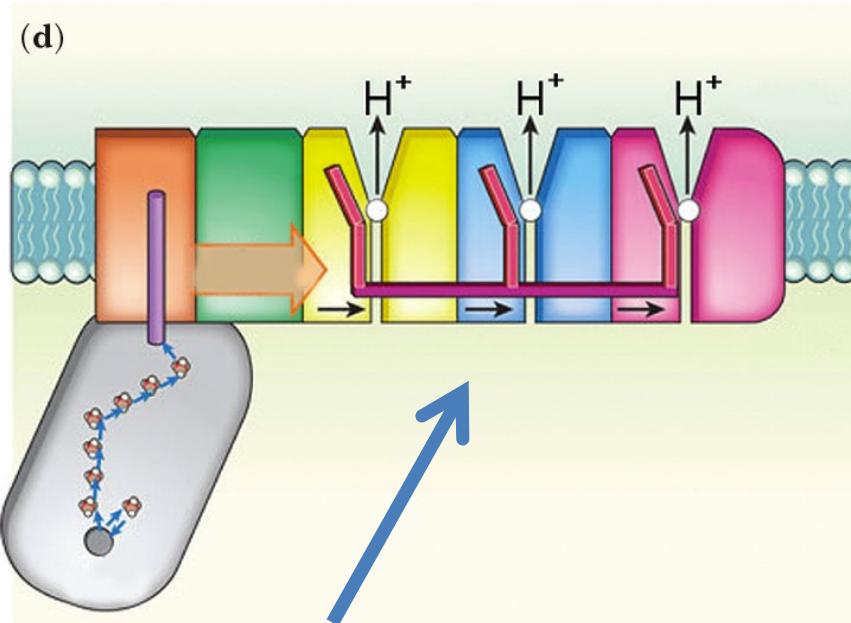
Co-enzyme Q

- Co-Q can accept 2 e⁻ from the Fe-S clusters

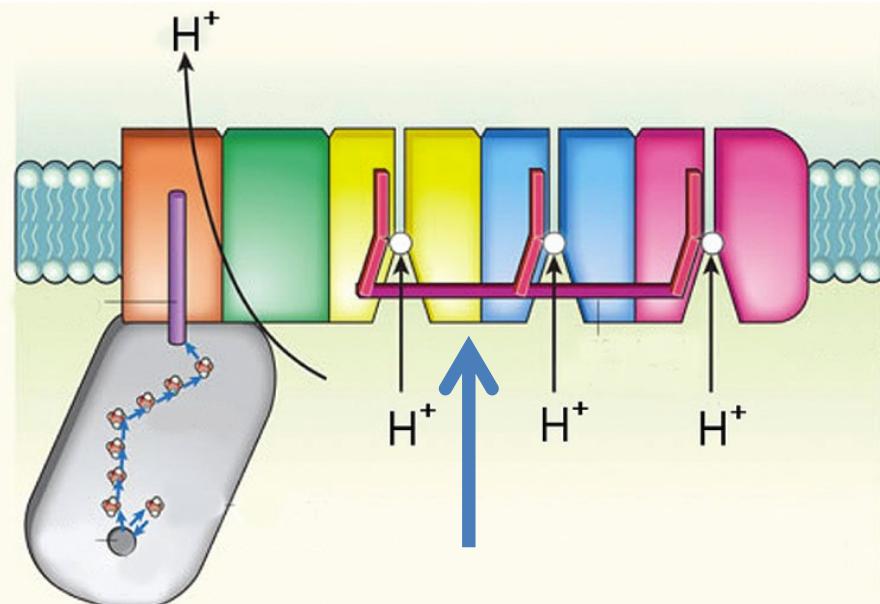


- Mobile e⁻ carrier
- Hydrophobic isoprenoid tail wriggles into hydrophobic interior of inner mitochondrial membrane
- Shuttles e⁻ from complex I and II to complex III

Conformational changes allow bound protons to cross the membrane



e⁻ transfer from
NADH through Fe-
S clusters to CoQ

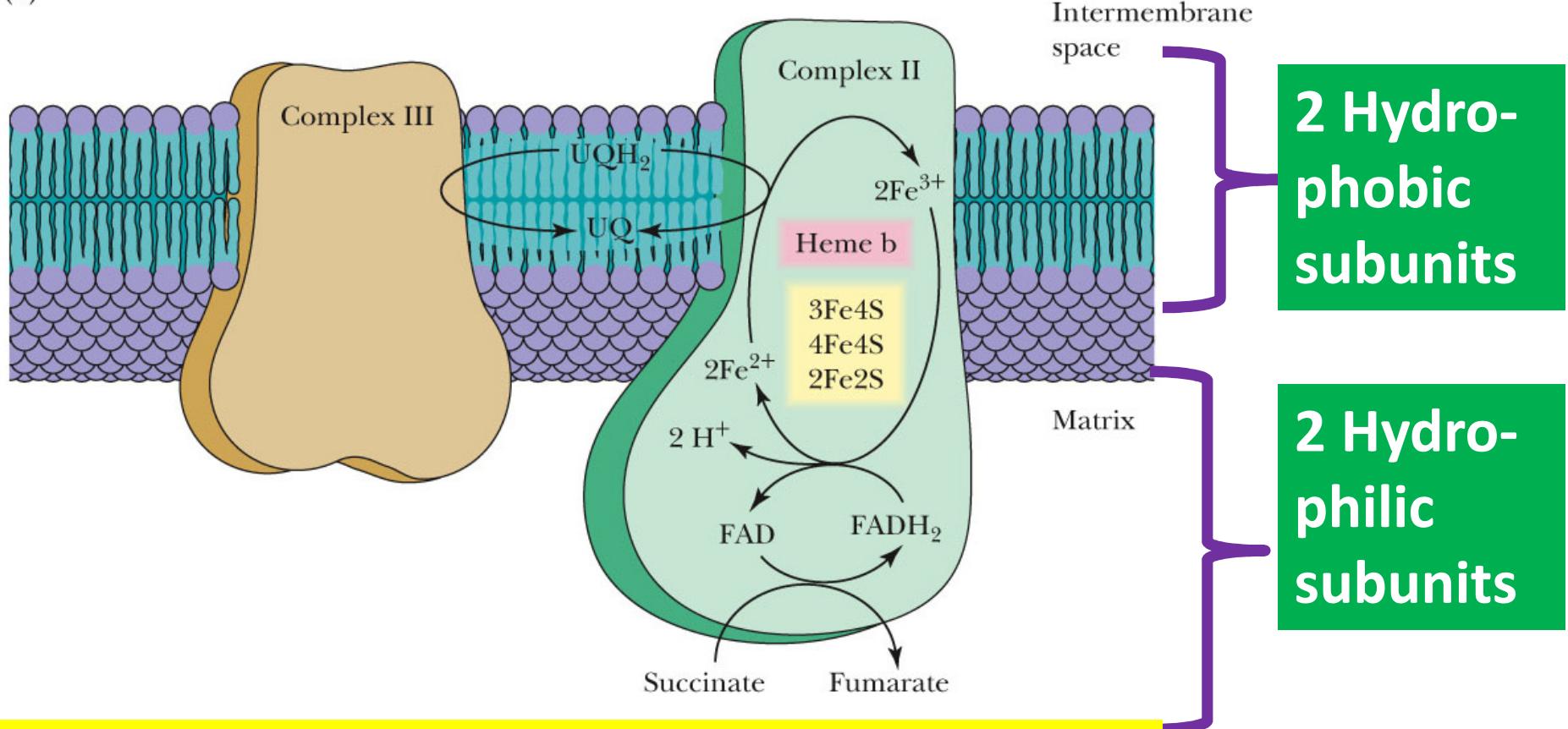


Long parallel helix functions as a rod to open and close the H^+ channels

Complex II oxidises succinate and reduces CoQ

Succinate-UQ oxidoreductase (succinate dehydrogenase)

(a)



Electrons: succinate \rightarrow FADH₂ \rightarrow Fe-S \rightarrow UQ

$\text{UQH}\cdot + \text{H}^+ + e^- \longrightarrow \text{UQH}_2$ (UQ = coenzyme Q)	0.190
$\text{UQ} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{UQH}_2$	0.060
$\text{Cytochrome } b_H(\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } b_H(\text{Fe}^{2+})$	0.050
$\text{Fumarate} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{succinate}$	0.031
$\text{UQ} + \text{H}^+ + e^- \longrightarrow \text{UQH}\cdot$	0.030
$\text{Cytochrome } b_5(\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } b_5(\text{Fe}^{2+})$	0.020
$[\text{FAD}] + 2 \text{H}^+ + 2 e^- \longrightarrow [\text{FADH}_2]$	0.003–0.091*
$\text{Cytochrome } b_L(\text{Fe}^{3+}) + e^- \longrightarrow \text{cytochrome } b_L(\text{Fe}^{2+})$	-0.100
$\text{Oxaloacetate} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{malate}$	-0.166
$\text{Pyruvate} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{lactate}$	-0.185
$\text{Acetaldehyde} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{ethanol}$	-0.197
$\text{FMN} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{FMNH}_2$	-0.219
$\text{FAD} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{FADH}_2$	-0.219
$\text{Glutathione (oxidized)} + 2 \text{H}^+ + 2 e^- \longrightarrow 2 \text{ glutathione (reduced)}$	-0.230
$\text{Lipoic acid} + 2 \text{H}^+ + 2 e^- \longrightarrow \text{dihydrolipoic acid}$	-0.290
$1,3\text{-Bisphosphoglycerate} + 2 \text{H}^+ + 2 e^- \longrightarrow$ $\text{glyceraldehyde-3-phosphate} + \text{P}_i$	-0.290
$\text{NAD}^+ + 2 \text{H}^+ + 2 e^- \longrightarrow \text{NADH} + \text{H}^+$	-0.320
$\text{NADP}^+ + 2 \text{H}^+ + 2 e^- \longrightarrow \text{NADPH} + \text{H}^+$	-0.320

Complex III mediates electron transport from CoQ to Cyt c

- UQ-cytochrome c oxidoreductase
- 3 different cytochromes (haemes)
- 1 Fe-S protein

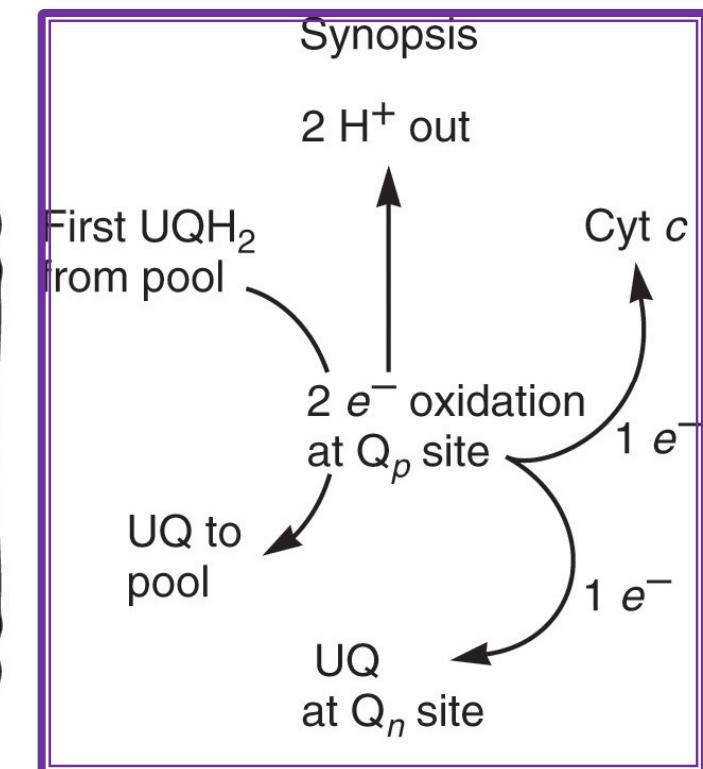
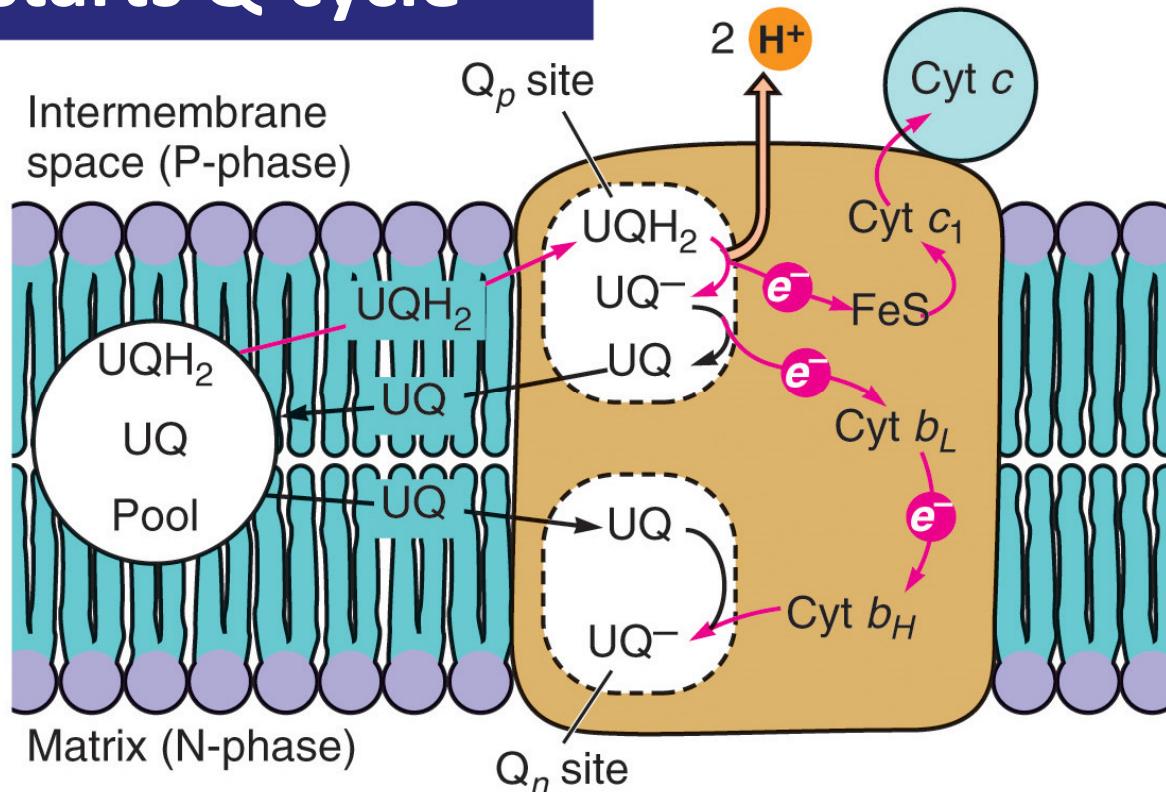
Electrons: $\text{UQH}_2 \rightarrow \text{Cyt c}$

- The Q-cycle is accompanied by **proton transfer** across inner mitochondrial membrane
- **Q_p** site on cytosolic side binds UQH₂ and releases UQ
- **Q_n** site on matrix side of complex III binds UQ and releases UQH₂

The Q-cycle: 1st half

UQH_2 to Q_p site:
starts Q-cycle

UQH_2 oxidised in 2 steps

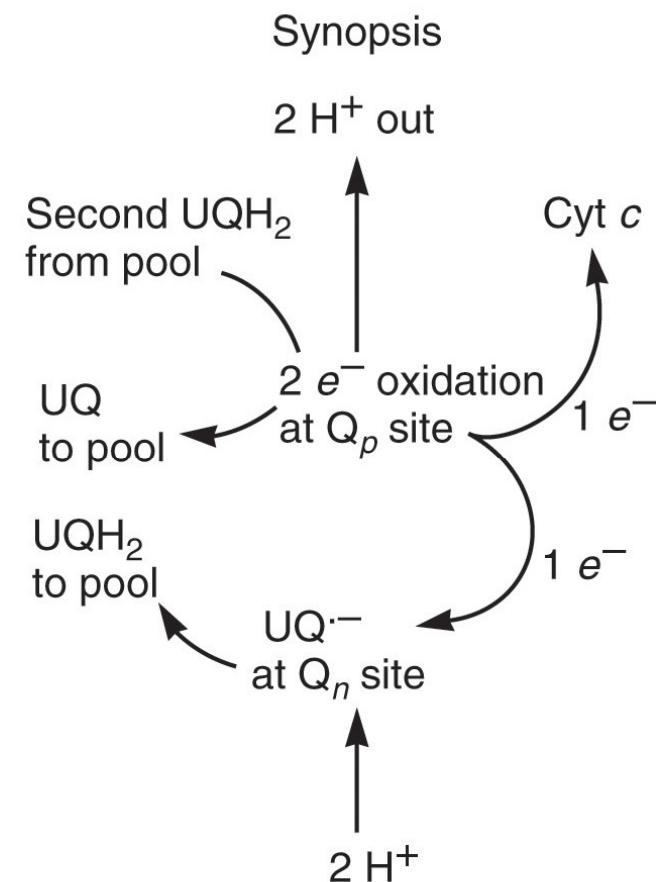
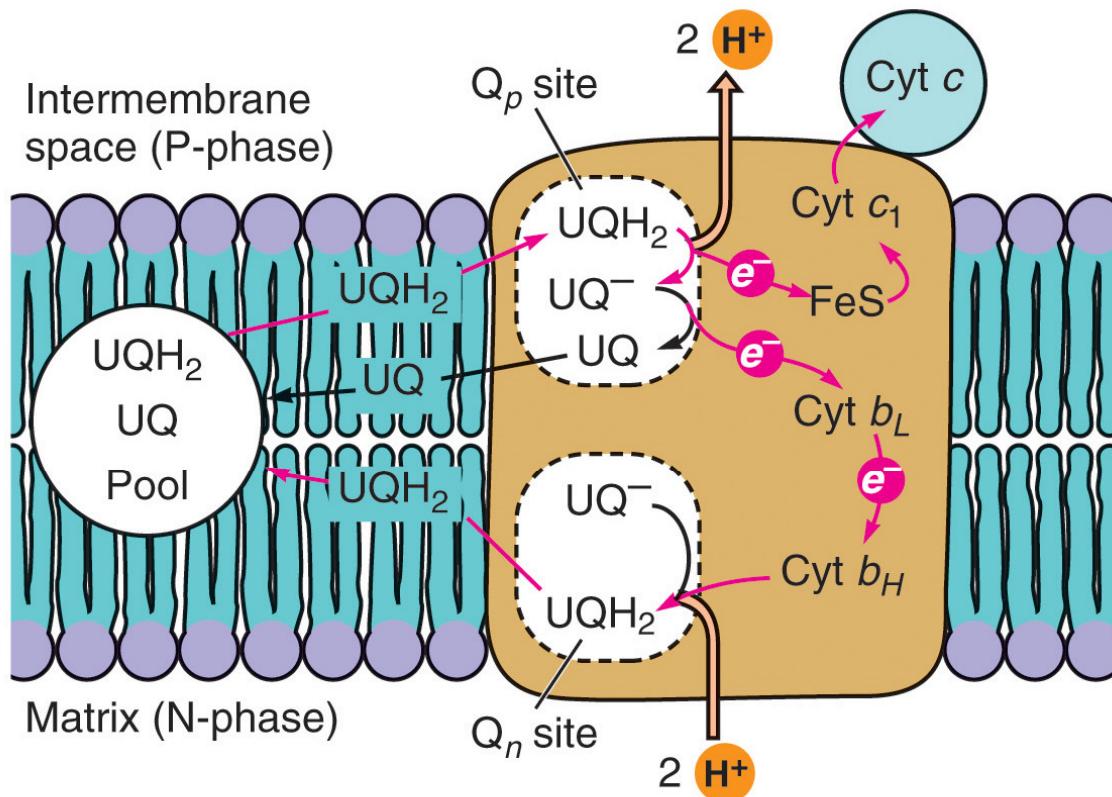


The Q-cycle: 2nd half

$1 e^-$ to Cyt c + $2 H^+$ to cytoplasm

$1 e^-$ to UQ⁻ → completely reduced UQH₂

$2 H^+$ taken up from matrix

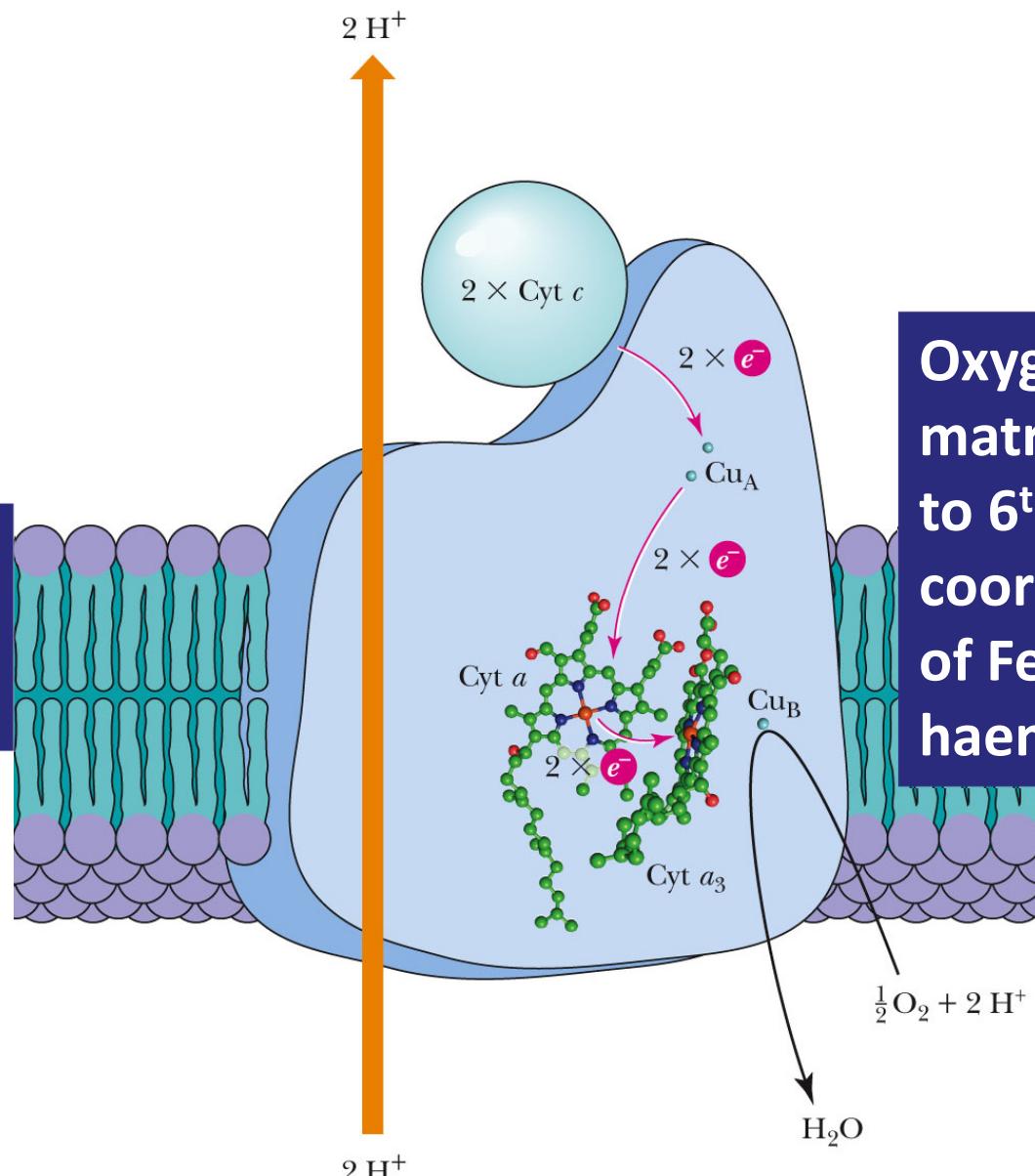


Write down the net reaction for the Q-cycle and indicate from which half of the cycle each of the components were derived:

Complex IV transfers electrons from Cytc to oxygen

- Cytochrome *c* oxidase
- 2 different cytochromes
- 3 copper ions
- Accepts e⁻ from Cytc and directs them to oxygen to form water
- 4 e⁻ reduction $4 \text{ Cytc}(\text{Fe}^{2+}) + 4 \text{ H}^+ + \text{O}_2 \rightarrow 4 \text{ Cytc}(\text{Fe}^{3+}) + 2 \text{ H}_2\text{O}$
- 4 **protons transferred** across the inner mitochondrial membrane

Proton channels transfer protons via proton-jump



Oxygen from matrix side binds to 6th coordination site of Fe atom in the haeme of cyta₃

Cytc → Cu_A → cyt a → Cu_B /cyt a₃ → O₂

Model for electron transport pathway in inner mitochondrial membrane

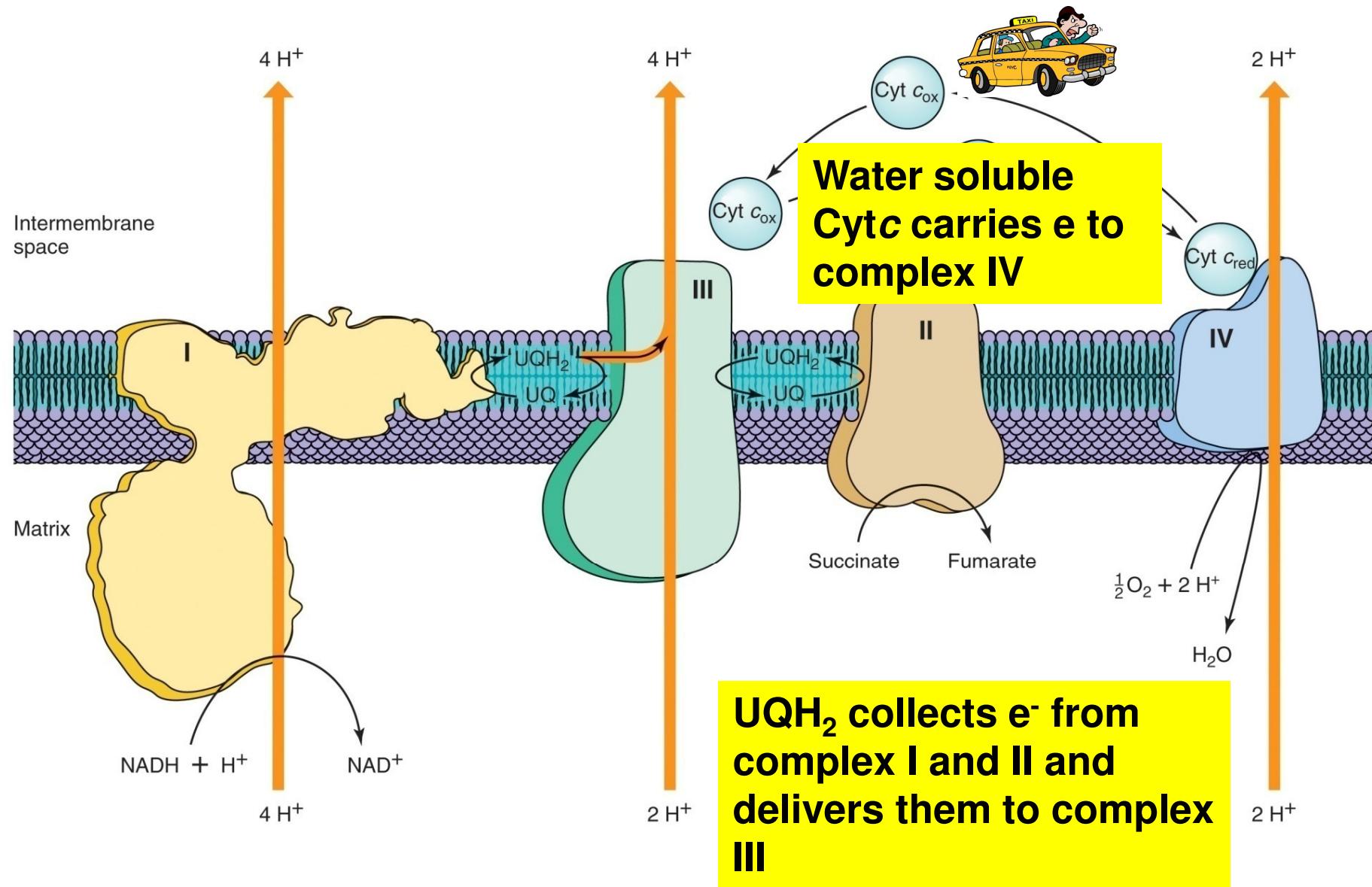


TABLE 20.2 Protein Complexes of the Mitochondrial Electron-Transport Chain

Complex	Mass (kD)	Subunits	Prosthetic Group	Binding Site for:
NADH-UQ reductase	980	≥45	FMN Fe-S	NADH (matrix side) UQ (lipid core)
Succinate-UQ reductase	140	4	FAD Fe-S	Succinate (matrix side) UQ (lipid core)
UQ-Cyt <i>c</i> reductase	250	9–10	Heme <i>b</i> _L Heme <i>b</i> _H Heme <i>c</i> ₁ Fe-S	Cyt <i>c</i> (intermembrane space side)
Cytochrome <i>c</i>	13	1	Heme <i>c</i>	Cyt <i>c</i> ₁ Cyt <i>a</i>
Cytochrome <i>c</i> oxidase	162	13	Heme <i>a</i> Heme <i>a</i> ₃ Cu _A Cu _B	Cyt <i>c</i> (intermembrane space side)

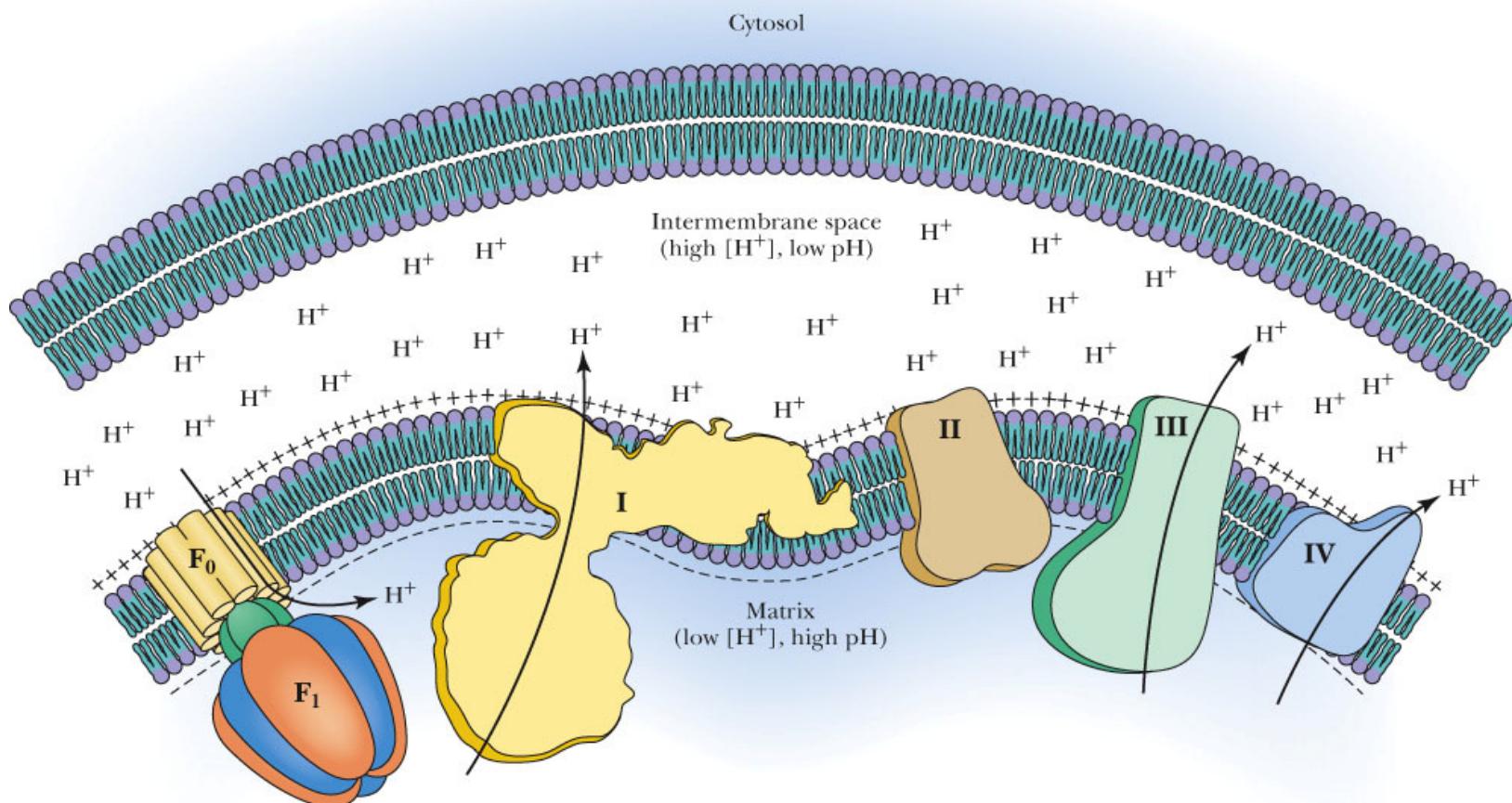
The Mitchell hypothesis

Energy from e^- transfer is stored in the proton gradient

- Protons driven across membrane:
- What happens to the pH in the matrix?
- Both **electrochemical** and **pH** gradient across membrane

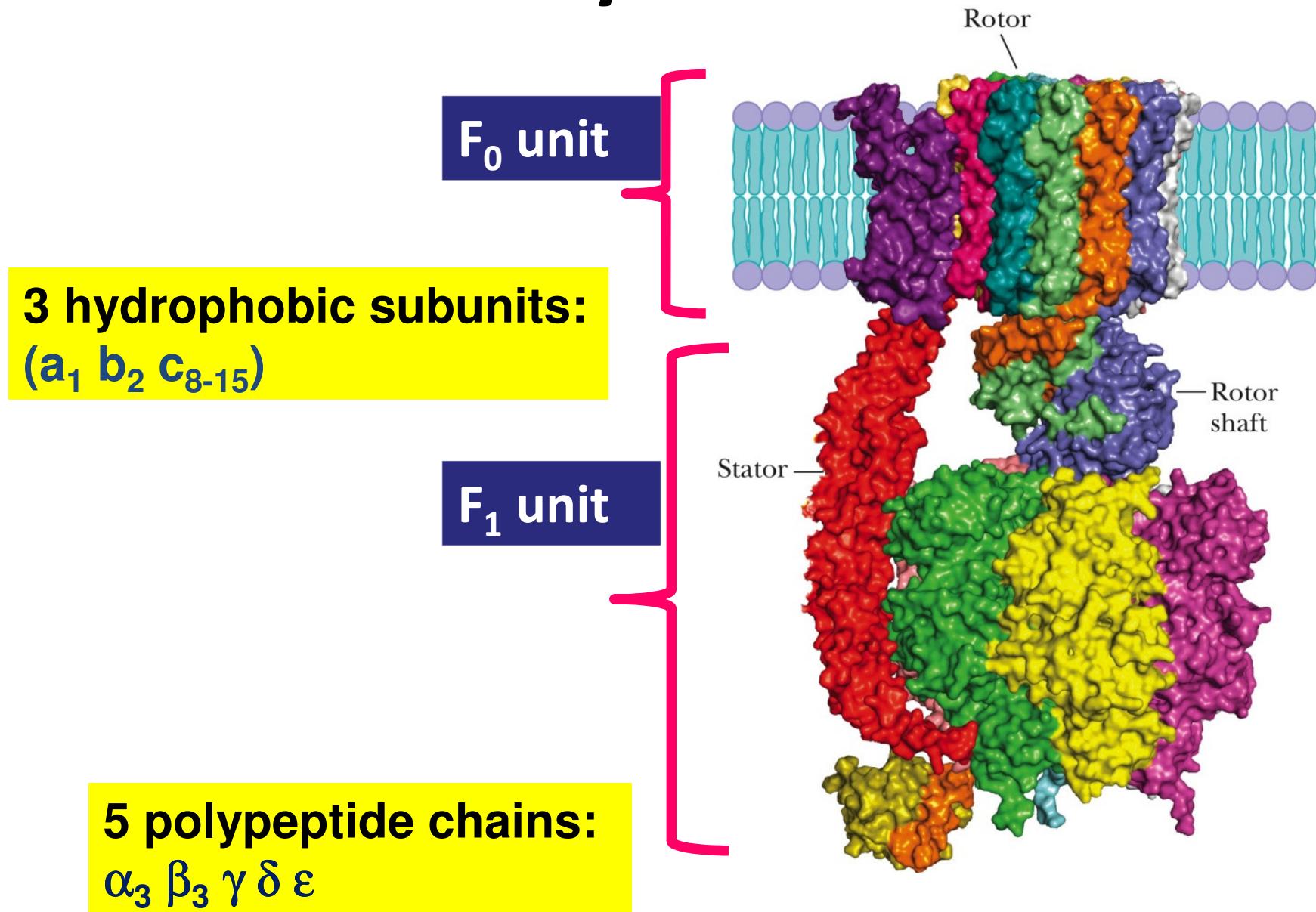
**Write down why both the
electrochemical and the pH
gradient will tend to cause protons
to move back into the matrix**

Proton & electrochemical gradients across inner mitochondrial membrane



How does the proton gradient drive ATP synthesis?

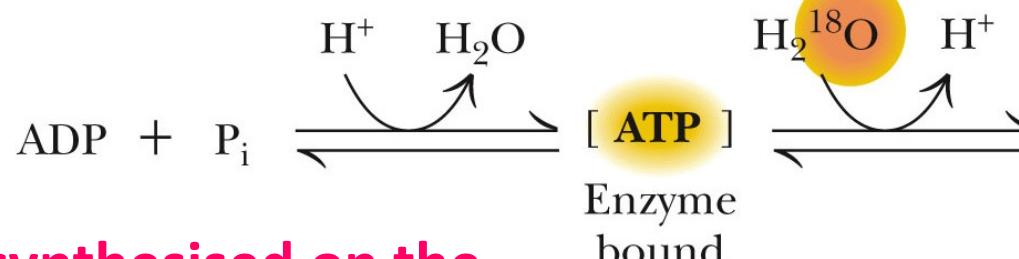
ATP synthase



Mechanism of ATP synthesis

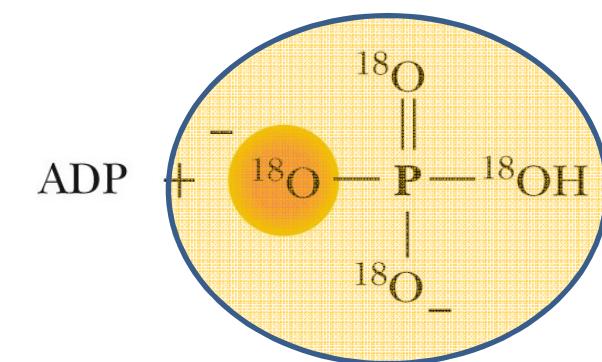
- ^{18}O exchange experiment by Boyer:

Labelled water oxygen
incorporated into phosphate upon
(protein-bound) ATP hydrolysis



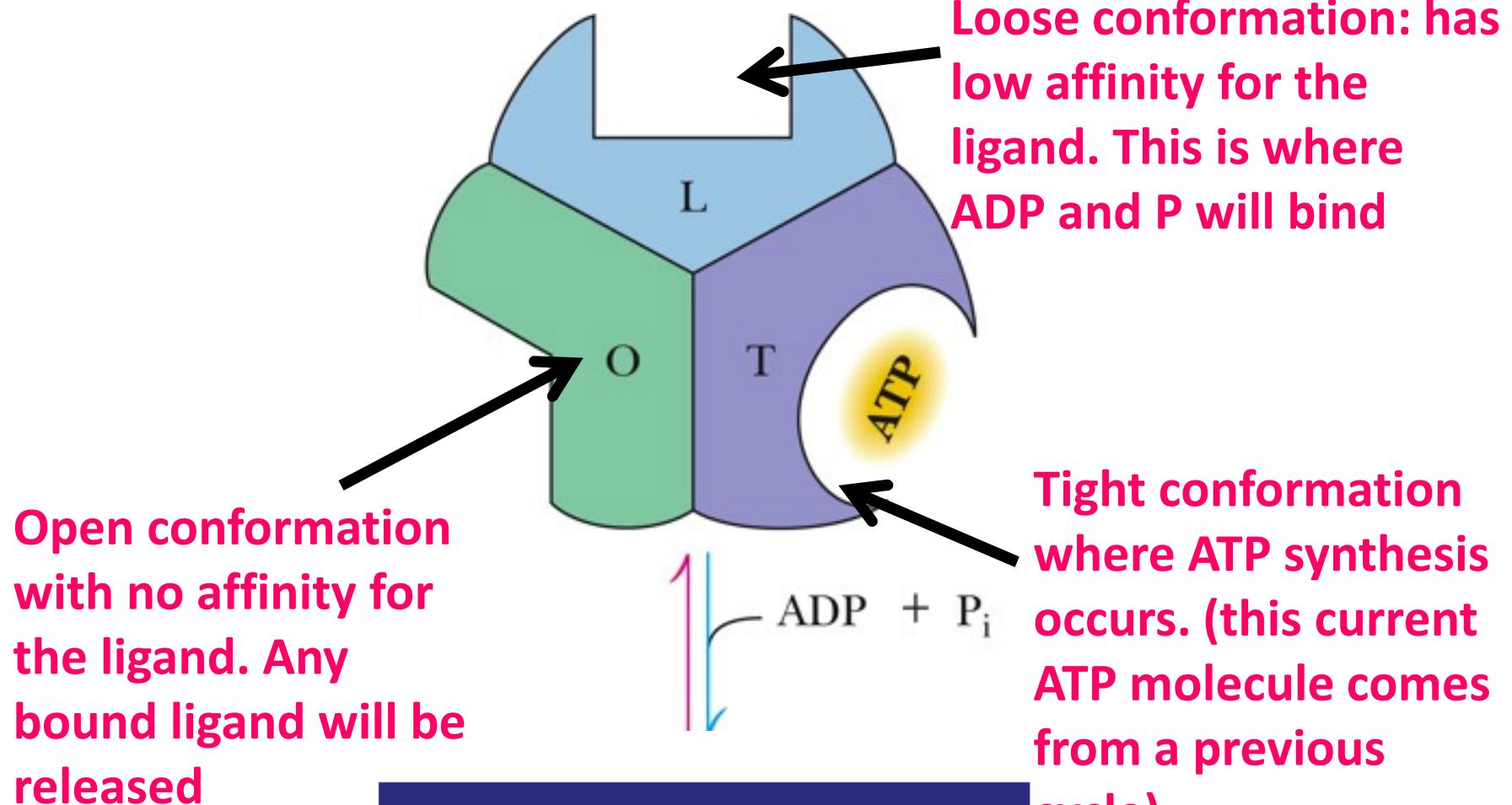
ATP synthesised on the
enzyme from ADP and
phosphate

This happens in the absence
of a proton gradient



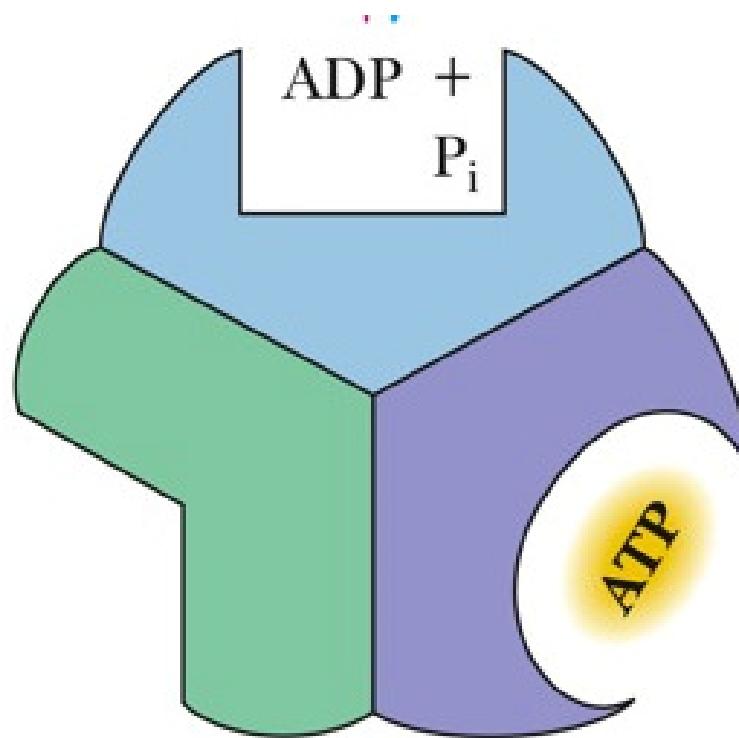
Label incorporated into
phosphate easily \therefore ATP
can be formed on the
enzyme

Binding change mechanism for ATP synthesis



Step 1: ADP and P_i bind to L conformation

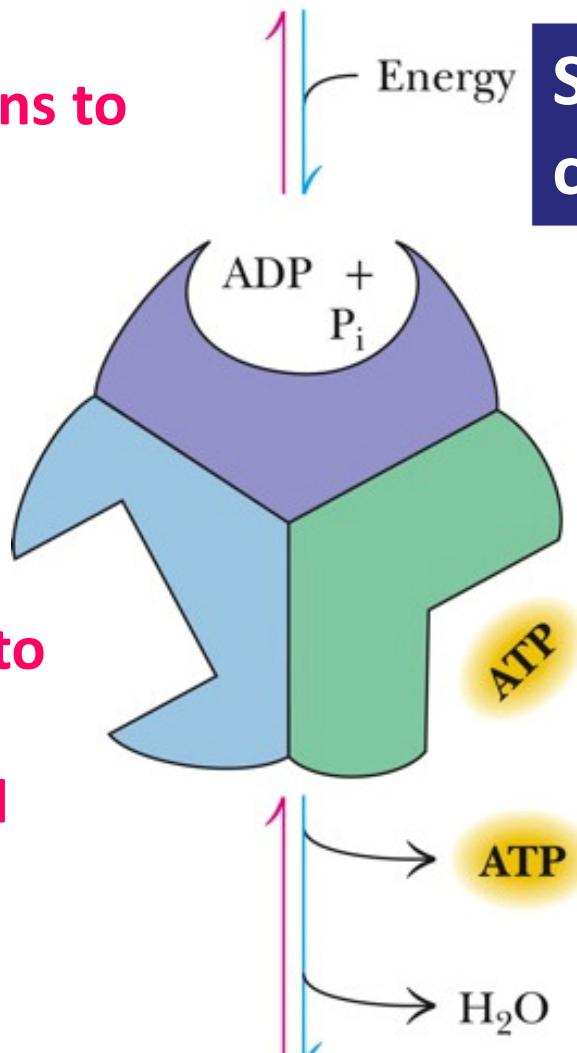
Binding change mechanism for ATP synthesis



Binding change mechanism for ATP synthesis

L conformation turns to T conformation

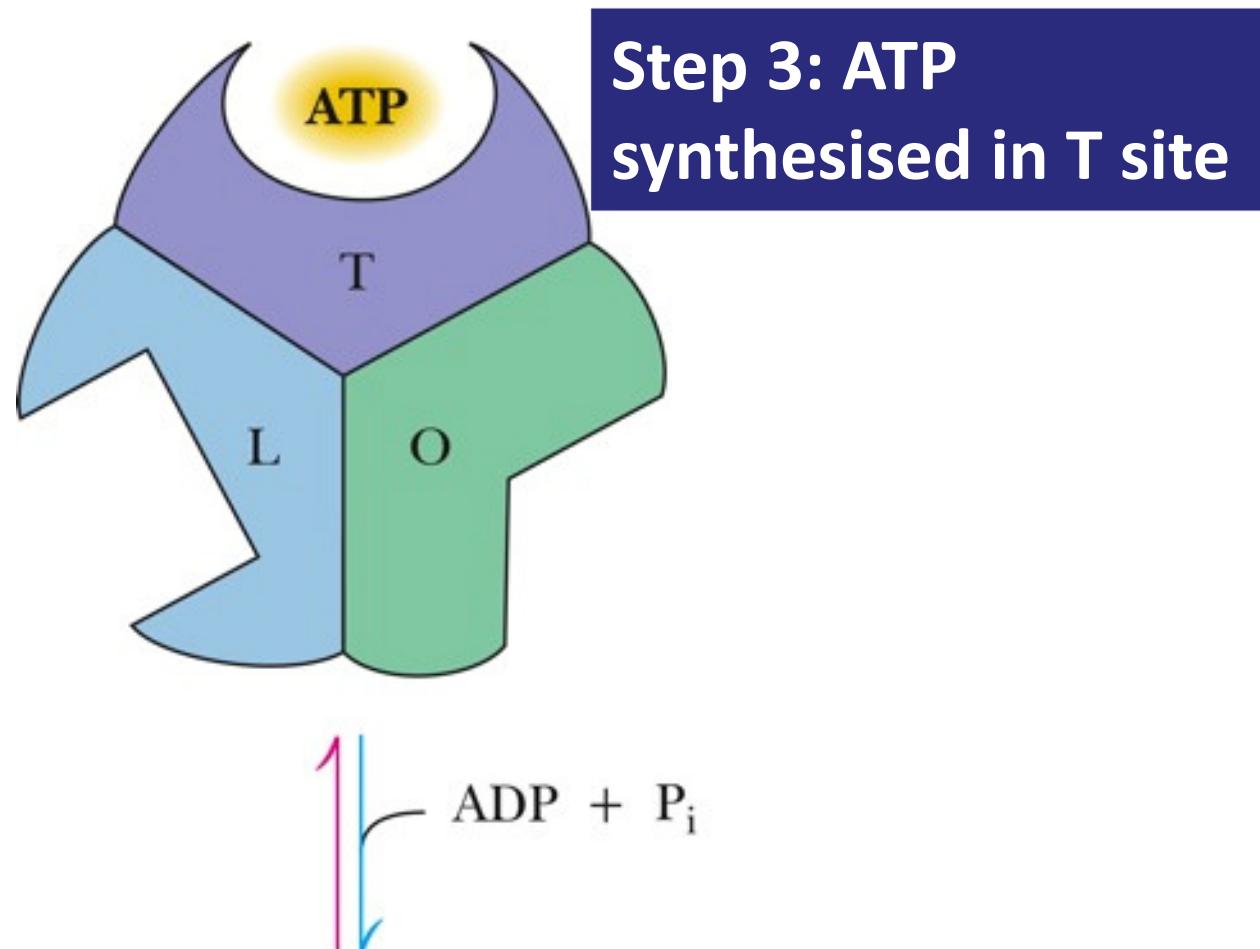
O conformation turns to L conformation and becomes ready to bind ADP



Step 2: Conformational change to all 3 subunits

T conformation turns to O conformation and releases previously bound ATP

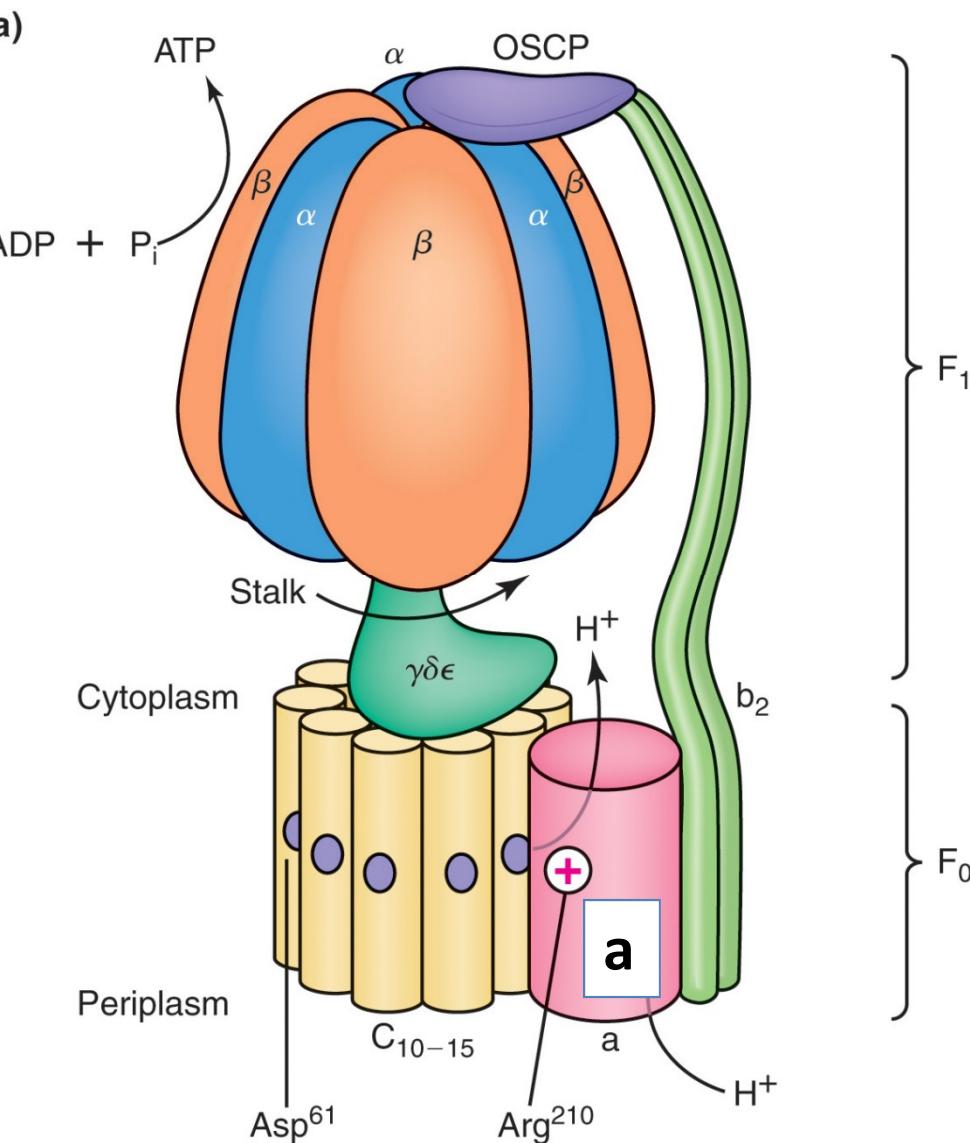
Binding change mechanism for ATP synthesis



The cycle repeats

ATP synthase mechanism of action

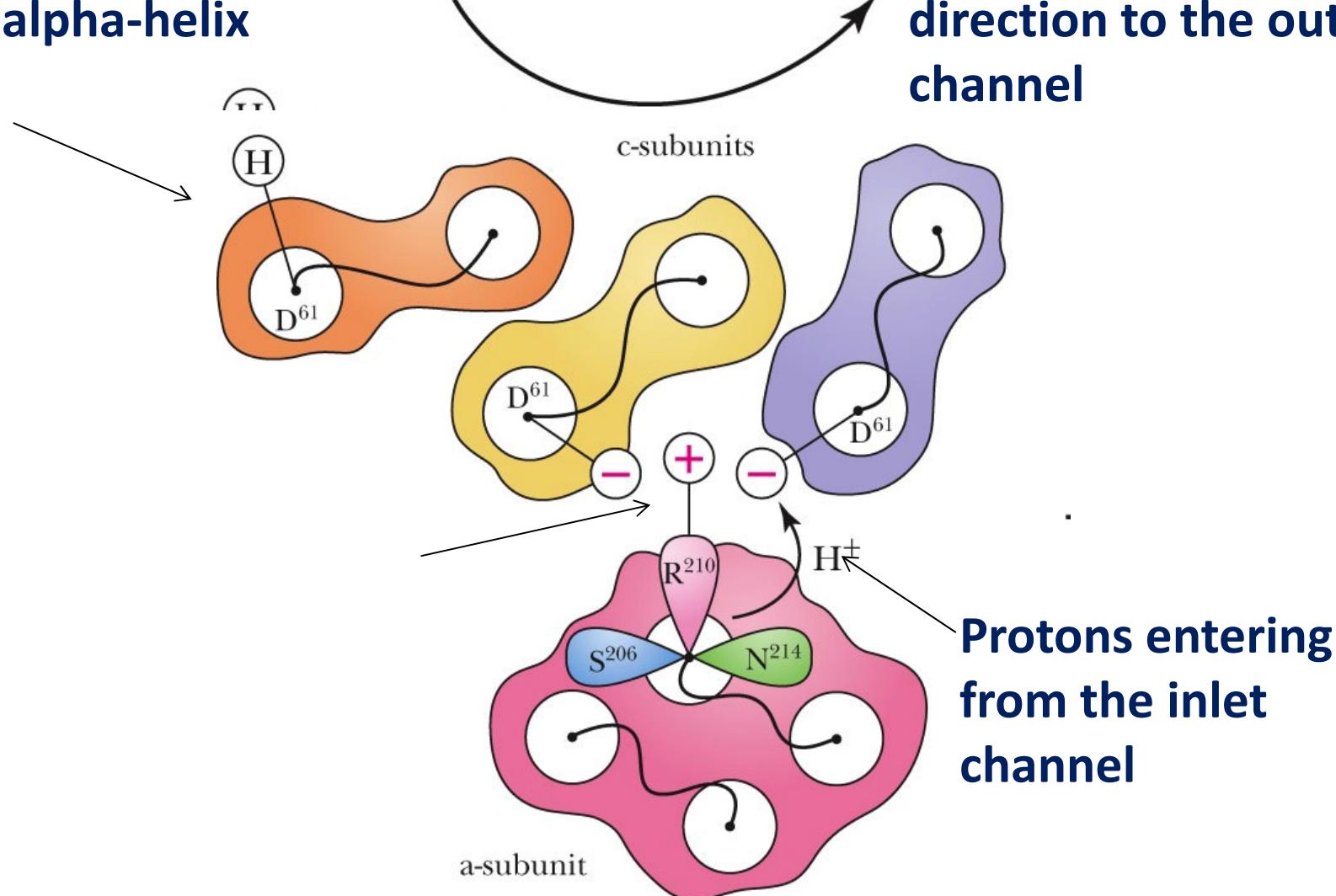
- **a subunit** – 5 hydrophobic helices
- 2 half channels



Bound proton is buried via clockwise rotation the alpha-helix

Each c-ring remains protonated when in the membrane

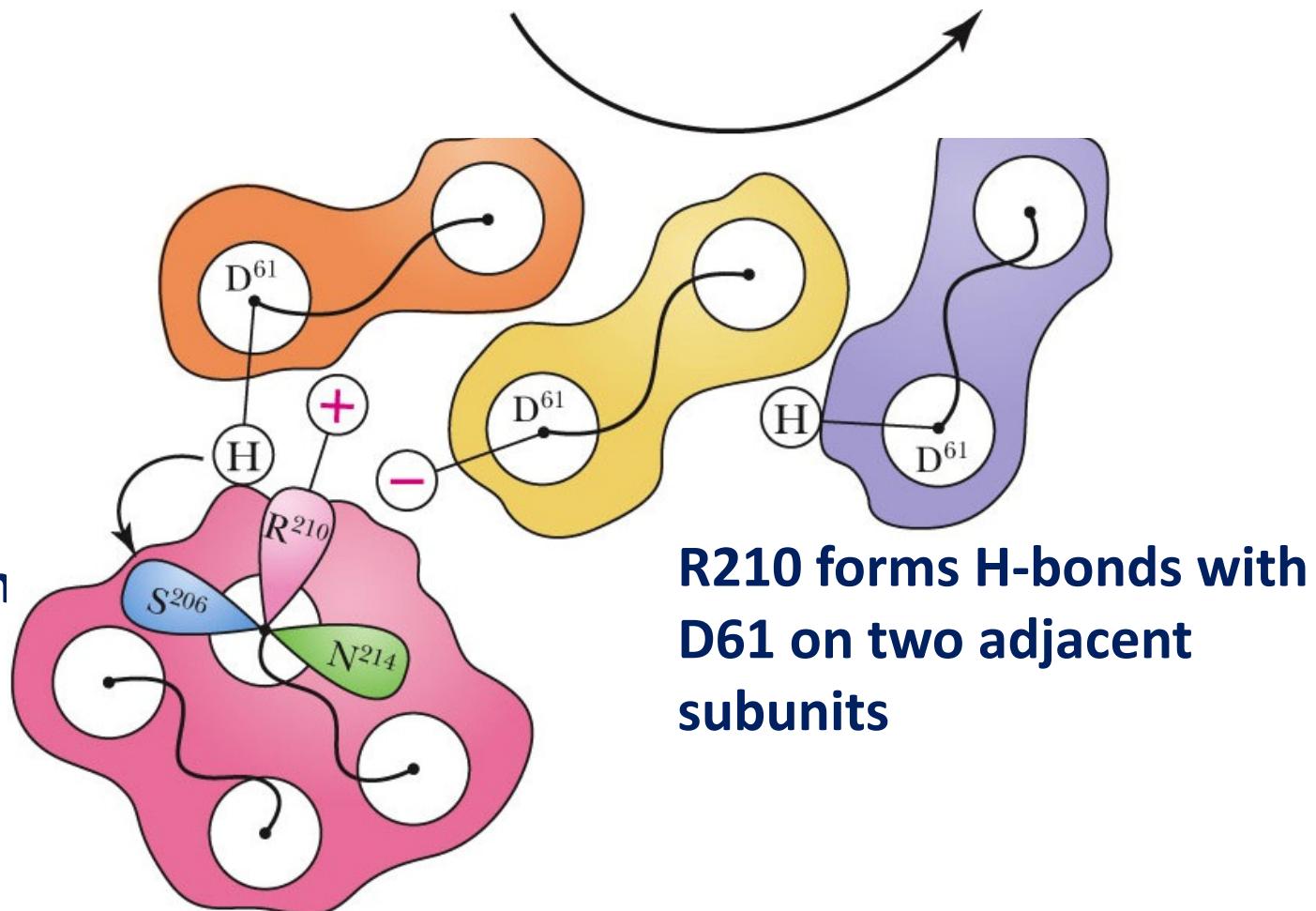
Protons ride the c subunit rotor round the ring in an anticlockwise direction to the outlet channel



Protons entering from the inlet channel

R210 promotes the transfer of the entering protons from the a-subunit N214 to the c-subunit D61 and the transfer of the exiting protons from D61 to S206

Inlet ends in N214 and outlet ends in S206

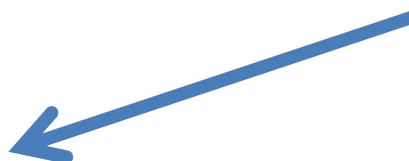


How are the electrons of cytosolic NADH fed into the ETC?

- 2 NADH per glucose produced in the **cytosol** via glyceraldehyde 3-P dehydrogenase
- NADH must be oxidised to regenerate NAD^+
- Shuttle systems

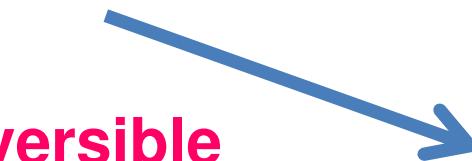
Irreversible

Glycerophosphate

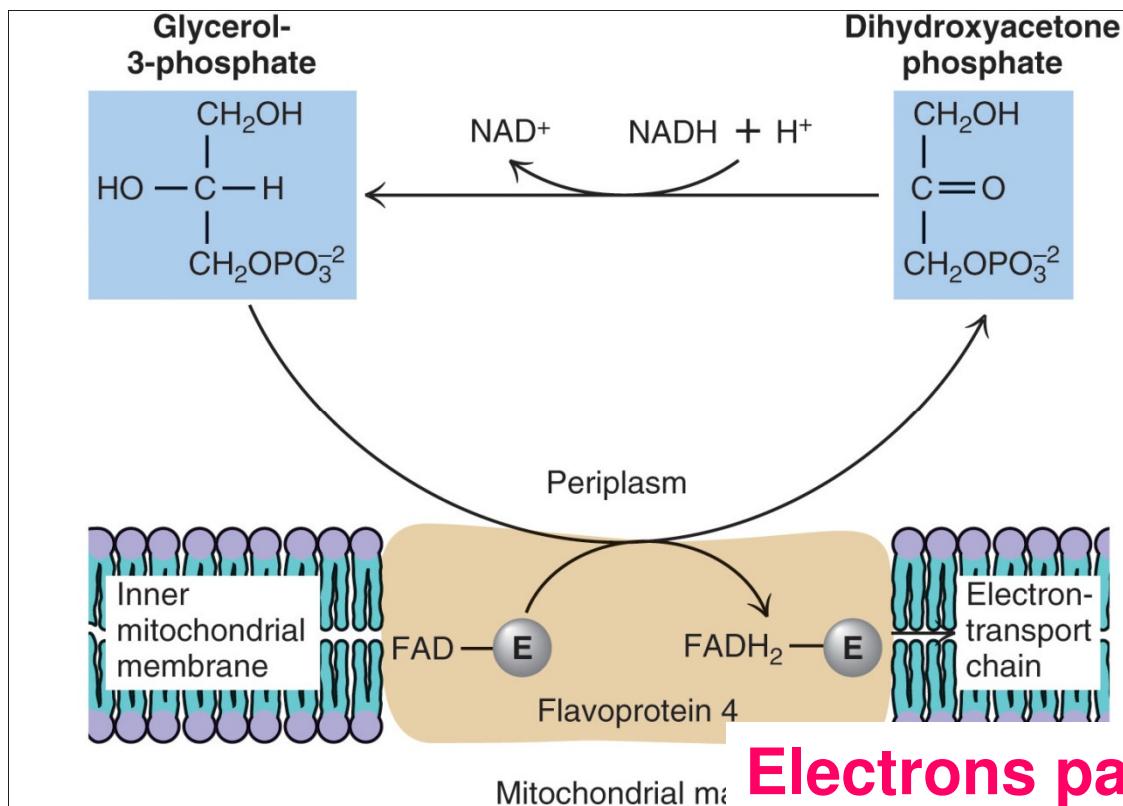


Reversible

Malate-Aspartate



Glycerophosphate shuttle



Electrons pass directly to
UQ → UQH₂ in ETC

Malate-Aspartate shuttle

