Lecture 5 - Oxidative Phosphorylation

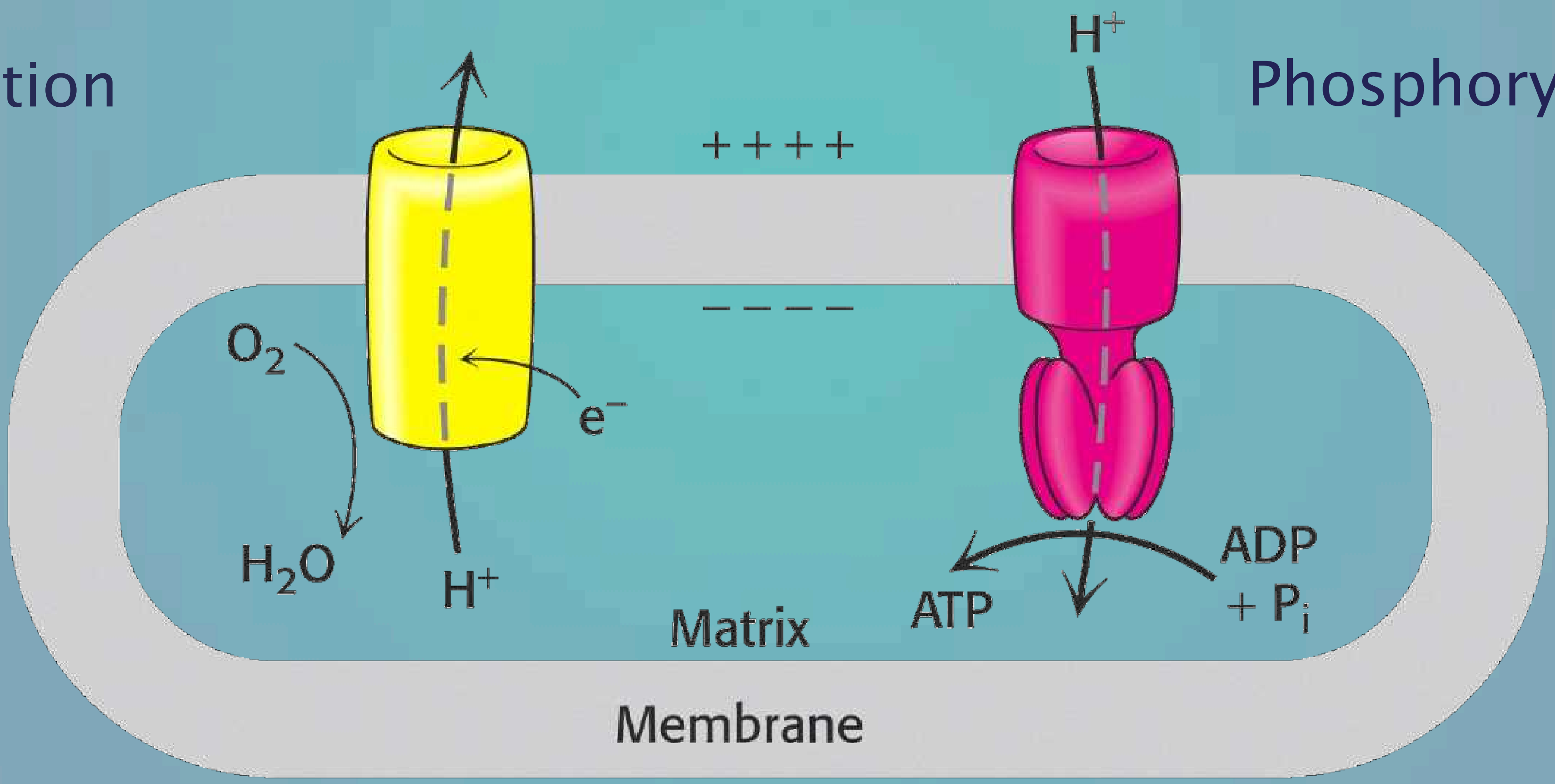
Chem 454: Regulatory Mechanisms in Biochemistry
University of Wisconsin-Eau Claire

Introduction

Oxidation and Phosphorylation are coupled by transmembrane proton fluxes:

Oxidation

Phosphorylation



1. Mitochondria

Oval-shaped organelles

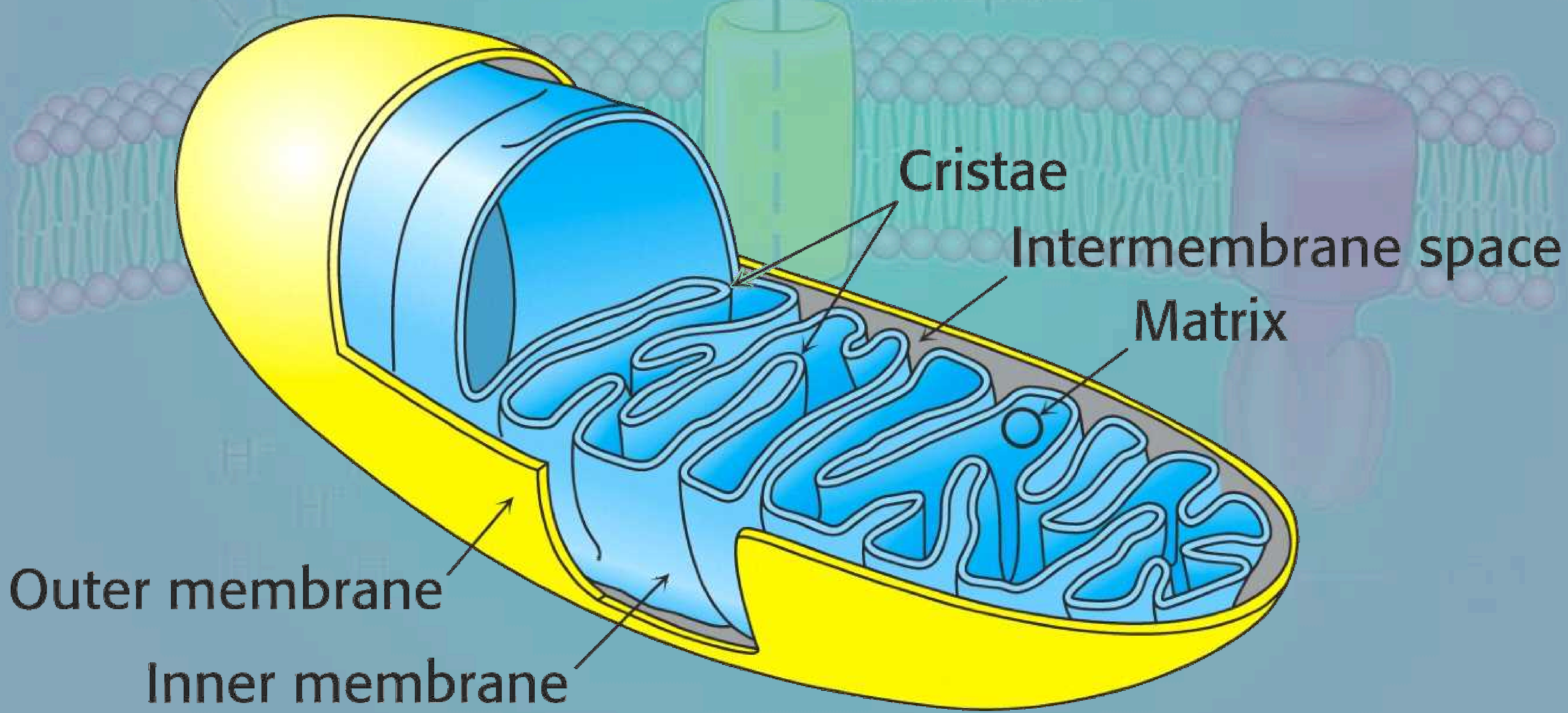
- 0.5 μm X 2 μm

Contain

- Citric acid cycle enzymes
- Fatty acid oxidation enzymes
- respiratory assembly

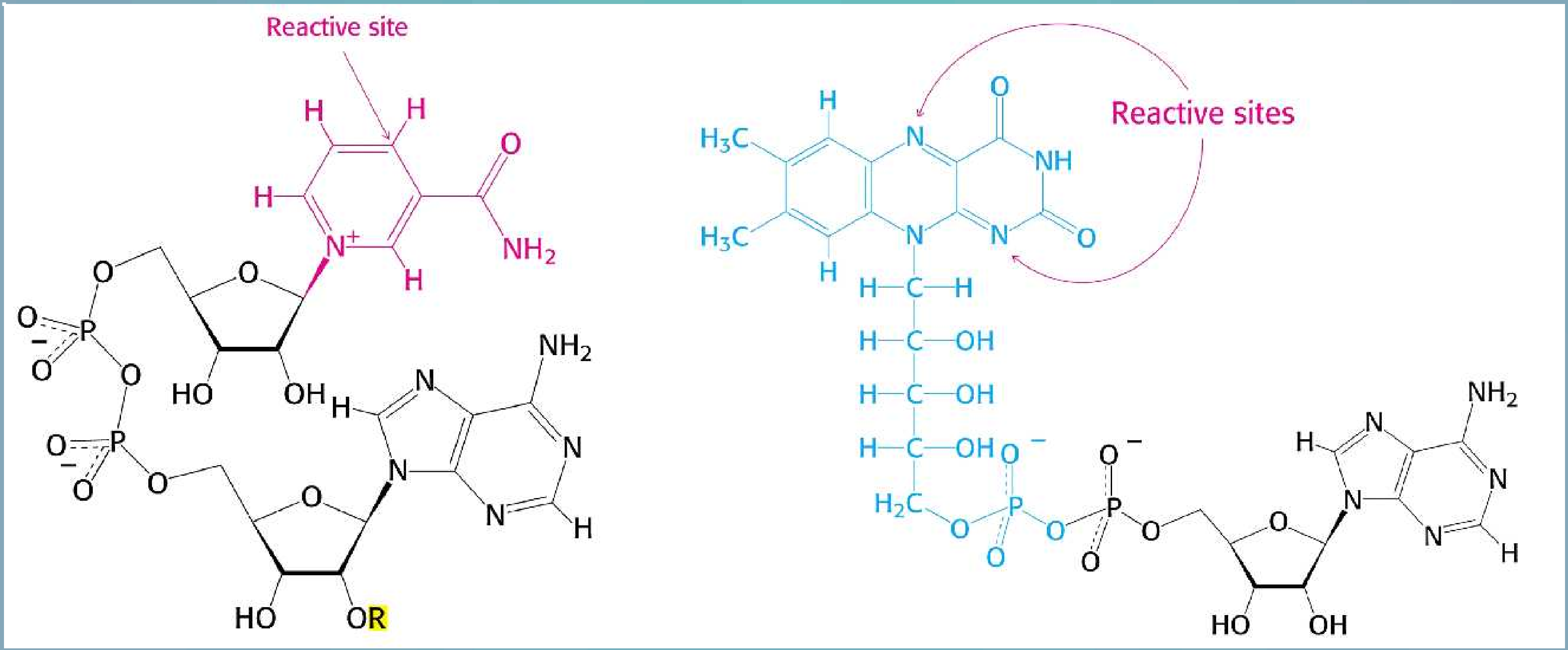
1.1 Structure of Mitochondria

Mitochondria are bound by a double membrane



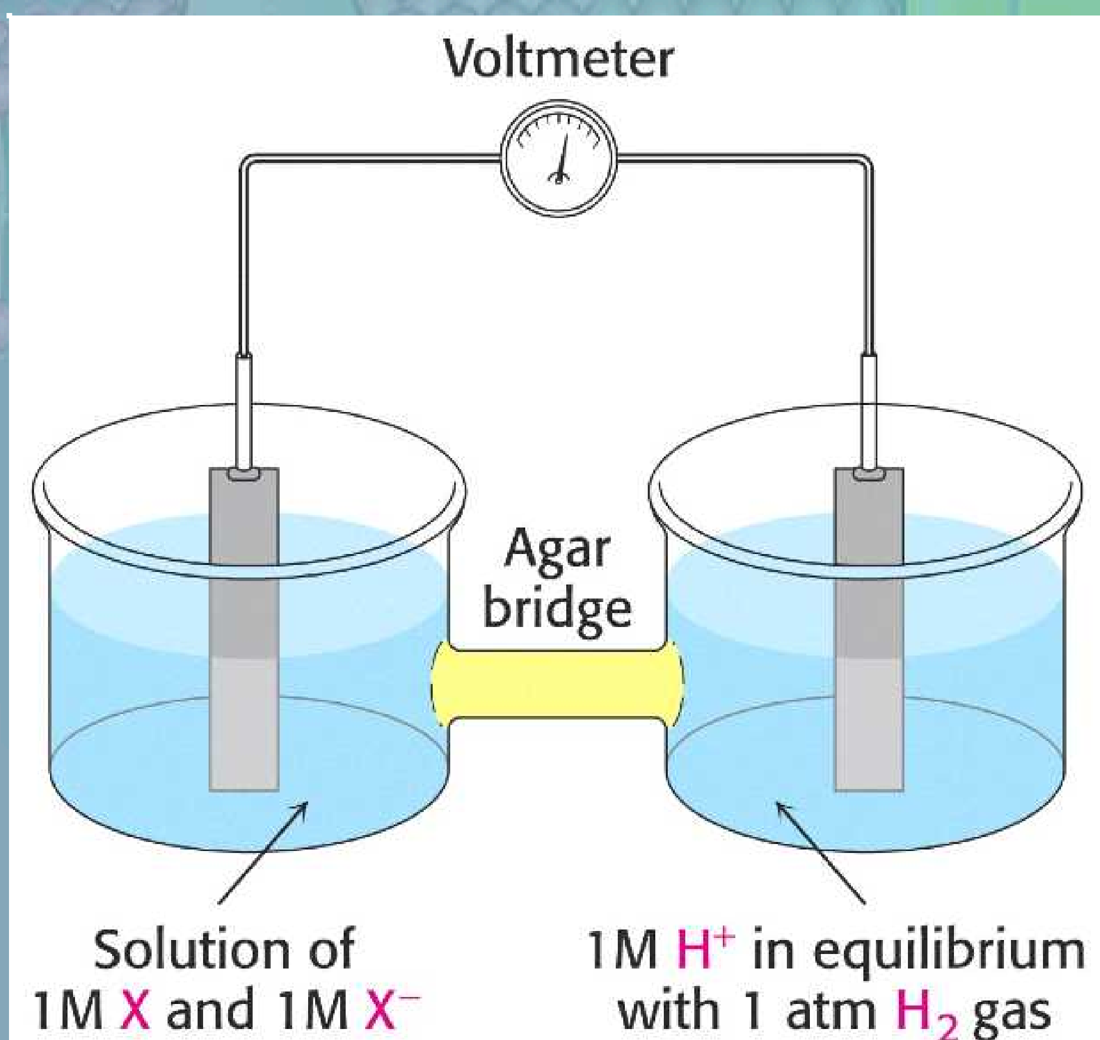
2. Electron Transfer

The reoxidation of NADH and FADH2 by molecular oxygen is highly exergonic.



2.1 High Energy Electrons

The ability of a substance to participate in an oxidation/reduction reaction is measured by its reduction potential.



$$\Delta G^{0'} = -n\mathcal{F}\,\Delta E'_{o}$$

2.1 Standard Reduction Potentials

TABLE 18.1 Standard reduction potentials of some reactions

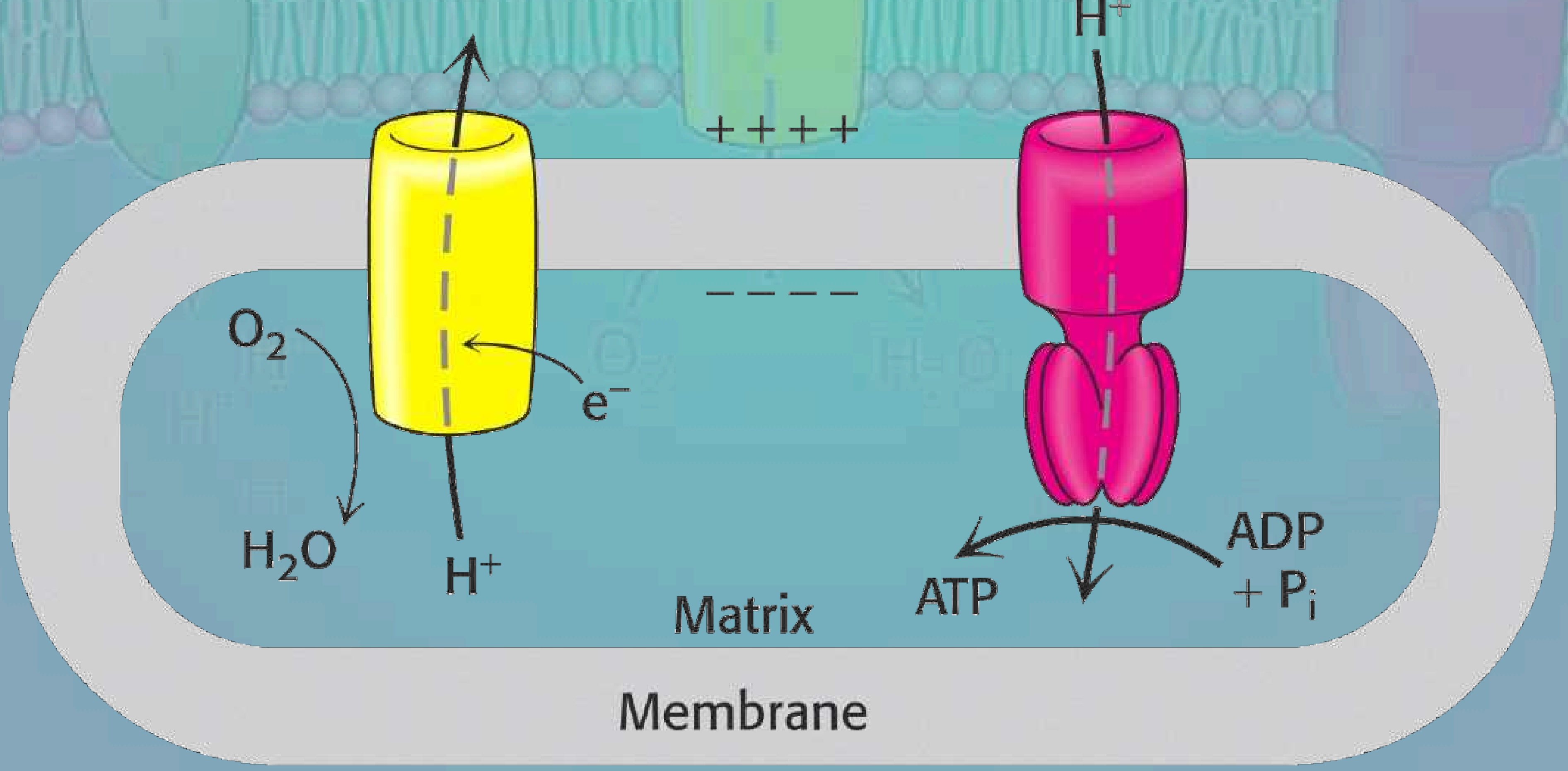
Oxidant	Reductant	*n*	E'_0 (V)
Succinate + CO_2	α-Ketoglutarate	2	−0.67
Acetate	Acetaldehyde	2	−0.60
Ferredoxin (oxidized)	Ferredoxin (reduced)	1	−0.43
2 H^+	H_2	2	−0.42
NAD^+	NADH + H^+	2	−0.32
$NADP^+$	NADPH + H^+	2	−0.32
Lipoate (oxidized)	Lipoate (reduced)	2	−0.29
Glutathione (oxidized)	Glutathione (reduced)	2	−0.23
FAD	$FADH_2$	2	−0.22
Acetaldehyde	Ethanol	2	−0.20
Pyruvate	Lactate	2	−0.19
Fumarate	Succinate	2	0.03
Cytochrome *b* (+3)	Cytochrome *b* (+2)	1	0.07
Dehydroascorbate	Ascorbate	2	0.08
Ubiquinone (oxidized)	Ubiquinone (reduced)	2	0.10
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	0.22
Fe (+3)	Fe (+2)	1	0.77
$\frac{1}{2}O_2 + 2H^+$	H_2O	2	0.82

Note: E'_0 is the standard oxidation–reduction potential (pH 7, 25°C) and *n* is the number of electrons transferred. E'_0 refers to the partial reaction written as

$$\text{Oxidant} + e^- \longrightarrow \text{reductant}$$

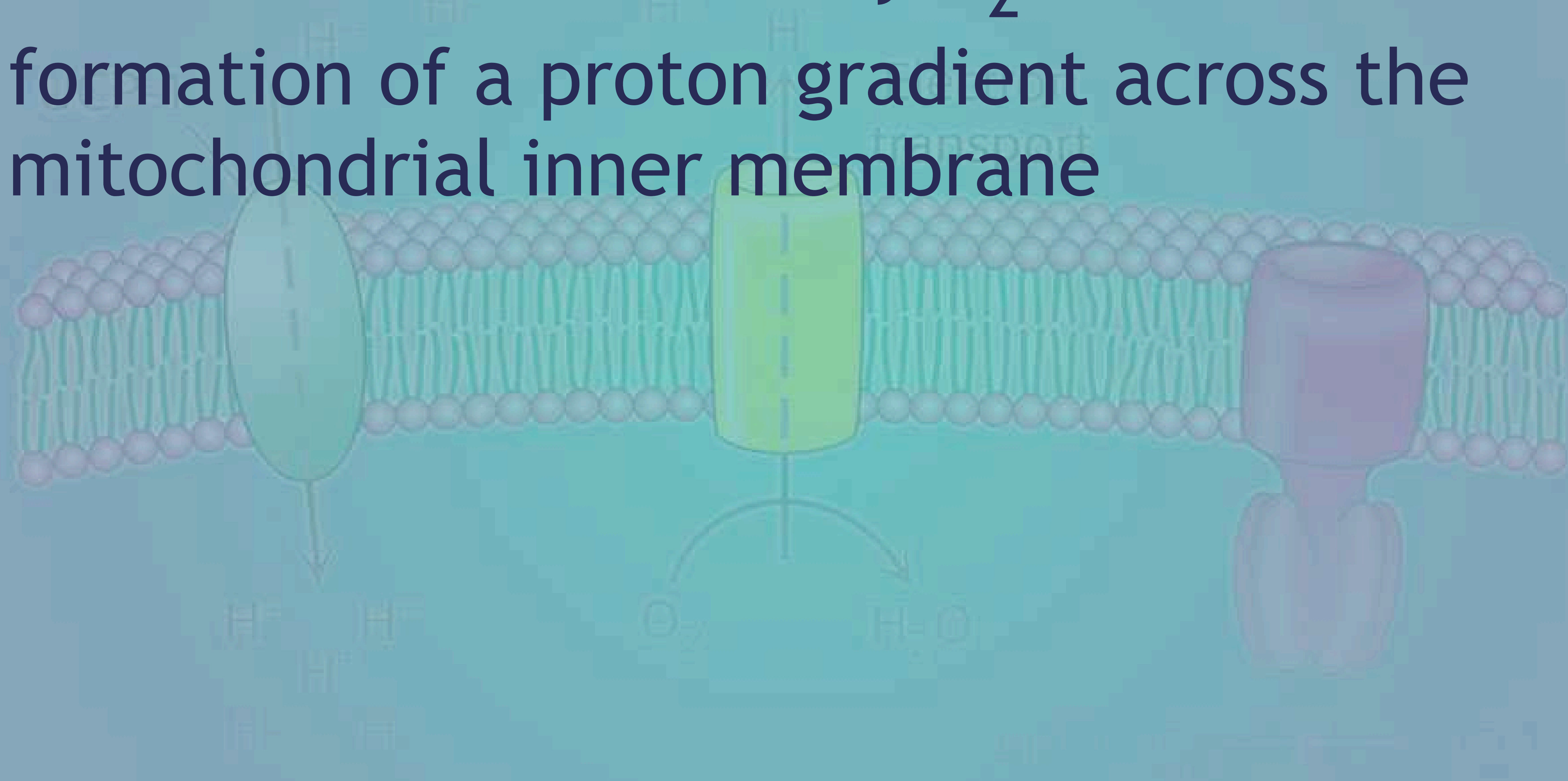
Problem

Using the data given in Table 18.1, calculate the standard free energy change for transferring 2 electrons from NADH to $1/2O_2$ to form water.



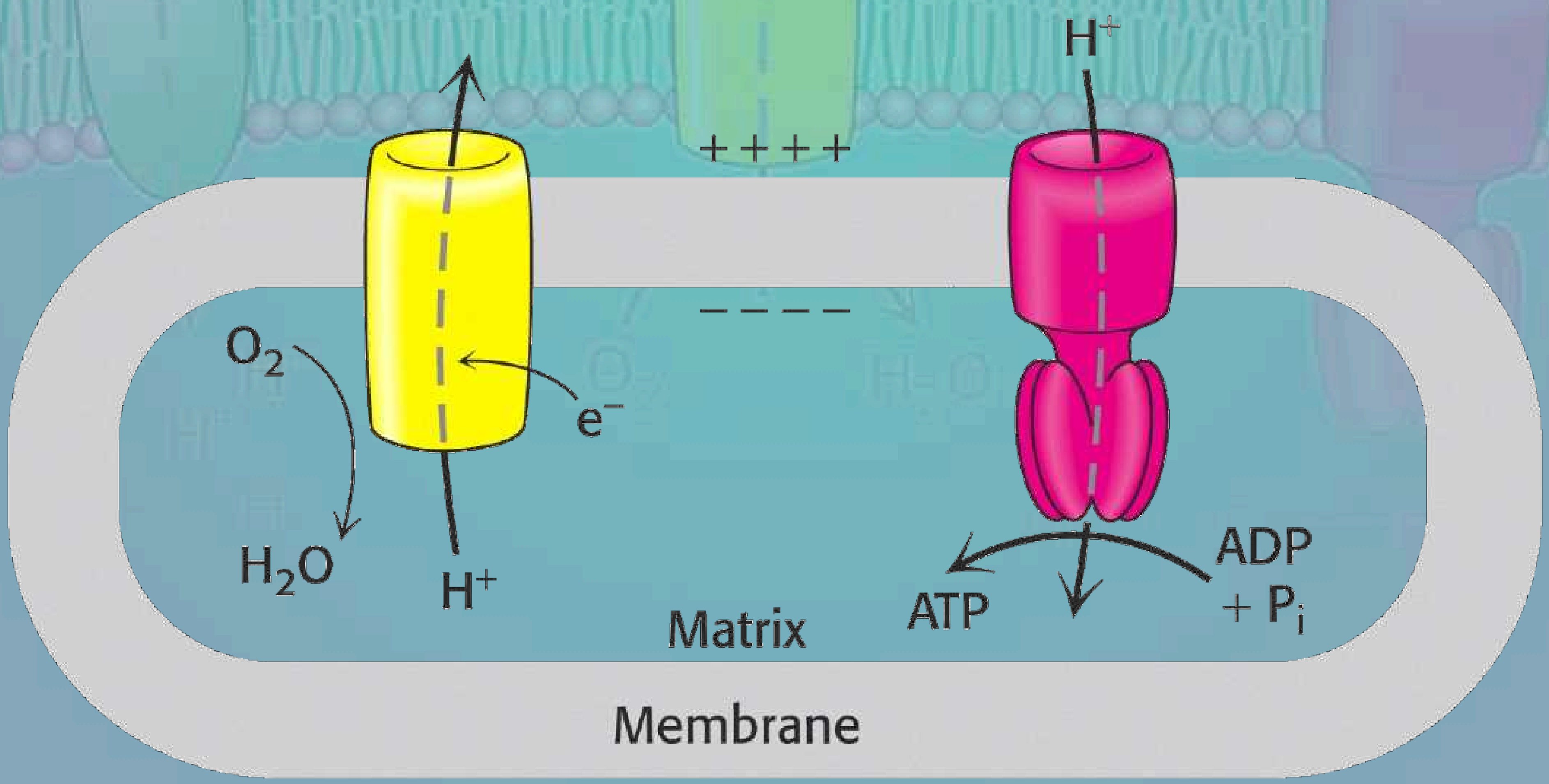
2.2 Formation of Proton Gradient

The oxidation of NADH by O_2 drives formation of a proton gradient across the mitochondrial inner membrane



Problem

If the *pH* of the mitochondrial intermembrane space is 6.8 while the *pH* of the mitochondrial matrix is 8.2, what the free energy change for transporting one proton (H^+) out of the mitochondrial matrix if the membrane potential is 0.14 V?

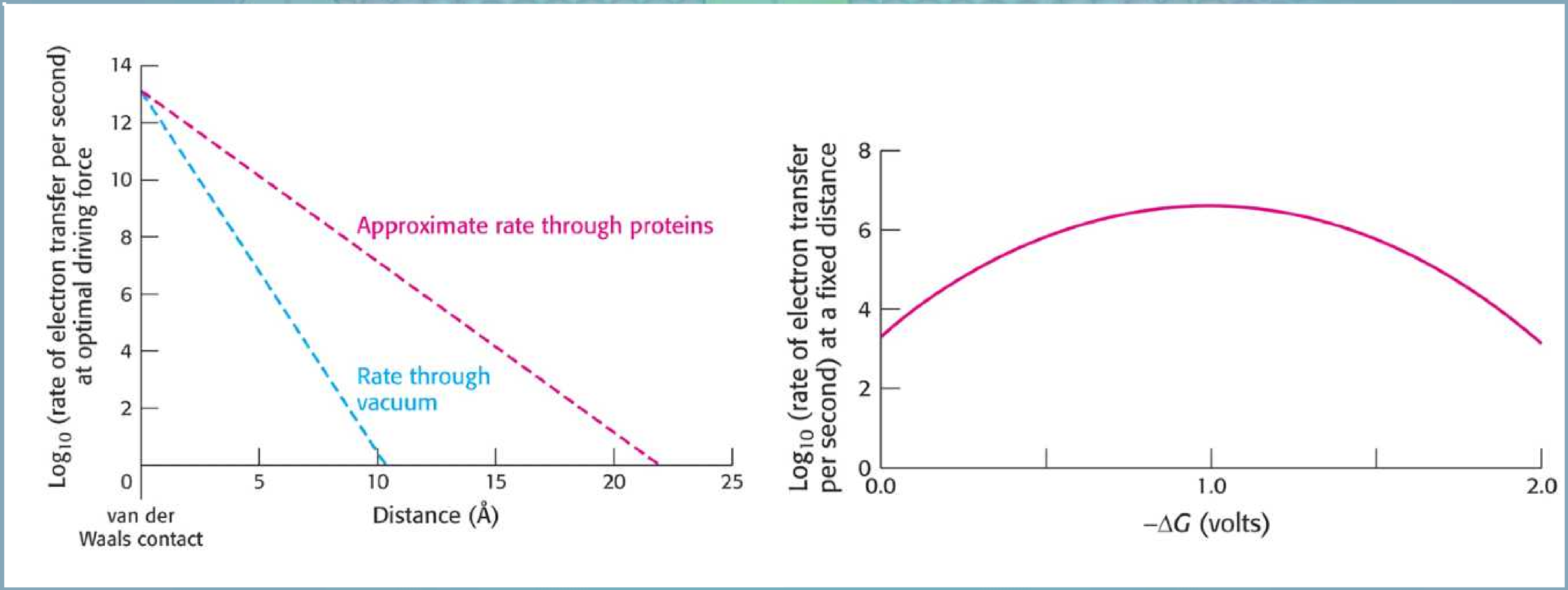


2.3 Electron Transfer

The rate of electron transfer is dependent up two factors:

Distance

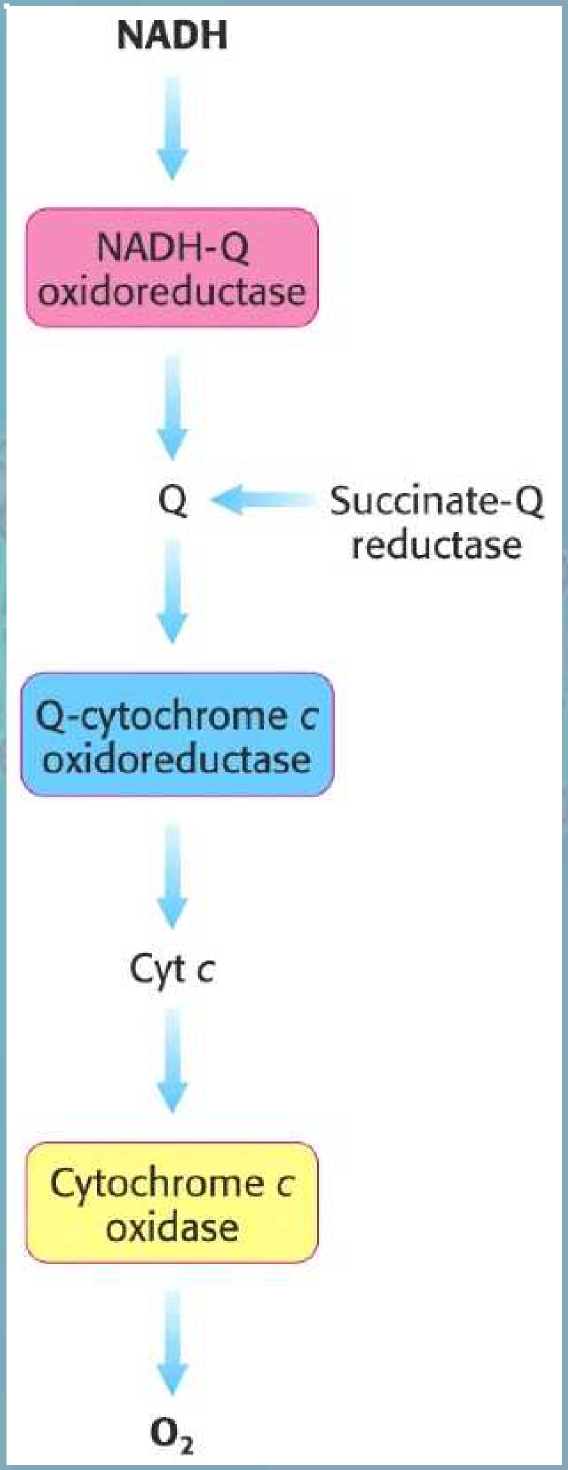
Driving Force



3. Respiratory Chain

The respiratory chain consists of four complexes

- Three proton pumps
- Physical link to the citric acid cycle



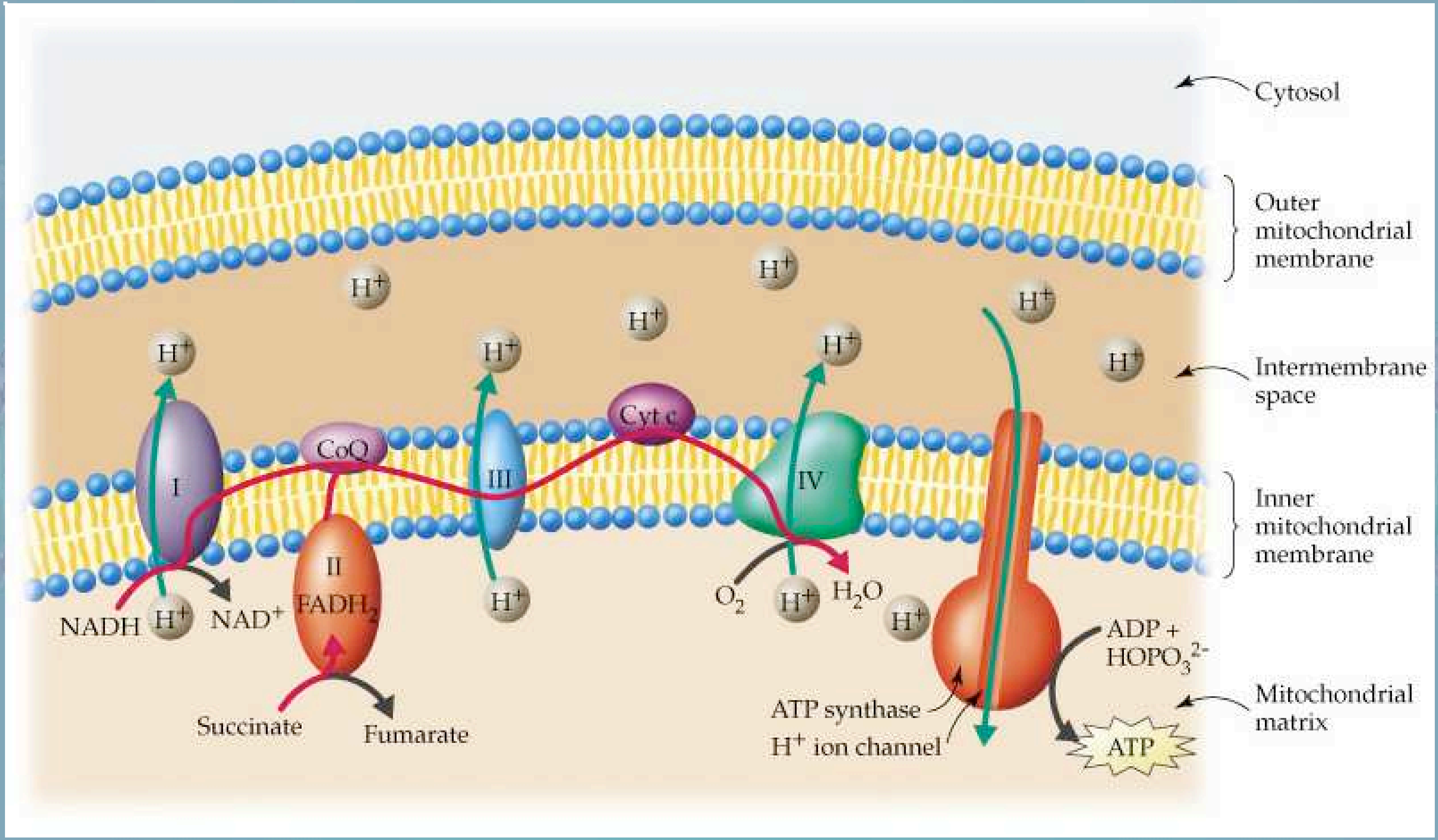
3. Respiratory Chain

TABLE 18.2 Components of the mitochondrial electron-transport chain

Enzyme complex	Mass (kd)	Subunits	Prosthetic group	Oxidant or reductant: Matrix side	Oxidant or reductant: Membrane core	Oxidant or reductant: Cytosolic side
NADH-Q oxidoreductase	880	≥ 34	FMN Fe-S	NADH	Q	
Succinate-Q reductase	140	4	FAD Fe-S	Succinate	Q	
Q-cytochrome *c* oxidoreductase	250	10	Heme b_H Heme b_L Heme c_1 Fe-S		Q	Cytochrome *c*
Cytochrome *c* oxidase	160	10	Heme *a* Heme a_3 Cu_A and Cu_B			Cytochrome *c*

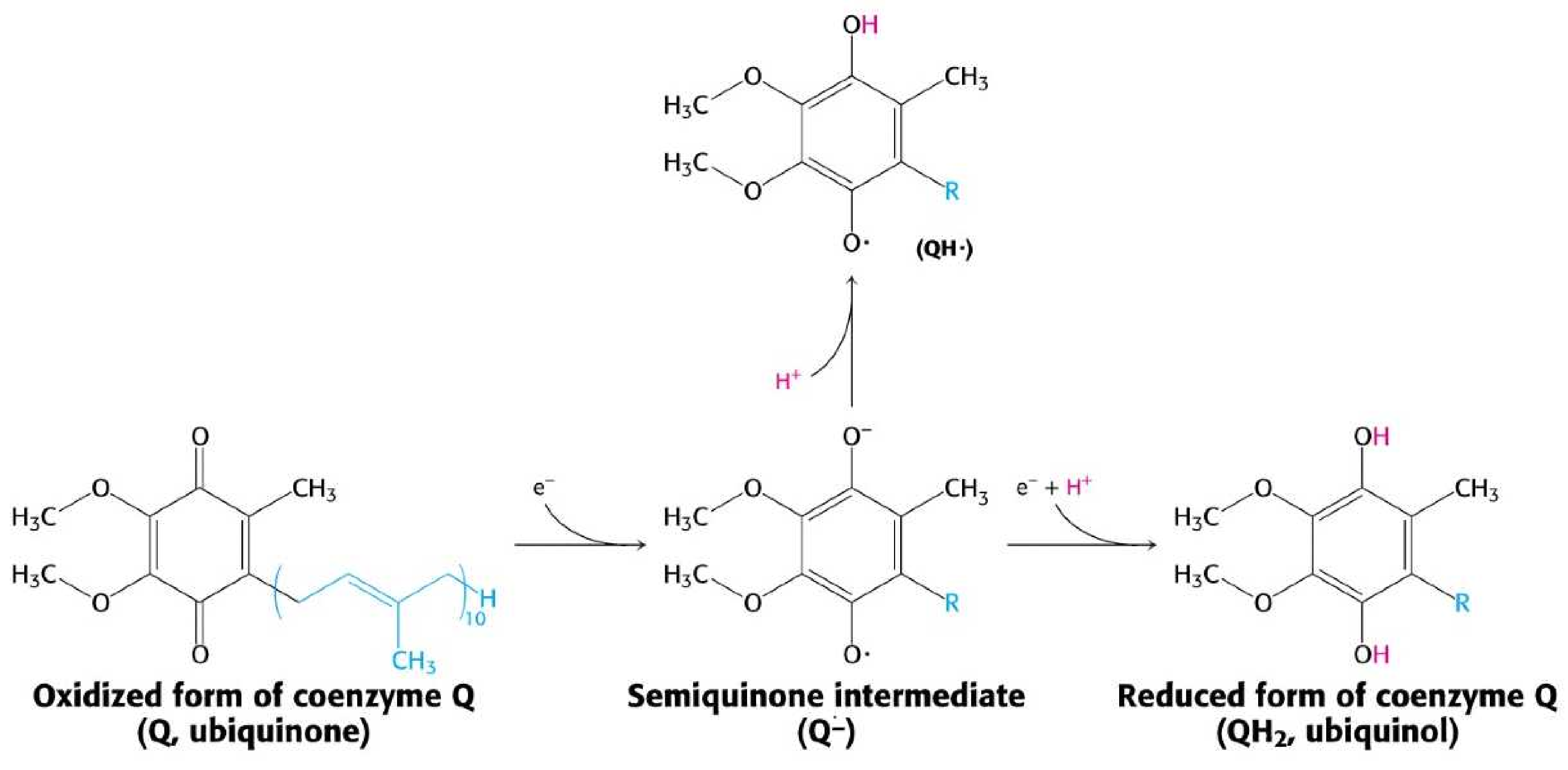
Sources: J. W. DePierre and L. Ernster, *Annu. Rev. Biochem.* 46(1977):215; Y. Hatefi, *Annu Rev. Biochem.* 54(1985):1015; and J. E. Walker, *Q. Rev. Biophys.* 25(1992):253.

3. Respiratory Chain



3. Carriers Between Complexes

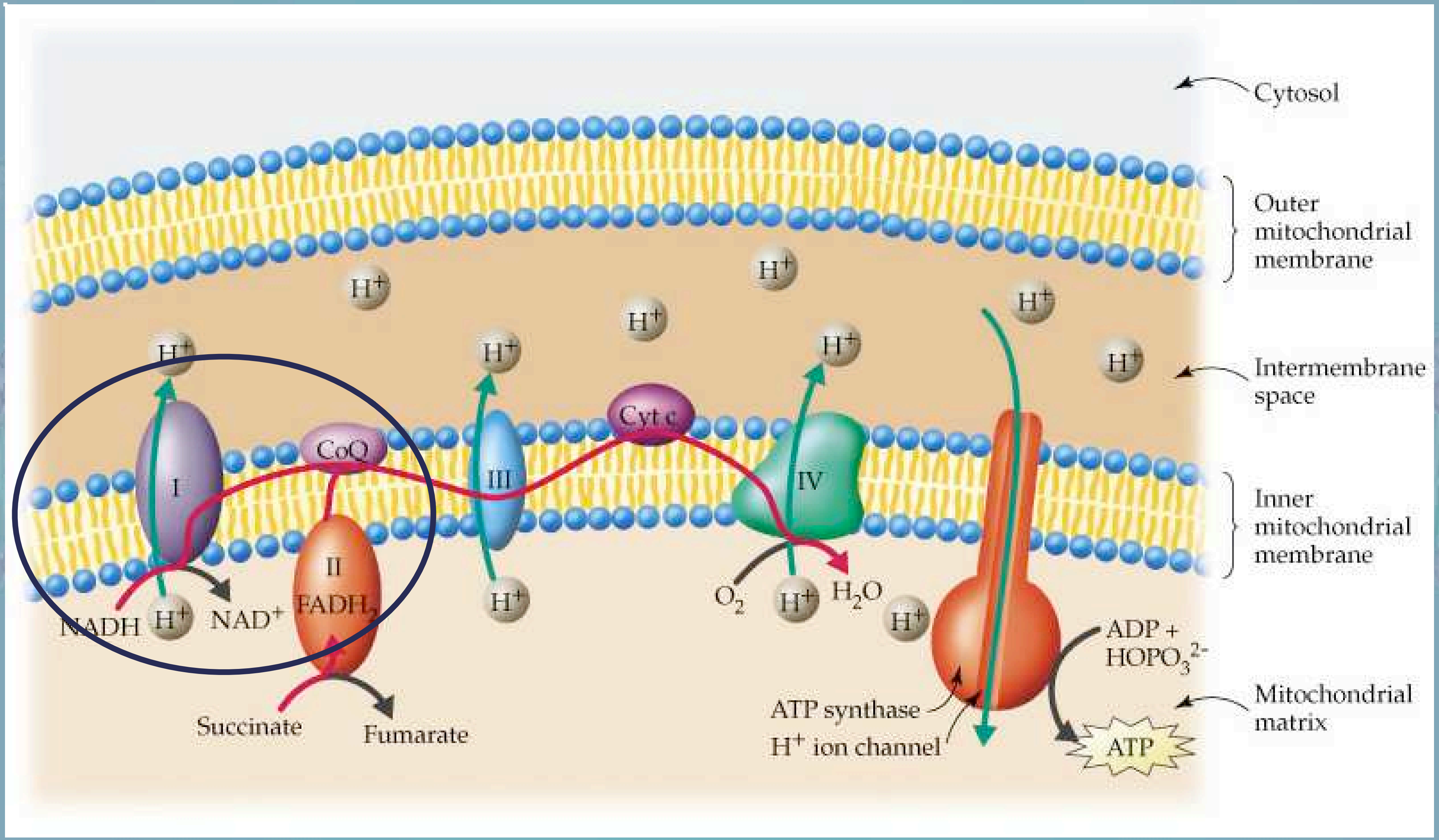
Coenzyme Q (Ubiquinone) carries the electrons from Complexes I & II to Complex III



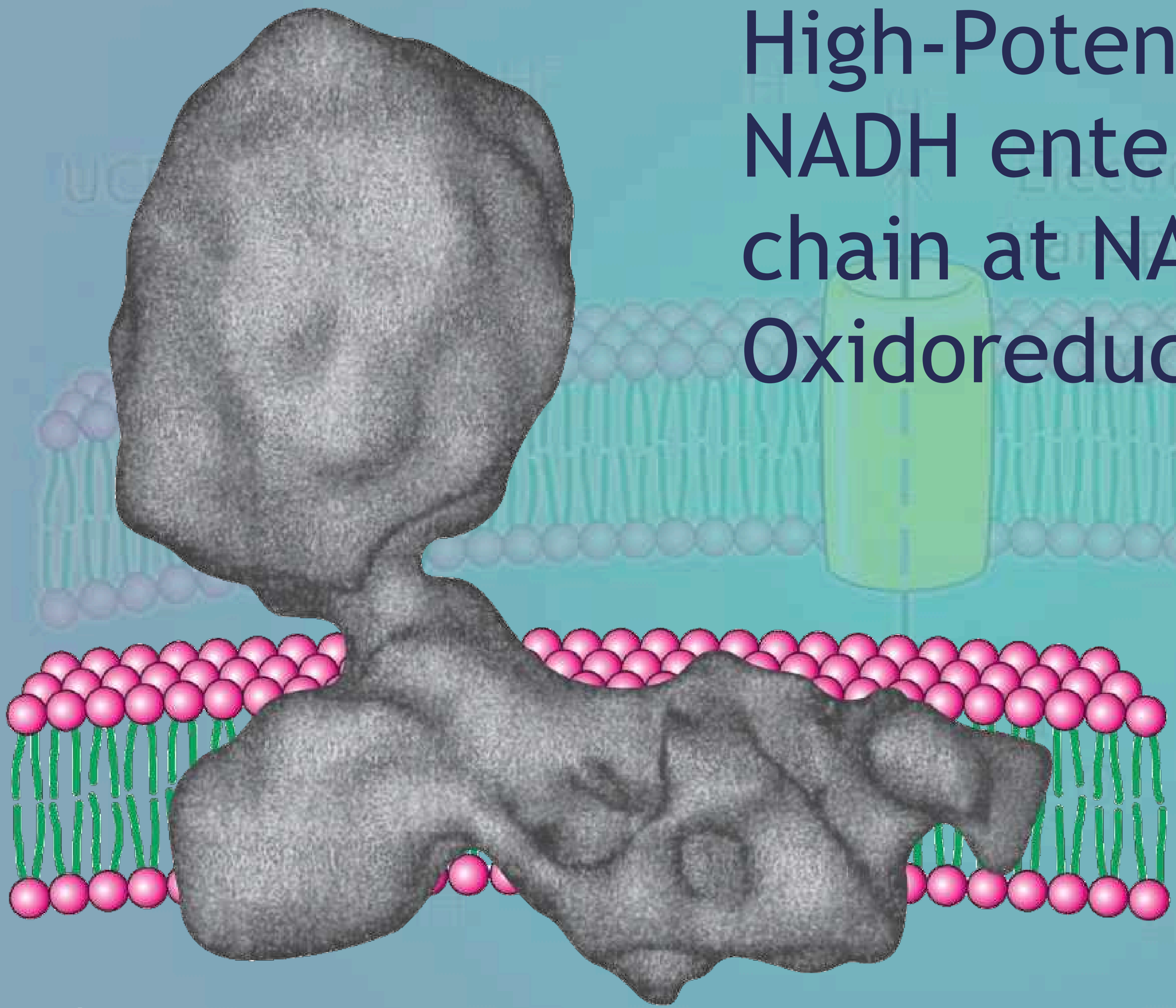
3. Carriers Between Complexes

Cytochrome c is a small heme protein that carries the electrons from Complex III to Complex IV

3.1 NADH-Q Oxidoreductase



3.1 NADH-Q Oxidoreductase

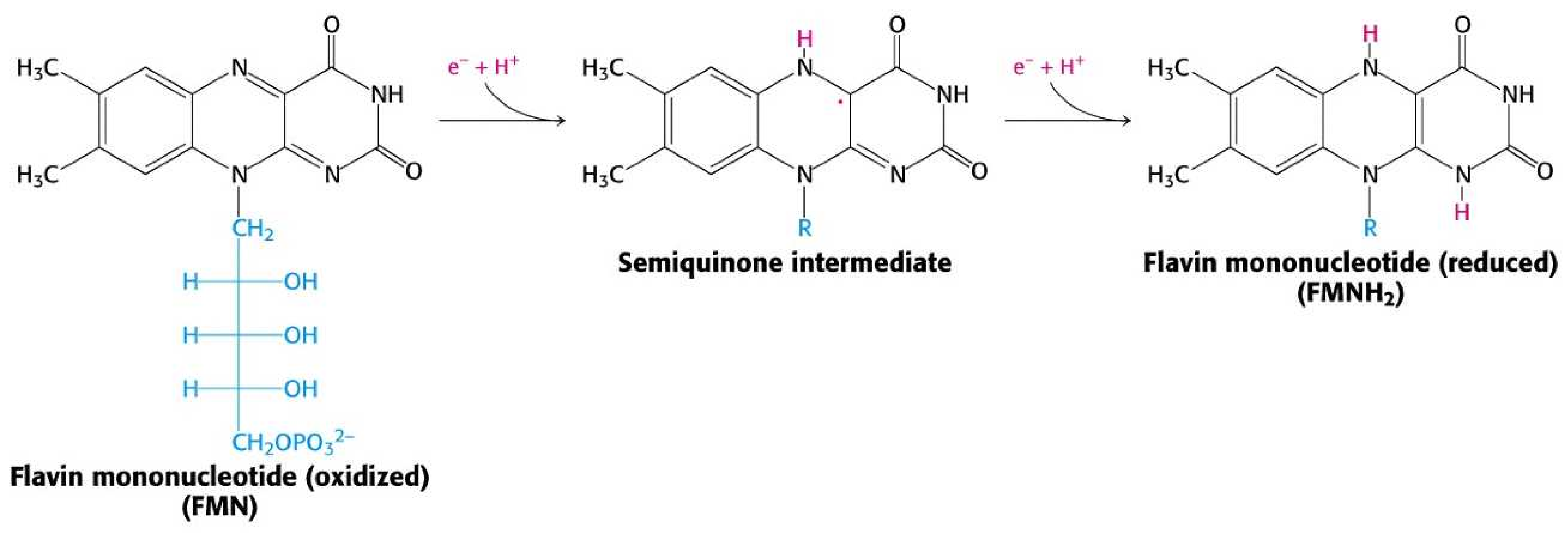


High-Potential electrons of NADH enter the respiratory chain at NADH-Q Oxidoreductase

$$NADH + Q + 5\,H^{+}_{matrix} \longrightarrow NAD^{+} + QH_2 + 4\,H^{+}_{cytosol}$$

3.1 NADH-Q Oxidoreductase

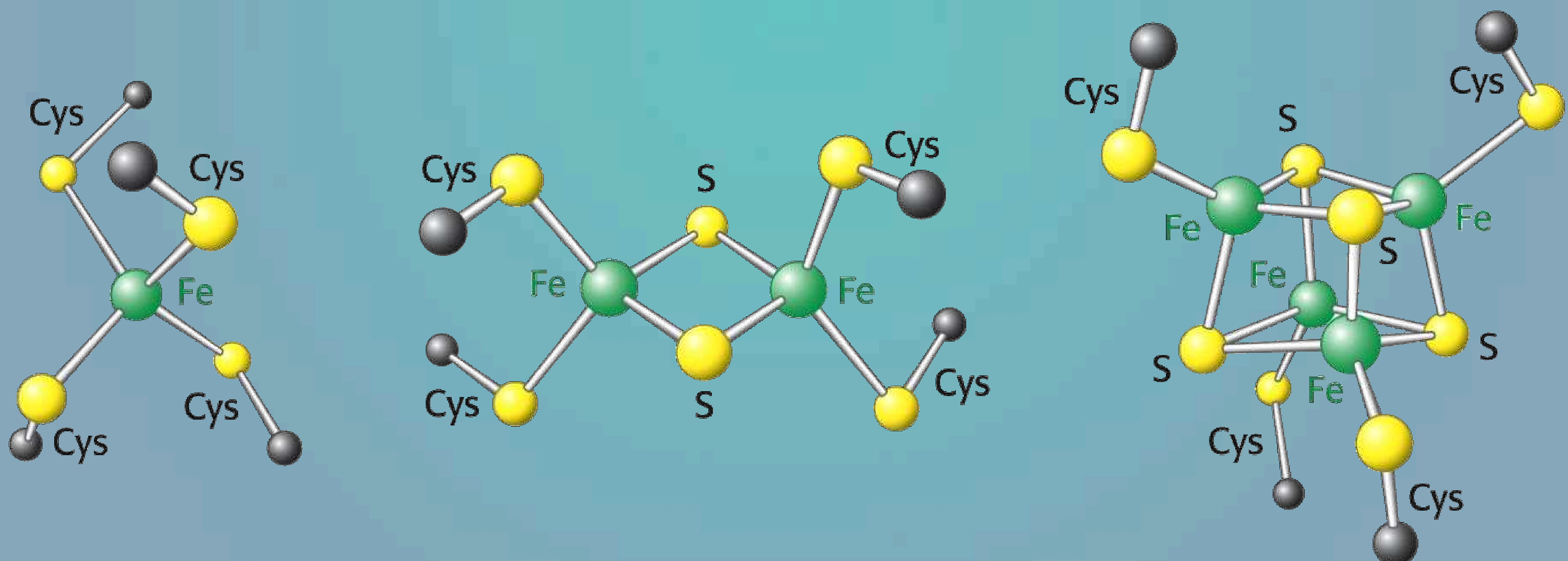
The electrons from NADH are transferred to a bound FMN



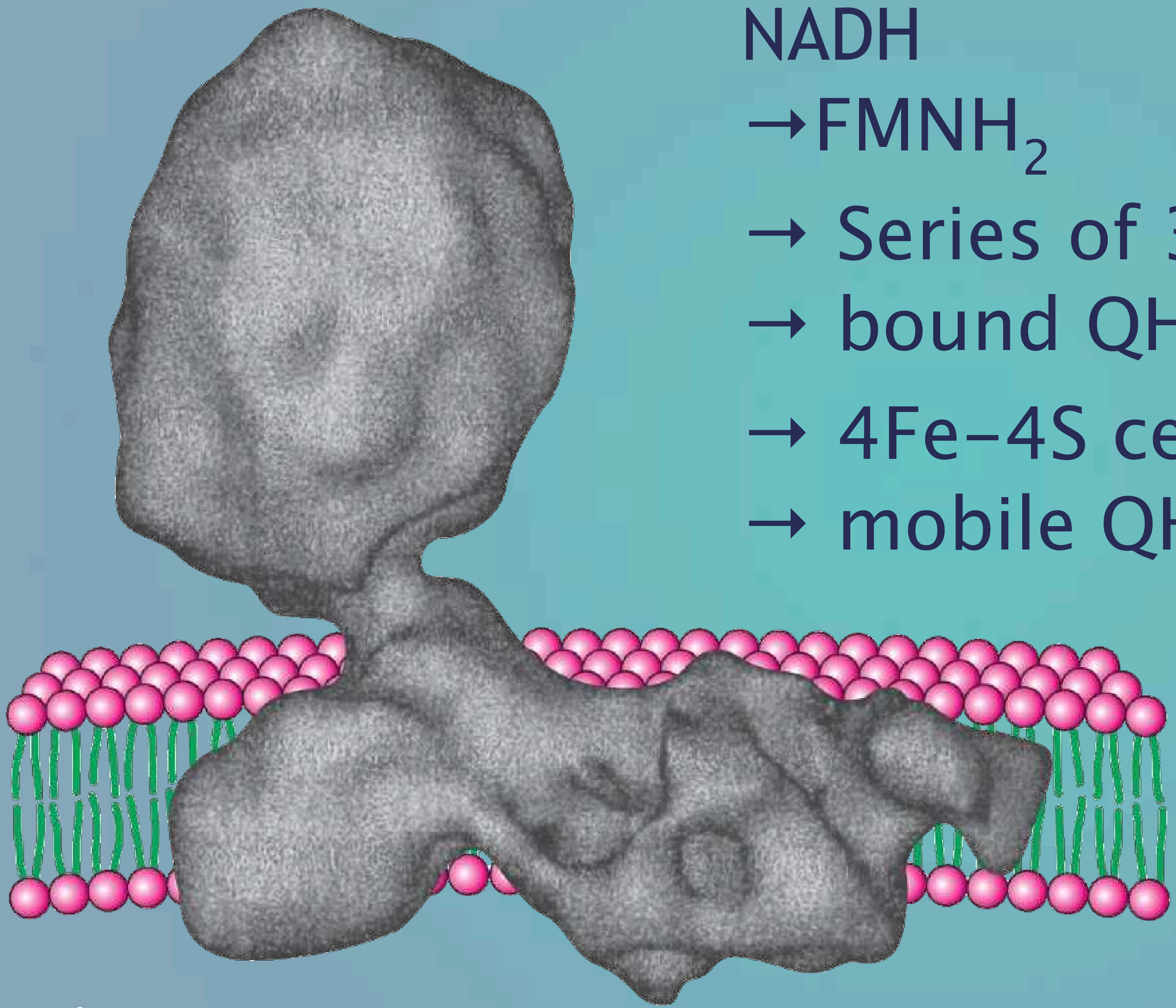
3.1 NADH-Q Oxidoreductase

The electrons from $FMNH_2$ are then transferred to a series of iron-sulfur centers.

Single Iron   2Fe–2S   4Fe–4S



3.1 NADH-Q Oxidoreductase

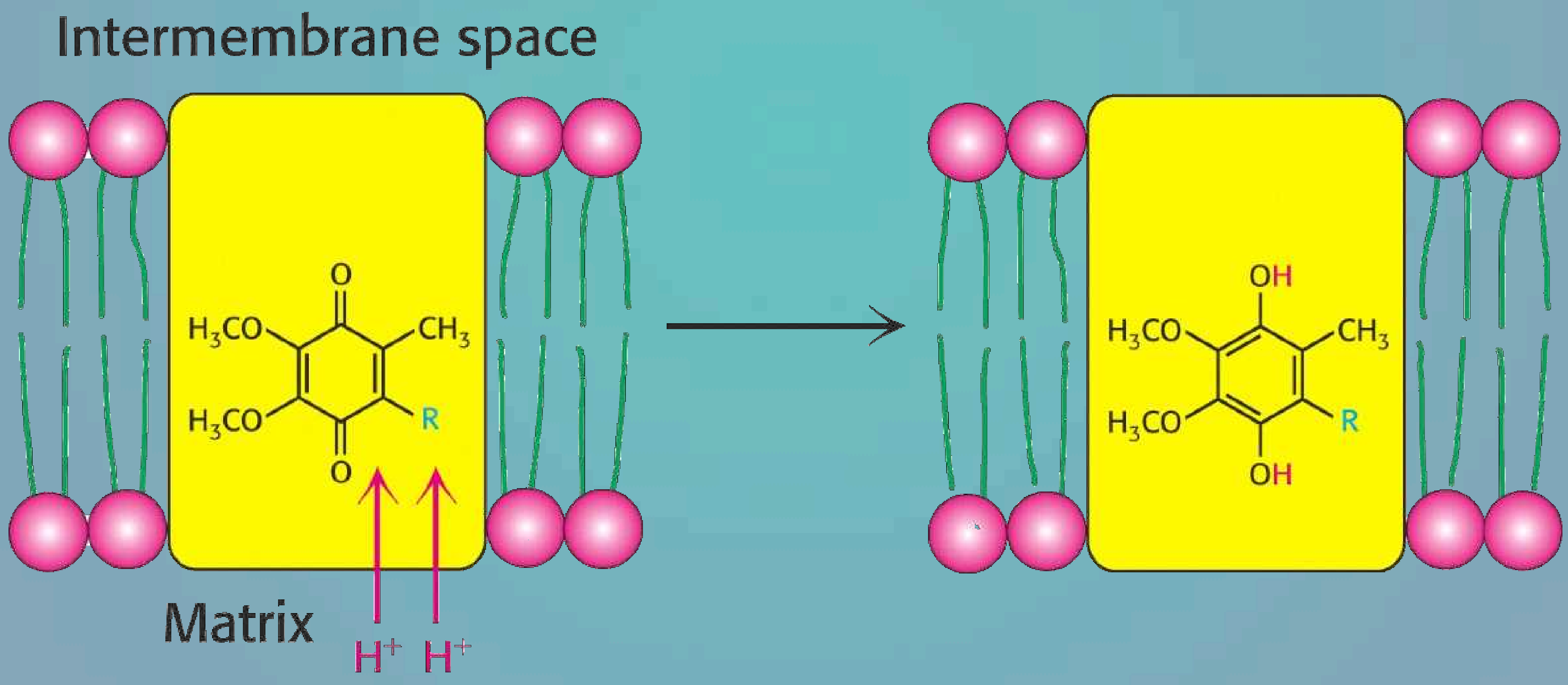


NADH
→ $FMNH_2$
→ Series of 3 4Fe–4S centers
→ bound QH_2
→ 4Fe–4S center
→ mobile QH_2

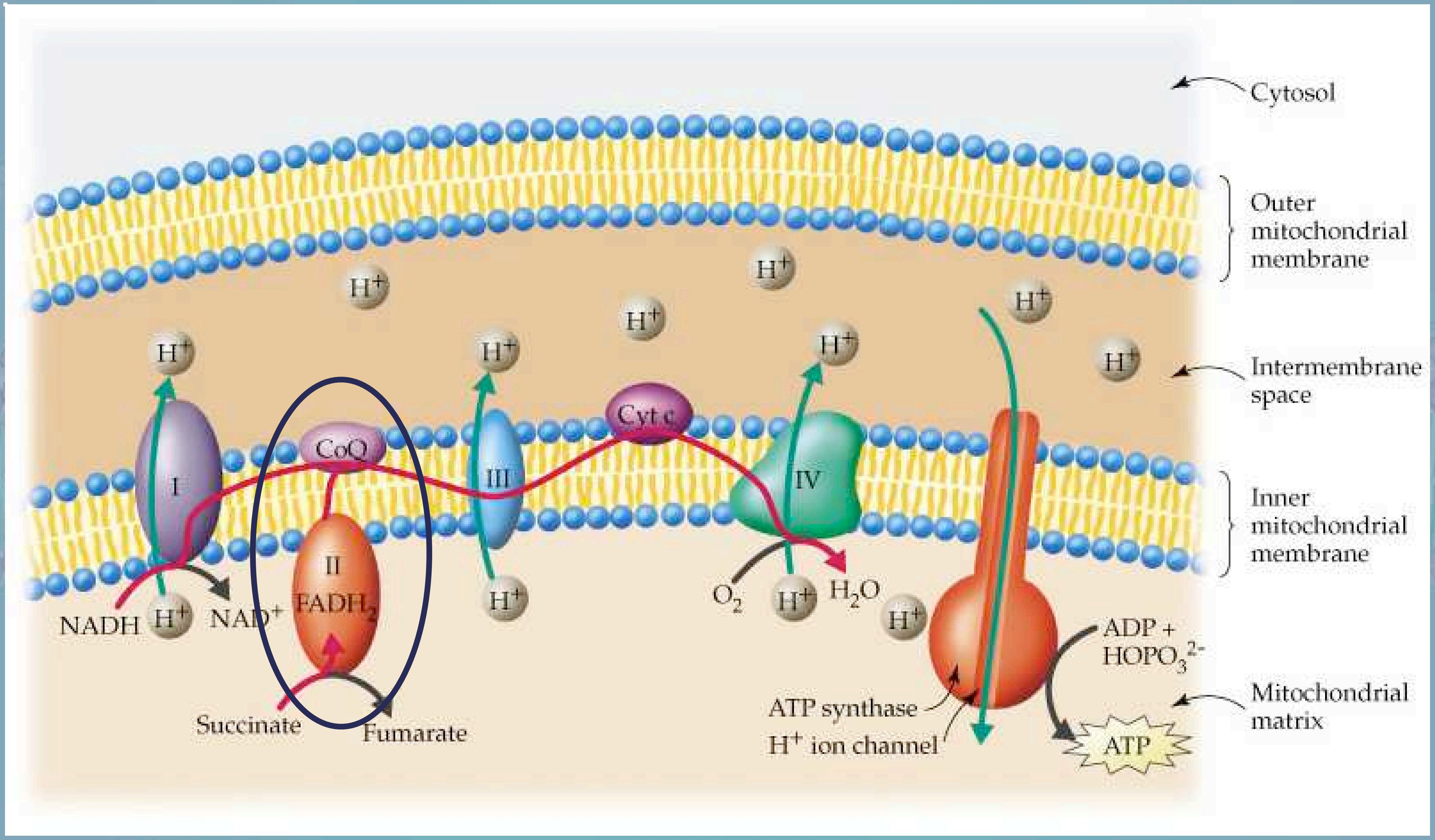
$$NADH + Q + 5\ H^+_{matrix} \longrightarrow NAD^+ + QH_2 + 4\ H^+_{cytosol}$$

3.1 NADH-Q Oxidoreductase

The electrons from 4Fe-4S centers are transferred to Q.

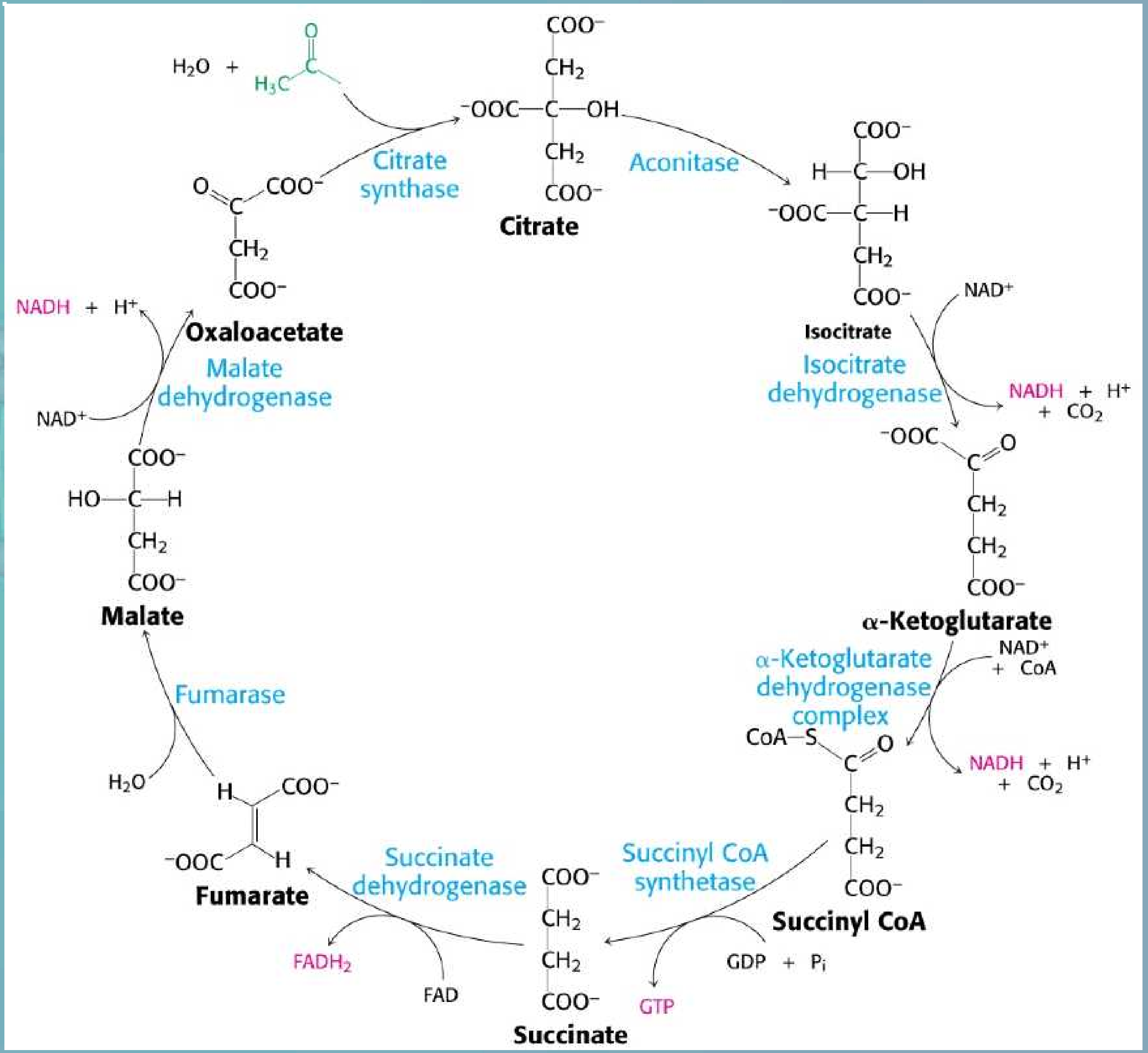


3.2 Succinate-Q Reductase Complex



3.2 Succinate-Q Reductase Complex

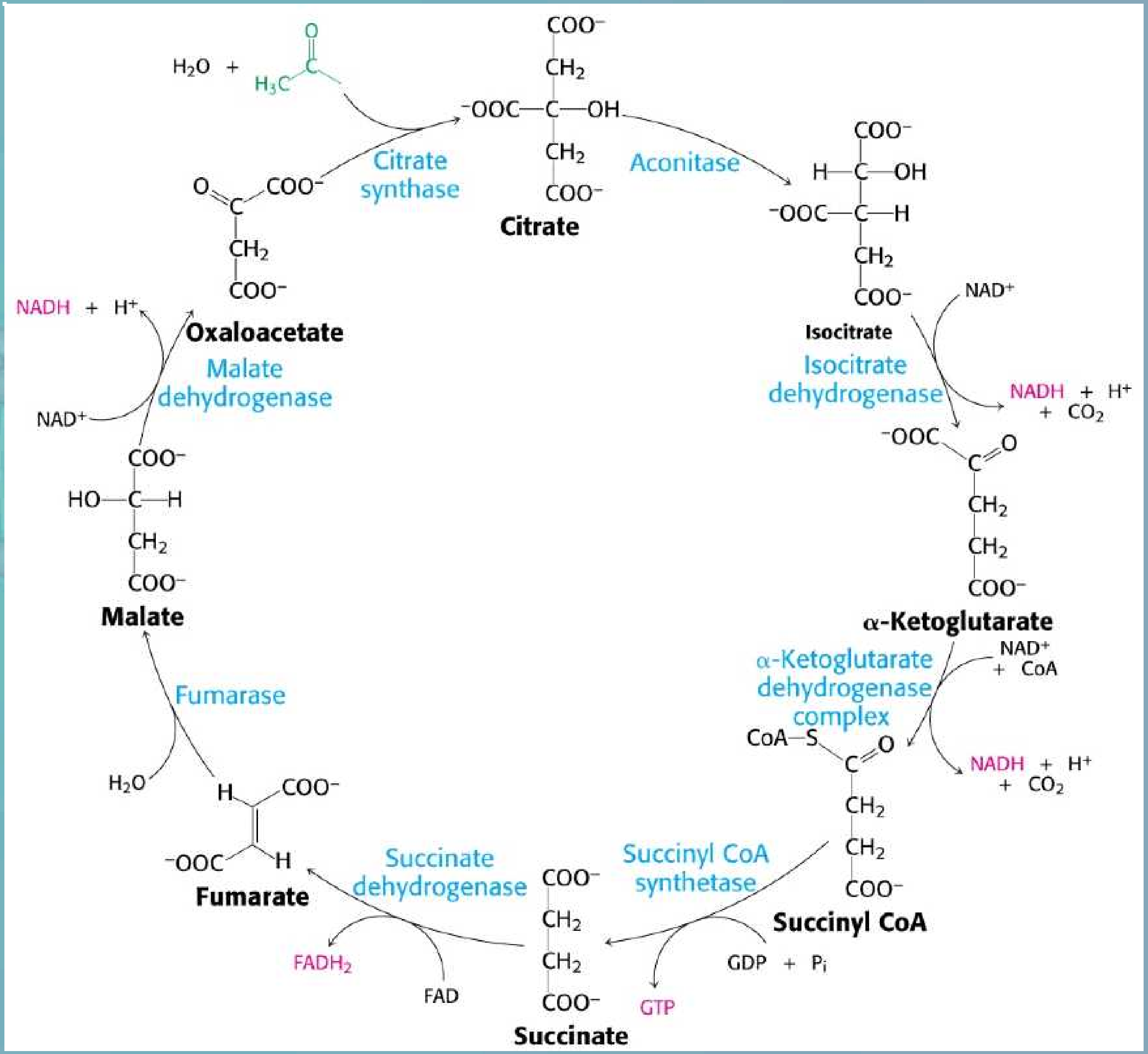
Succinate dehydrogenase from the citric acid cycle is a component of the Succinate-Q Reductase complex



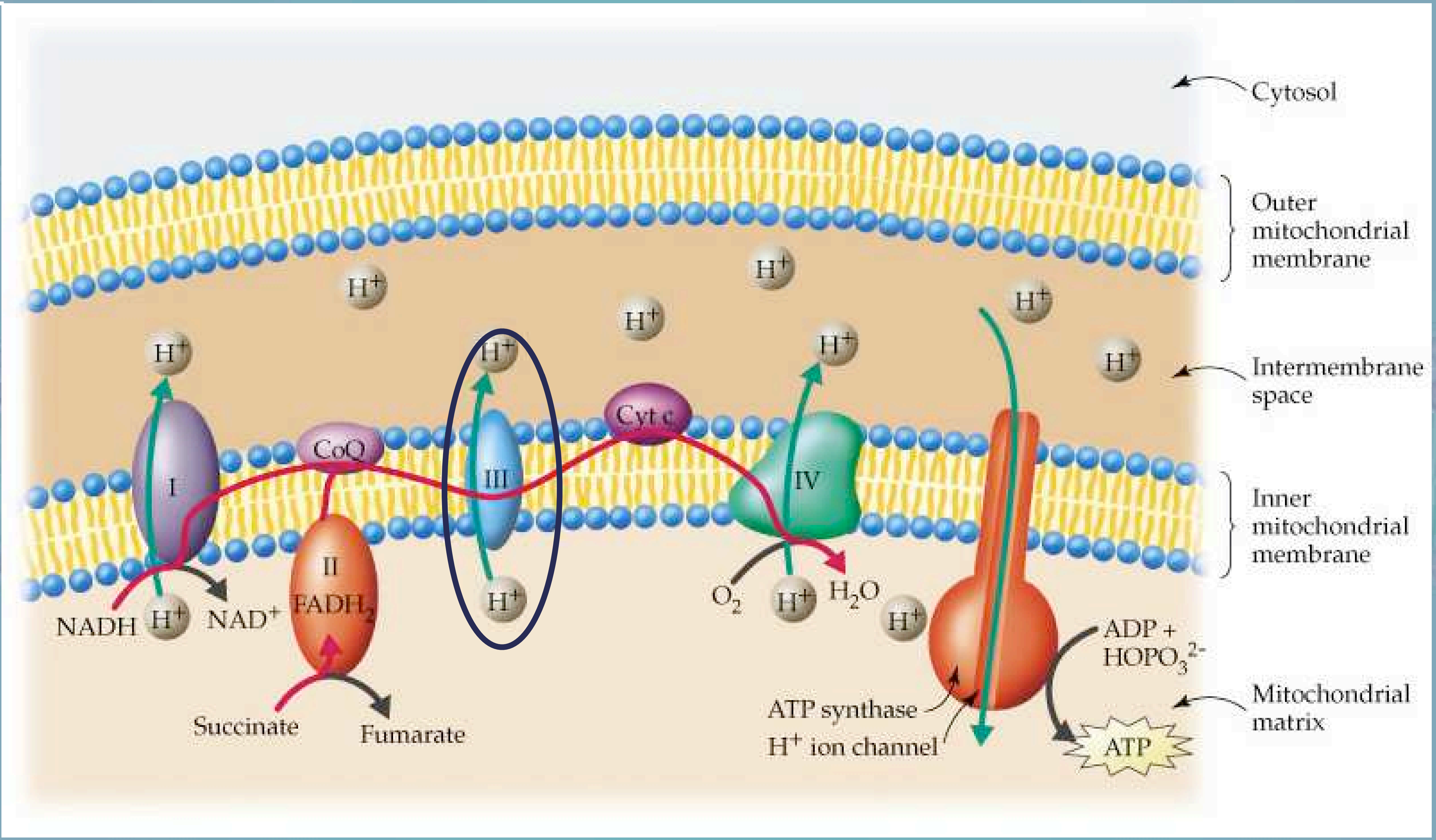
3.2 Succinate-Q Reductase Complex

$FADH_2$
→ Fe–S centers
→ mobile QH_2

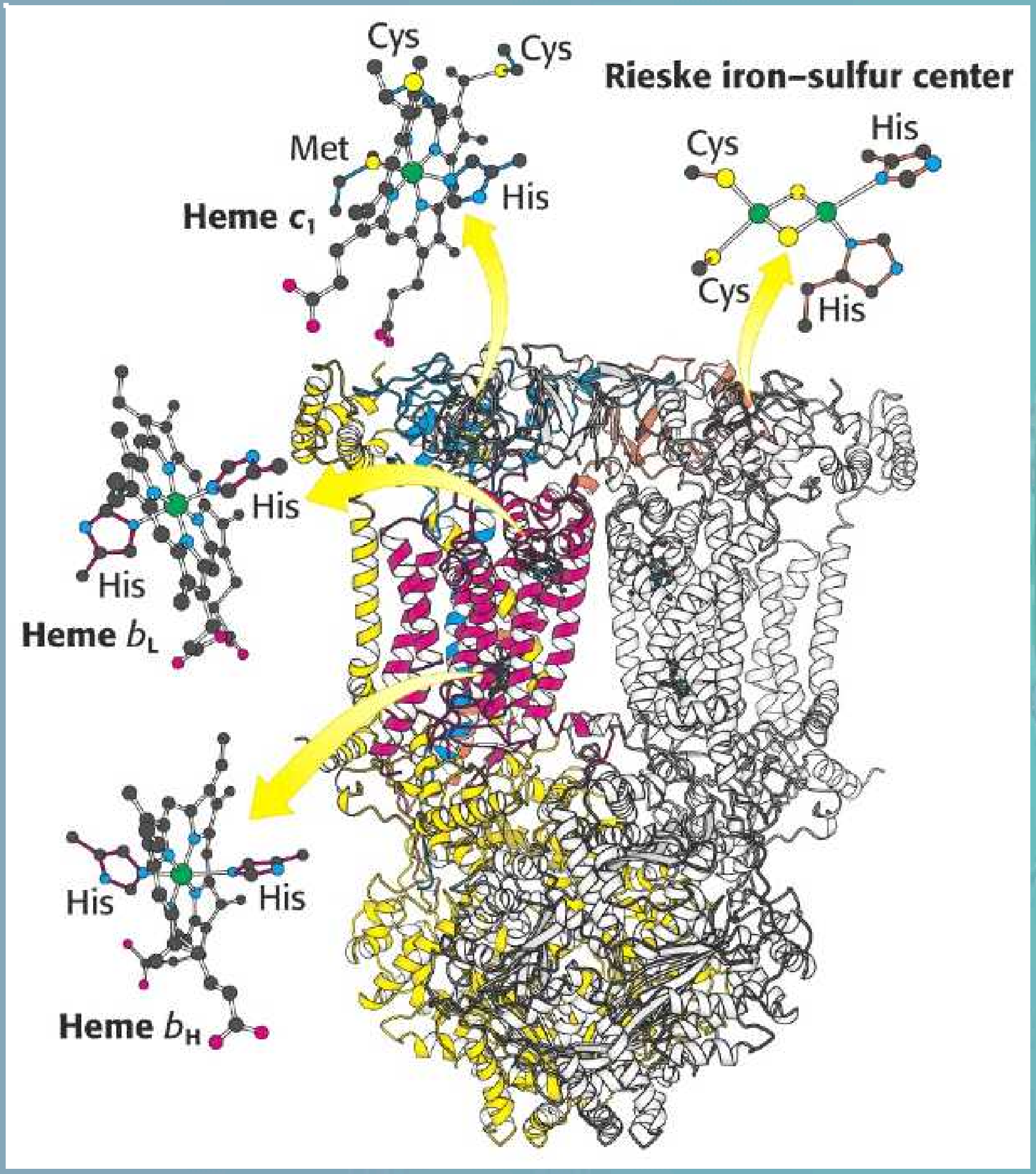
No hydrogens are pumped out of the mitochondrial matrix



3.3 Q-Cytochrome c Oxidoreductase



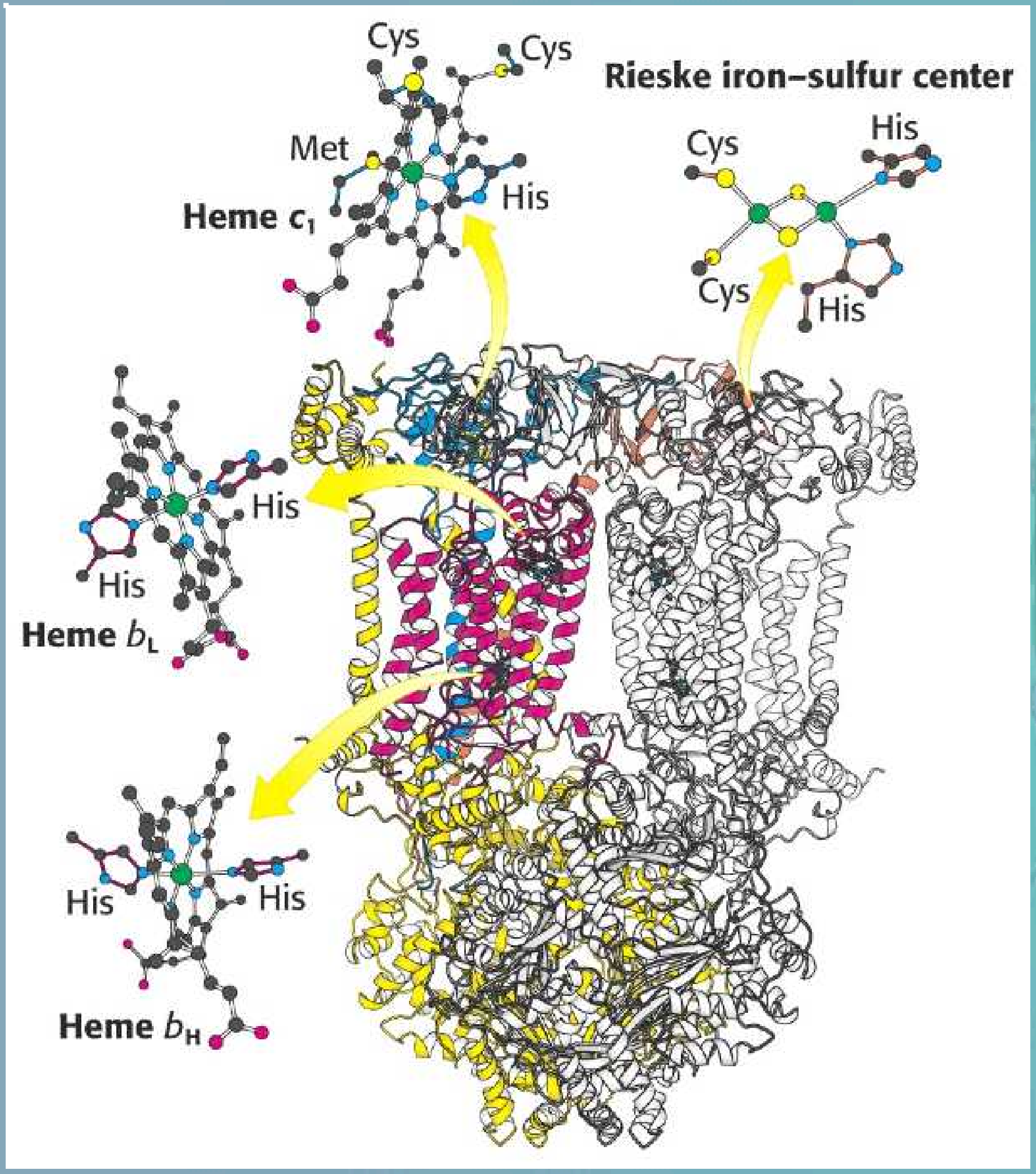
3.3 Q-Cytochrome c Oxidoreductase



Electron flow from ubiquinol to cytochrome c through Q-cytochrome c oxidoreductase

$$QH_2 + 2\ \text{Cyt}\ c_{ox} + 2\ H^+_{matrix} \longrightarrow Q + 2\ \text{Cyt}\ c_{red} + 4\ H^+_{cytosol}$$

3.3 Q-Cytochrome c Oxidoreductase



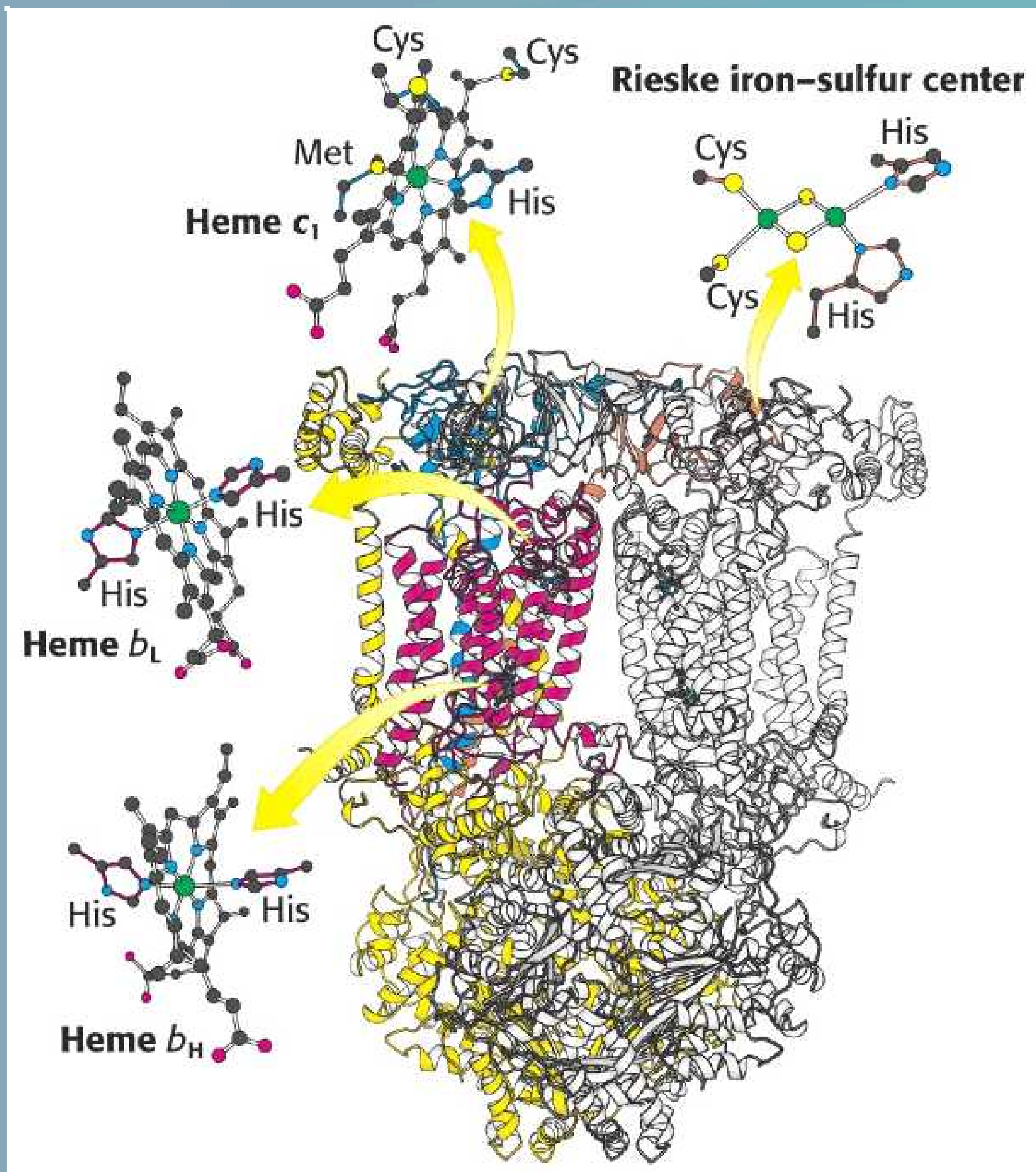
First QH_2

Electron 1
→ 2Fe–2S center
→ cyt c_1
→ mobile cyt c

Electron 2
→ cyt b_L
→ cyt b_L
→ $Q\bullet^-$

$$QH_2 + 2\ Cyt\ c_{ox} + 2\ H^+_{matrix} \longrightarrow Q + 2\ Cyt\ c_{red} + 4\ H^+_{cytosol}$$

3.3 Q-Cytochrome c Oxidoreductase



$$QH_2 + 2\ \text{Cyt}\ c_{ox} + 2\ H^+_{matrix} \longrightarrow Q + 2\ \text{Cyt}\ c_{red} + 4\ H^+_{cytosol}$$

Second QH_2

Electron 1

→ 2Fe–2S center

→ cyt c_1

→ mobile cyt c

Electron 2

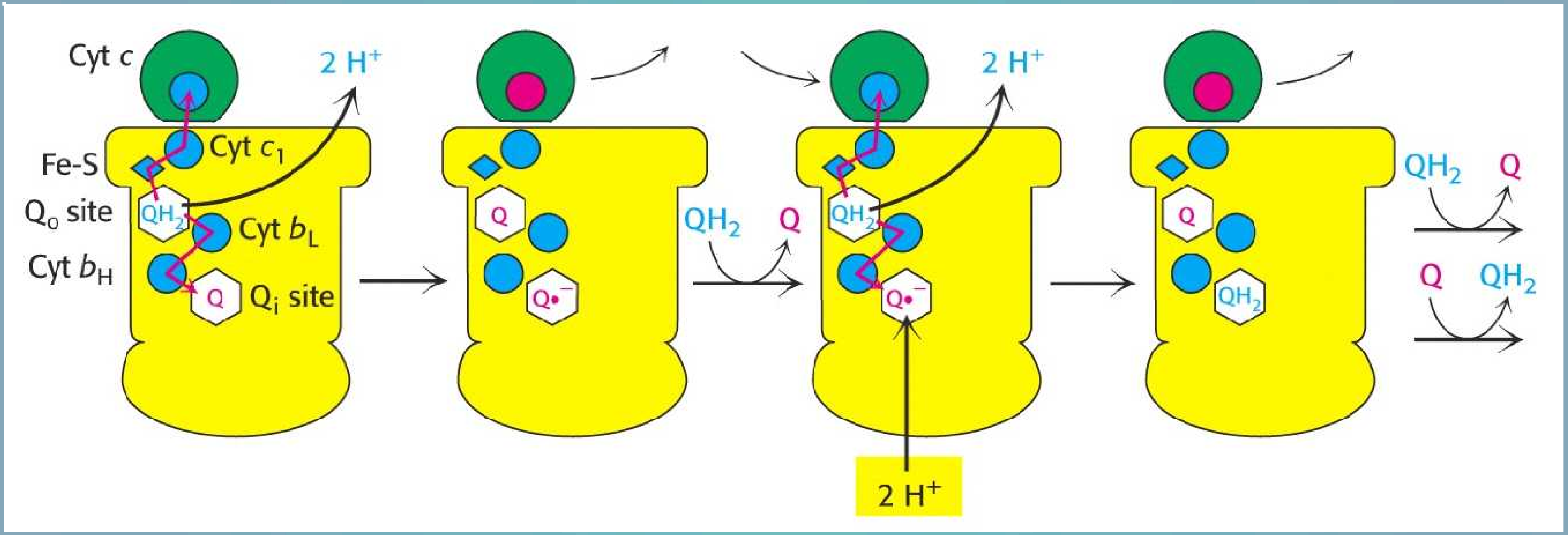
→ cyt b_L

→ cyt b_L

→ QH_2

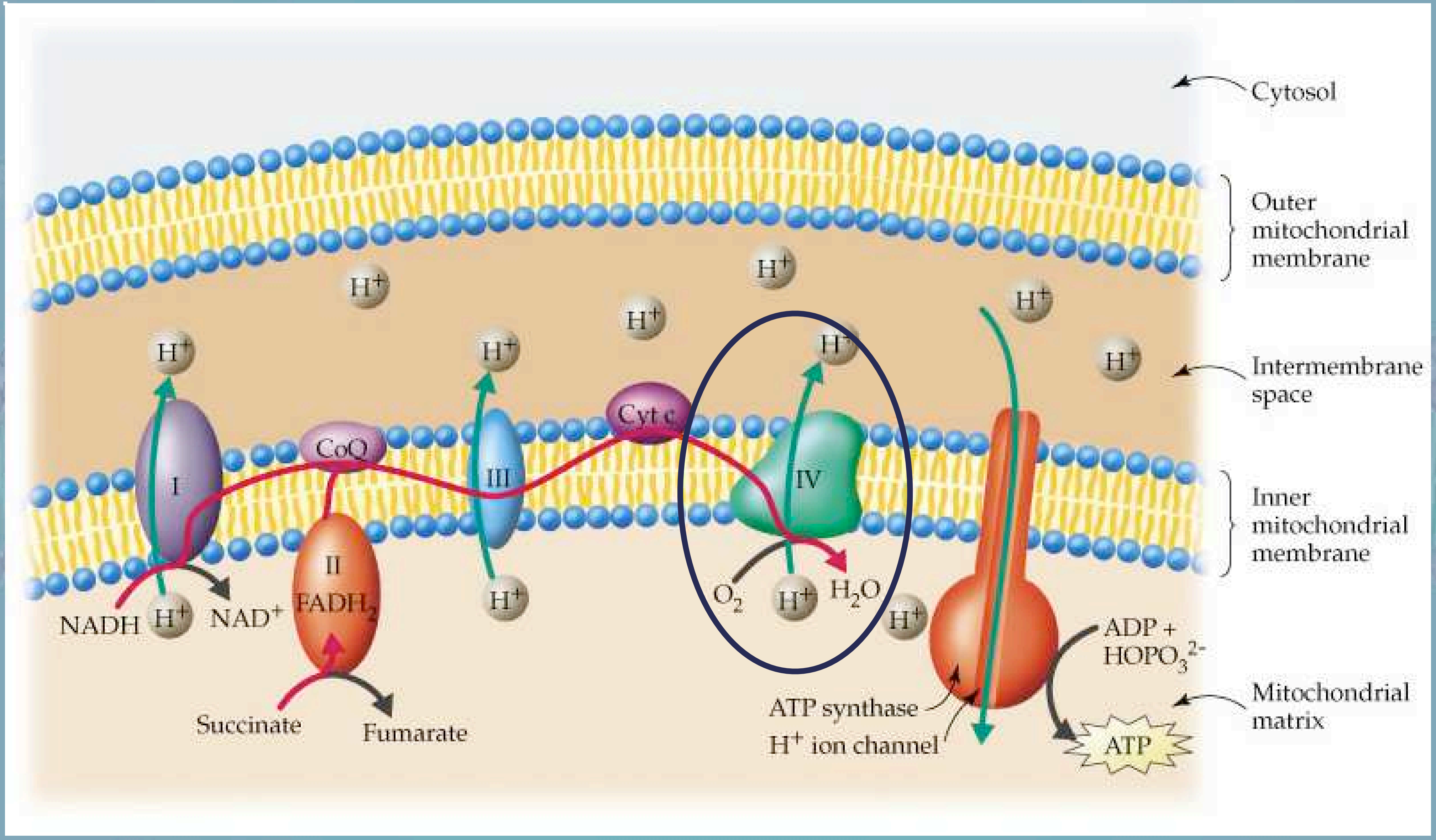
3.3 Q-Cytochrome c Oxidoreductase

The Q cycle:

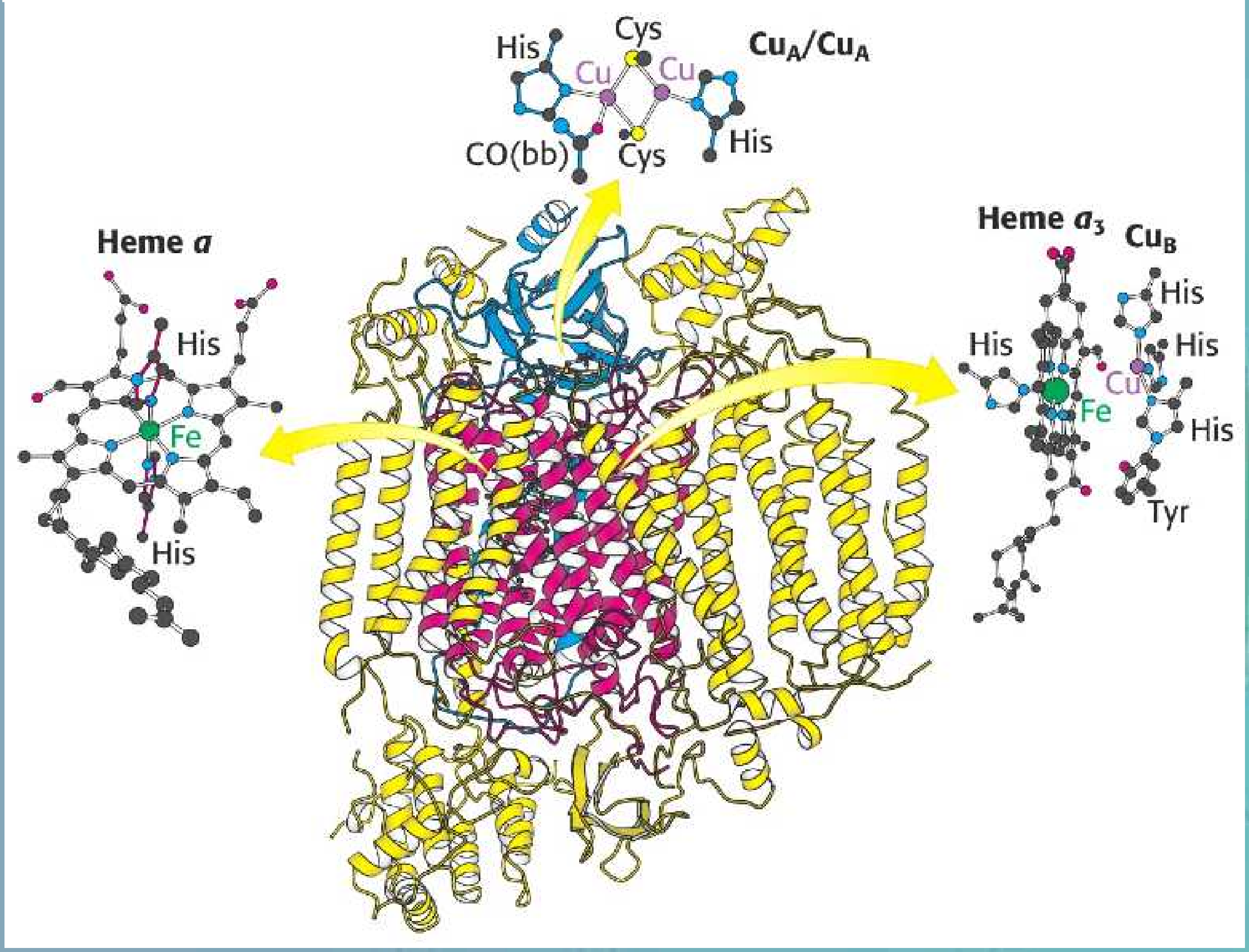


$$QH_2 + 2\ Cyt\ c_{ox} + 2\ H^+_{matrix} \longrightarrow Q + 2\ Cyt\ c_{red} + 4\ H^+_{cytosol}$$

3.5 Cytochrome c Oxidase



3.5 Cytochrome c Oxidase

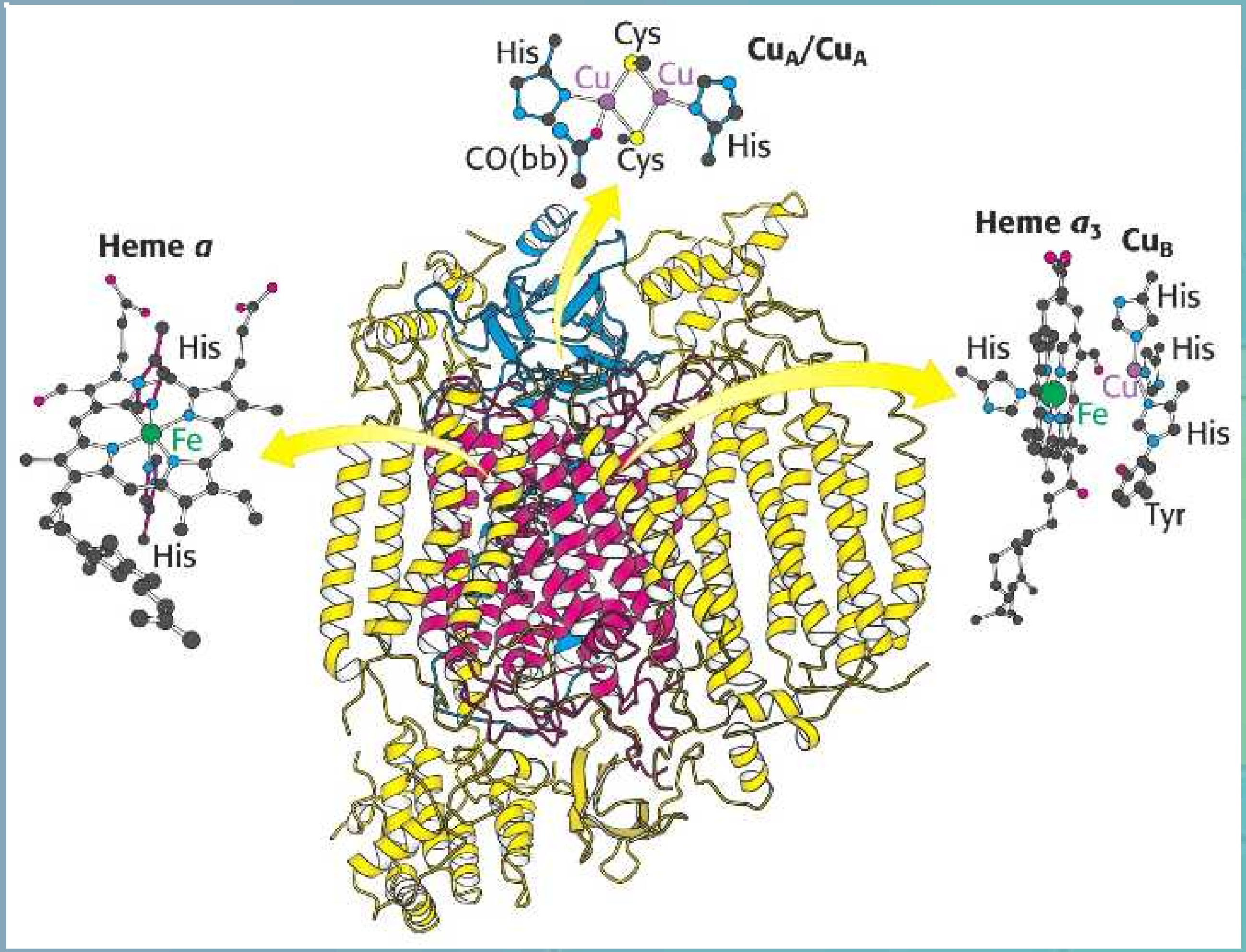


Cytochrome c Oxidase catalyzes the reduction of molecular oxygen to water

Cu_A/Cu_A
Heme a
Heme a_3/Cu_B
Fe(3+)/Cu(+2)

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

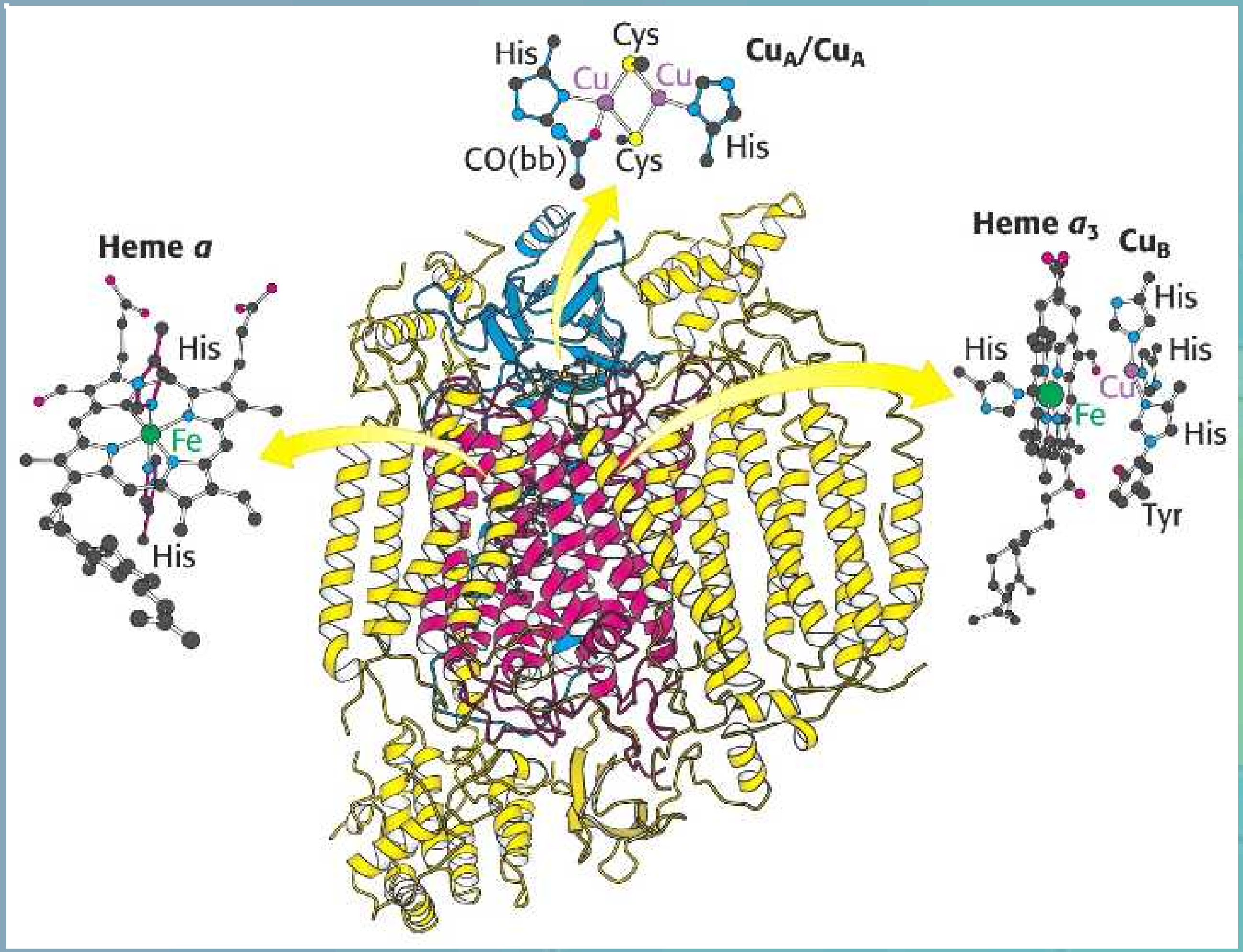


First cyt c

→ Cu_A/Cu_A
→ Heme a
→ Heme a_3/Cu_B
  $Fe(3+)/Cu(+1)$

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

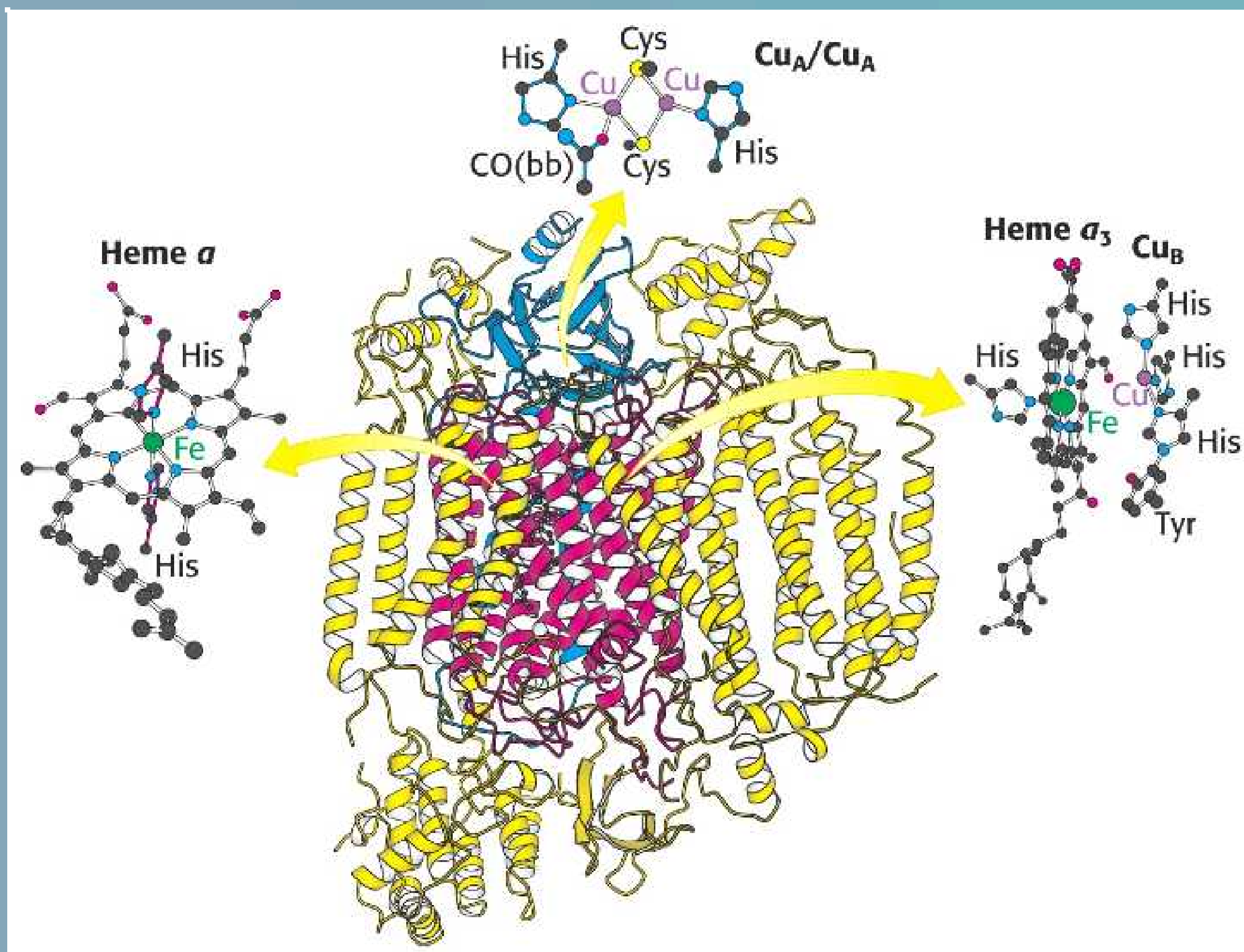


Second cyt c

→ Cu_A/Cu_A
→ Heme a
→ Heme a_3/Cu_B
Fe(2+)/Cu(+1)

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

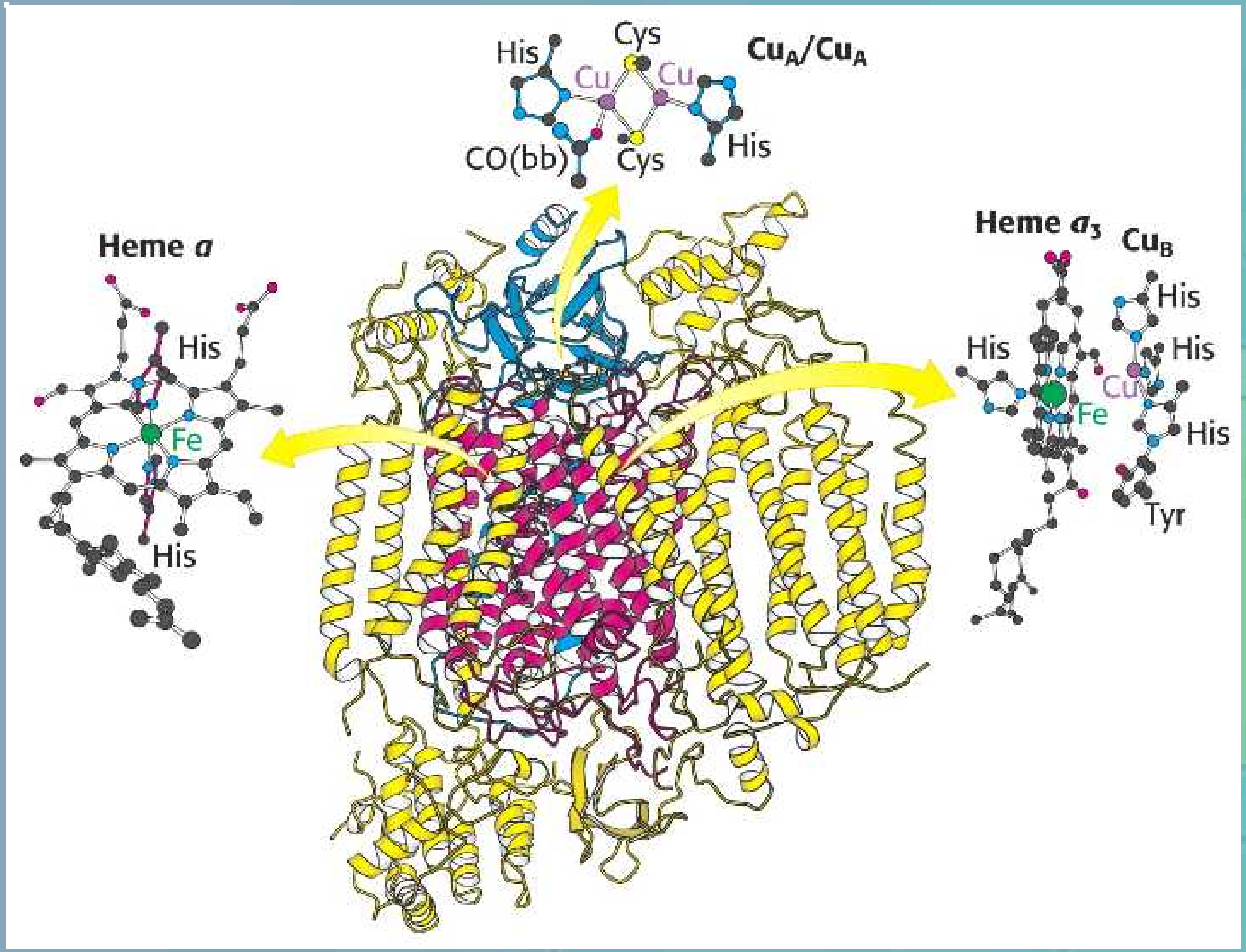


- → Cu_A/Cu_A
- → Heme a
- → Heme a_3/Cu_B

  $Fe(2+)O{=}O\ Cu(+1)$

$$4\ \text{Cyt}\ c_{red} + 4\ H^{+}_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

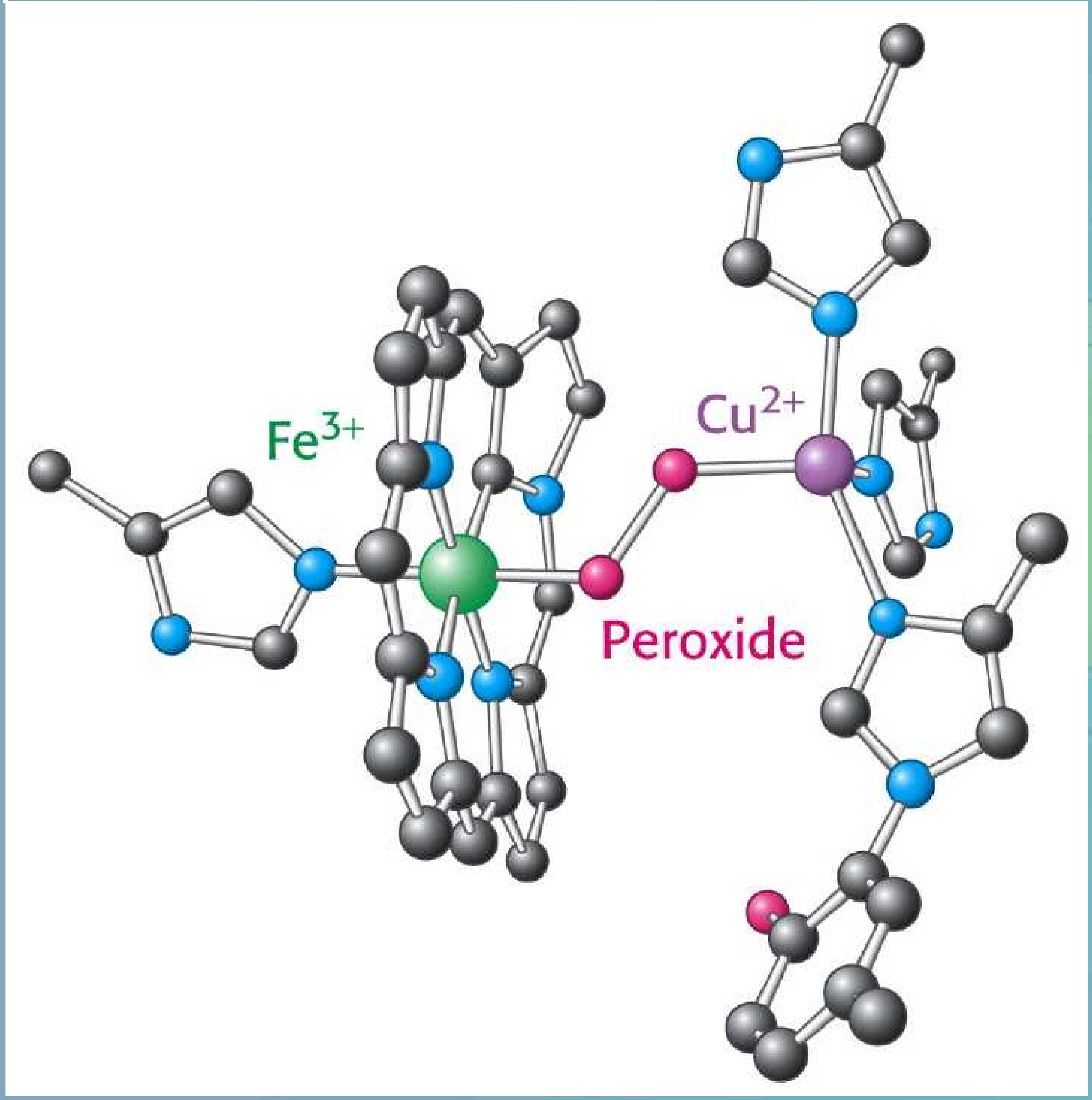


- → Cu_A/Cu_A
- → Heme a
- → Heme a_3/Cu_B

  Fe(3+)-O-O-Cu(+2)

$$4\ Cyt\ c_{red} + 4\ H^{+}_{matrix} + O_2 \longrightarrow 4\ Cyt\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

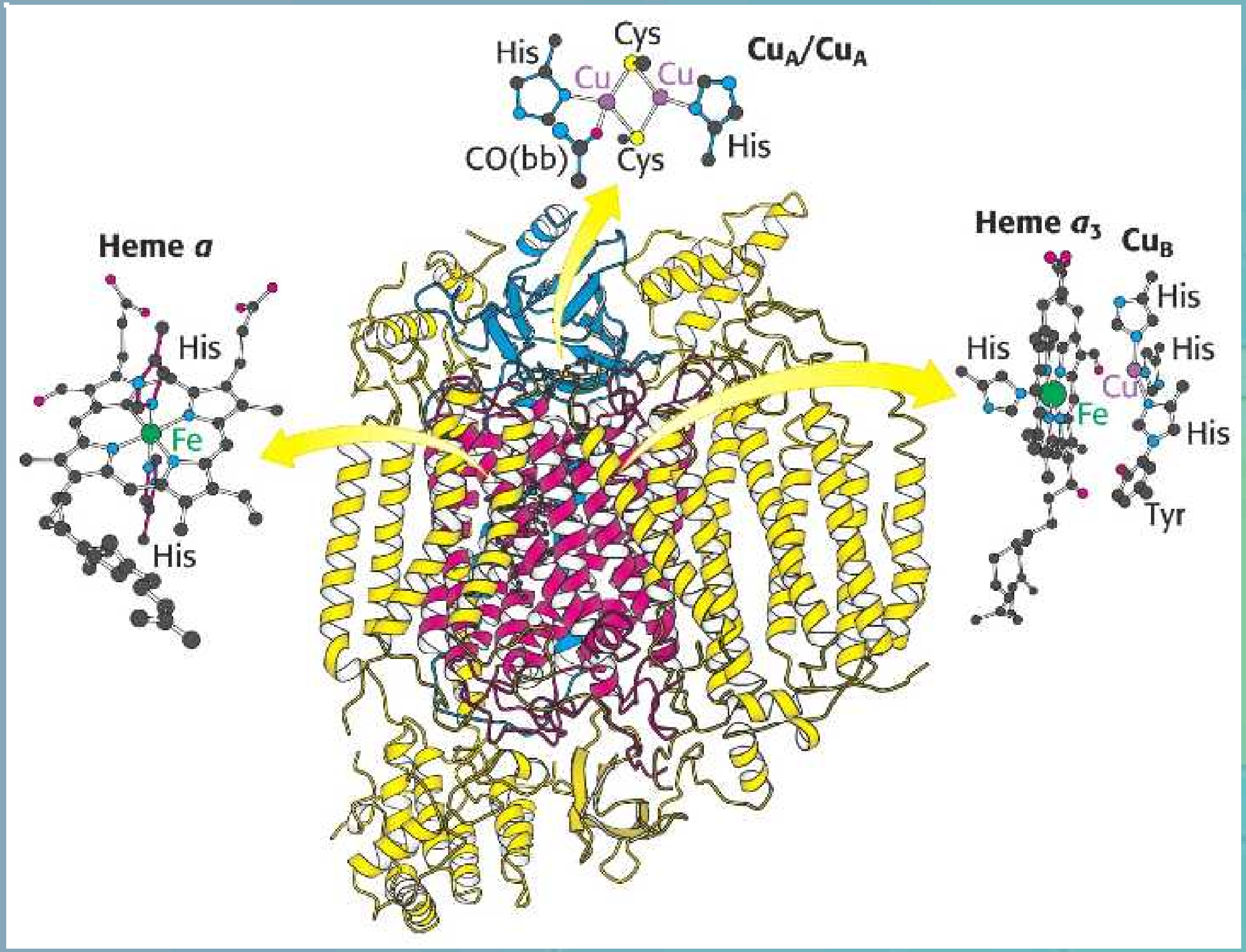


- → Cu_A/Cu_A
- → Heme a
- → Heme a_3/Cu_B

  Fe(3+)–O–O–Cu(+2)

$$4\ \text{Cyt}\ c_{red} + 4\ H^{+}_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase



Third cyt c + H^+

→ Cu_A/Cu_A

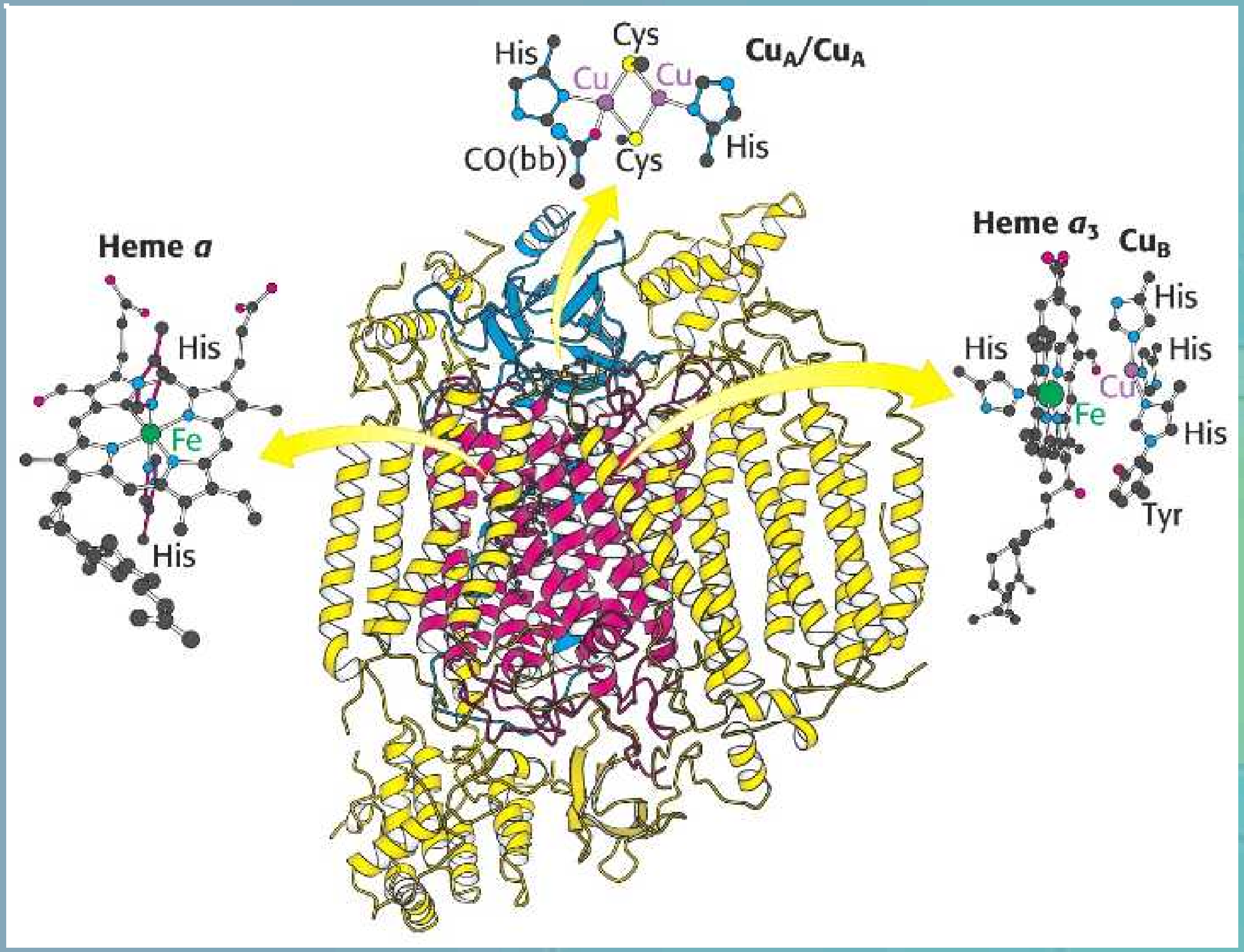
→ Heme a

→ Heme a_3/Cu_B

Fe(4+)=O HO–Cu(+2)

$$4\ \text{Cyt}\ c_{red} + 4\ H^{+}_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase

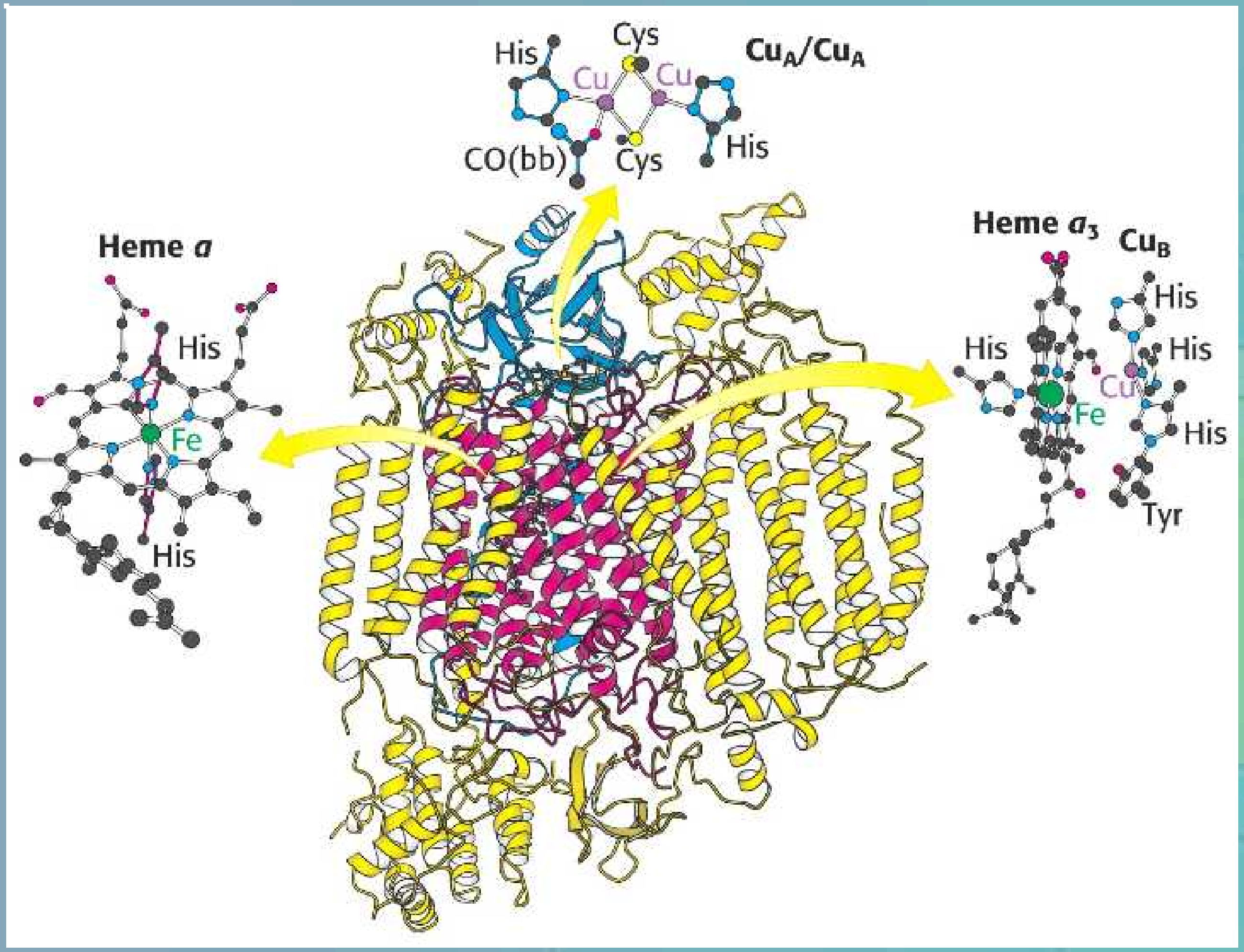


Fourth cyt c + H^+

→ Cu_A/Cu_A
→ Heme a
→ Heme a_3/Cu_B
Fe(3+)–OH HO–Cu(+2)

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

3.5 Cytochrome c Oxidase



$+ 2\ H^+$

→ Cu_A/Cu_A

→ Heme a

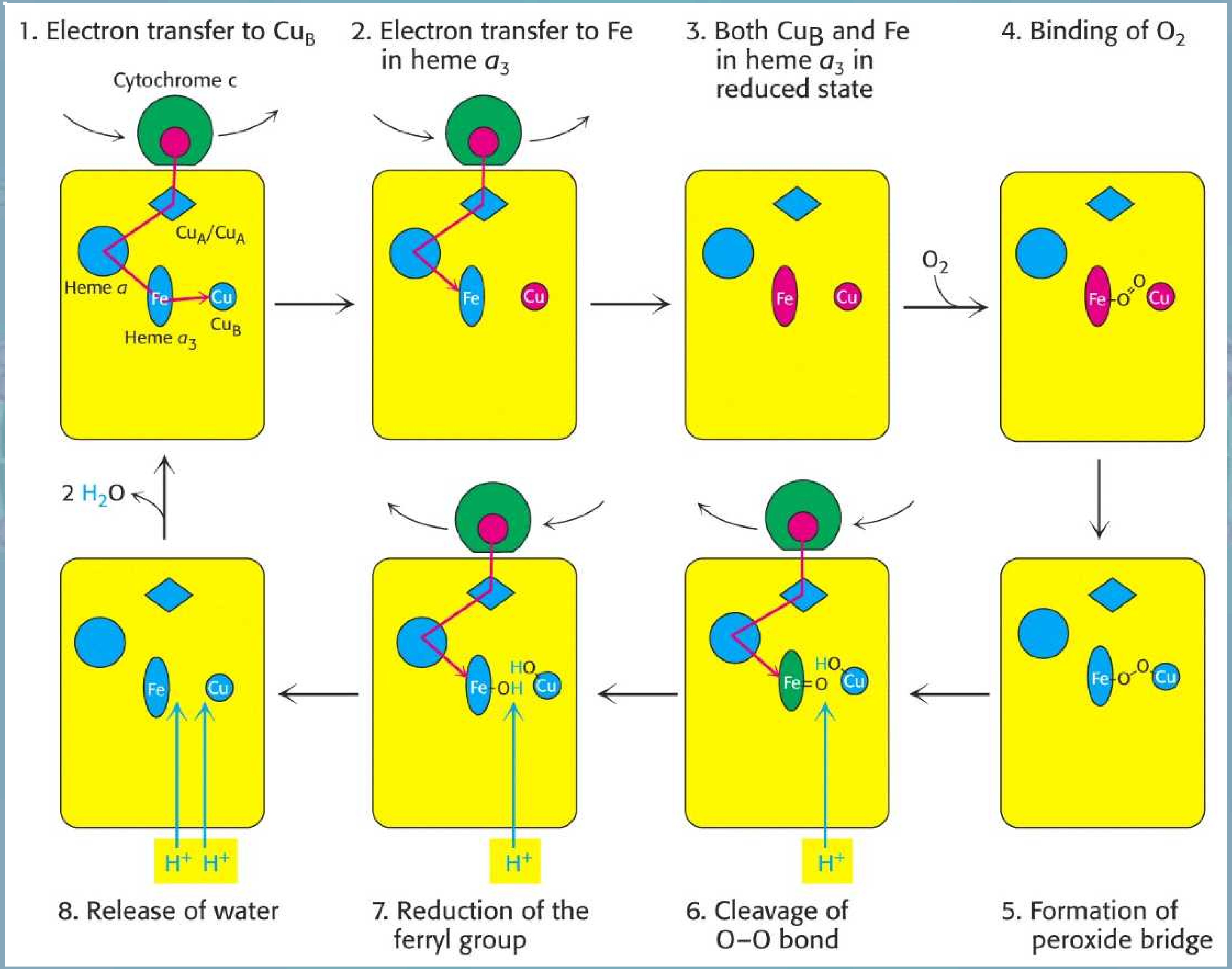
→ Heme a_3/Cu_B

Fe(3+)/Cu(+2)

→ 2 H_2O

$$4\ \text{Cyt}\ c_{red} + 4\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O$$

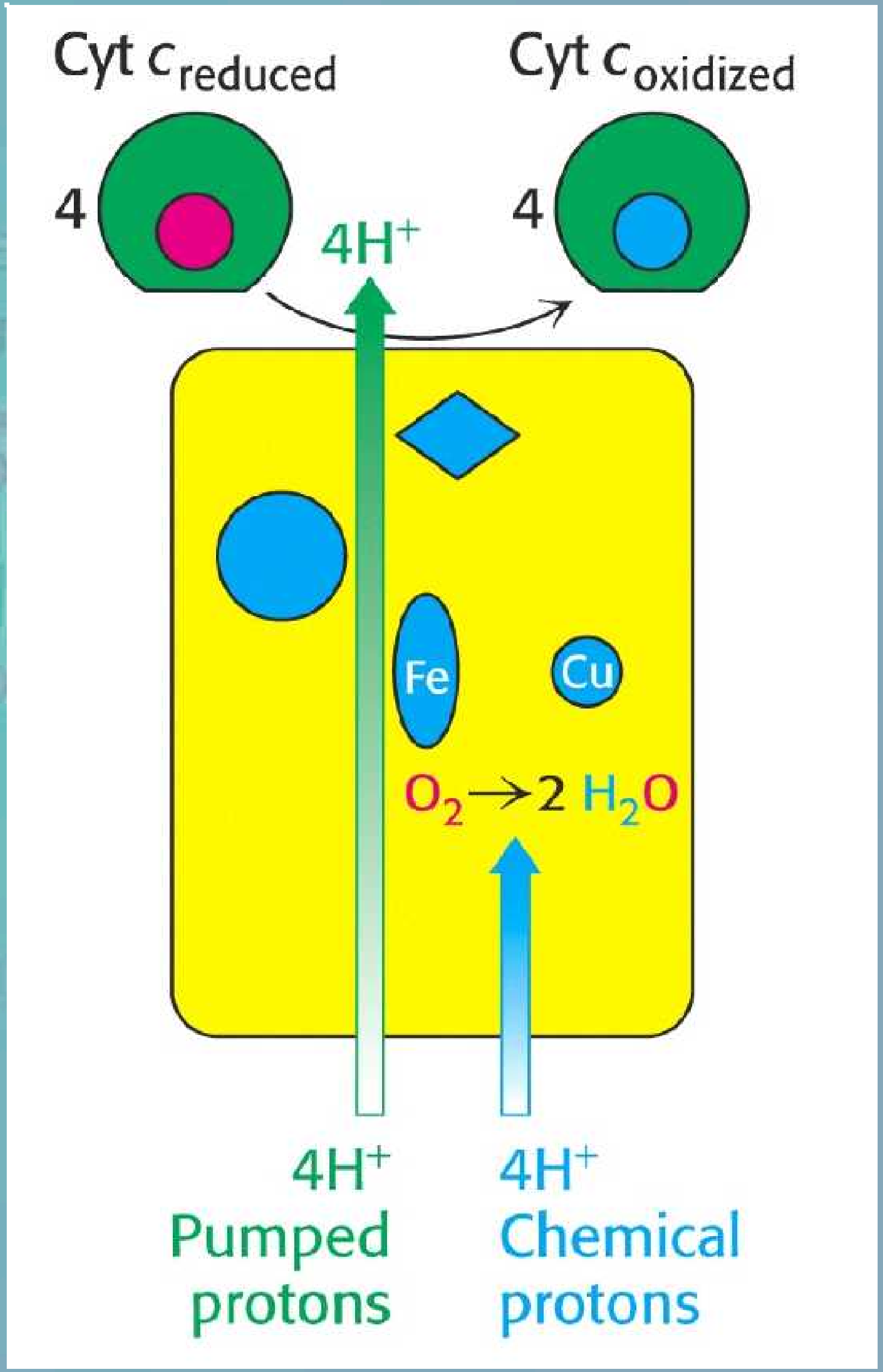
3.5 Cytochrome c Oxidase



3.5 Cytochrome c Oxidase

In addition to the 4 H+ that are taken up from the matrix side to make the 2 H2O, 4 H+'s are also pumped across the membrane.

$$4\ \text{Cyt}\ c_{red} + 8\ H^+_{matrix} + O_2 \longrightarrow 4\ \text{Cyt}\ c_{ox} + 2\ H_2O + 4\ H^+_{matrix}$$



3.6 Protective Enzymes

- The ability of the Fe/Cu center to hold the partially reduced oxygen intermediates is important because of the cellular toxicity of these intermediates:

  $O_2 + e^- \rightarrow O_2\bullet^-$ (superoxide radical)

  $O_2\bullet^- + e^- \rightarrow O_2^{2-}$ (peroxide ion)

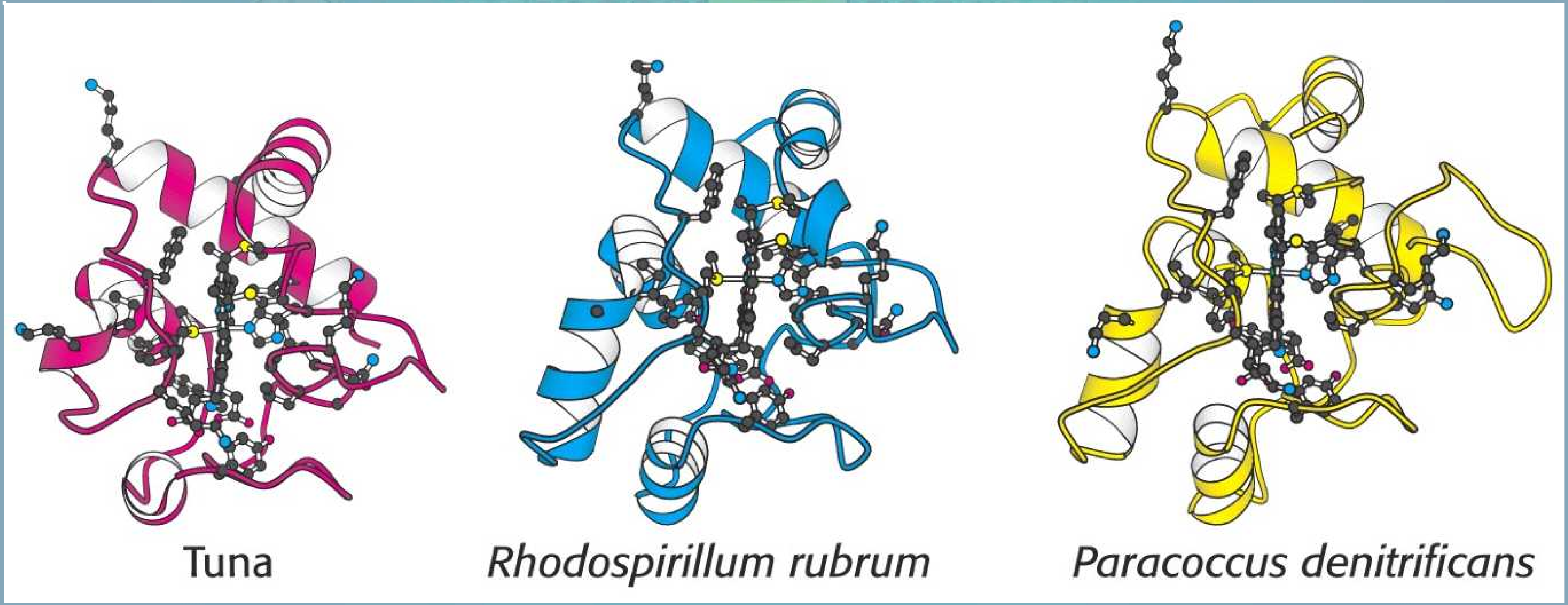
- These toxic intermediates are scavanged by the enzymes superoxide dismutase and catalase

  $2\ O_2\bullet^- + H^+ \rightarrow O_2 + H_2O_2$ (superoxide dismutase, SOD)

  $2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$ (catalase)

3.7 Conformation of Cytochrome c Oxidase

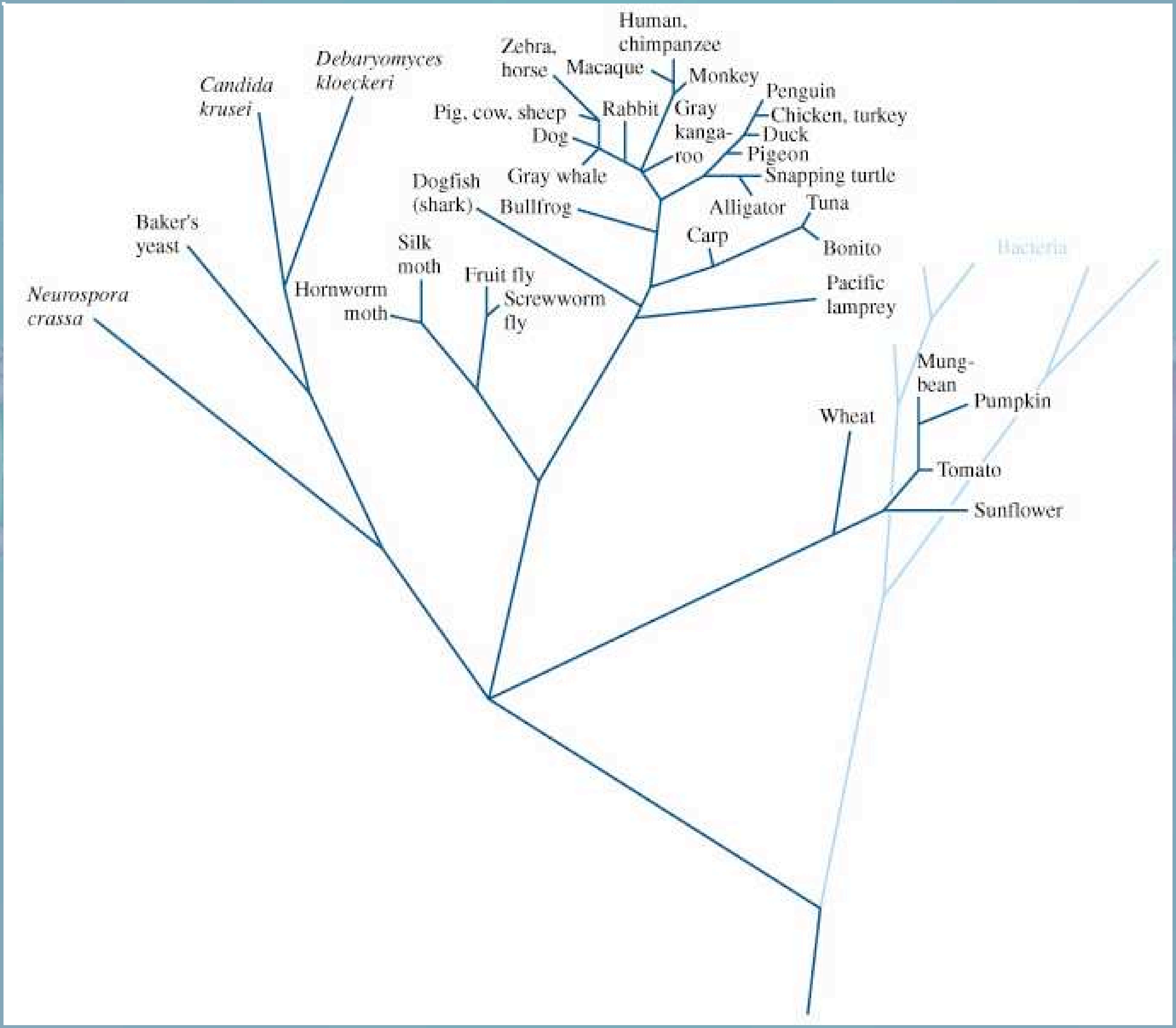
Throughout evolution the structure of cytochrome c has been highly conserved.



3.7 Conformation of Cytochrome c Oxidase

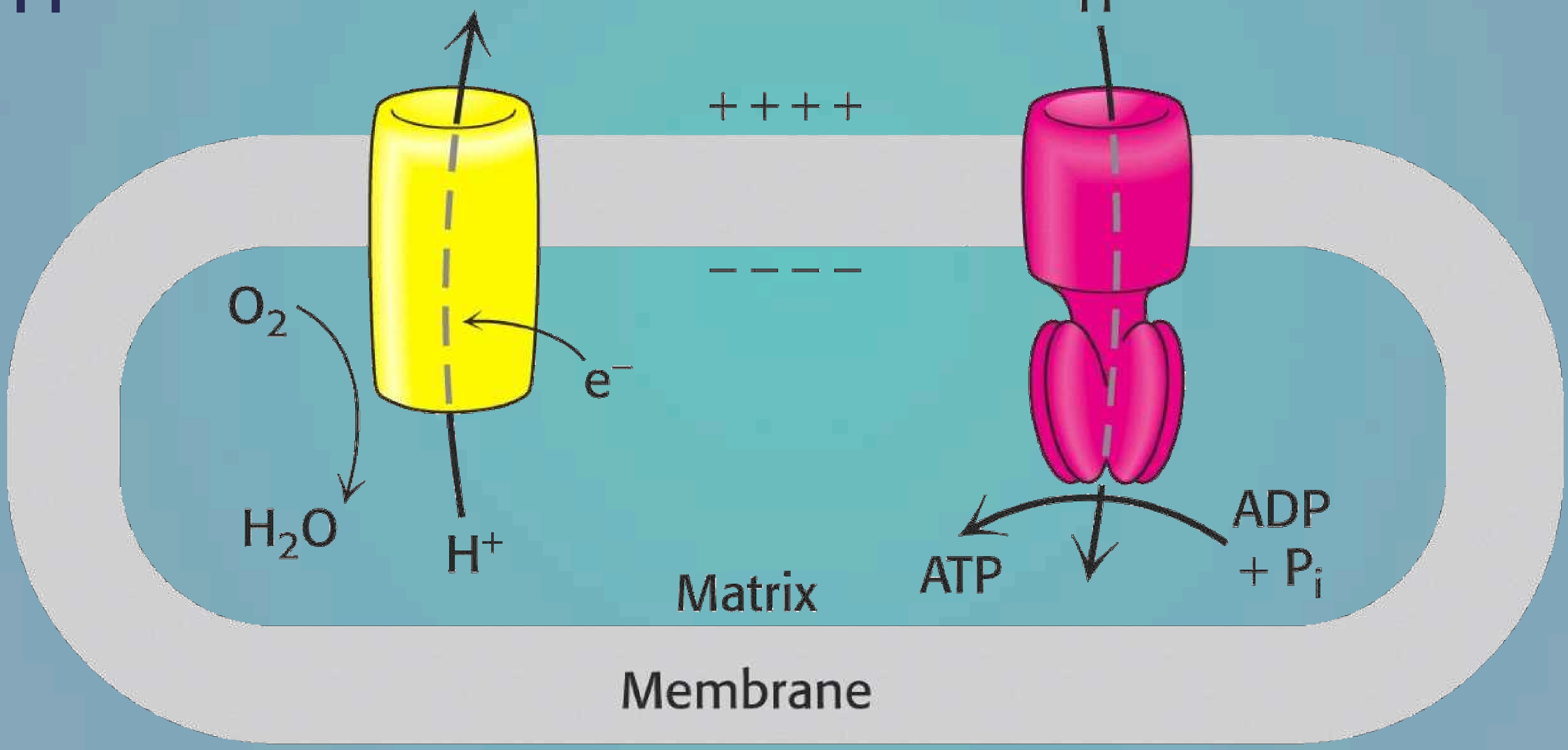
	10	20	30	40	50	60	70	80	90	100	
Human	GDVEKGKKIF	IMKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGYSYTA	ANKNKGIIWG	EDTLMEYLEN	PKKYIPGTKM	IFVGIKKKEE	RADLIAYLKK	ATNE
Chimpanzee	GDVEKGKKIF	IMKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGYSYTA	ANKNKGIIWG	EDTLMEYLEN	PKKYIPGTKM	IFVGIKKKEE	RADLIAYLKK	ATNE
Spider monkey	GDVFKGKRIF	IMKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQASGFTYTE	ANKNKGIIWG	EDTLMEYLEN	PKKYIPGTKM	IFVGIKKKEE	RADLIAYLKK	ATNE
Macaque	GDVEKGKKIF	IMKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGYSYTA	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFVGIKKKEE	RADLIAYLKK	ATNE
Cow	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGFSYTD	ANKNKGITWG	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKGE	REDLIAYLKK	ATNE
Dog	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGFSYTD	ANKNKGITWG	EETLMEYLEN	PKKYIPGTKM	IFAGIKKTGE	RADLIAYLKK	ATKE
Gray whale	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAVGFSYTD	ANKNKGITWG	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKGE	RADLIAYLKK	ATNE
Horse	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGFTYTD	ANKNKGITWK	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKTE	REDLIAYLKK	ATNE
Zebra	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAPGFSYTD	ANKNKGITWK	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKTE	REDLIAYLKK	ATNE
Rabbit	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAVGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKDE	RADLIAYLKK	ATNE
Kangaroo	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGIFGRKT	GQAPGFTYTD	ANKNKGIIWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKGE	RADLIAYLKK	ATNE
Duck	GDVEKGKKIF	VQKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAEGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKSE	RADLIAYLKD	ATAK
Turkey	GDIEKGKKIF	VQKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAEGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKSE	RVDLIAYLKD	ATSK
Chicken	GDIEKGKKIF	VQKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAEGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKSE	RVDLIAYLKD	ATSK
Pigeon	GDIEKGKKIF	VQKCSQCHTV	EKGGKHKTGP	NLHGLFGRKT	GQAEGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKAE	RADLIAYLKQ	ATAK
King penguin	GDIEKGKKIF	VQKCSQCHTV	EKGGKHKTGP	NLHGIFGRKT	GQAEGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKSE	RADLIAYLKD	ATSK
Snapping turtle	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLNGLIGRKT	GQAEGFSYTE	ANKNKGITWG	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKAE	RADLIAYLKD	ATSK
Alligator	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKTGP	NLHGLIGRKT	GQAPGFSYTE	ANKNKGITWG	EETLMEYLEN	PKKYIPGTKM	IFAGIKKKPE	RADLIAYLKE	ATSN
Bull frog	GDVEKGKKIF	VQKCAQCHTV	EKGGKHKVGP	NLYGLIGRKT	GQAAGFSYTD	ANKNKGITWG	EDTLMEYLEN	PKKYIPGTKM	IFAGIKKKGE	RQDLIAYLKS	ACSK
Tuna	GDVAKGKKTF	VQKCAQCHTV	ENGGKHKVGP	NLWGLFGRKT	GQAEGYSYTD	ANKSKGIVWN	ENTLMEYLEN	PKKYIPGTKM	IFAGIKKKGE	RQDLVAYLKS	ATS
Dogfish	GDVEKGKKVF	VQKCAQCHTV	ENGGKHKTGP	NLSGLFGRKT	GQAQGFSYTD	ANKSKGITWQ	QETLRIYLEN	PKKYIPGTKM	IFAGIKKKSE	RQDLIAYLKK	TAAS
Starfish	GQVEKGKKIF	VQRCAQCHTV	EKAGKHKTGP	NLNGILGRKT	GQAAGFSYTD	ANRNKGITWK	NETLFEYLEN	PKKYIPGTKM	VFAGLKKQKE	RQDLIAYLEA	ATK
Fruit fly	GDVEKGKKLF	VQRCAQCHTV	EAGGKHKVGP	NLHGLIGRKT	GQAAGFAYTD	ANKAKGITWN	EDTLFEYLEN	PKKYIPGTKM	IFAGLKKPNE	RGDLIAYLKS	ATK
Silkmoth	GNAENGKKIF	VQRCAQCHTV	EAGGKHKVGP	NLHGFYGRKT	GQAPGFSYSN	ANKAKGITWG	DDTLFEYLEN	PKKYIPGTKM	VFAGLKKANE	RADLIAYLKE	STK
Pumpkin	GNSKAGEKIF	KTKCAQCHTV	DKGAGHKQGP	NLNGLFGRQS	GTTPGYSYSA	ANKNRAVIWE	EKTLYDYLLN	PKKYIPGTKM	VFPGLKKPQD	RADLIAYLKE	ATA
Tomato	GNPKAGEKIF	KTKCAQCHTV	EKGAGHKEGP	NLNGLFGRQS	GTTAGYSYSA	ANKNMAVNWG	ENTLYDYLLN	PKKYIPGTKM	VFPGLKKPQE	RADLIAYLKE	ATA
Arabidopsis	GDAKKGANLF	KTRCAQCHTL	KAGEGNKIGP	ELHGLFGRKT	GSVAGYSYTD	ANKQKGIEWK	DDTLFEYLEN	PKKYIPGTKM	AFGGLKKPKD	RNDLITFLEE	ETK
Mung bean	GNSKSGEKIF	KTKCAQCHTV	DKGAGHKQGP	NLNGLIGRQS	GTTAGYSYST	ANKNMAVIWE	EKTLYDYLLN	PKKYIPGTKM	VFPGLKKPQD	RADLIAYLKE	STA
Wheat	GNPDAGAKIF	KTKCAQCHTV	DAGAGHKQGP	NLHGLFGRQS	GTTAGYSYSA	ANKNKAVEWE	ENTLYDYLLN	PKKYIPGTKM	VFPGLKKPQD	RADLIAYLKK	ATSS
Sunflower	GNPTTGEKIF	KTKCAQCHTV	EKGAGHKQGP	NLNGLFGRQS	GTTAGYSYSA	GNKNKAVIWE	ENTLYDYLLN	PKKYIPGTKM	VFPGLKKPQE	RADLIAYLKT	STA
Yeast	GSAKKGATLF	KTRCLQCHTV	EKGGPHKVGP	NLHGIFGRHS	GQAEGYSYTD	ANIKKNVLWD	ENNMSEYLTN	PKKYIPGTKM	AFGGLKKEKD	RNDLITYLKK	ACE
Debaryomyces	GSEKKGANLF	KTRCLQCHTV	EKGGPHKVGP	NLHGVVGRTS	GQAQGFSYTD	ANKKKGVEWT	EQDLSDYLEN	PKKYIPGTKM	AFGGLKKAKD	RNDLITYLVK	ATK
Candida	GSEKKGATLF	KTRCLQCHTV	EKGGPHKVGP	NLHGVFGRKS	GLAEGYSYTD	ANKKKGVEWT	EQTMSDYLEN	PKKYIPGTKM	AFGGLKKPKD	RNDLVTYLKK	ATS
Aspergillus	GDAK-GAKLF	QTRCAQCHTV	EAGGPHKVGP	NLHGLFGRKT	GQSEGYAYTD	ANKQAGVTWD	ENTLFSYLEN	PKKFIPGTKM	AFGGLKKGKE	RNDLITYLKE	STA
Rhodomicrobium	GDPVKGEQVF	KQ-CKICHQV	GPTAKNGVGP	EQNDVFGQKA	GARPGFNYSD	AMKNSGLTWD	EATLDKYLEN	PKAVVPGTKM	VFVGLKNPQD	RADVIAYLKQ	LSGK
Nitrobacter	GDVEAGKAAF	NK-CKACHEI	GESAKNKVGP	ELDGLDGRHS	GAVEGYAYSP	ANKASGITWT	EAEFKEYIKD	PKAKVPGTKM	VFAGIKKDSE	LDNLWAYVSQ	FDKD
Agrobacterium	GDVAKGEAAF	KR-CSACHAI	GEGAKNKVGP	QLNGIIGRTA	GGDPDYNYSN	AMKKAGLVWT	PQELRDFLSA	PKKKIPGNKM	ALAGISKPEE	LDNLIAYLIF	SASSK
Rhodopila	GDPVEGKHLF	HTICILICHT-	DIKGRNKVGP	SLYGVVGRHS	GIEPGYNYSE	ANIKSGIVWT	PDVLFKYIEH	PQKIVPGTKM	GYPG-QPDQK	RADIIAYLET	LK

3.7 Conformation of Cytochrome c Oxidase

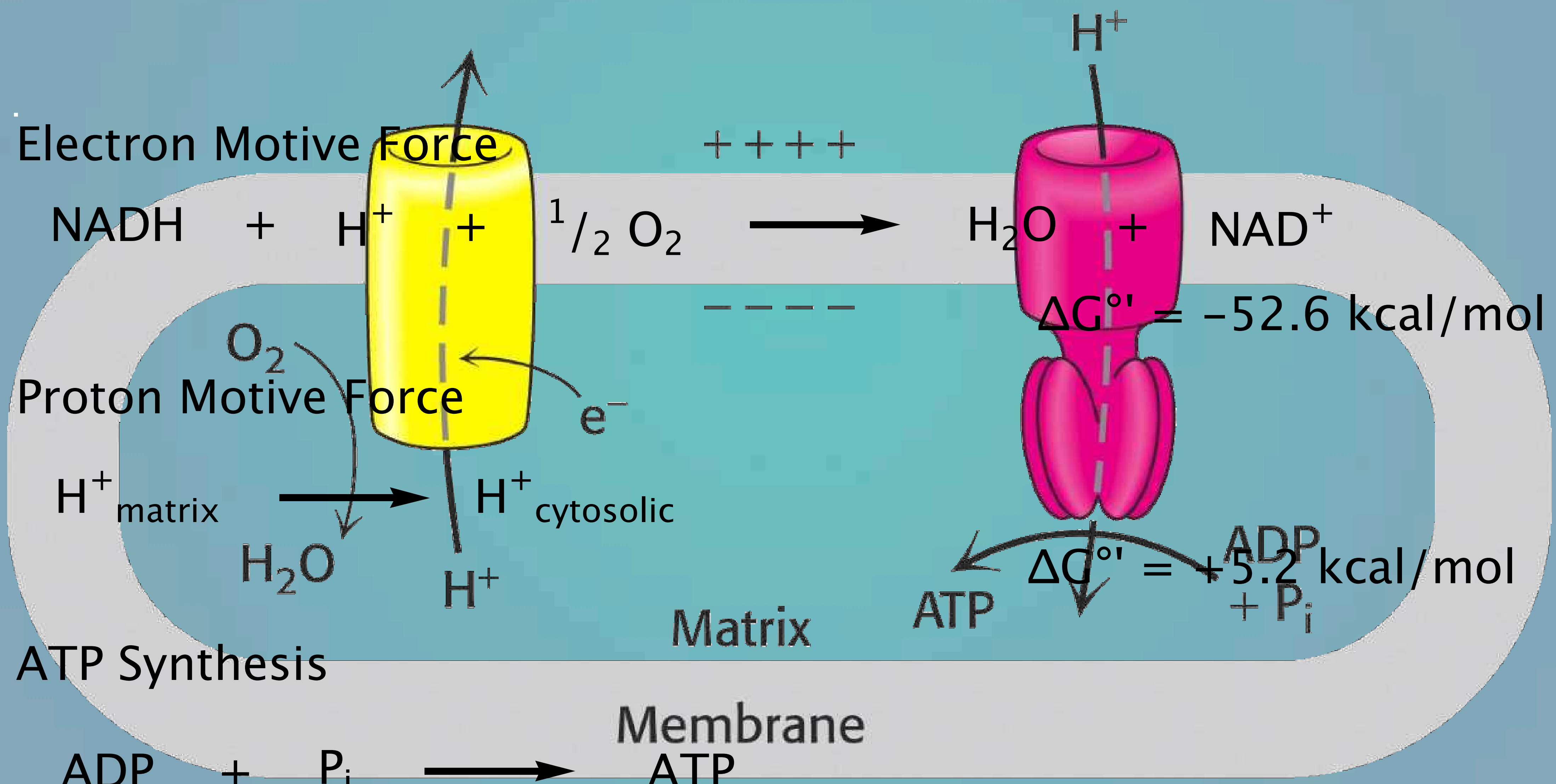


4. Proton Gradient and ATP Synthesis

A proton gradient powers the synthesis of ATP



4. Proton Gradient



Electron Motive Force

$$NADH + H^+ + \frac{1}{2} O_2 \rightarrow H_2O + NAD^+$$

$\Delta G°' = -52.6$ kcal/mol

Proton Motive Force

$$H^+_{matrix} \rightarrow H^+_{cytosolic}$$

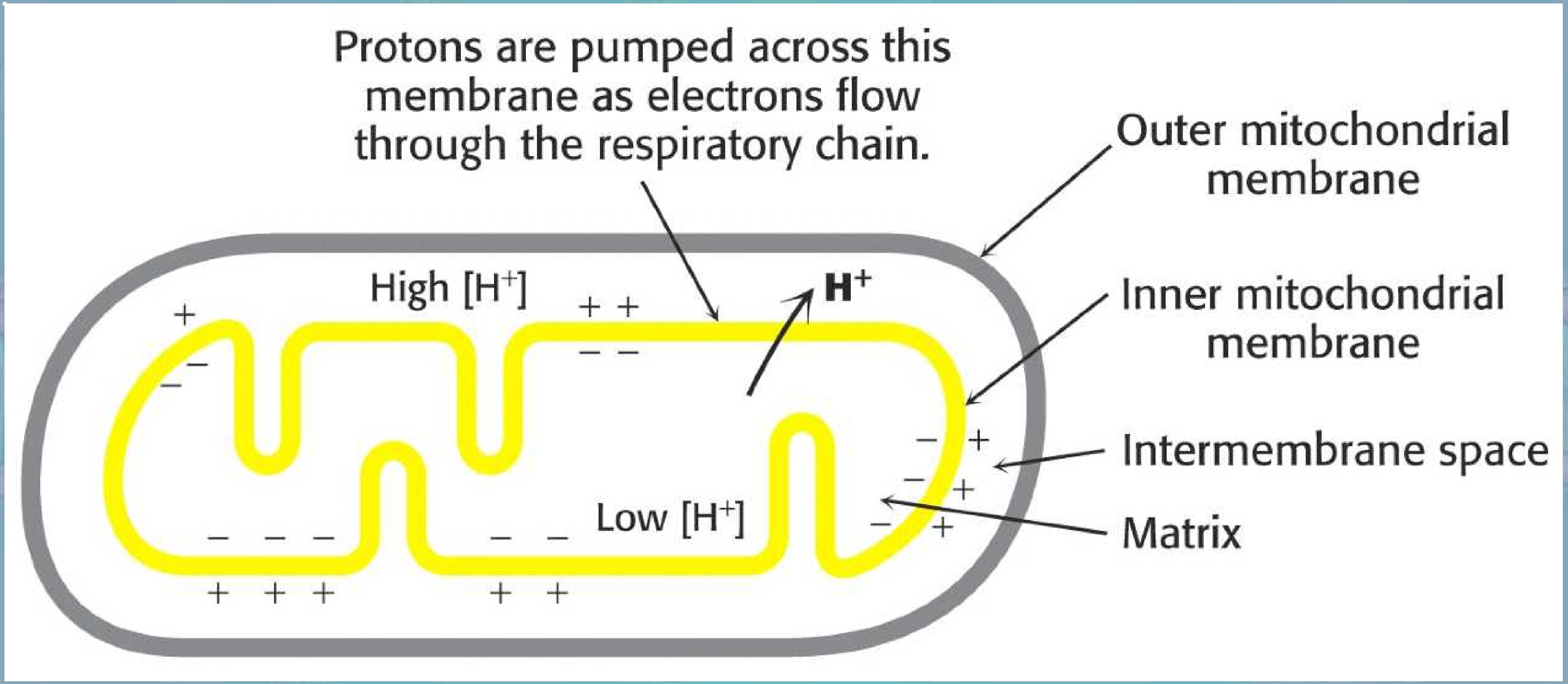
$\Delta G°' = +5.2$ kcal/mol

ATP Synthesis

$$ADP + P_i \rightarrow ATP$$

$\Delta G°' = +7.3$ kcal/mol

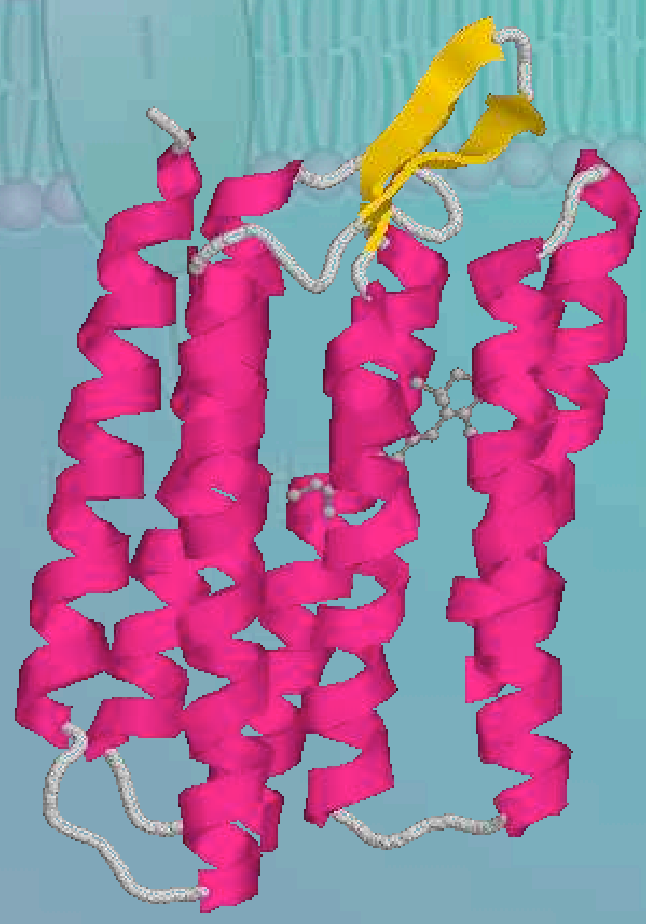
4. Proton Gradient

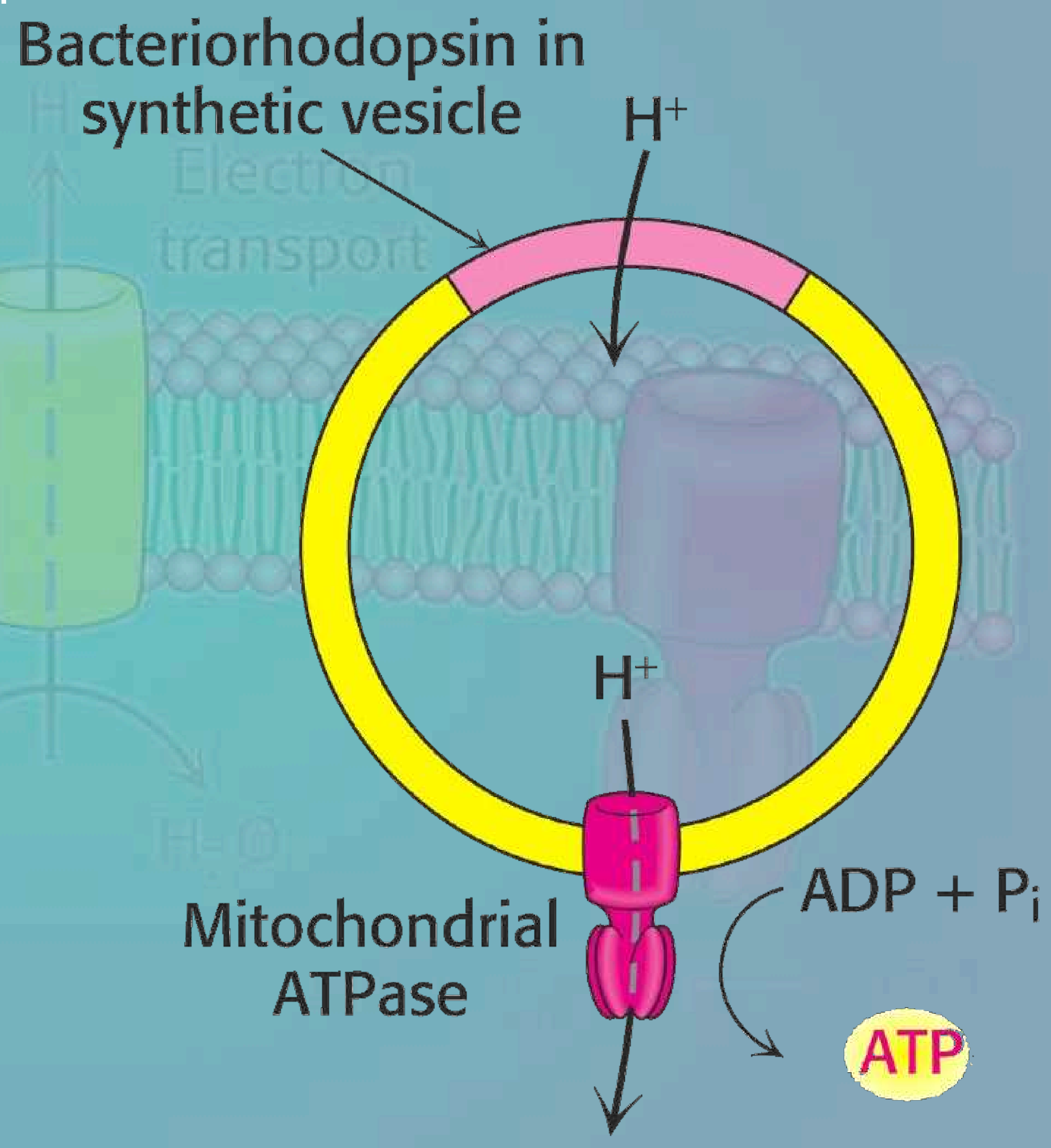


4. Proton Gradient

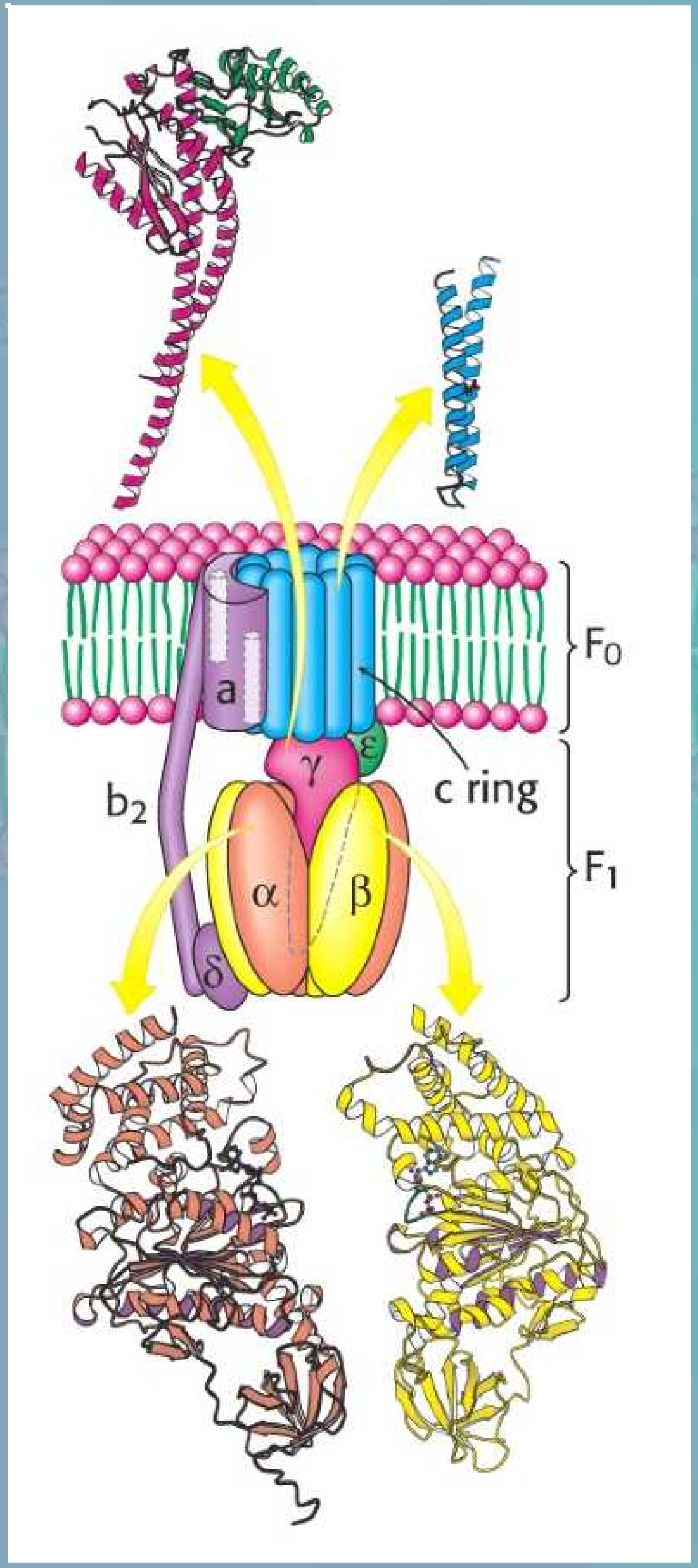
The Chemiosmotic Theory of Peter Mitchell

- Proposed in 1961

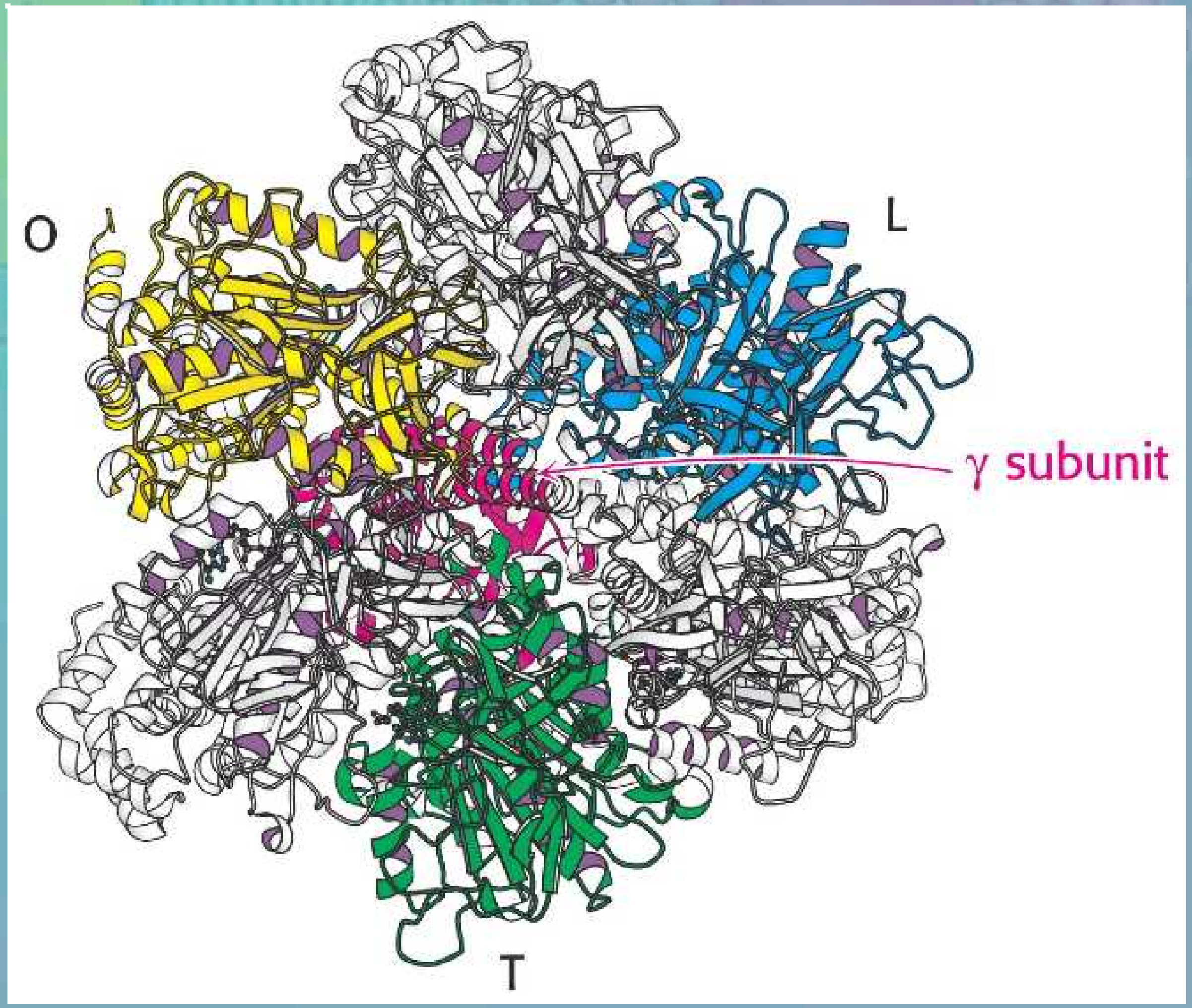




4.1 Structure of ATP Synthase

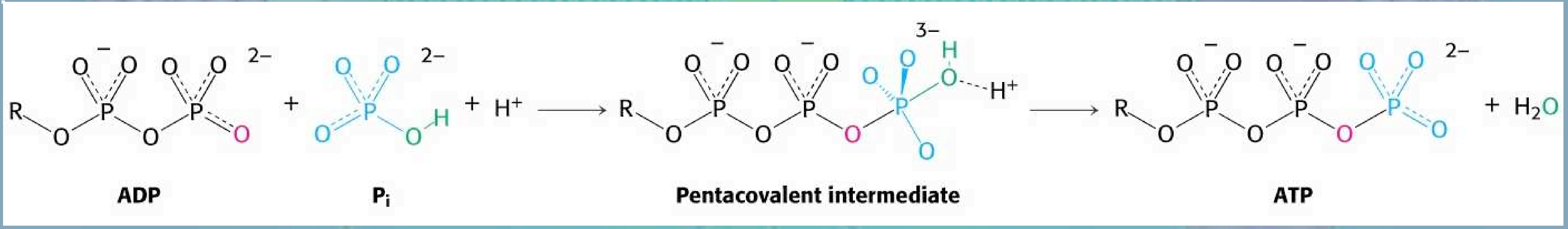


ATP synthase is composed of a proton-conducting unit and a catalytic unit



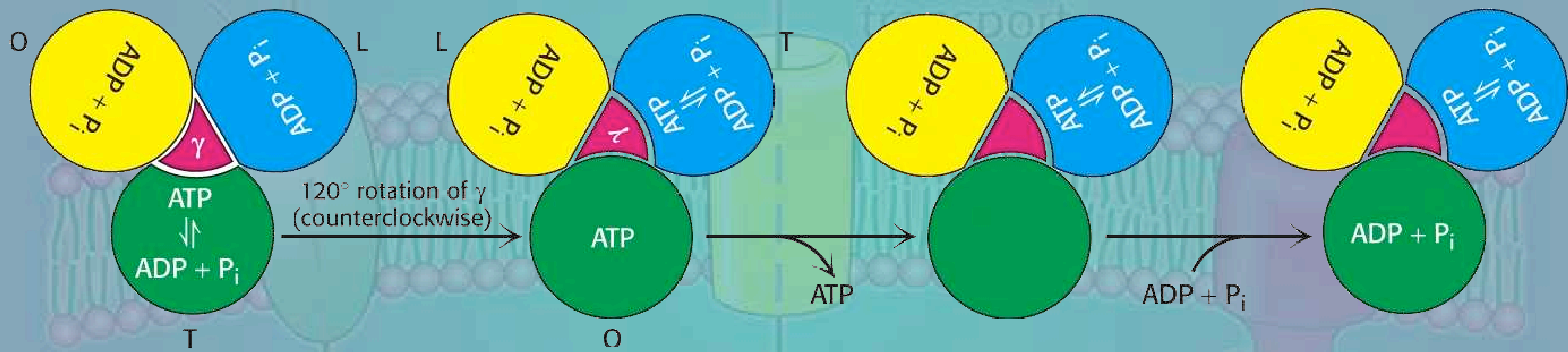
4.2 ATP Synthase Binding-Change Mechanism

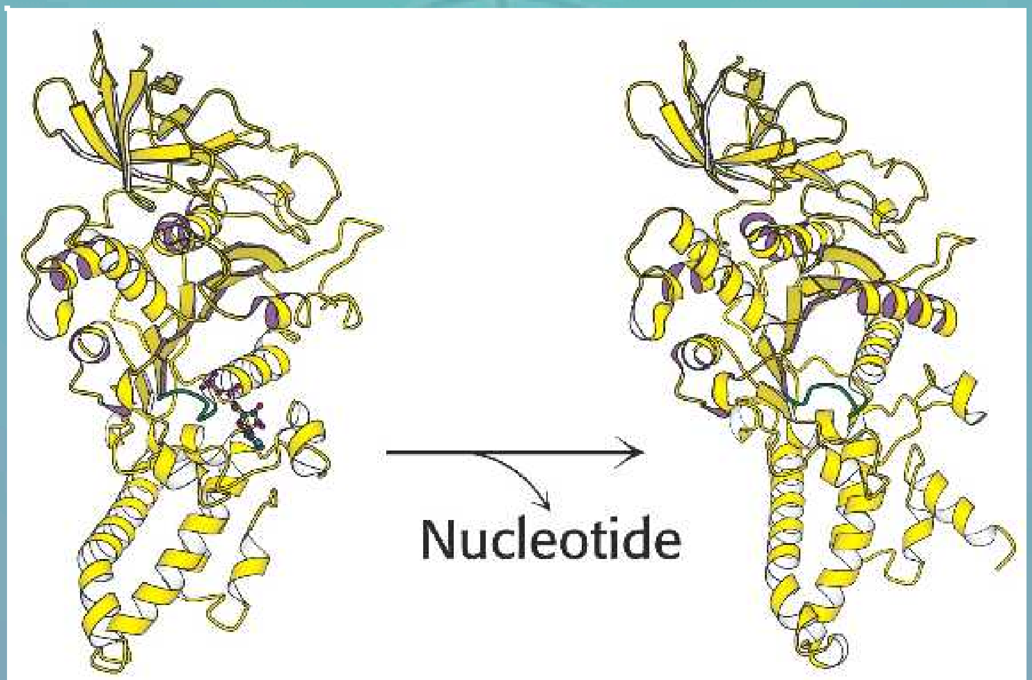
ATP is synthesized on β subunit without independent of the proton-motive force.



4.2 ATP Synthase Binding-Change Mechanism

- Proton flow through ATP synthase leads to the release of tightly bound ATP

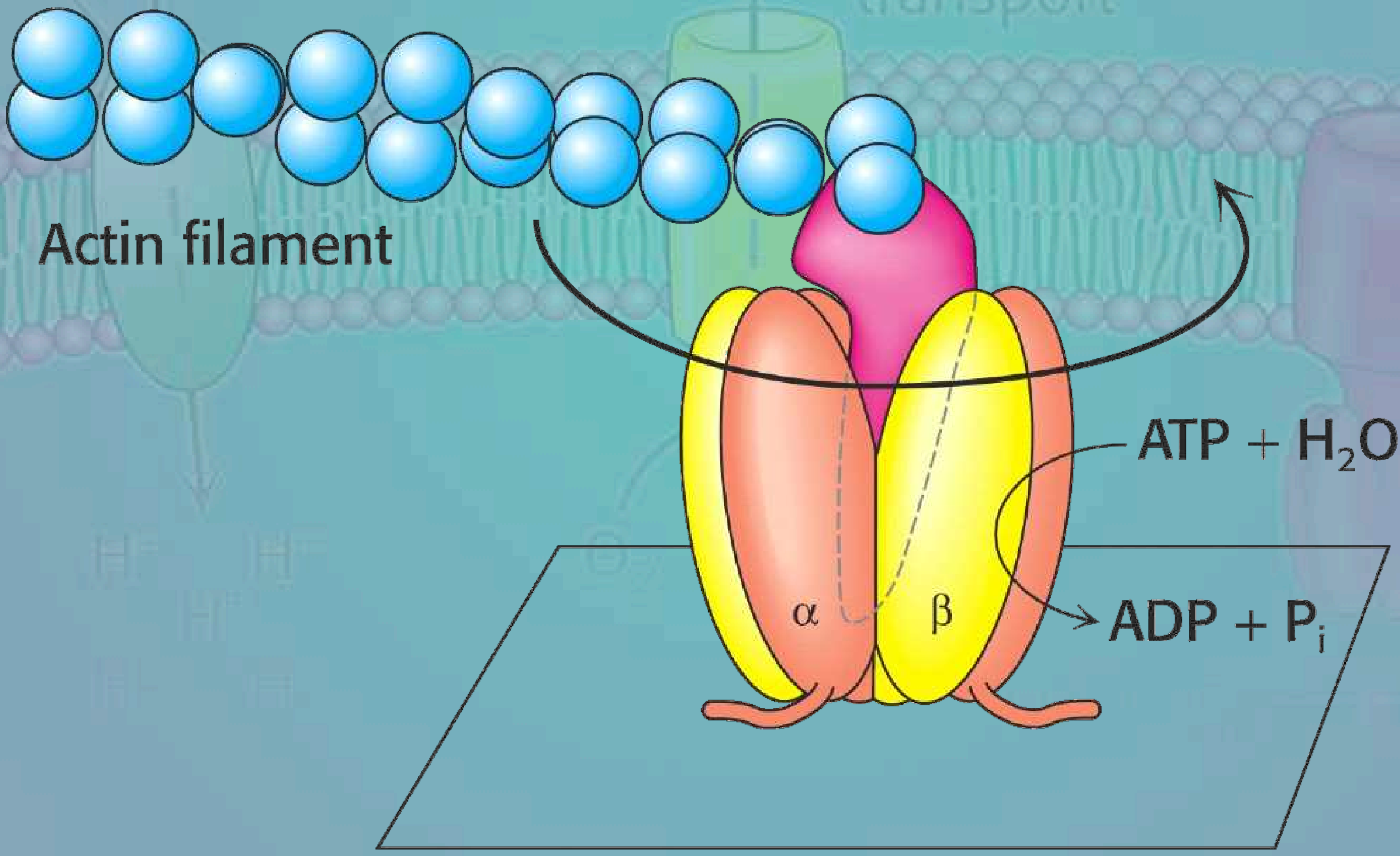




4.3 Molecular Motors

The world's smallest molecular motor

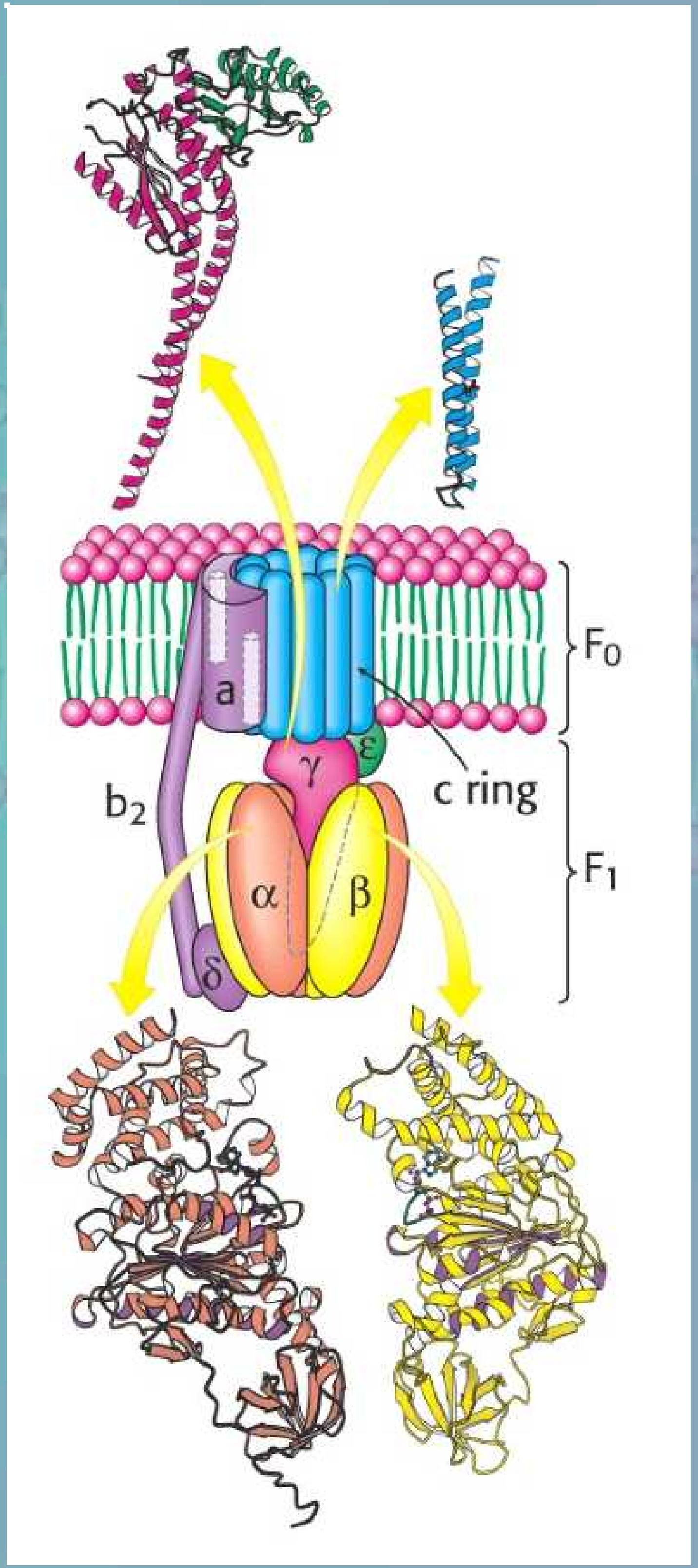
- Rotational catalysis



4.4 ATP Synthesis and Proton Flow

Proton flow around the c Ring powers ATP synthesis.

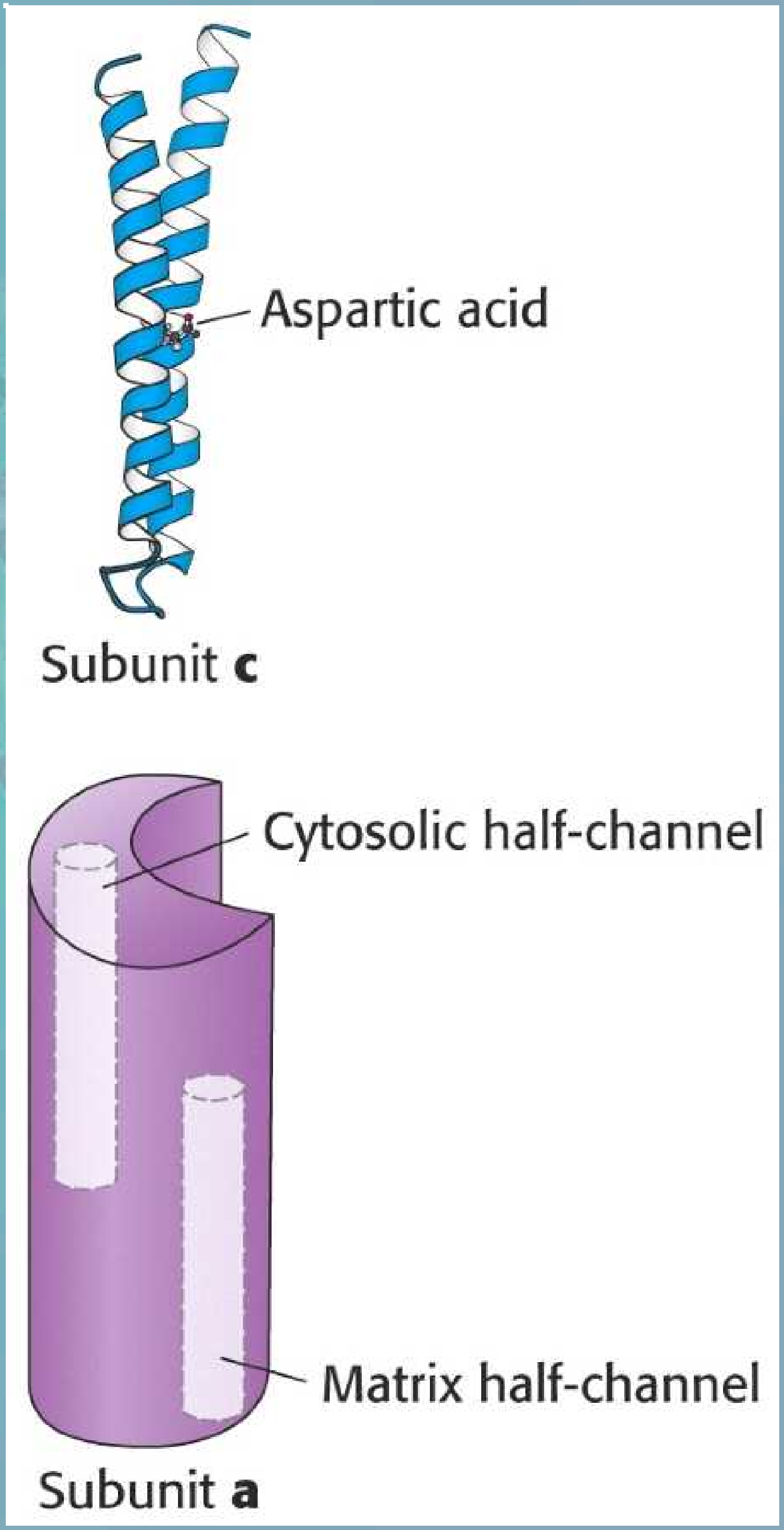
- c, γ and ϵ subunits constitute the **rotor**.
- a, b2 and δ subunites constitute the **stator**



4.4 ATP Synthesis and Proton Flow

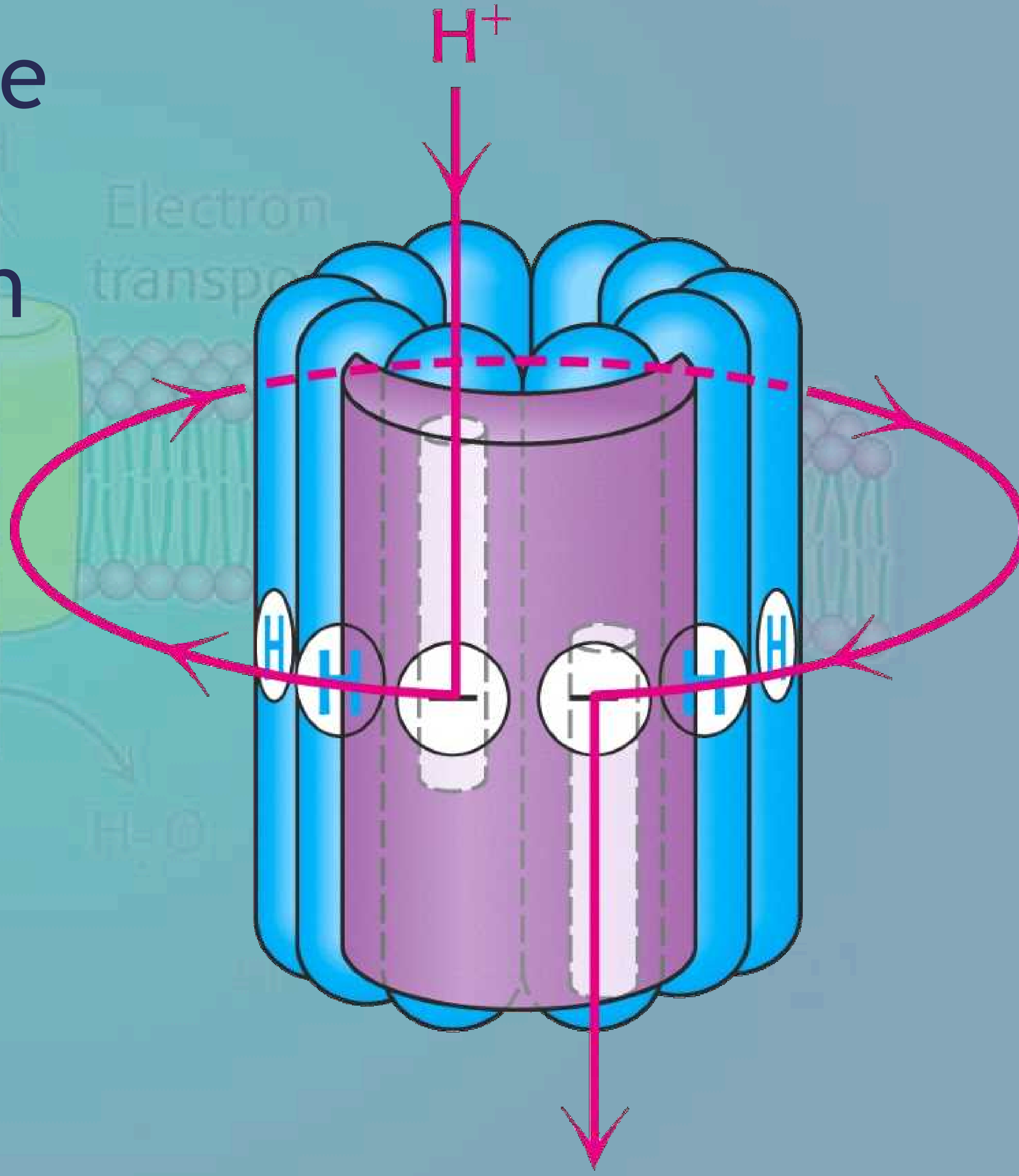
Proton flow around the c Ring powers ATP synthesis.

- c, γ and ϵ subunits constitute the **rotor**.
- a, b2 and δ subunites constitute the **stator**

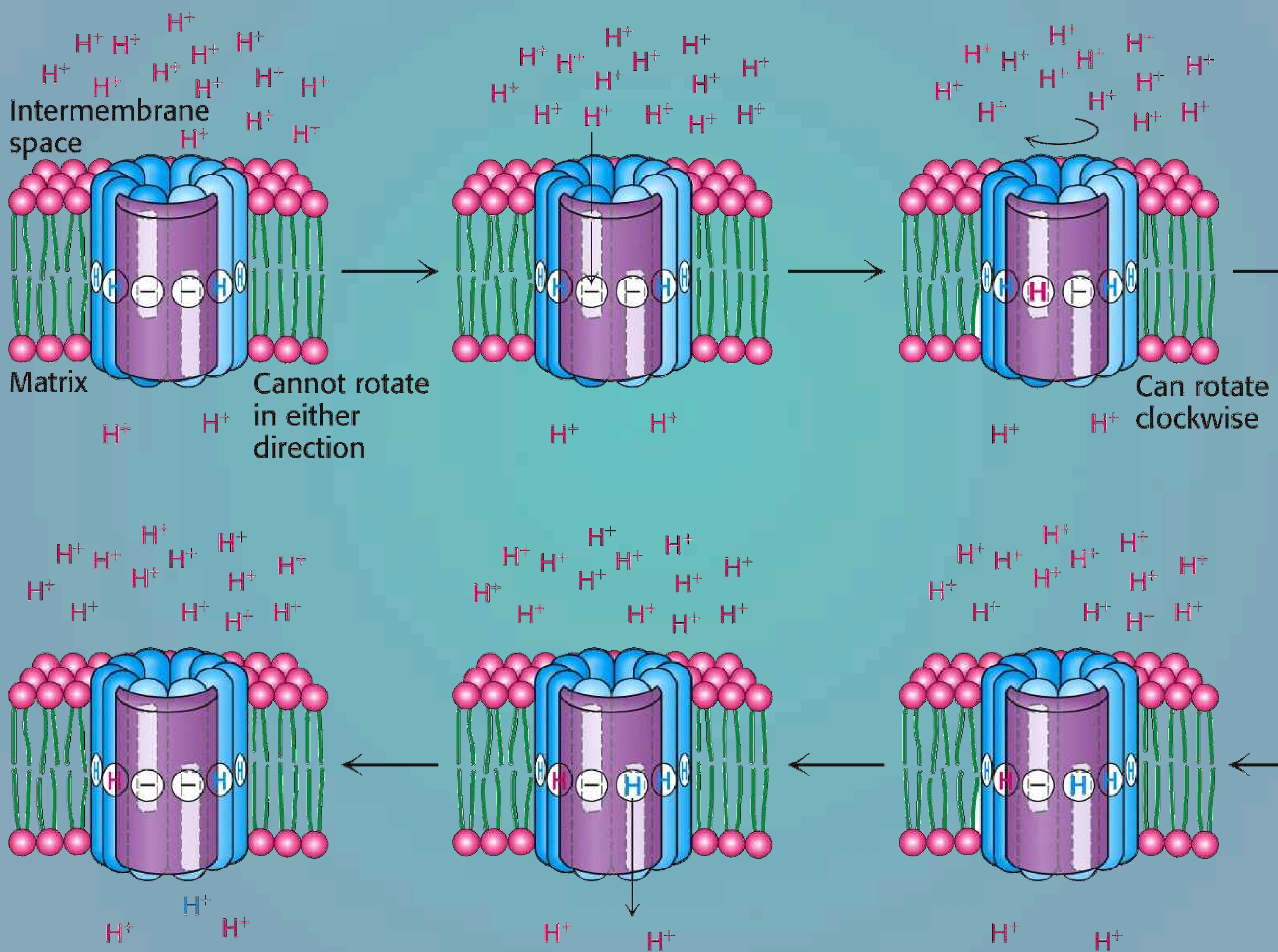


4.4 ATP Synthesis and Proton Flow

The combination of the **a** and **c** subunits provide a path through the membrane



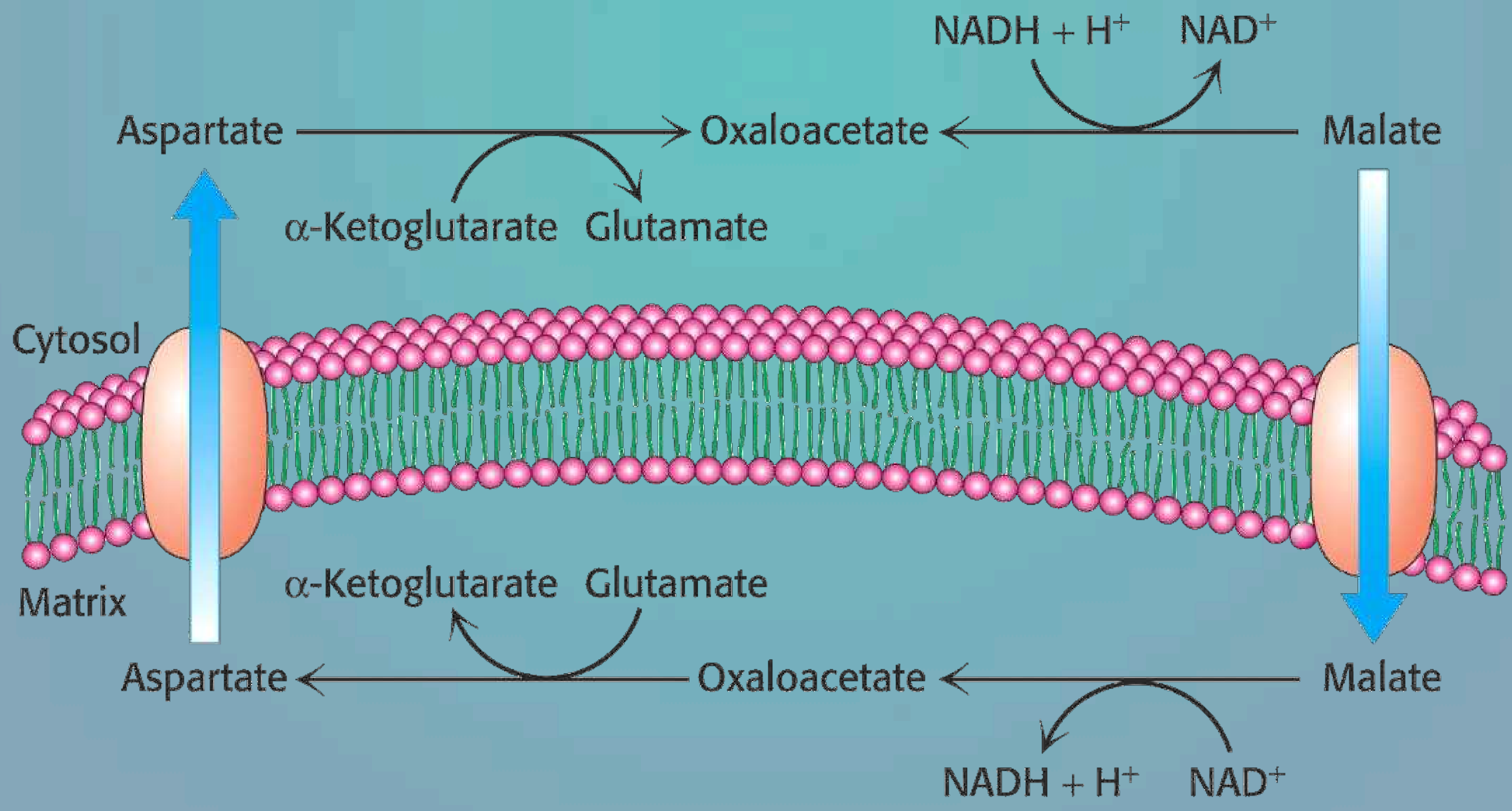
4.4 ATP Synthesis and Proton Flow



5. Shuttles

The inner mitochondrial membrane is impermeable to most substances

- Shuttles are required to move materials into and out of the mitochondrial matrix.

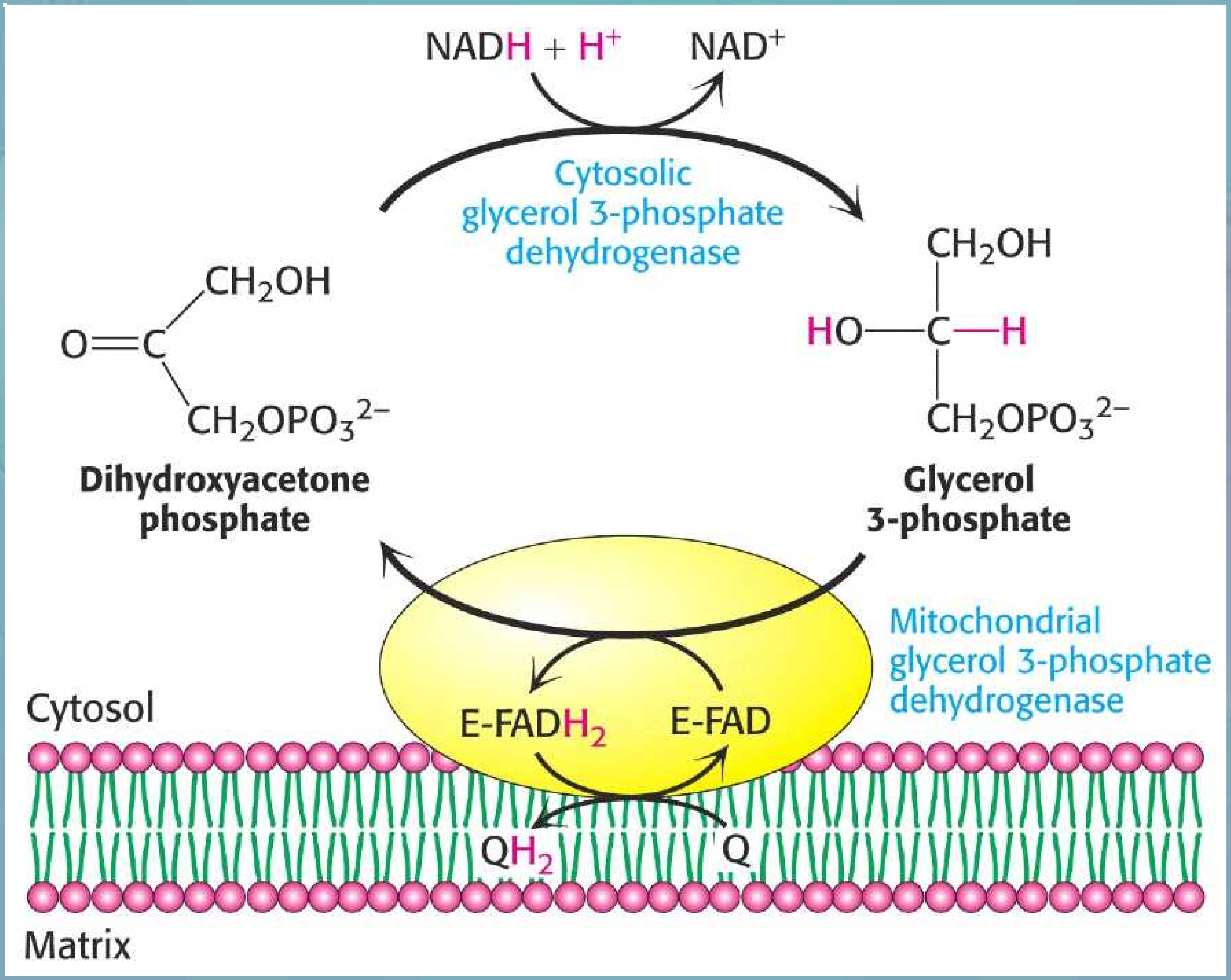


5.1 Electrons from Cytosolic NADH

- The NADH produced in glycolysis is on the wrong side of the inner mitochondrial membrane.
- There are two ways to get these electrons into mitochondrial matrix where they can feed into the electron transport chain
  - Glycerol 3-phosphate shuttle
  - Malate/Aspartate shuttle

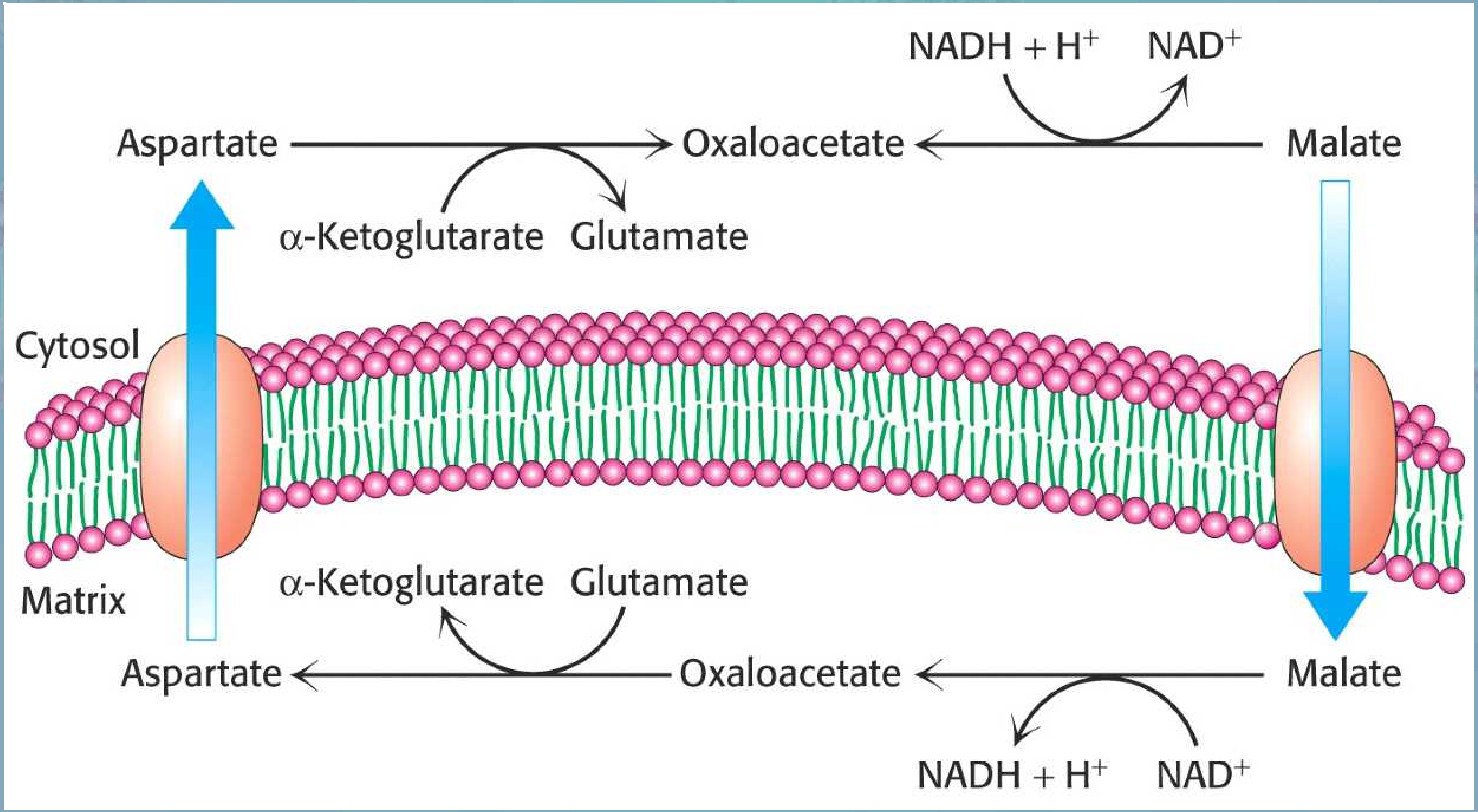
5.1 Electrons from Cytosolic NADH

Glycerol 3-phosphate shuttle



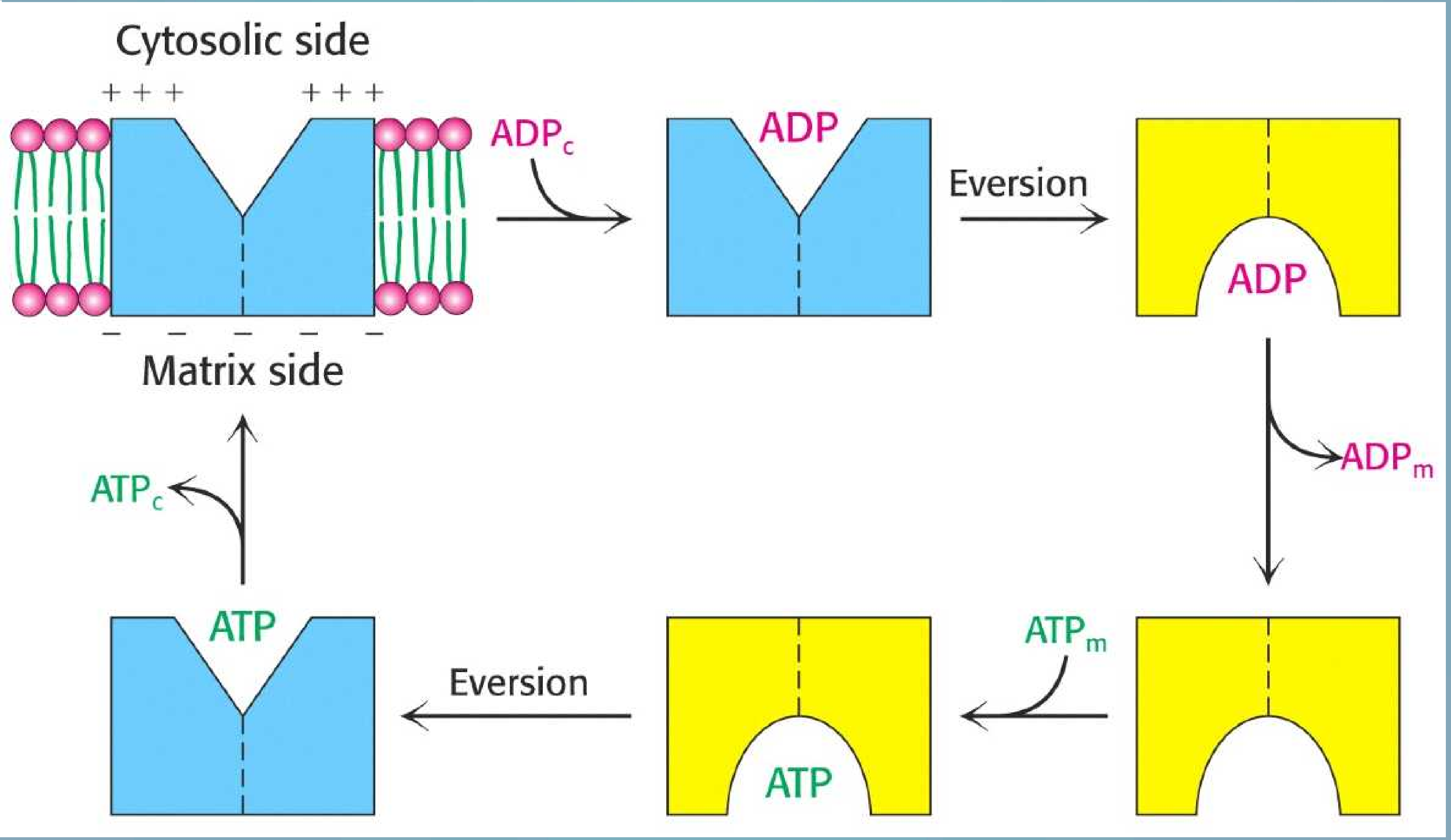
5.1 Electrons from Cytosolic NADH

Malate/Aspartate Shuttle



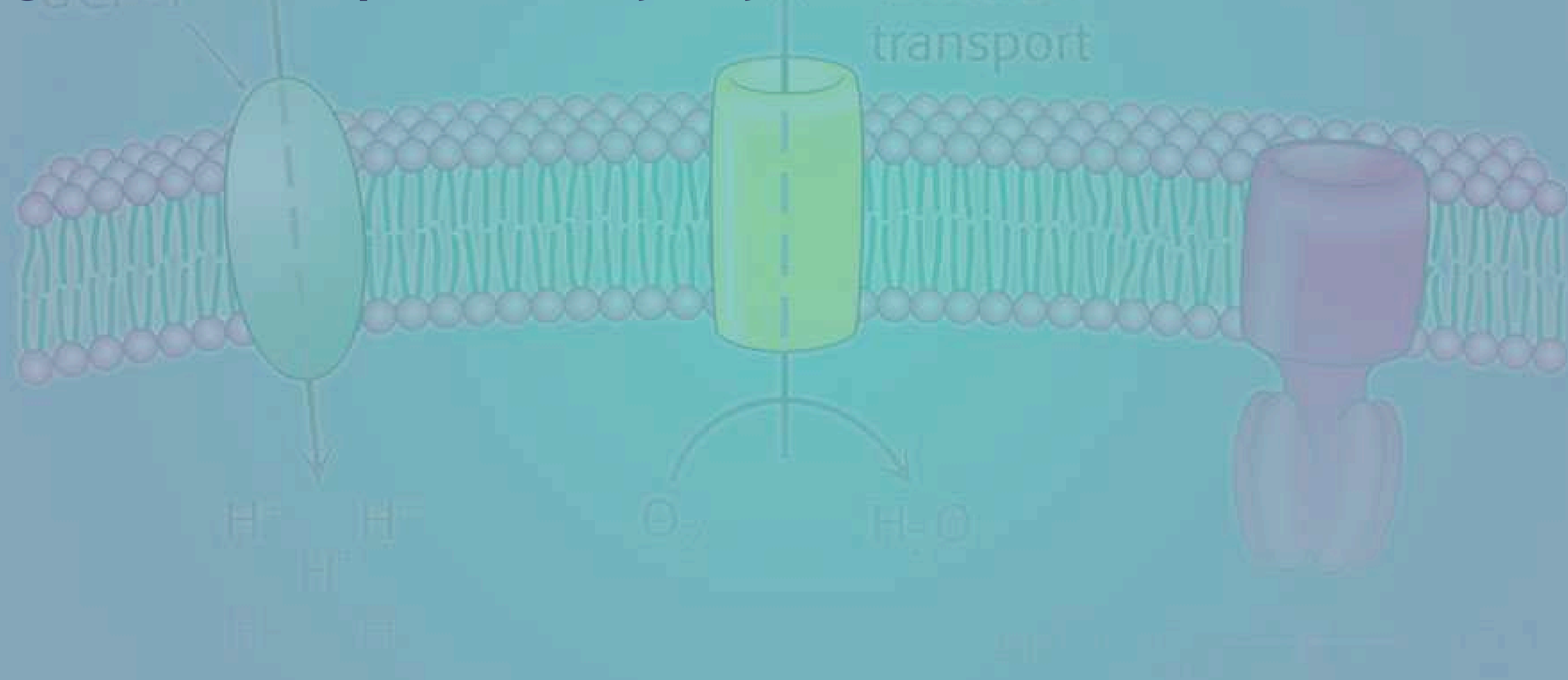
5.2 ATP-ADP Translocase

ATP-ADP translocase is an antiporter



6. Regulation

The regulation of cellular respiration is governed primarily by the need for ATP



6.1 ATP Yield

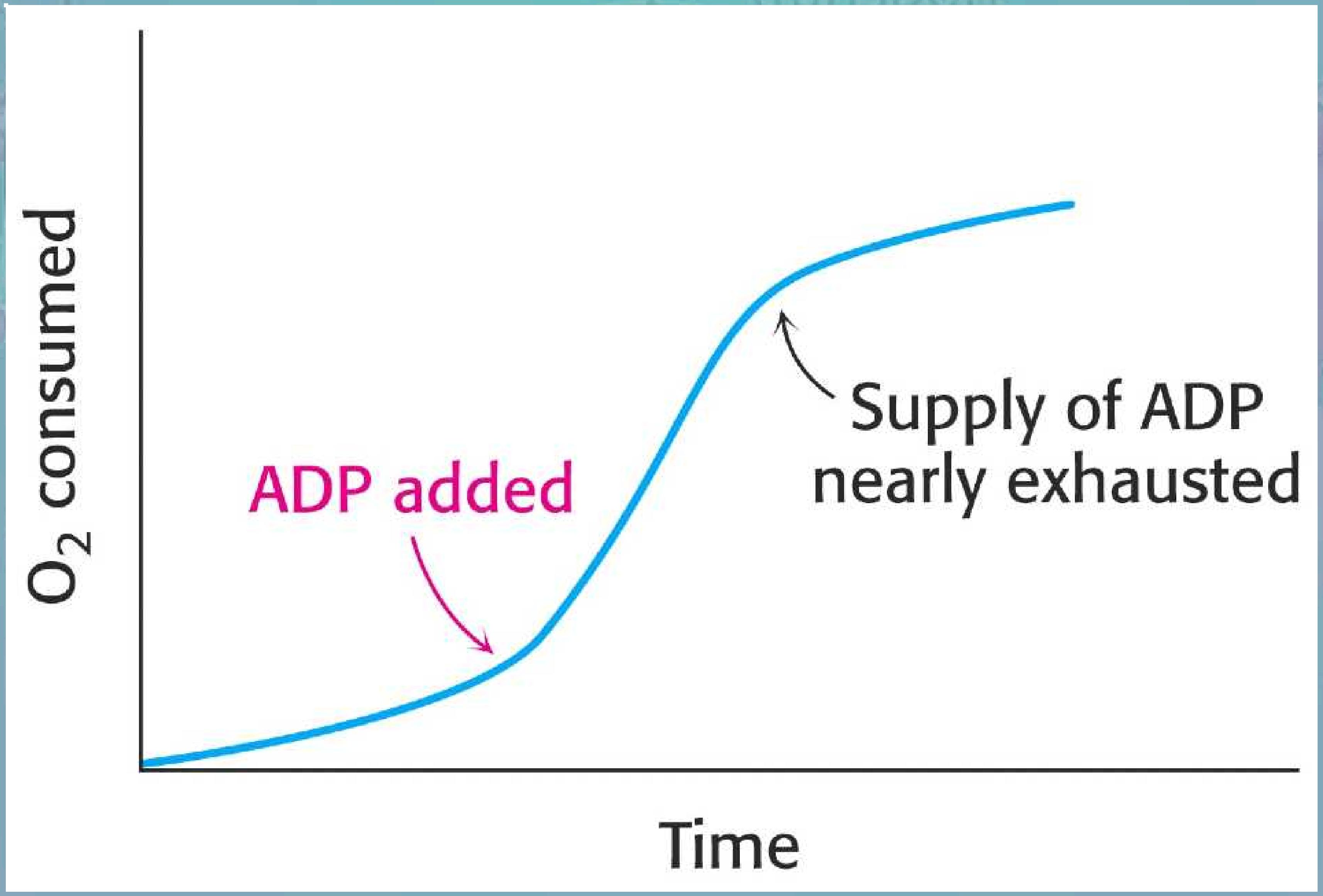
ATP yield for complete oxidation is approximately 30

TABLE 18.4 **ATP yield from the complete oxidation of glucose**

Reaction sequence	ATP yield per glucose molecule
Glycolysis: Conversion of glucose into pyruvate (in the cytosol)	
Phosphorylation of glucose	−1
Phosphorylation of fructose 6-phosphate	−1
Dephosphorylation of 2 molecules of 1,3-BPG	+2
Dephosphorylation of 2 molecules of phosphoenolpyruvate	+2
2 molecules of NADH are formed in the oxidation of 2 molecules of glyceraldehyde 3-phosphate	
Conversion of pyruvate into acetyl CoA (inside mitochondria)	
2 molecules of NADH are formed	
Citric acid cycle (inside mitochondria)	
2 molecules of guanosine triphosphate are formed from 2 molecules of succinyl CoA	+2
6 molecules of NADH are formed in the oxidation of 2 molecules each of isocitrate, α-ketoglutarate, and malate	
2 molecules of $FADH_2$ are formed in the oxidation of 2 molecules of succinate	
Oxidative phosphorylation (inside mitochondria)	
2 molecules of NADH formed in glycolysis; each yields 1.5 molecules of ATP (assuming transport of NADH by the glycerol 3-phosphate shuttle)	+3
2 molecules of NADH formed in the oxidative decarboxylation of pyruvate; each yields 2.5 molecules of ATP	+5
2 molecules of $FADH_2$ formed in the citric acid cycle; each yields 1.5 molecules of ATP	+3
6 molecules of NADH formed in the citric acid cycle; each yields 2.5 molecules of ATP	+15
NET YIELD PER MOLECULE OF GLUCOSE	+30

6.2 Rate of Oxidative Phosphorylation

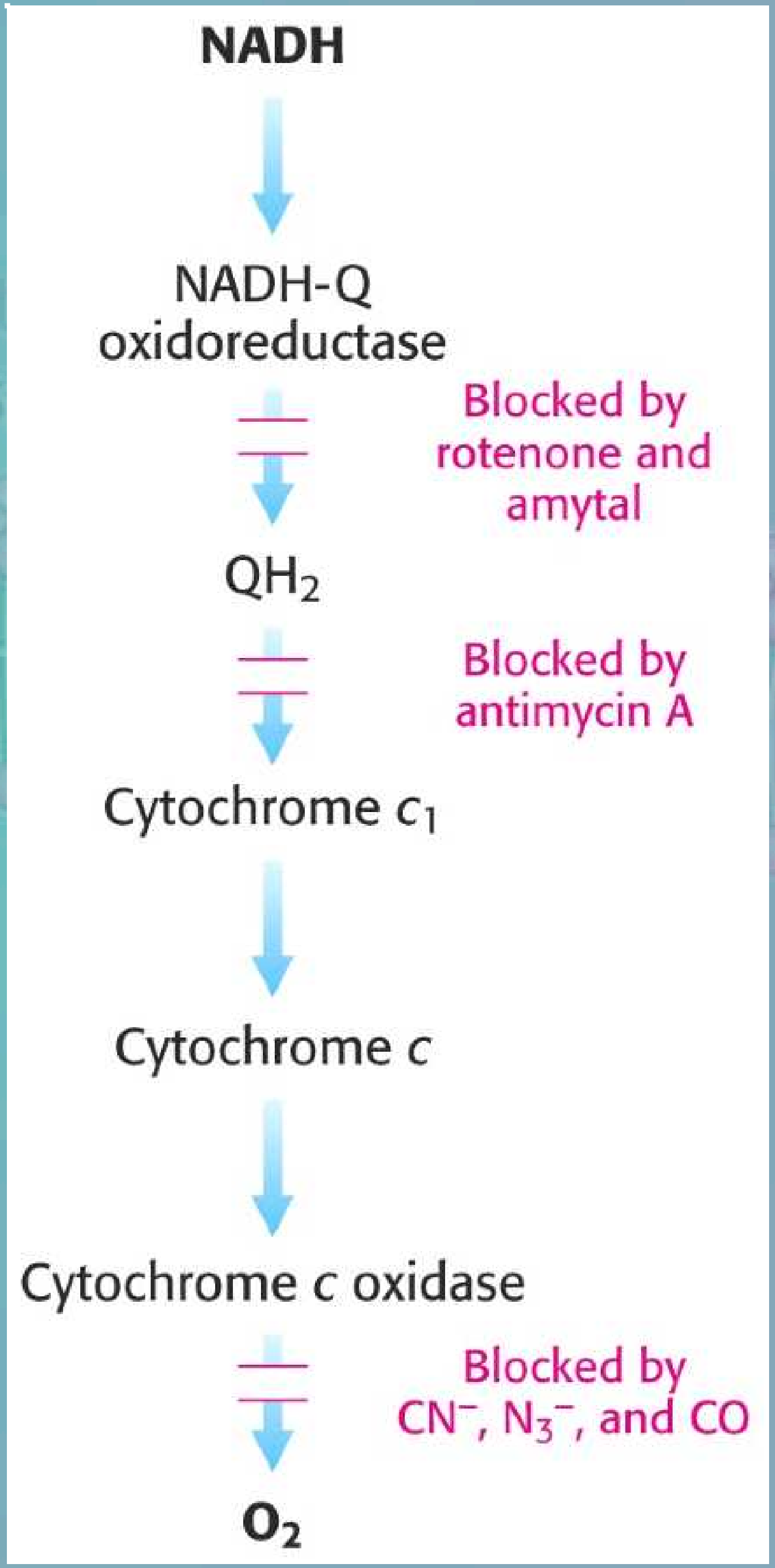
The rate of oxidative phosphorylation is determined by the need for ATP.



6.3 Inhibition of Oxidative Phosphorylation

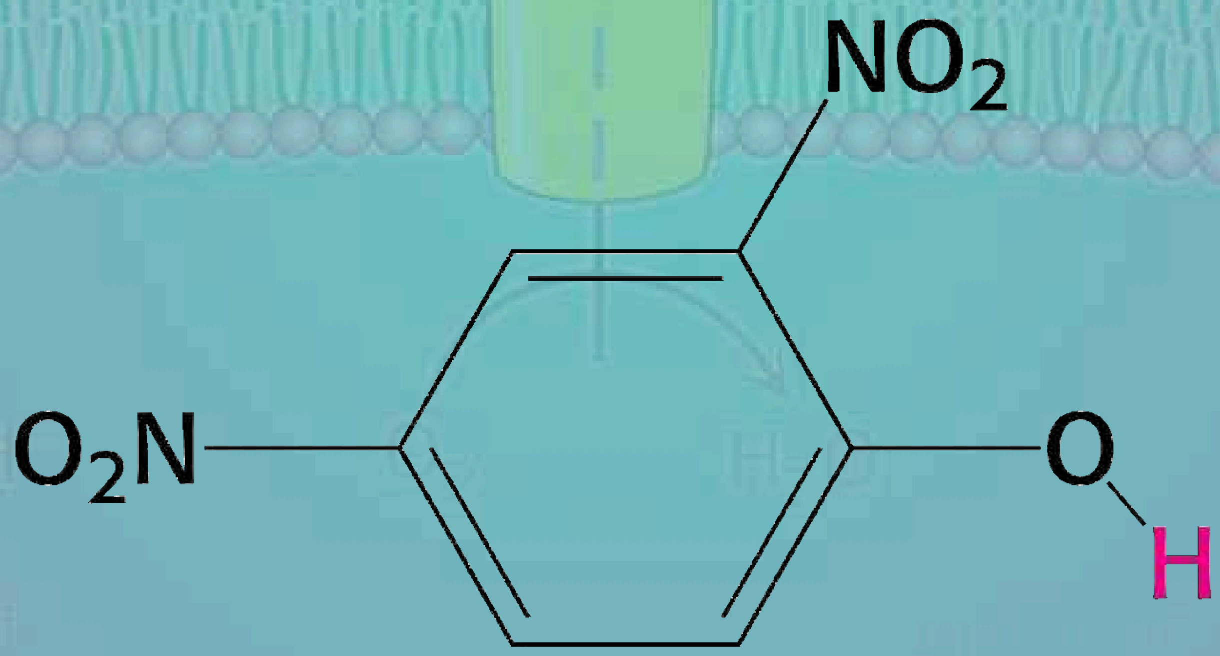
The electron transport chain can be blocked at several locations.

- ATP synthesis stops because the proton gradient can no longer be estabished.



6.3 Uncoupling of Oxidative Phosphorylation

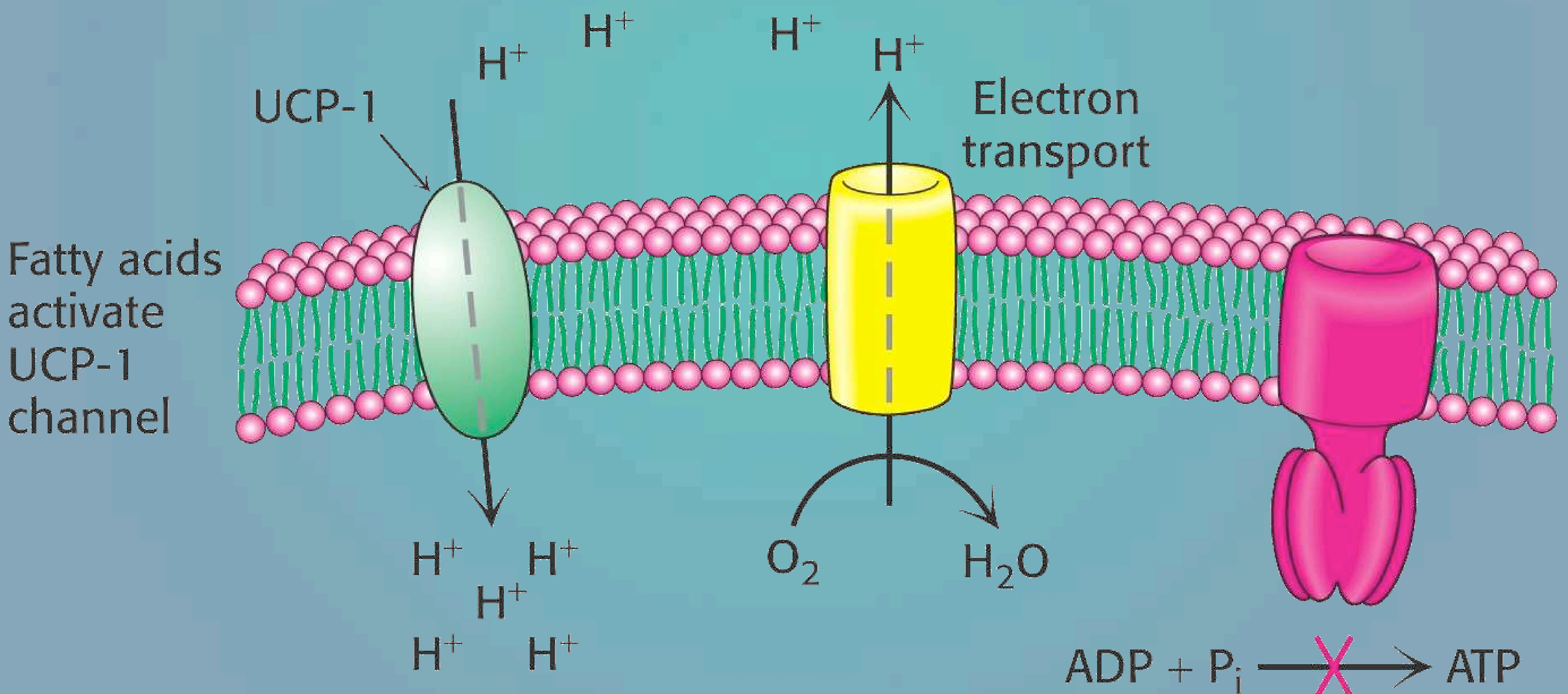
Electron transport can be uncoupled from ATP synthesis by allowing protons to move back into the matrix by alternative pathways.



2,4-Dinitrophenol (DNP)

6.4 Regulated Uncoupling

Sometimes uncoupling is done intentionally as a means for generating heat.



6.7 Power Transmission by Protein Gradients

Proton motive force is used to power many cellular processes.