Welcome to BCII lesson 5

Chimie Biologique II

Biological Chemistry II

BIO-213

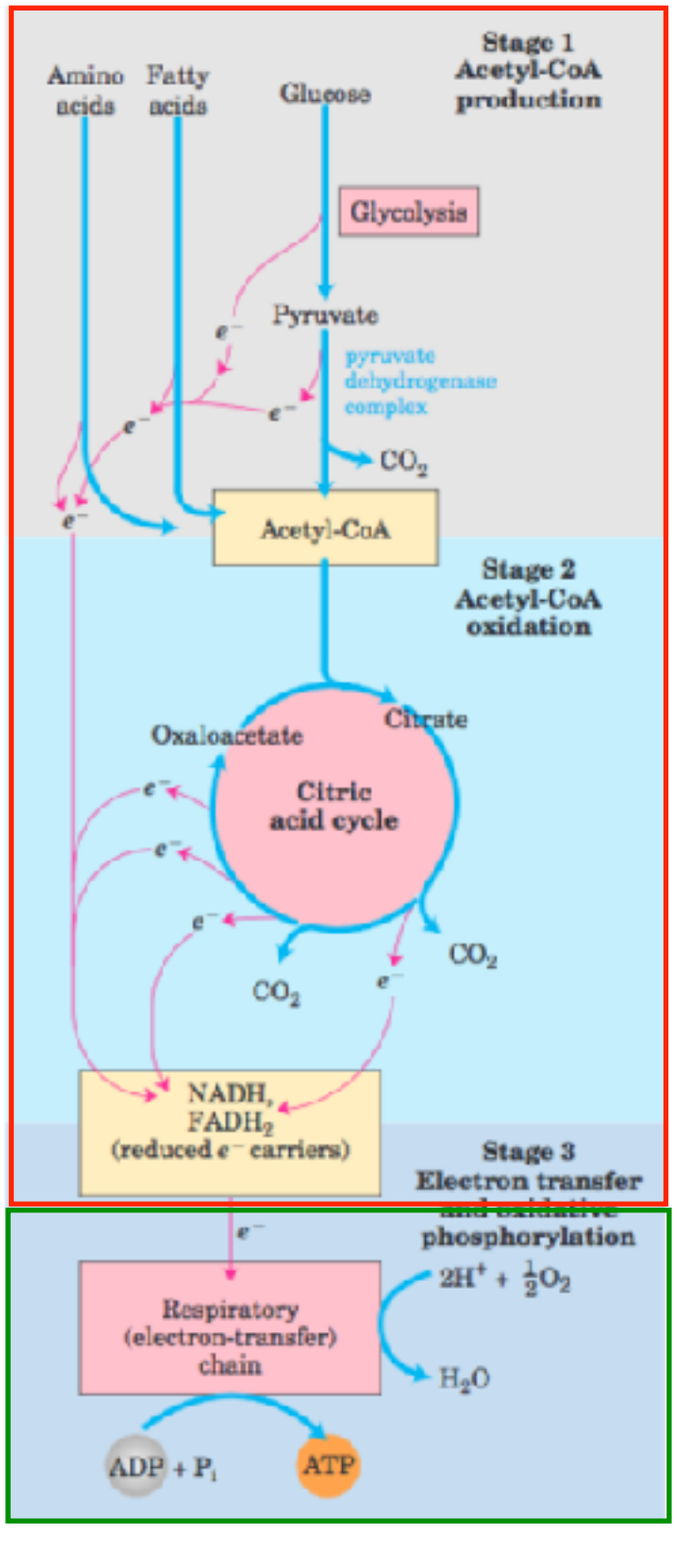
Teachers

Giovanni D’Angelo, IBI

Lecture 5

The oxidative phosphorylation

Catabolic Stages



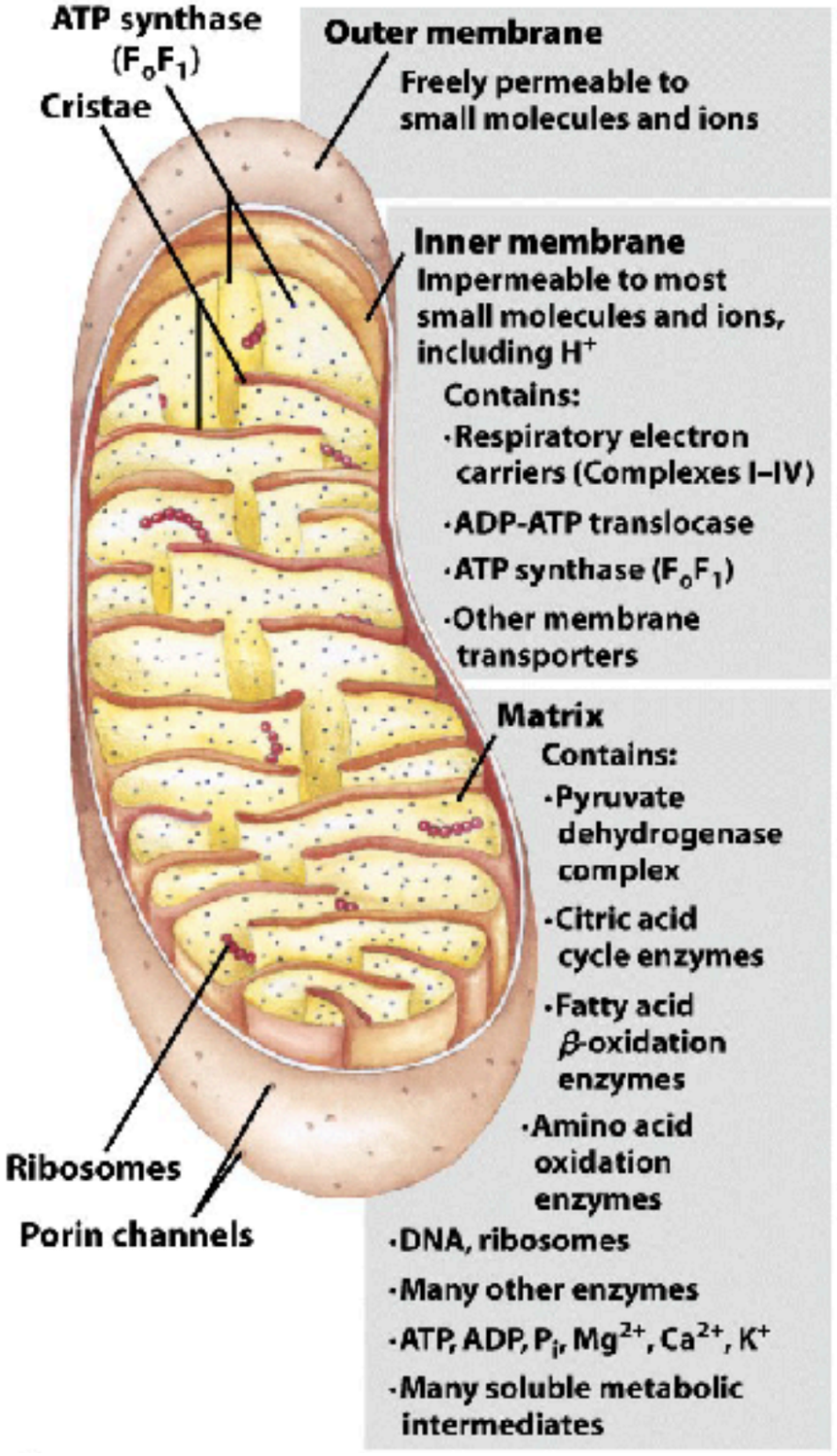
The three stages of the catabolism of proteins fatty acids and carbohydrates.

Stage 1: The oxidation of fatty acids, glucose and some amino acids yields Acetyl-CoA

Stage 2: Oxidation of the acetyl groups in the citric acid (or Kerbs or tricarboxylic acids or TCA) cycle includes 4 steps where electrons are abstracted and transferred to electron carriers

Stage 3: Electrons carried by NADH and $FADH_2$ are funnelled into a chain of mitochondrial (or plasma membrane in bacteria) electron carriers ultimately reducing O_2 to H_2O. This electron flow produces ATP

The mitochondrial compartmentalisation



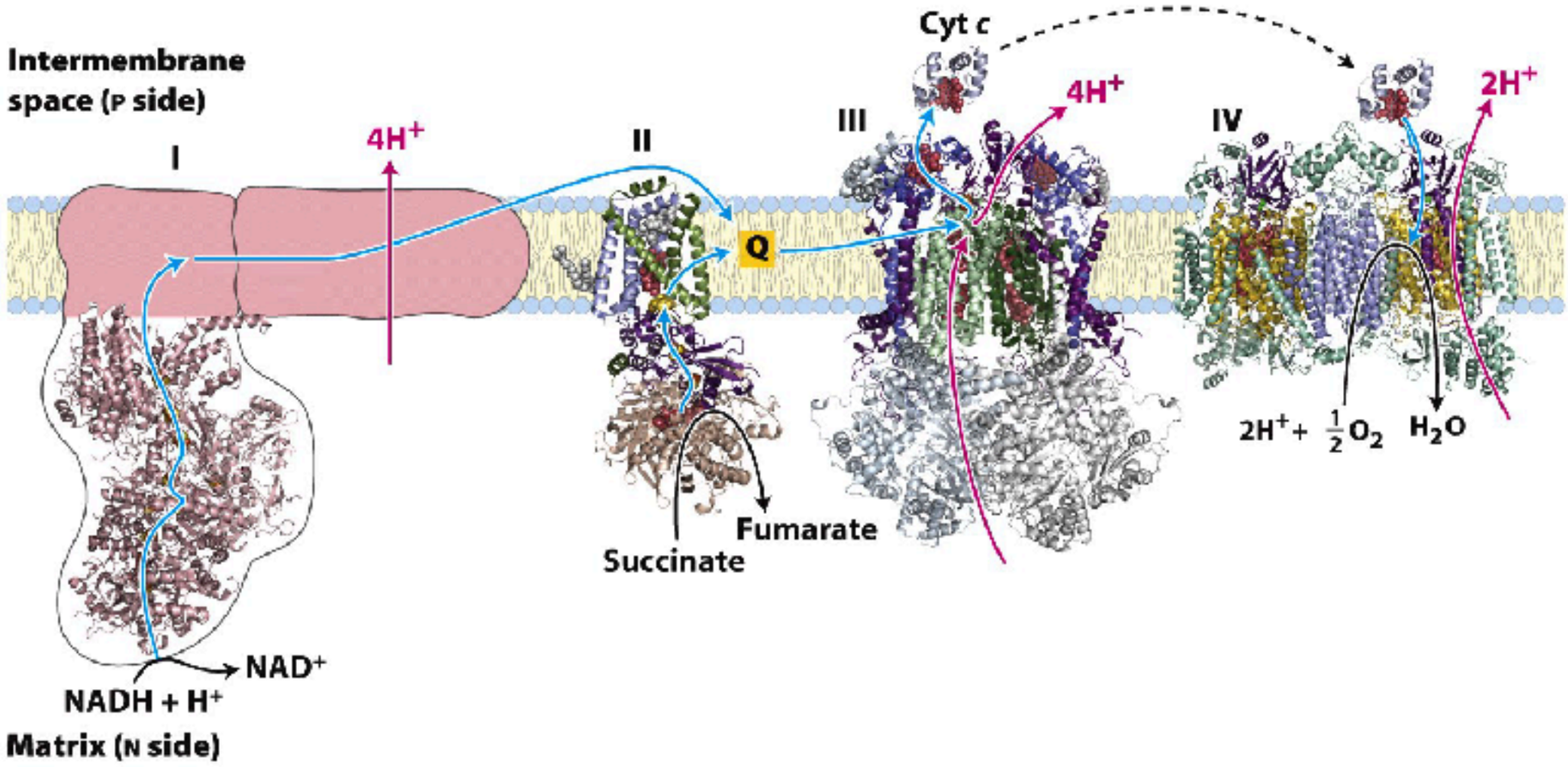
The process of aerobic respiration is completed in the mitochondria by oxidative phosphorylation.The NADH molecules formed in glycolysis as well as the NADH and FADH2 molecules formed in the TCA cycle, in Beta-oxidation and in amino acid oxidation are used to reduce oxygen to water and to produce ATP along the electron transport chain (ETC).

The ETC is a series of protein complexes that reside in the inner mitochondrial membrane. By transporting electrons the ETC creates an electrical current that is coupled to the pumping of H+ ions form the mitochondrial matrix to the inter-membrane mitochondrial space.

The outer mitochondrial membrane is permeable to the majority of small molecules and anions due to thee presence of mitochondrial porins

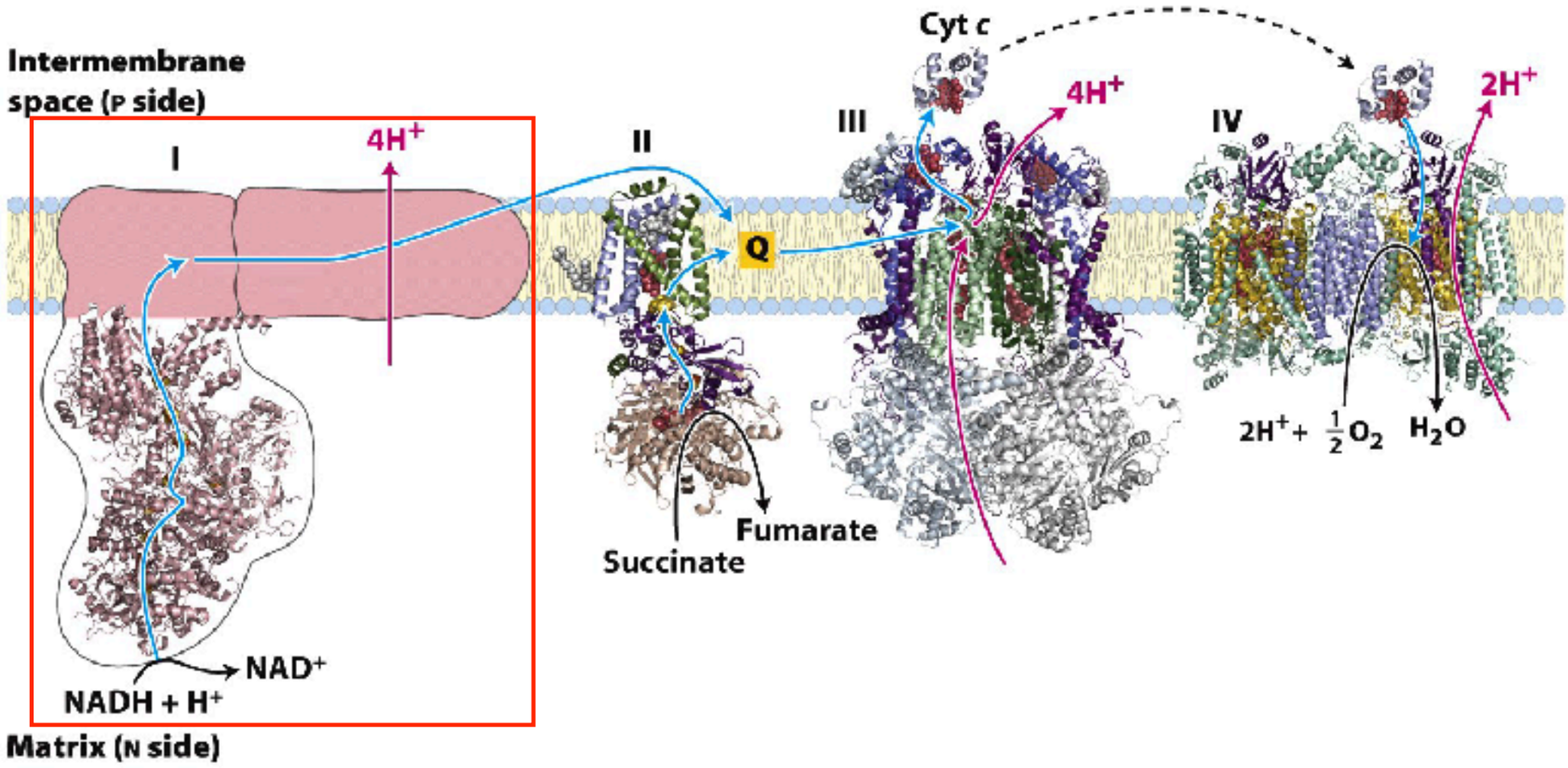
The inner membrane lacks porins and thus it is impermeable to ions and small metabolites that require specialised transporters to leave the mitochondrial matrix

The electron transport chain



The ETC consists of 4 protein complexes that receive high energy electrons from NADH and FADH2 and move this electrons along the inner mitochondrial membrane and onto the final electron acceptor, namely the oxygen. In the process a proton gradient is produced that is then used to produce ATP via the activity of a special enzyme called ATP synthase

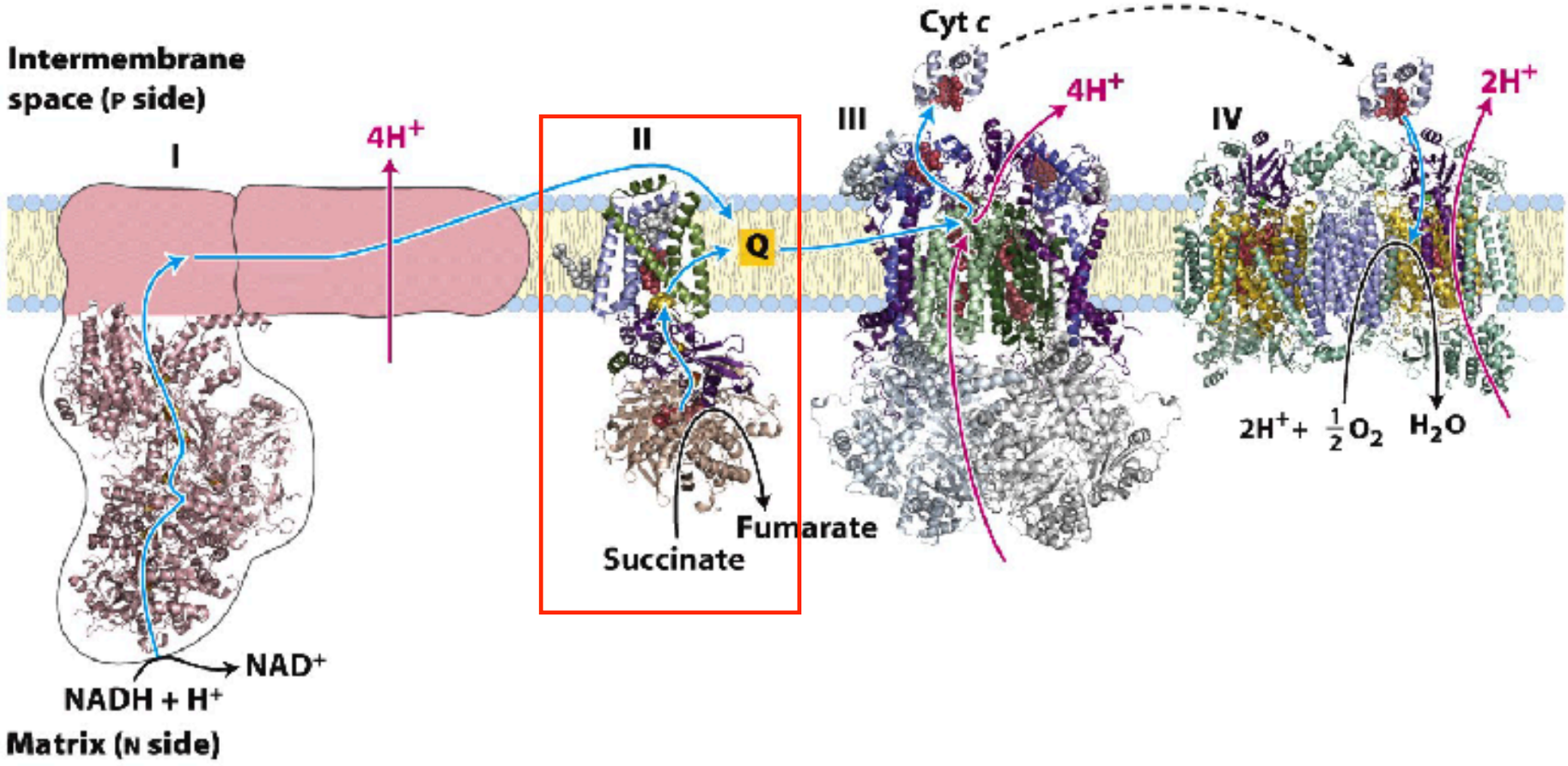
The electron transport chain



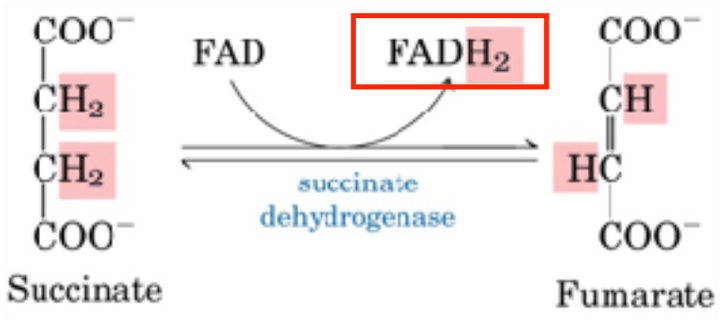
Complex I (aka NADH dehydrogenase or NADH oxidoreductase), is a very large protein complex comprising 46 polypeptides. It has a L shape with one arm lies within the inner membrane while the vertical component lies in the matrix.

Complex I receives high energy elections from **NADH**. It also acts as a proton pump that uses the movement of the electrons to move H+ ions in the intermembrane space

The electron transport chain

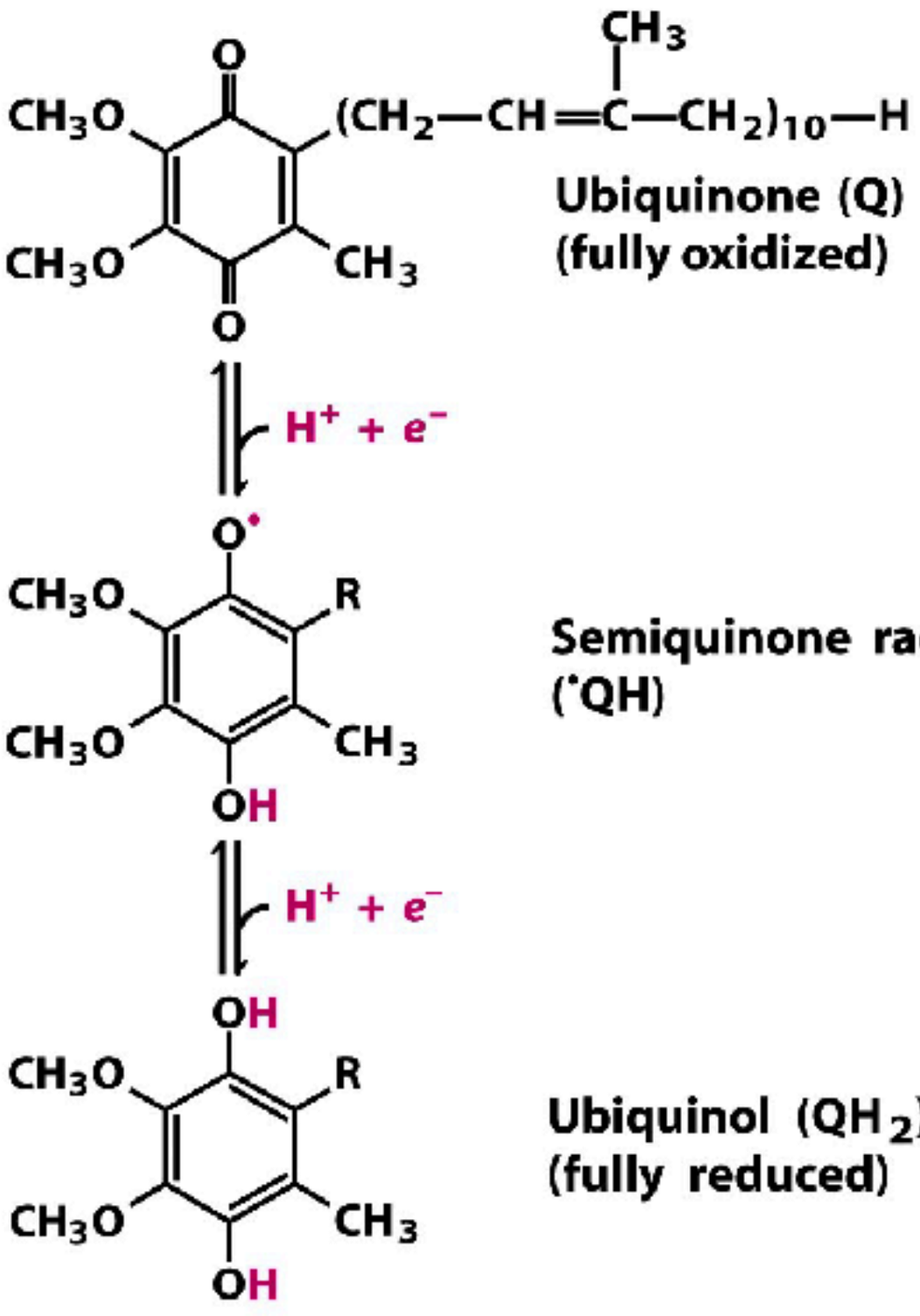


Complex II (aka succinate reductase), contains the succinate dehydrogenase used in the TCA cycle to form fumarate from succinate. <u>This complex is not a proton pump.</u>



Complex II receives high energy electrons from FADH2 and moves them to the hydrophobic electron carrier Ubiquinone (aka CoQ)

Coenzyme Q

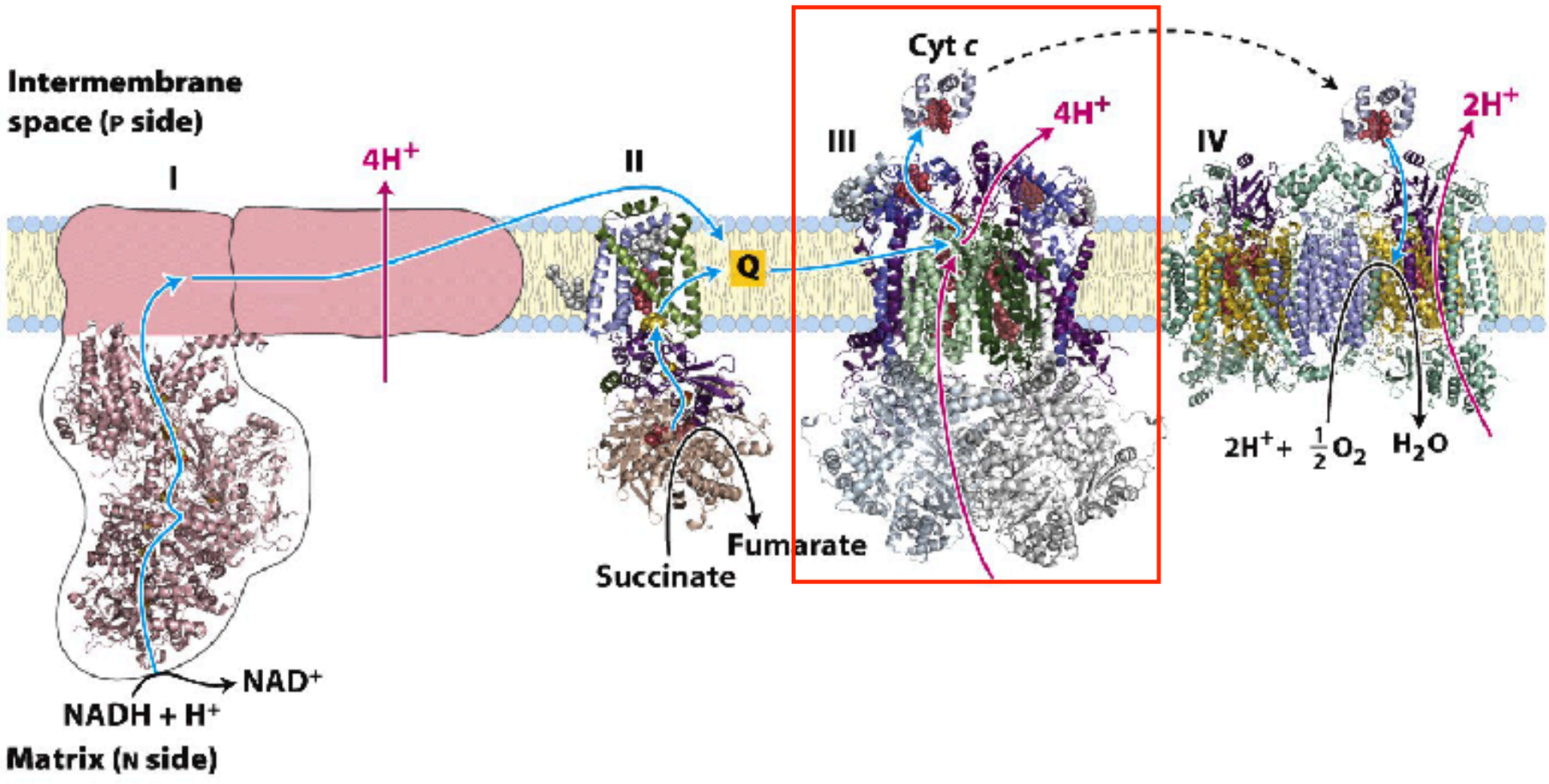


Coenzyme Q (aka Ubiquinone, or CoQ), is a small hydrophobic molecule that is dissolved in the inner mitochondrial membrane bilayer.

Coenzyme Q can accept and carry 1 or 2 electrons thus being reduced to Semiquinone / *CoQH and Ubiquinol/ CoQH2, respectively.

Coenzyme Q transports electrons from ETC Complex I/II to Complex III

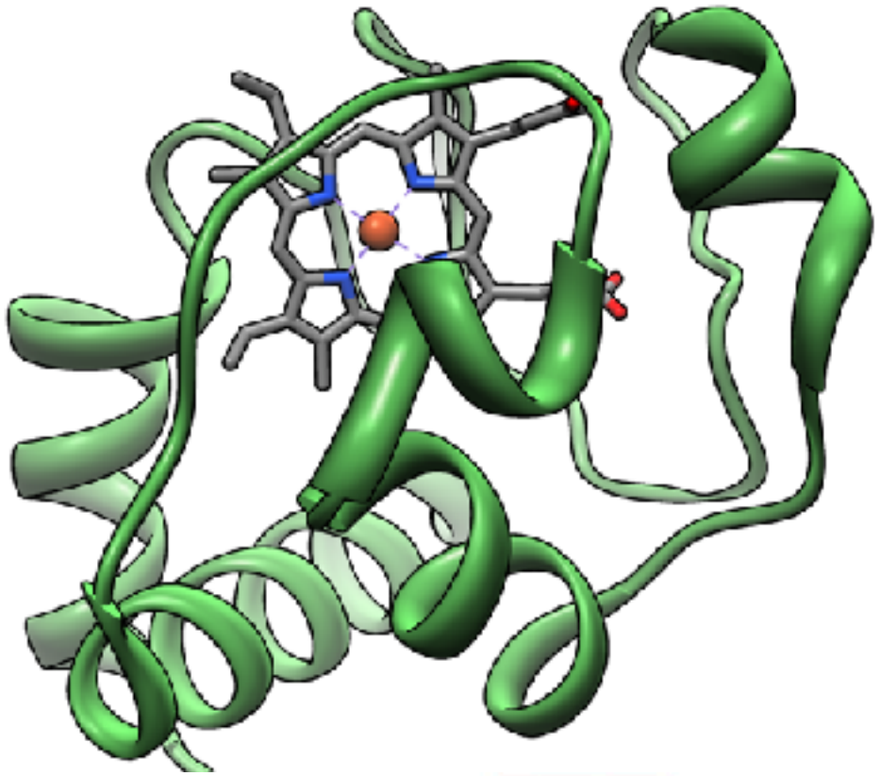
The electron transport chain

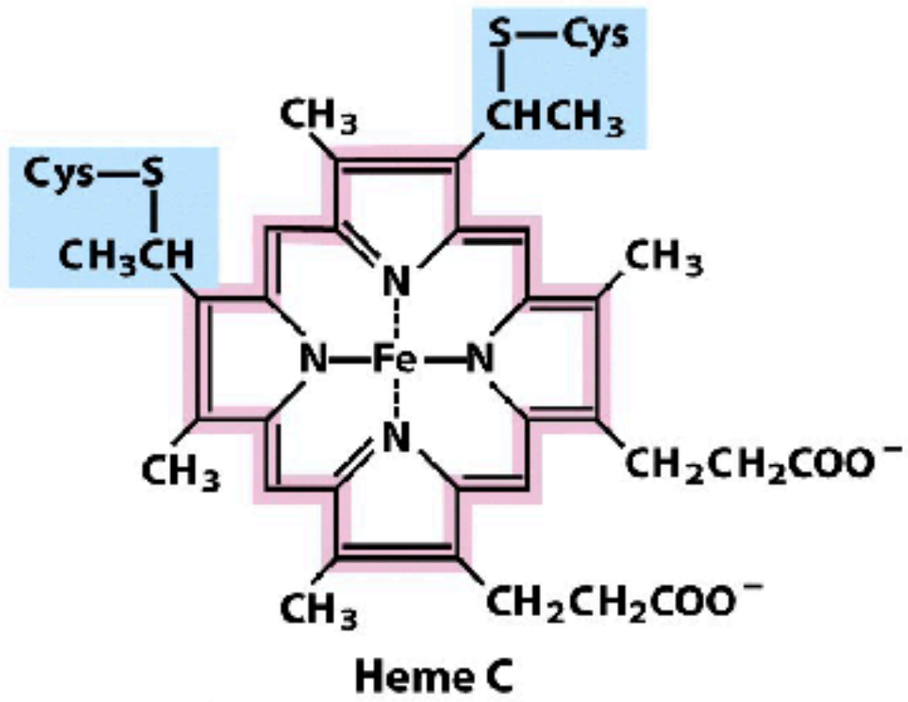


Complex III (aka cytochrome c oxidoreductase or cytochrome c reductase), accepts electrons from CoQH2 derived from complex I and II and transfers them to **Cytochrome c**.

Like Complex I this complex functions as a proton pump and helps generate a proton electrochemical gradient across the inner mitochondrial membrane.

Cytochrome C



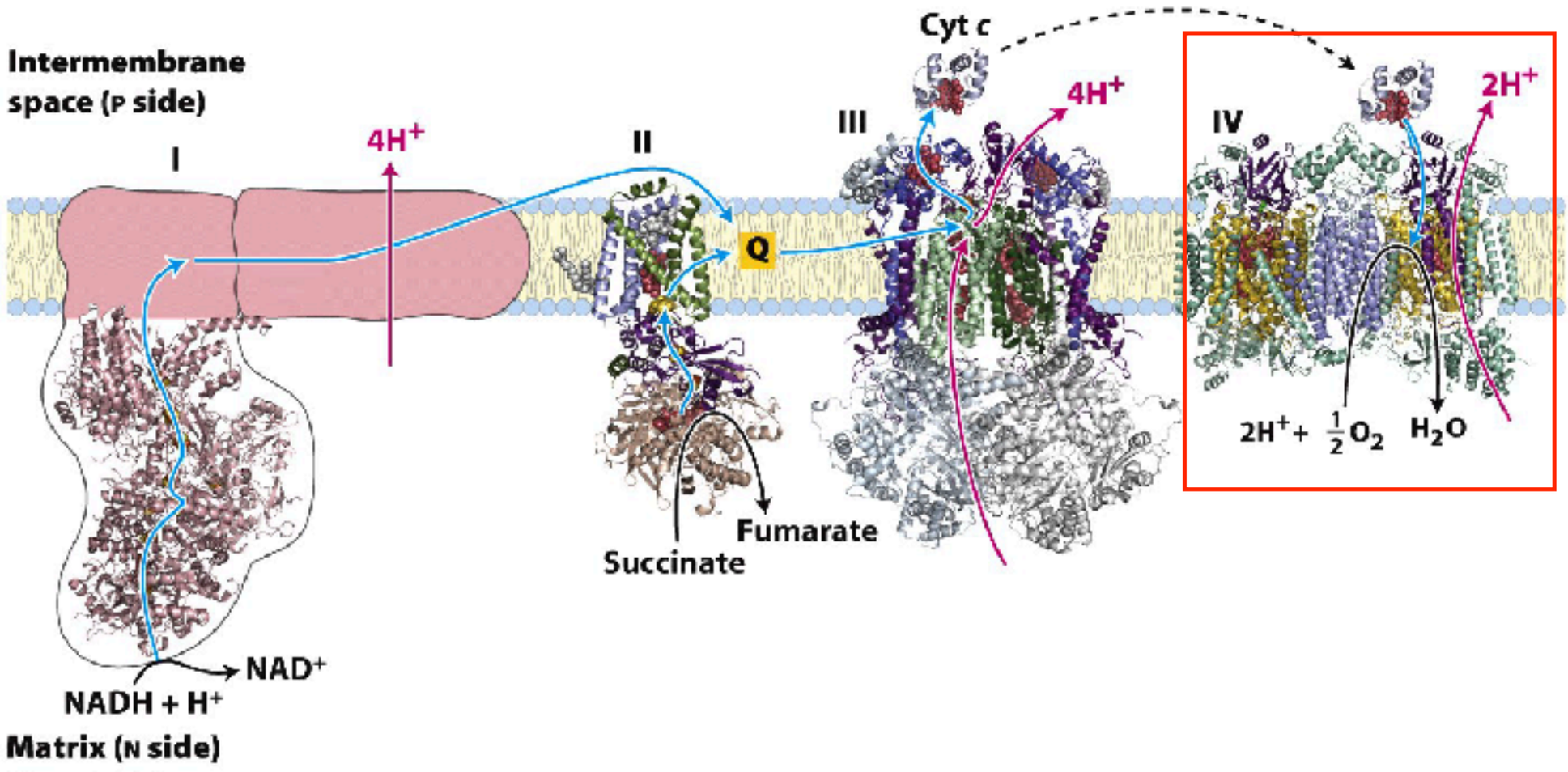


Heme C

Cytochrome *c* is a small hemeprotein found loosely associated with the inner membrane of the mitochondrion.

Cytochrome c is highly water-soluble, unlike other cytochromes, and is an essential component of the electron transport chain, where it carries one electron. It is capable of undergoing oxidation and reduction as its iron atom converts between the ferrous and ferric forms, but does not bind oxygen. It transfers electrons between Complexes III (Coenzyme Q – Cyt C reductase) and IV (Cyt C oxidase).

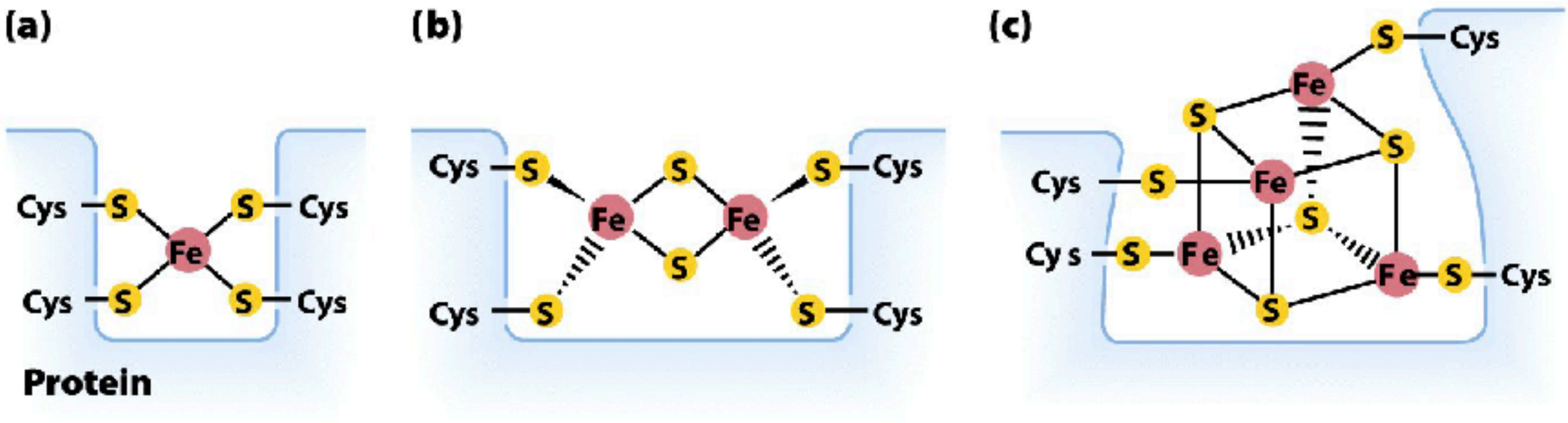
The electron transport chain



Complex IV (aka cytochrome c oxidase) accepts electrons from Cytochrome c and transfers them to Oxygen to produce water.

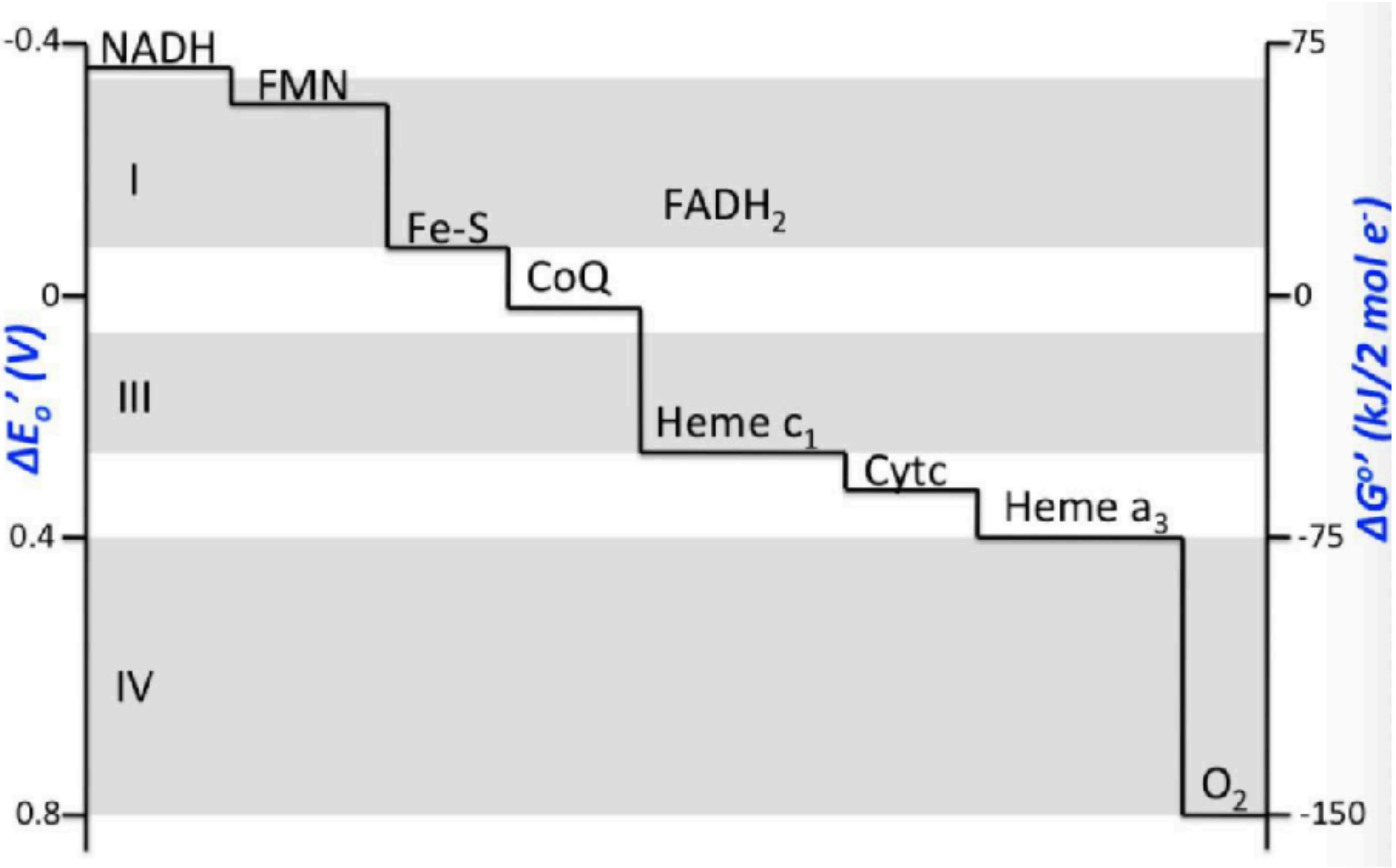
Like Complex I and III this complex functions as a proton pump and helps generate a proton electrochemical gradient across the inner mitochondrial membrane.

Fe-S clusters



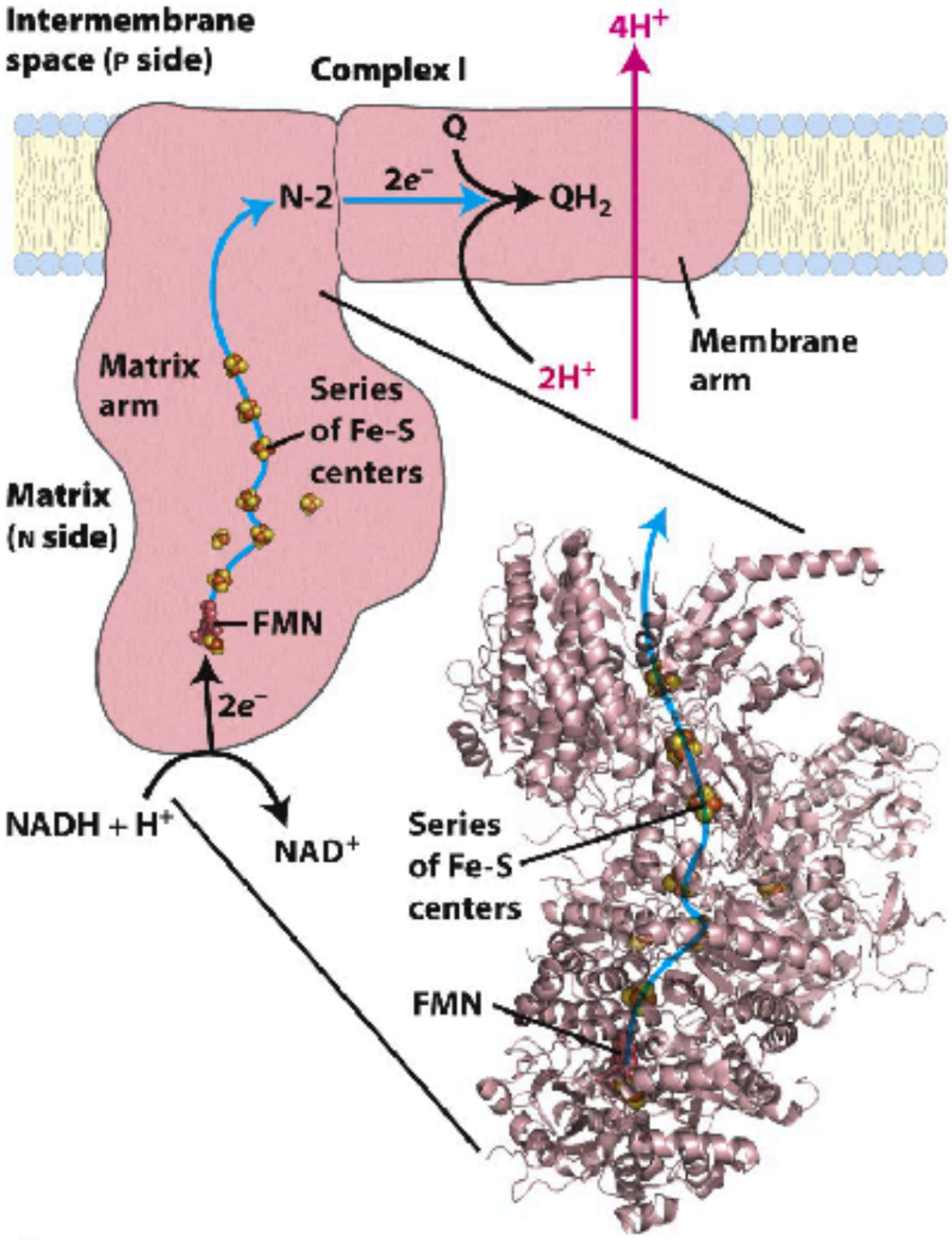
Iron–sulfur clusters occur in many biological systems, often as components of electron transfer proteins. They feature either 2Fe–2S or 4Fe–4S centers. They occur in all branches of life. Fe–S clusters can be classified according to their Fe:S stoichiometry [2Fe–2S], [4Fe–3S], [3Fe–4S], and [4Fe–4S].The relevant redox couple in all Fe–S proteins is Fe(II)/Fe(III)

The electron transport chain



Complex I

NADH dehydrogenase



NADH donates two electrons onto acceptor group found in the vertical component of Complex I (i.e., flavin mono nucleotide or **FMN**).

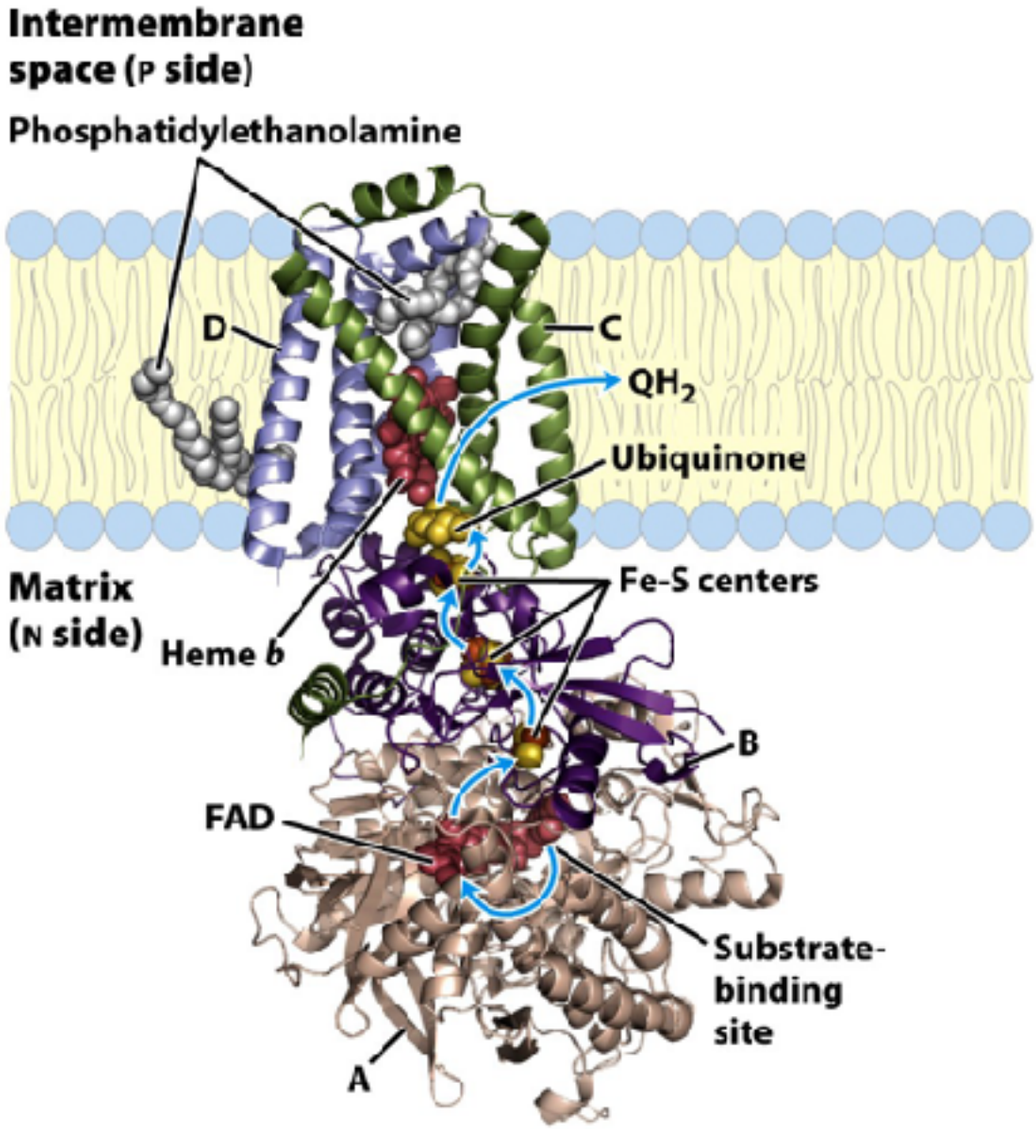
FMN is reduced to **FMNH2**.

FMNH2 donates electrons to a series of **Fe-S** clusters that ultimately transfer the two electrons to **CoQ** to produce **CoQH2**

As electrons move along the series of Fe-S clusters, the complex uses electrical power to pump H+ ions into the intermembrane space.

Complex II

succinate reductase



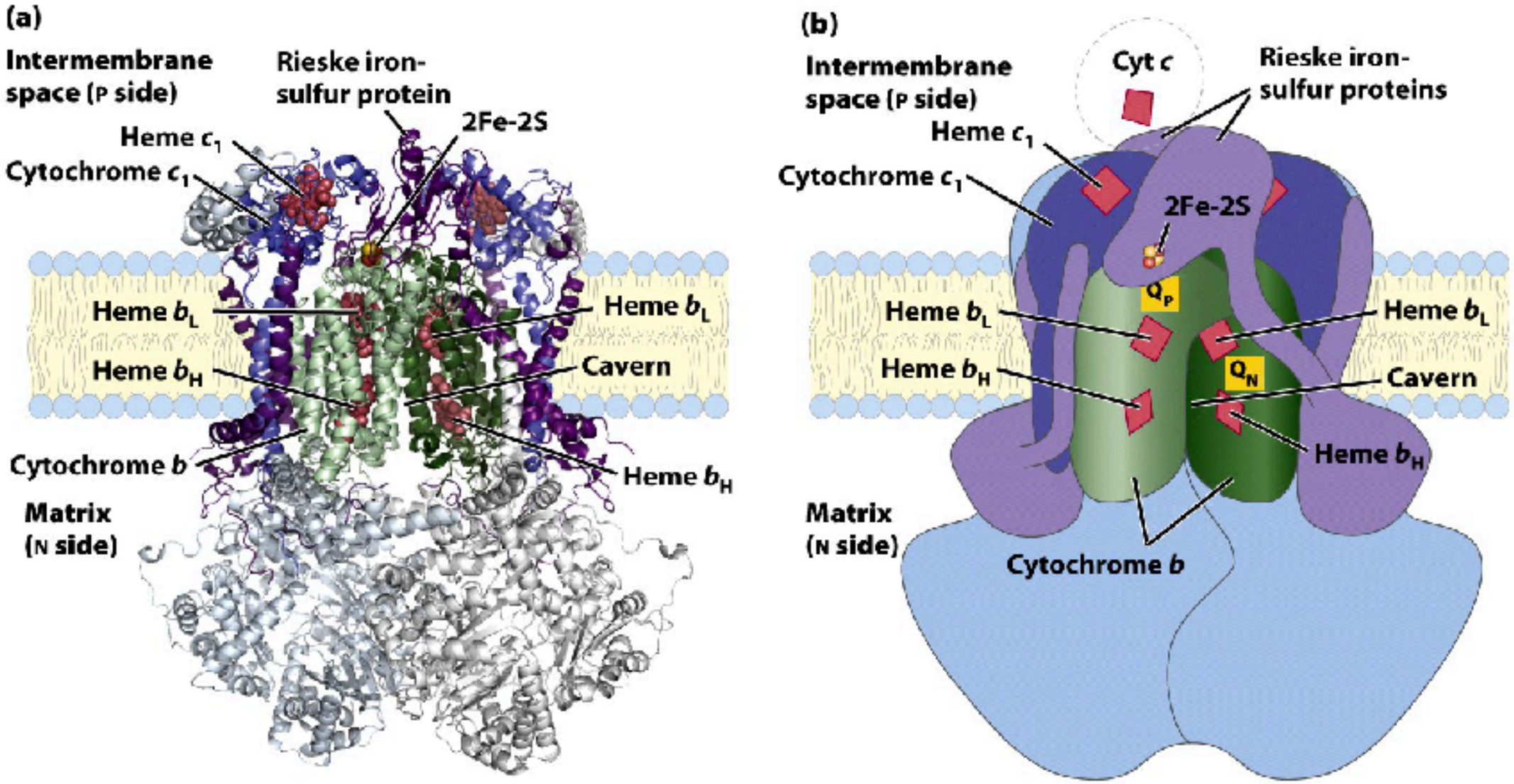
Complex II (that comprises succinate dehydrogenase) converts succinate to fumarate and produces **FADH2**.

FADH2 remains attached to the complex and gives off the 2 electrons to a series of **Fe-S** clusters that ultimately transfer these electrons to CoQ to produce **CoQH2.**

The Heme b prosthetic group does not appear to be part of electron transporting pathway within the complex II

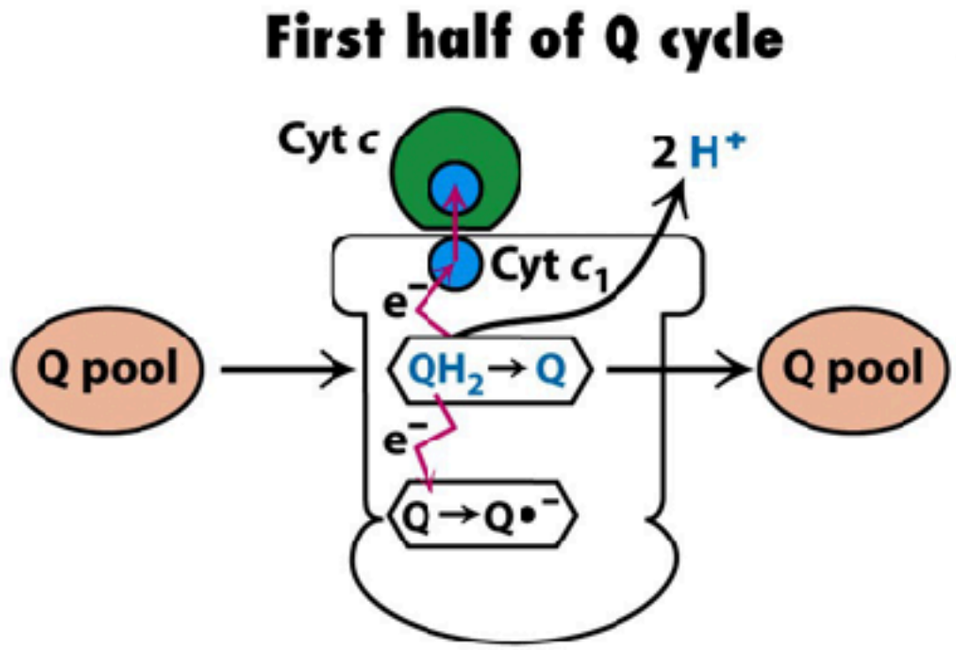
Complex III

cytochrome c reductase



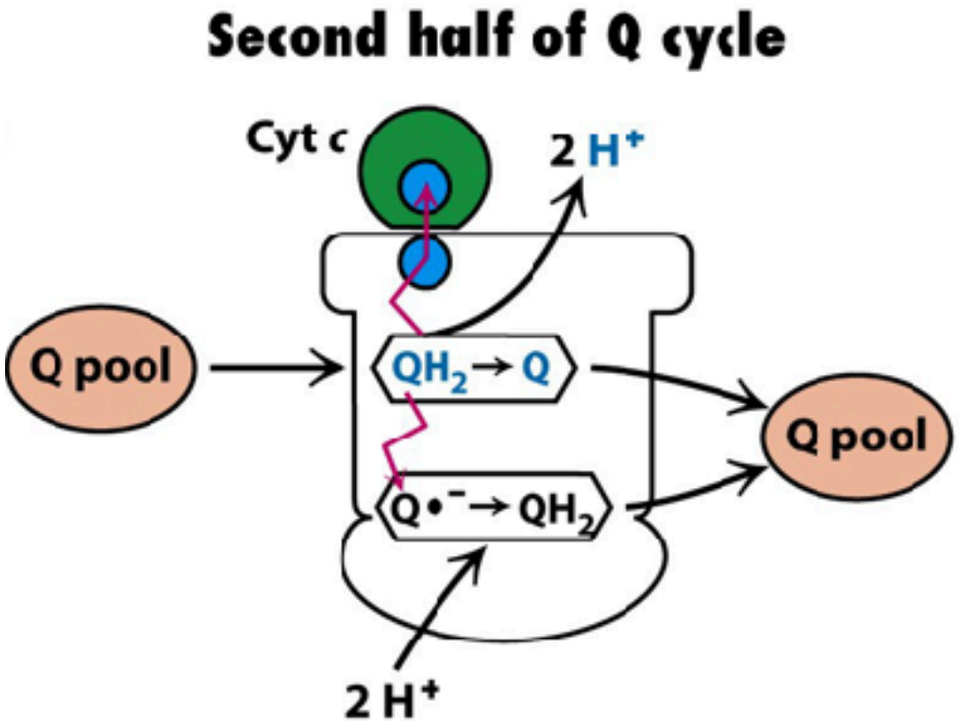
Complex III transfers electrons from **CoQH2** to **Cytochrome c**. Complex III contains several important structures for the electron tranfer: (1) Cytochrome c1 [1 heme]; (2) Cytochrome b [2 hemes]; Rieske center [2Fe-2S groups]. The process by which electrons are transferred form **CoQH2** to **Cytochrome c** is called the **Q cycle.**

The Q cycle



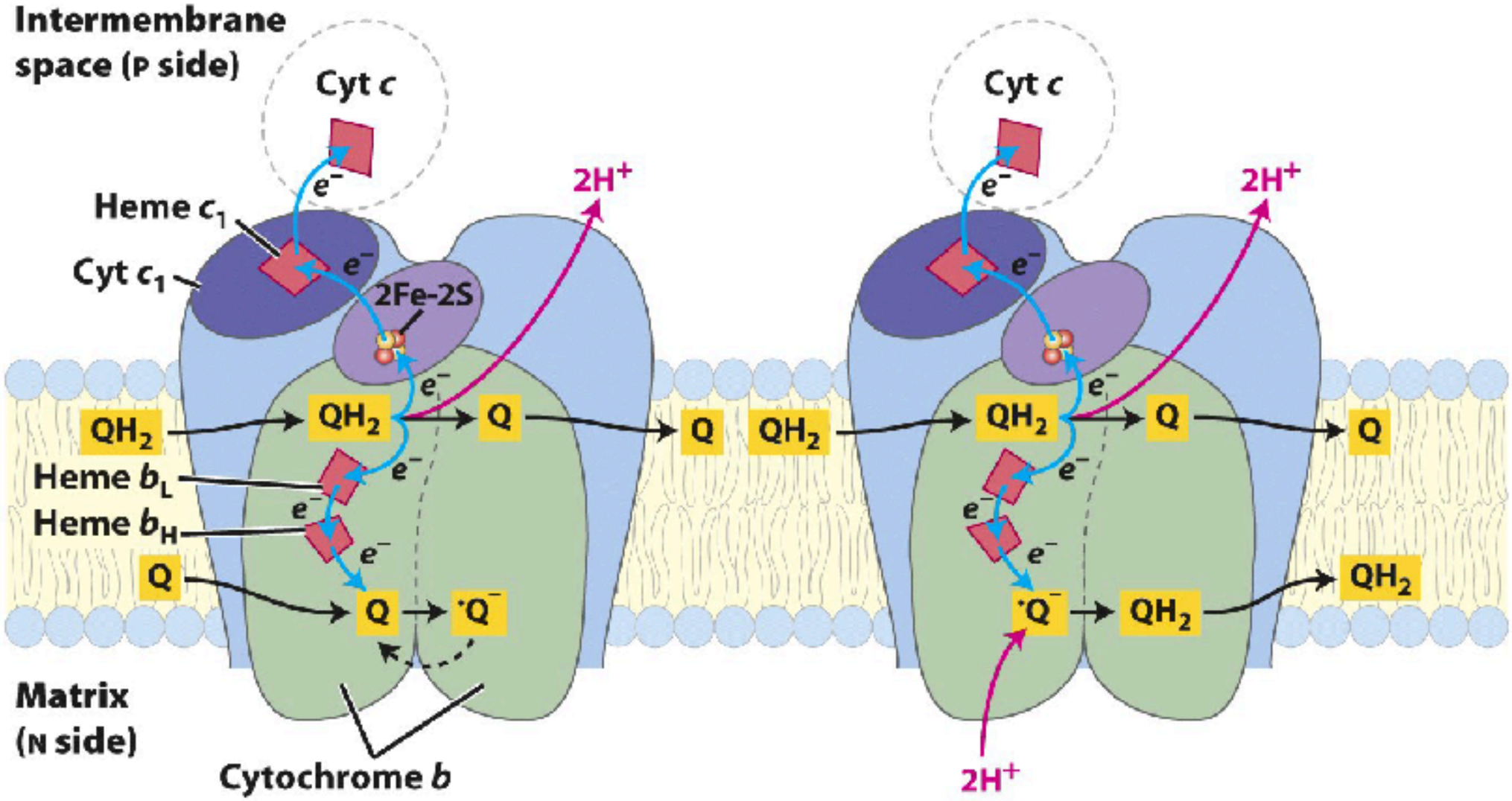
The Q cycle is divided into two half cycles

in the **first** half cycle **CoQH2** binds to complex III and transfers one electron to the **Rieske center** from where it is transferred to **Cyt c1** and than to **Cyt c**. the pumping of two H+ ions is coupled to these transfers. The second electron is transferred to **Cyt b** and from there to a **CoQ** molecule that is reduced to the state of semiquinone radical ***CoQH.**



in the **second** half cycle a new **CoQH2** binds to complex III transfers one electron to the **Rieske center** from where it is transferred to **Cyt c1** and than to **Cyt c**. the pumping of two H+ ions is coupled to these transfers. The second electron is transferred to **Cyt b** and from there to a ***CoQH** molecule that is reduced to the state of ubiquinoll **CoQH2.**

Complex III



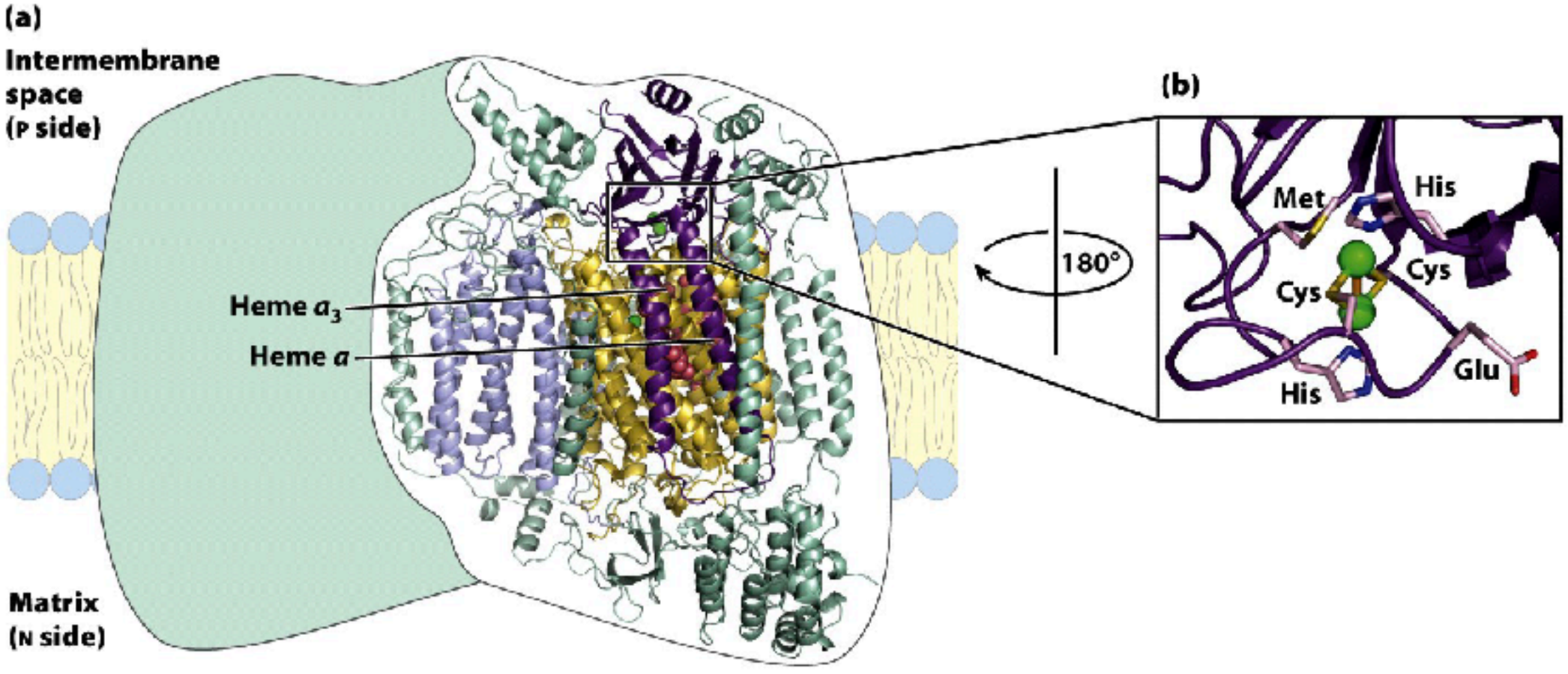
$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow {}^{\cdot}Q^- + Q + 2H_P^+ + \text{cyt } c_1 \text{ (reduced)}$

$QH_2 + {}^{\cdot}Q^- + 2H_N^+ + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow QH_2 + 2H_P^+ + Q + \text{cyt } c_1 \text{ (reduced)}$

Net equation: $QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H_N^+ \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H_P^+$

Complex IV

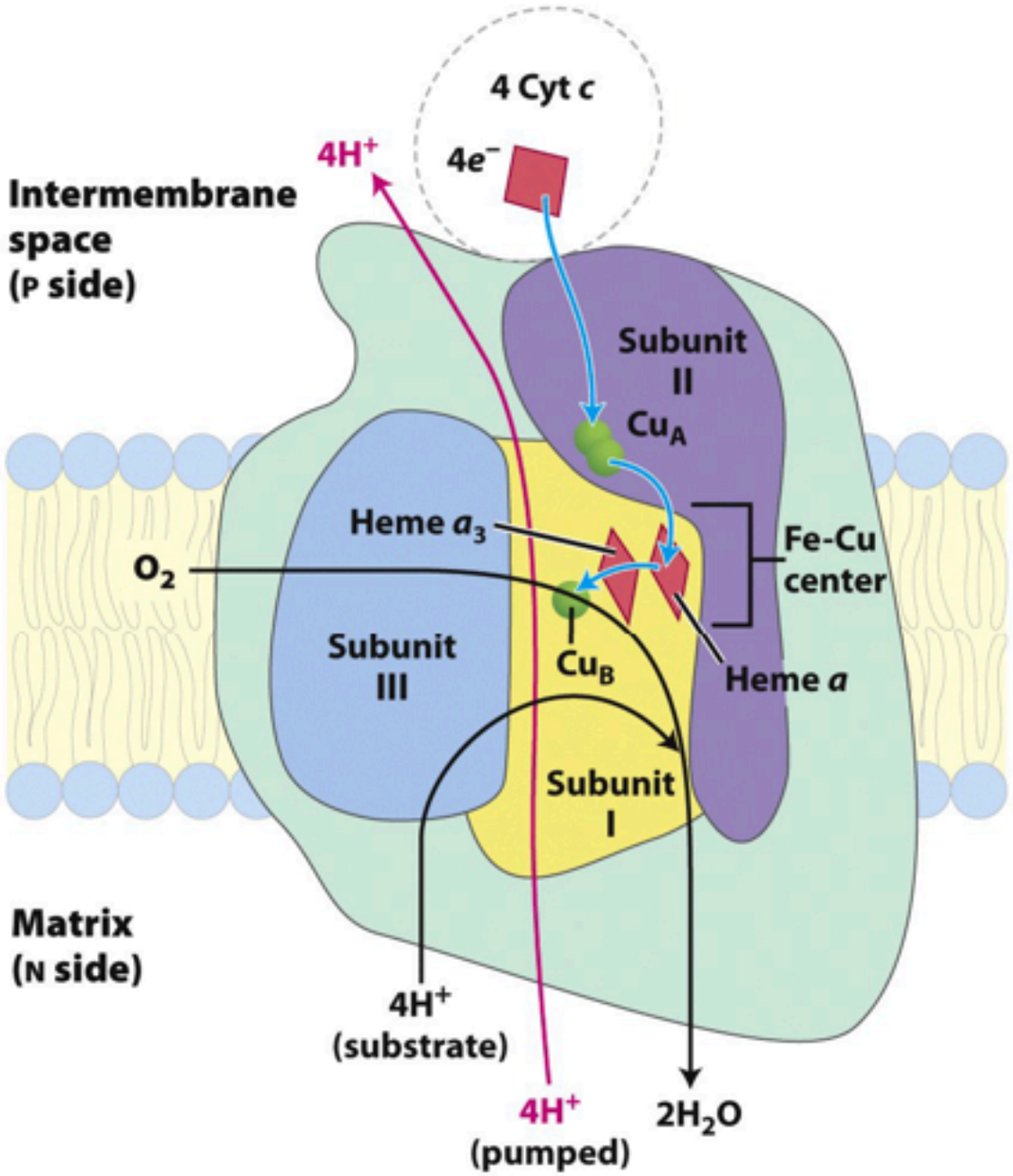
cytochrome c oxidase



Complex iV transfers electrons from reduced **Cytochrome c** to **oxygen**. Complex III contains two heme groups [heme a and heme a3] and three copper atoms Cu_A/Cu_A and Cu_B.

Cu2+ +e- +> Cu+

Complex IV



(1) Two reduced Cytochrome c molecules give off 2 electrons. One goes to Heme a3 the other to Cu_B.

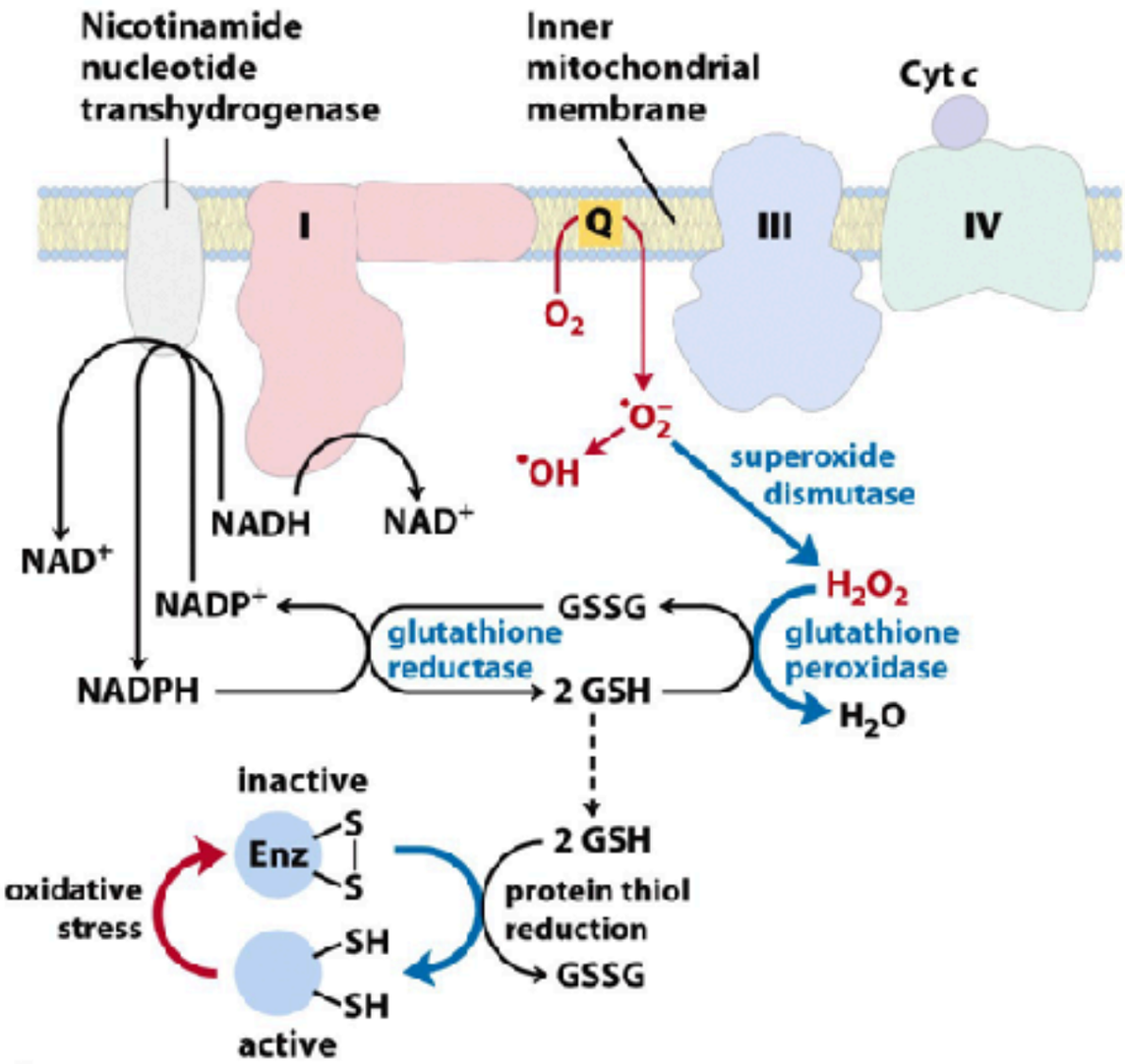
(2) Once Heme a3 and Cu_B are in their reduced forms, an O2 molecule can bind and abstract two electrons thus forming a peroxide bridge

(3) Two reduced Cytochrome c molecules are oxidised and transfer additional two e-; 2H+ are obtained from the matrix to break the peroxide bridge and form Heme a3-OH and Cu_B-OH

(4) Two more protons are obtained from the matrix that oxidise Heme a3 and Cu_B to their original states with release of water.

In the process 4 H+ are pumped in the intermembrane space.

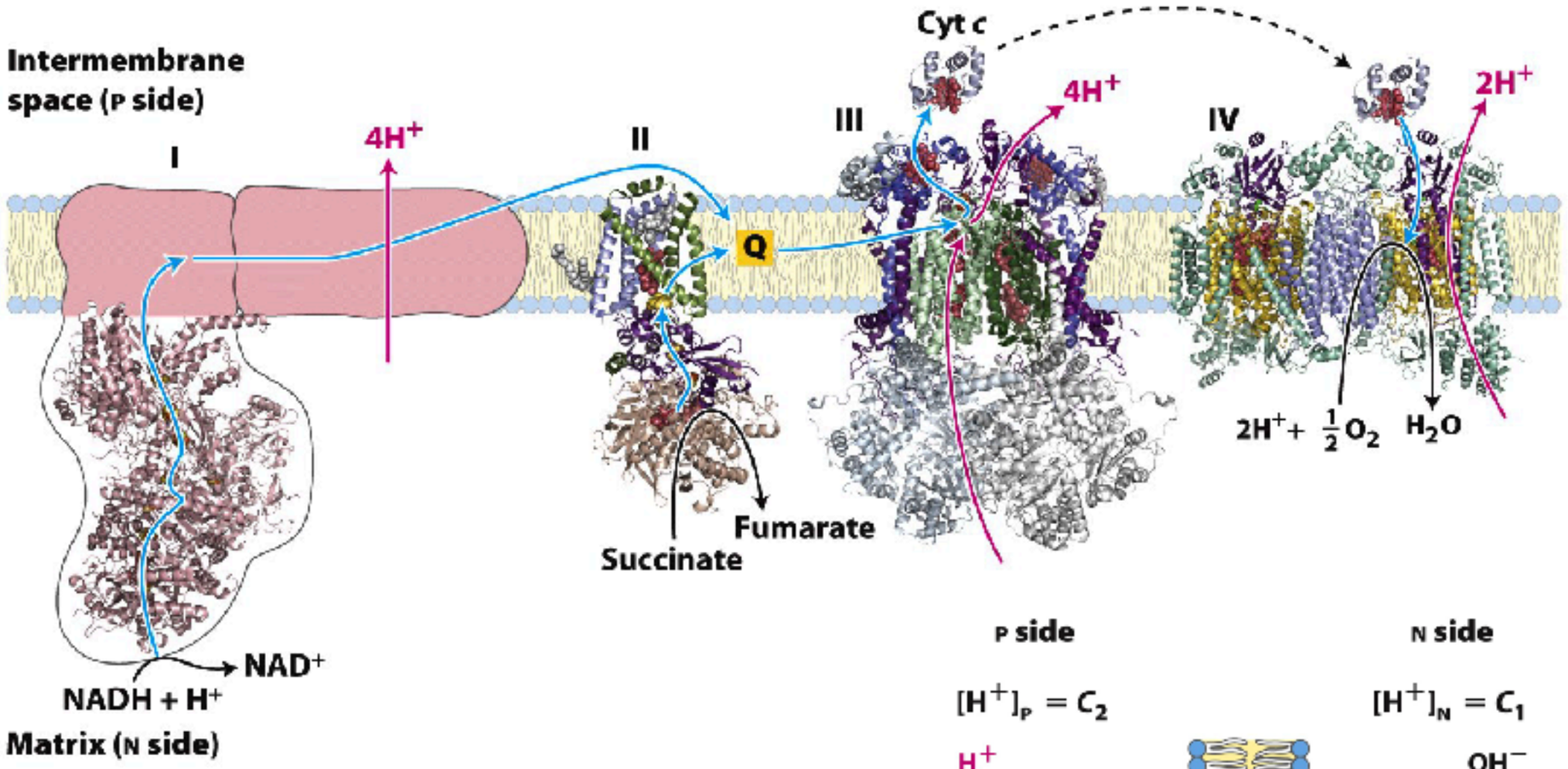
Reactive Oxygen Species (ROS)



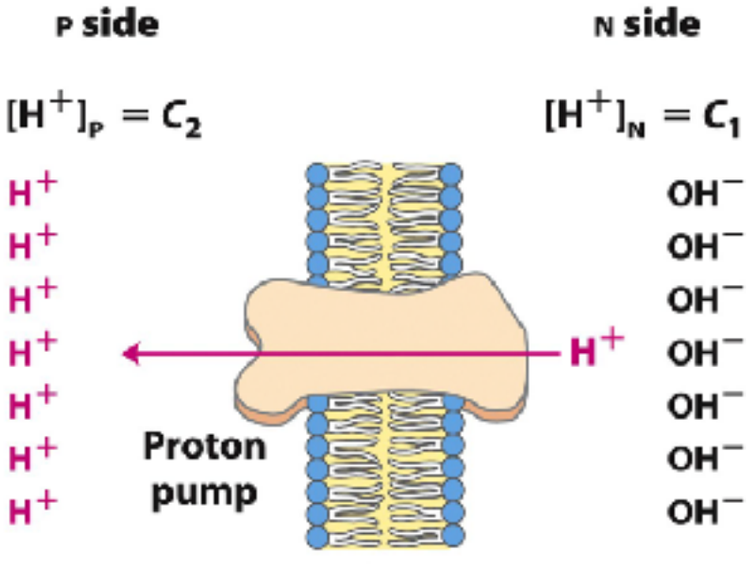
Reactive oxygen species (**ROS**) are chemically reactive chemical species containing oxygen. ROS are produced during a variety of biochemical reactions within the cell and within organelles such as mitochondria, peroxisomes, and endoplasmic reticulum.

In the electron transport chain, about 0.1–2% of electrons passing through the chain (this number derives from studies in isolated mitochondria, though the exact rate in live organisms is yet to be fully agreed upon), oxygen is instead prematurely and incompletely reduced to give the superoxide radical (·O-). Specific enzymes in our mitochondria detoxify superoxide radical by transforming it first into hydrogen peroxide (by superoxide disputasse; SOD) and then to water (by catalase or glutathione peroxidase)

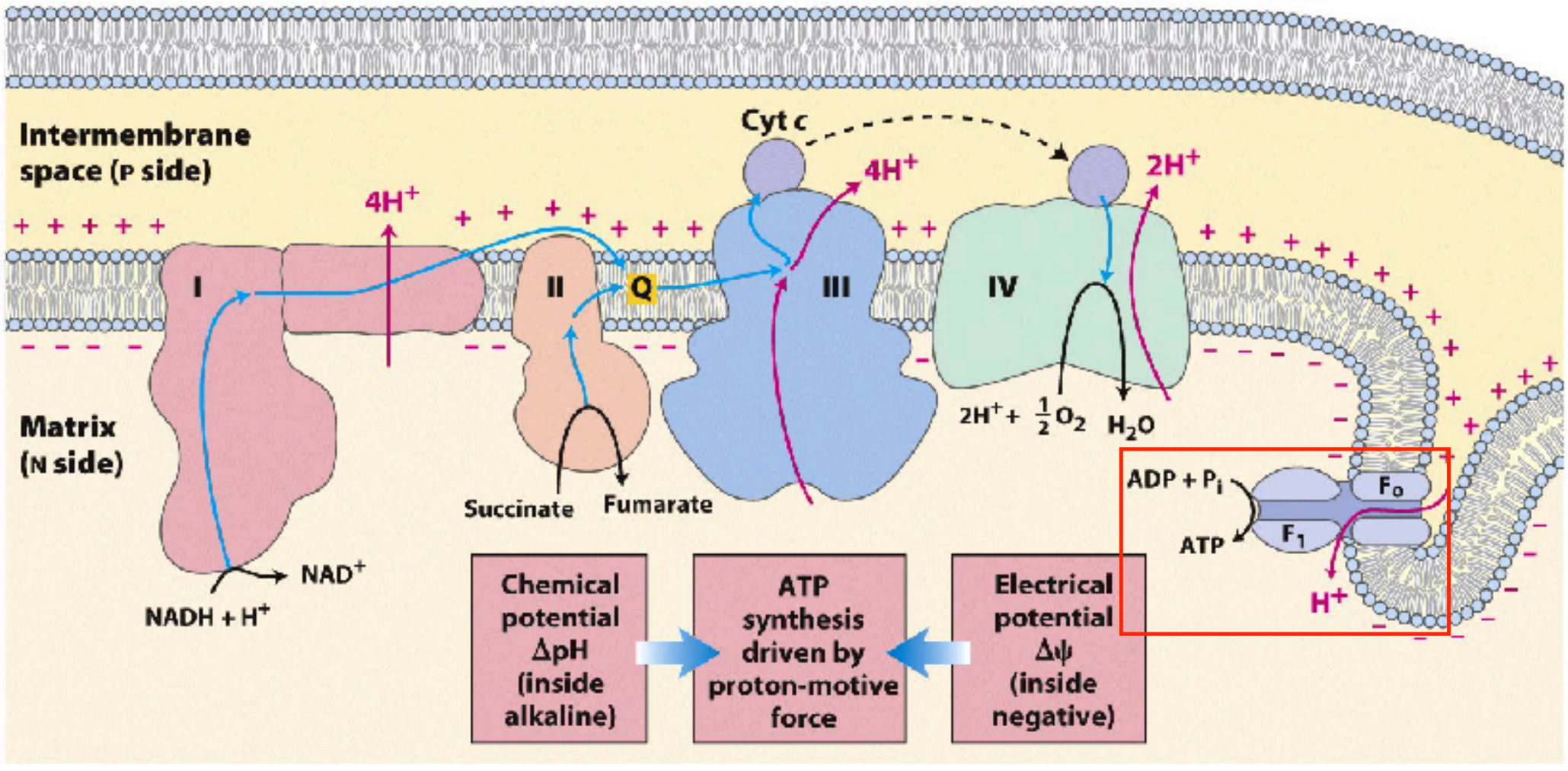
The electron transport chain



The net outcome of the operation transport chain is the production of a proton gradient between the intermembrane mitochondrial space (Positive [P] side) and the mitochondrial matrix (Negative [N] side)

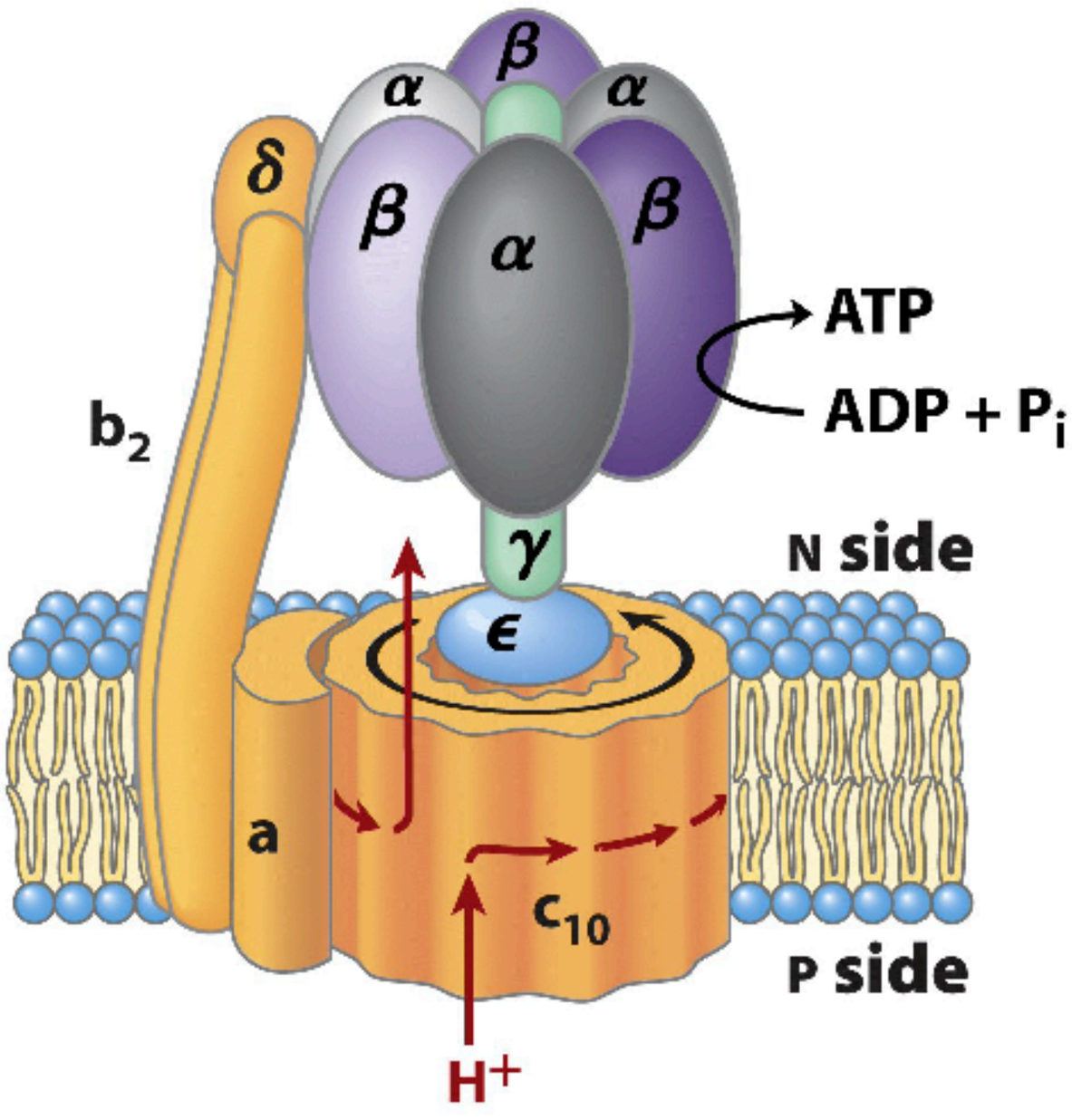


The electron transport chain



10’ Break

The ATP synthase



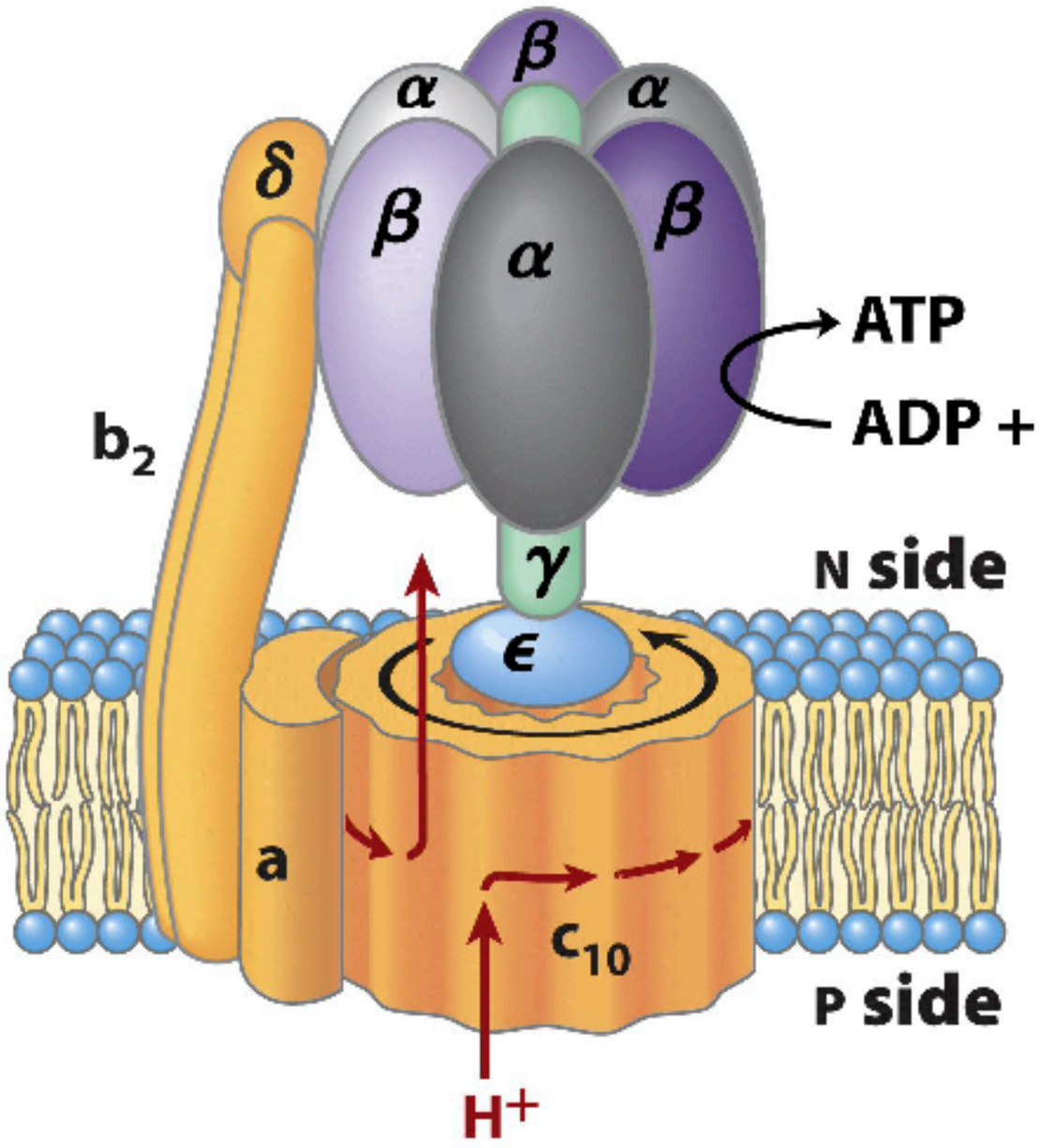
The ATP synthase (sometimes referred to as Complex V) generates ATP molecules and uses the proton motive force due to the H+ gradient to release ATP into the mitochondrial matrix.

The structure of ATP synthase consists of two major regions: The F0 and the F1.

The **F1 region** is the catalytic subunit, it lies in the matrix of the mitochondria and it is constituted by five polypeptides (alpha [3 copies], beta [3 copies], gamma, delta, epsilon)

The **F0 region** is hydrophobic and lies within the inner membrane. It consists of 10-14 **c subunits** organised in a ring; a single **a subunit** and two **b subunits**

The ATP synthase



The alpha and beta subunits form an hexametric hetero-oligomer having a central cavity.

the gamma and delta chains form what is called the central stalk that runs through the cavity of the alpha/beta hexamer

the delta submits holds the alpha/beta hexamer and keeps it from rotating

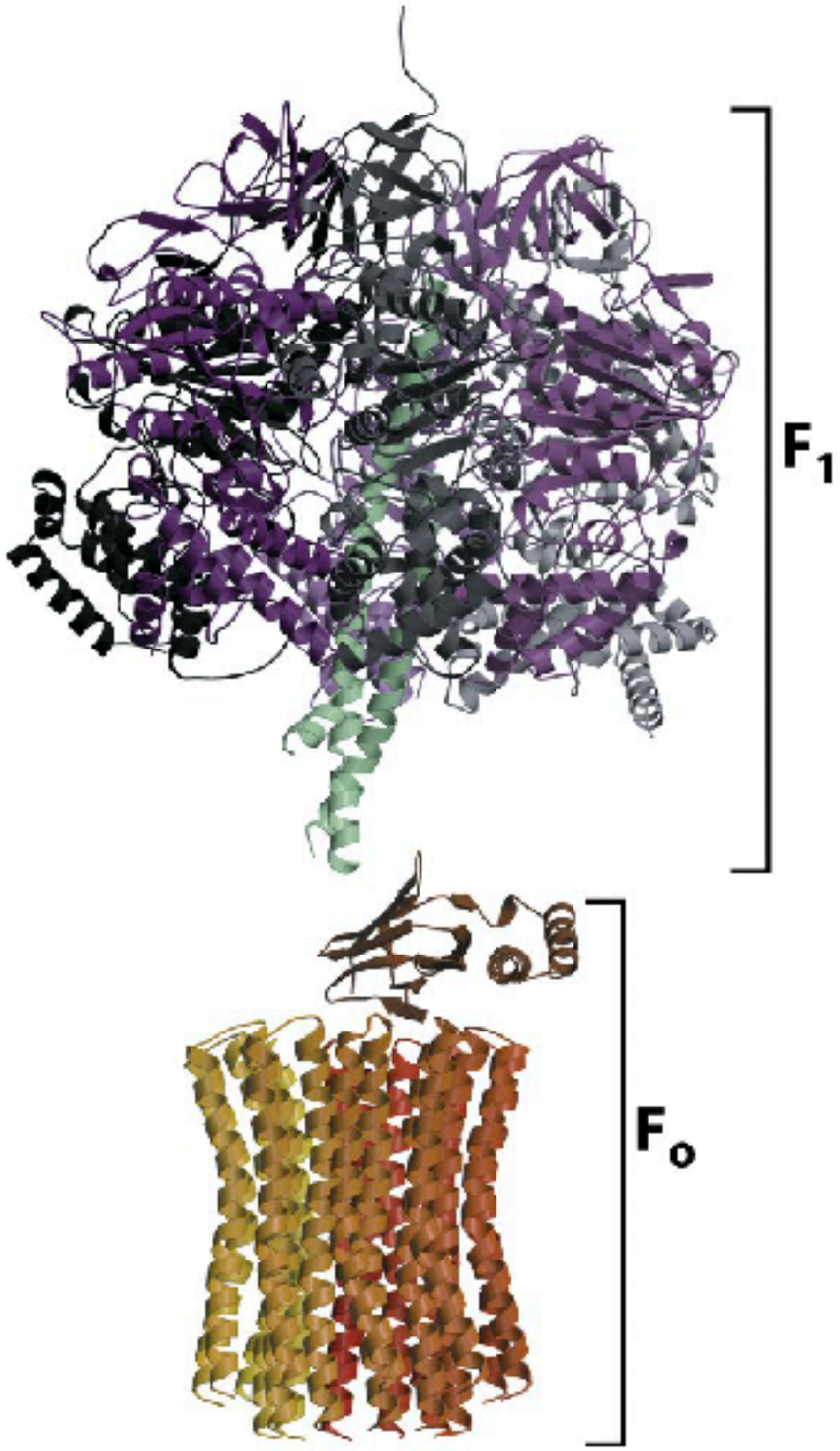
the c ring and the a subunit together work as a proton channel

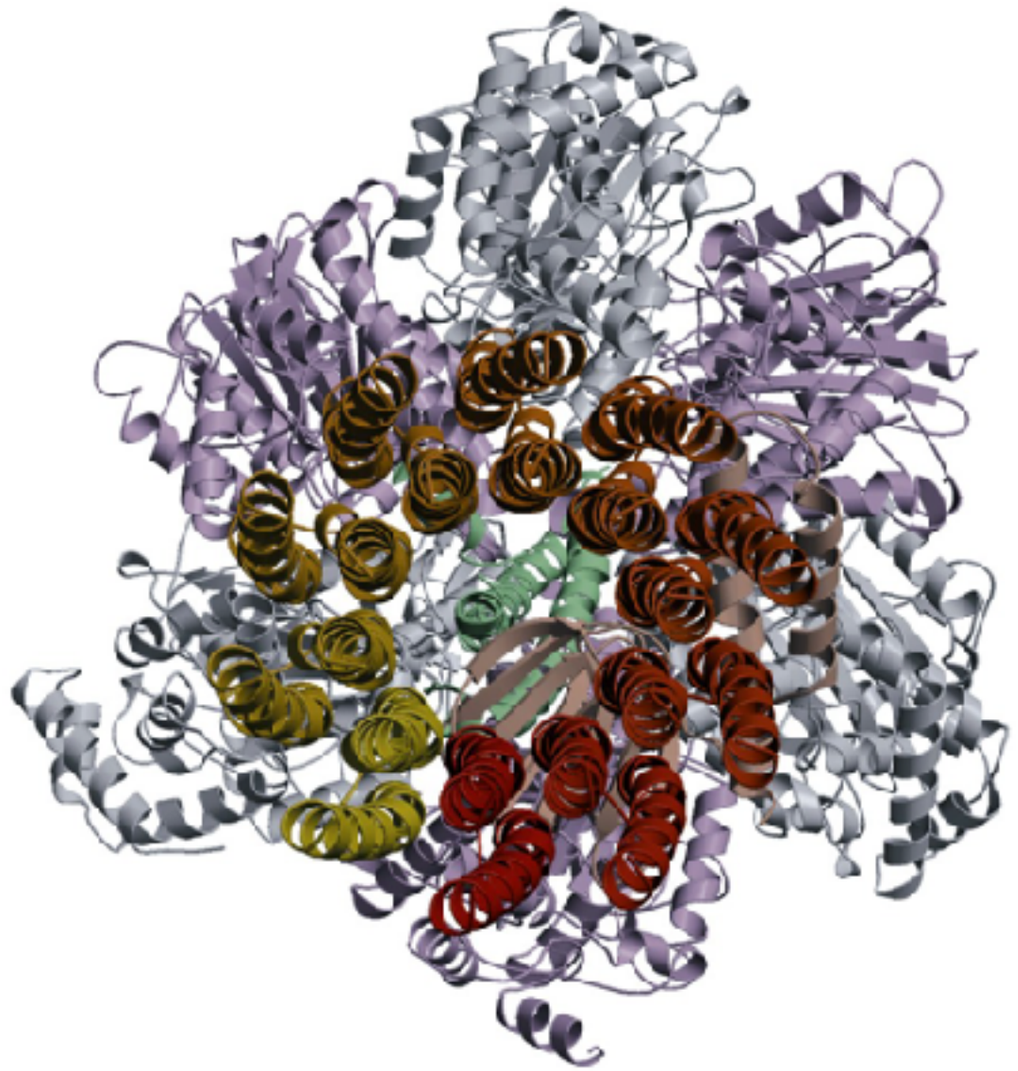
the two b subunits connect the a subunit in F0 to the F1

The c ring, and the central stalk are the rotating part of the complex

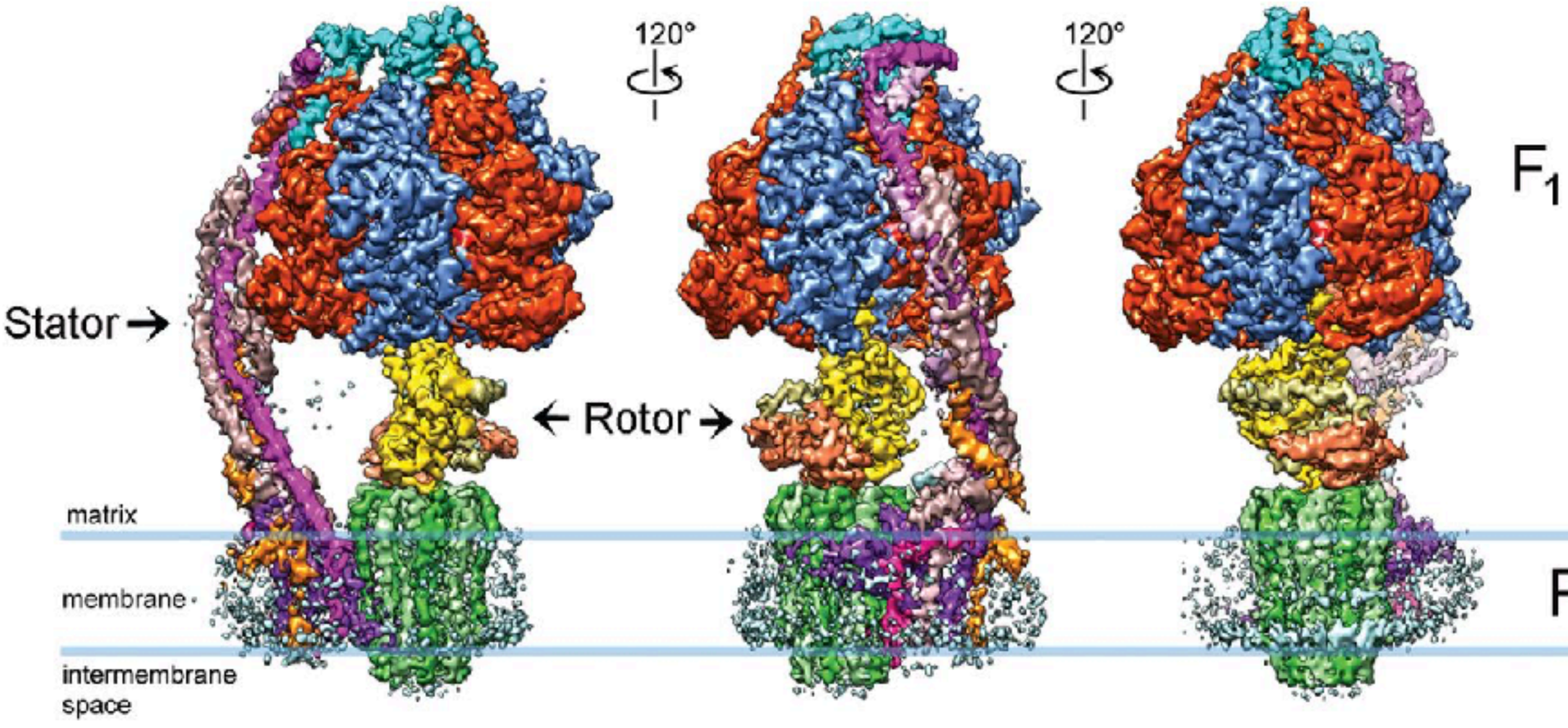
the a, b, delta, alpha and beta subunits do not rotate

The ATP synthase

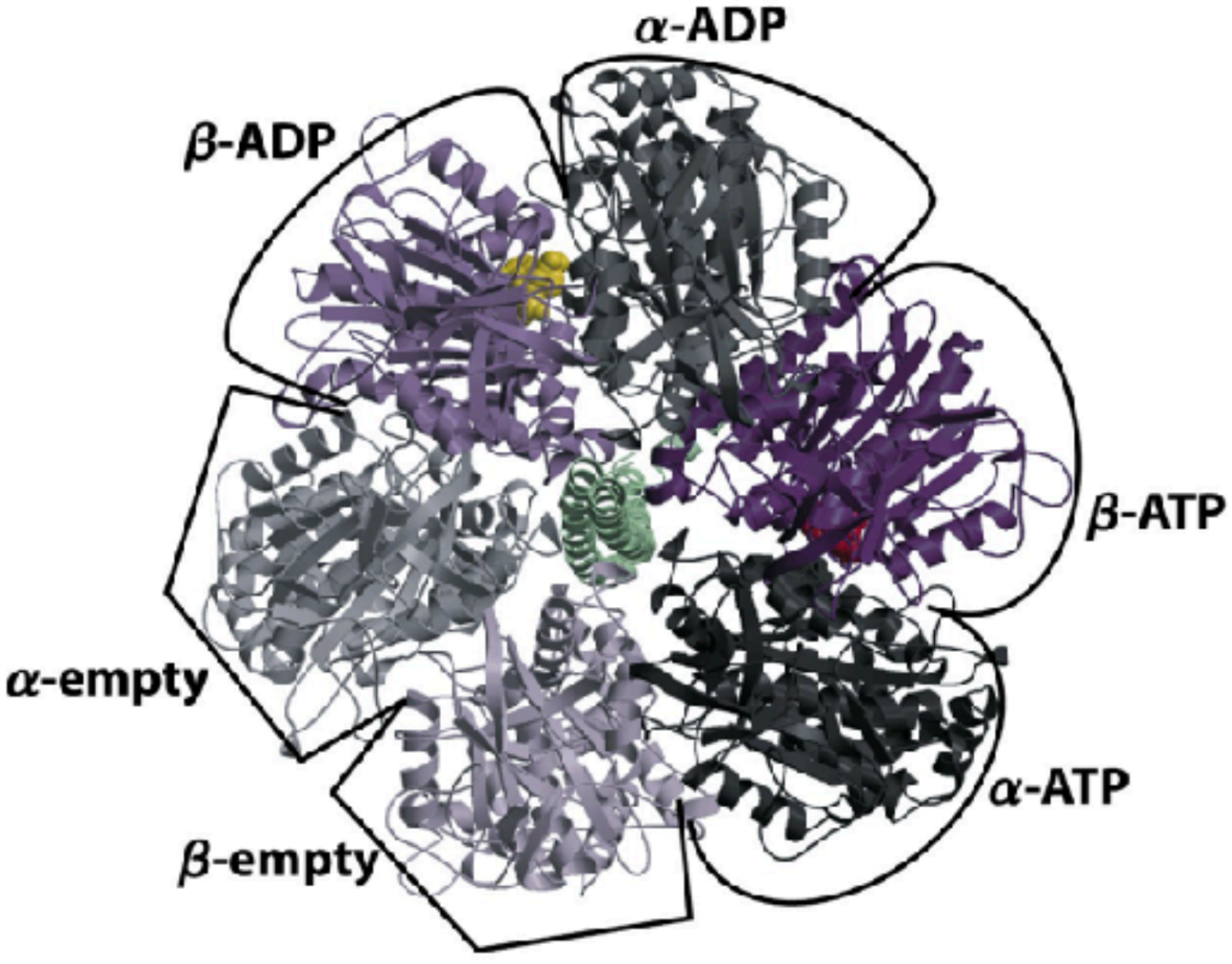




The ATP synthase



The ATP synthase



The alpha/beta hexamer is responsible for :

1) binding the ADP and Pi molecules
2) catalysing the synthesis of ATP
3) releasing the ATP molecule

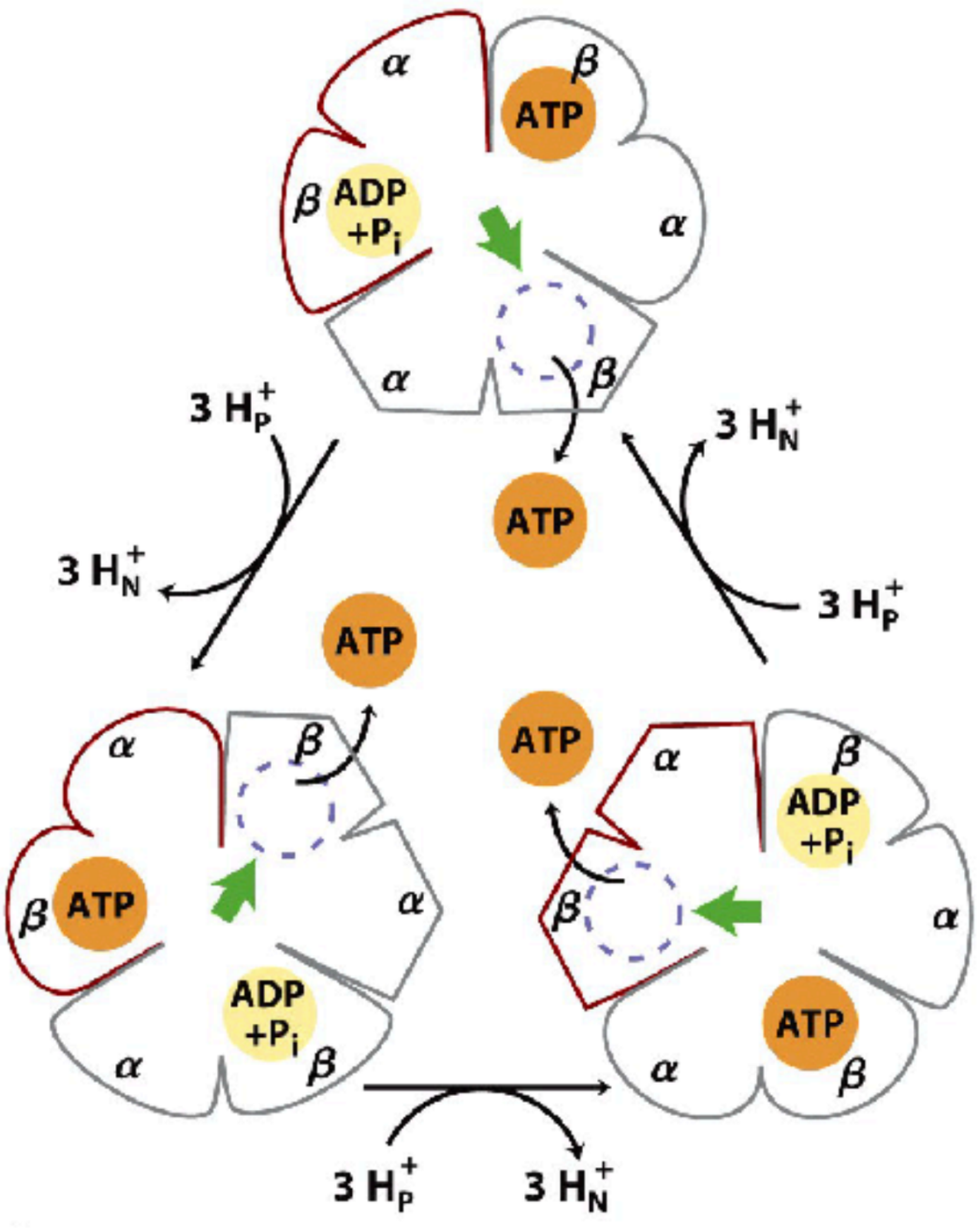
At any given moment each alpha-beta couple can be found in one of three distinct states:

Tense (T) state: ADP and Pi are brought close so that they can be combined into ATP

Open (O) state: The formed ATP is released and a new ADP/Pi set can bind

Loose (L) state: the bound ADP and Pi become trapped and cannot leave

The ATP synthase



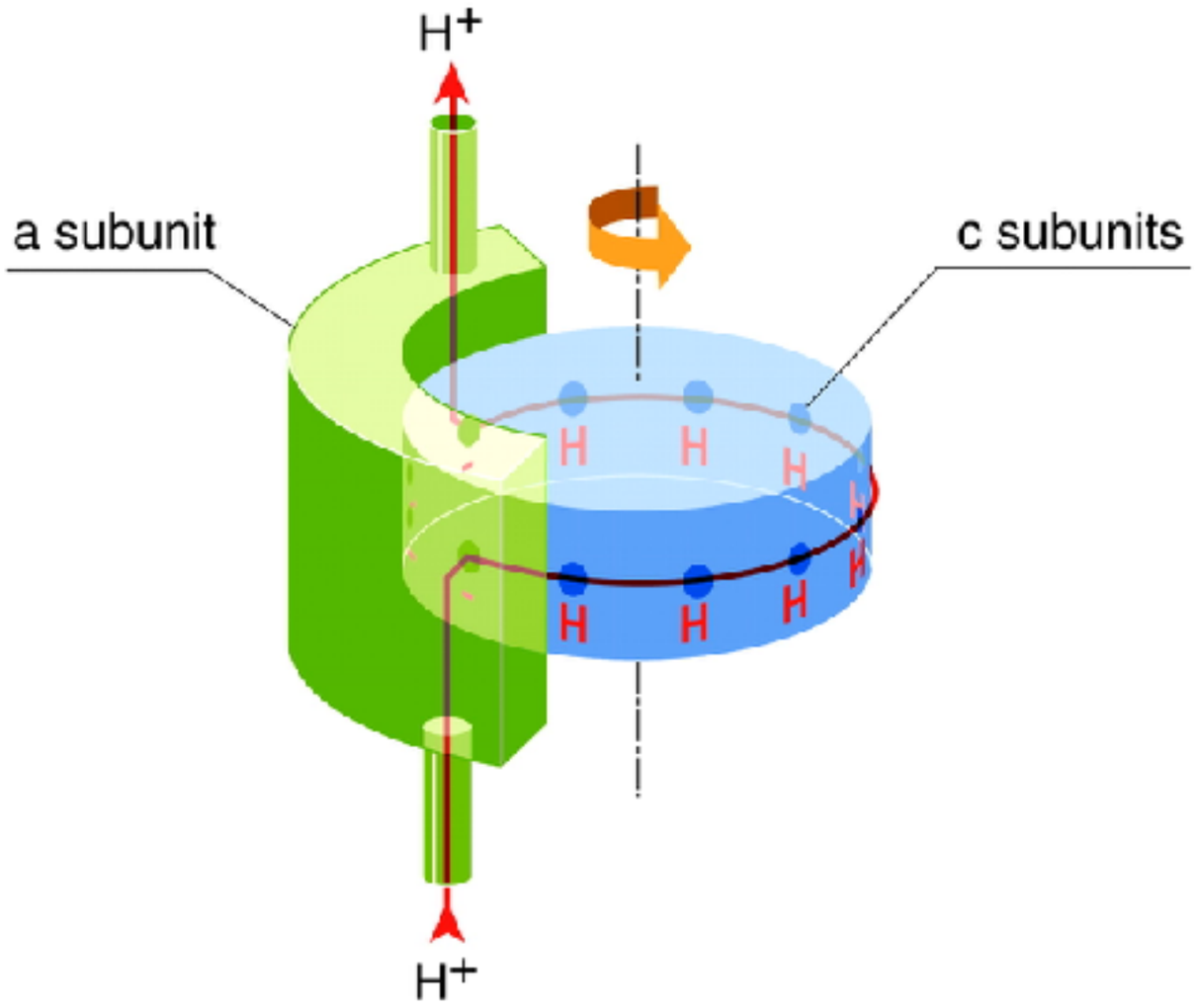
The **rotation of the gamma** subunit allows **the interconversion of the beta subunit from one state to another**.

Note that at any given time, all the beta subunits exist in a distinct state. That implies that any two beta subunits will never exist in the same identical state.

1) A rotation of the gamma subunit (120 degrees CCW) converts beta from the tense into the open state.
2) This relaxes beta and triggers the release of ATP
3) A new set of ADP/Pi enters the open beta and a further CCW turn of gamma will lock the reactants by foster the beta transition from open to loose

This model is known as the **binding-change mechanism**.

The ATP synthase



The a subunit contains 2 hydrophilic half channels that individually do not span the entire membrane thickness.

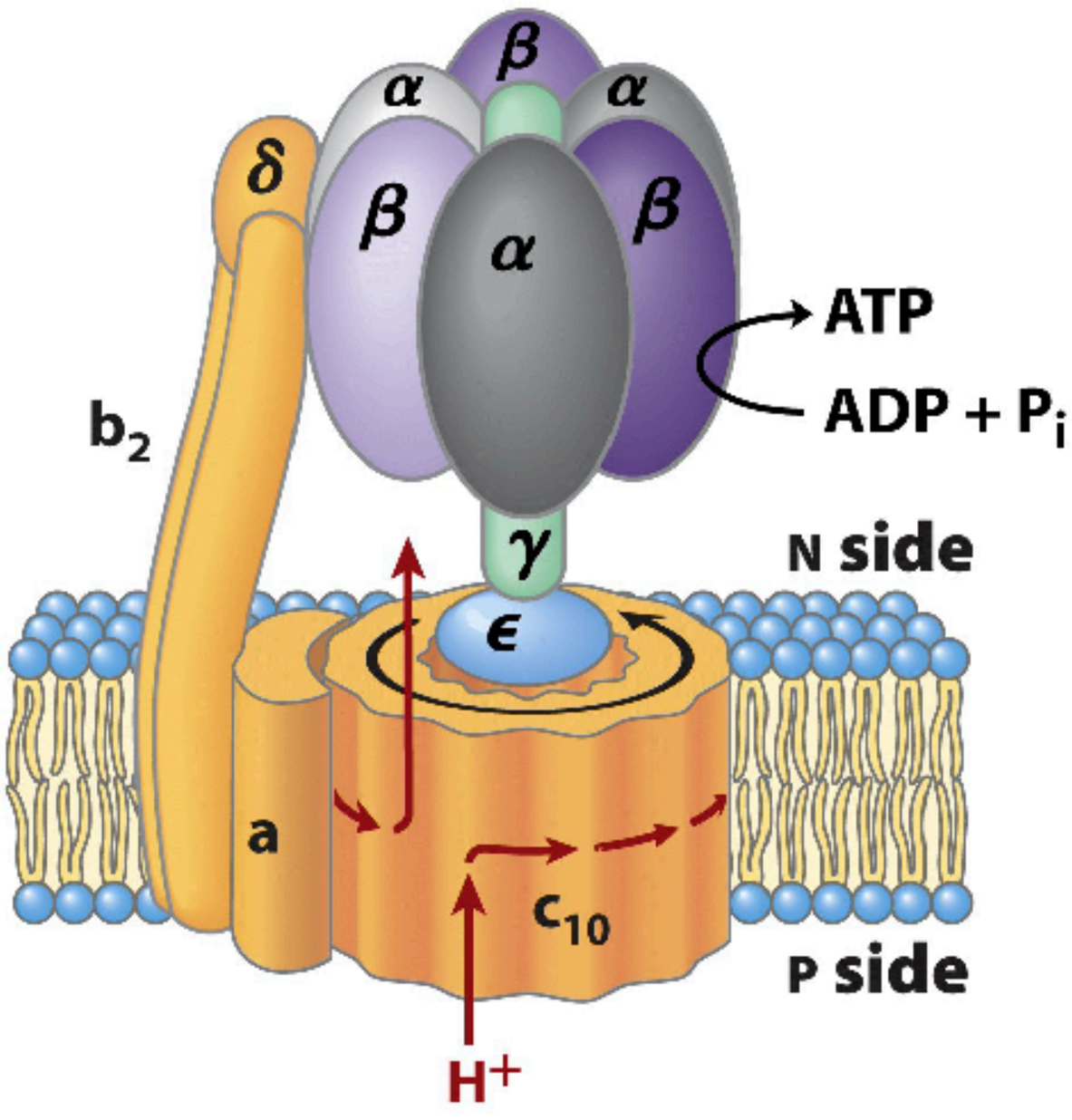
One of this half channels is open towards the matrix the other towards the intermembrane space.

The a subunit interacts with each subunit of the c ring. at the center of the c subunit there is an aspartate that binds H+ under acidic conditions.

Mechanism of proton movement:

1) an H+ enters the a subunit half channel from the matrix and binds to the aspartate residue in the closest c subunit
2) the entire c ring rotates until the H+ finds the other half channel facing the proton poor mitochondrial matrix
3) the H+ is released in the mitochondrial matrix

The ATP synthase



Therefore, the movement of the H+ through a subunit powers the rotation of the c ring.

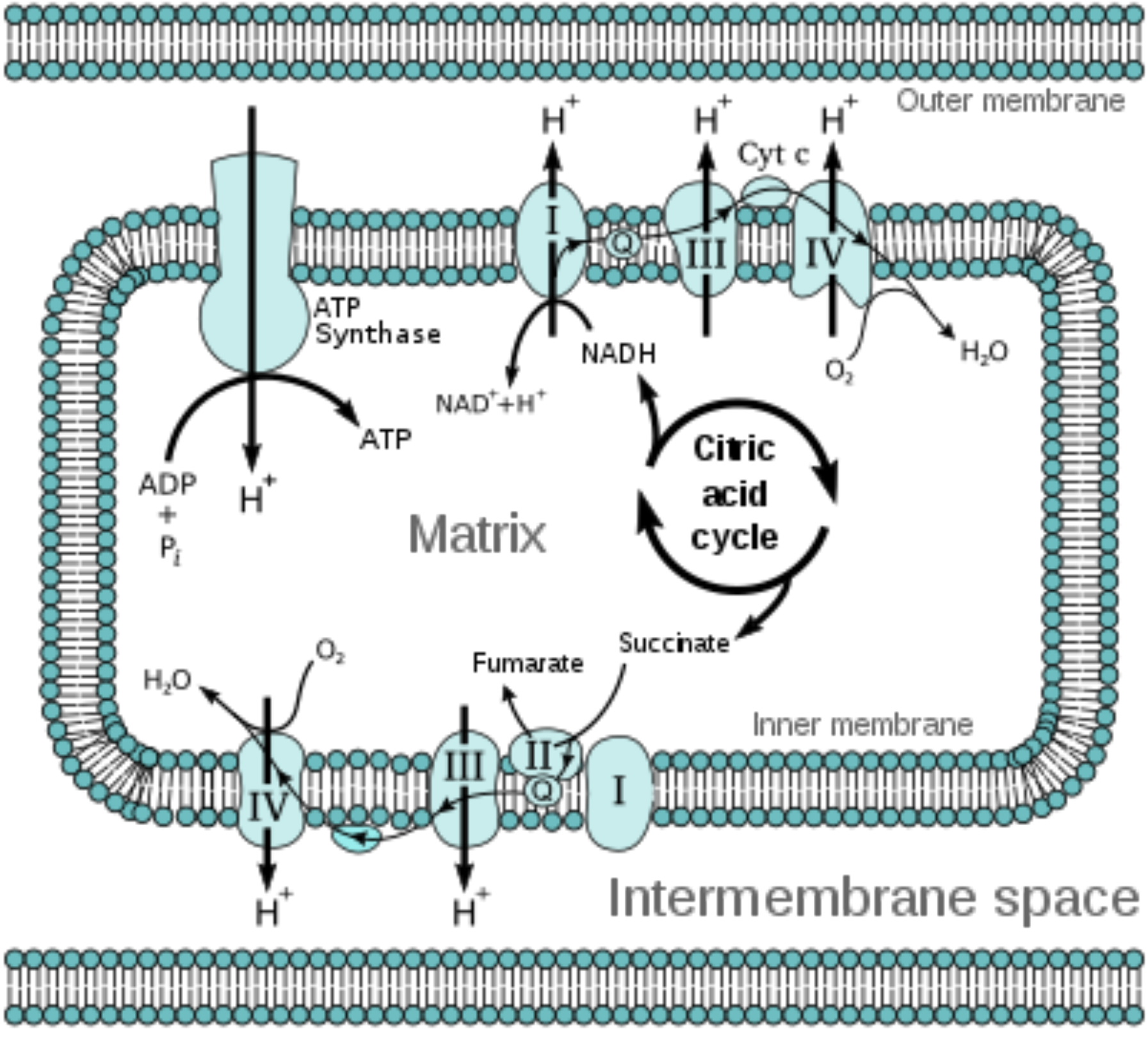
Since the c ring is connected to the epsilon and gamma submits this causes the central stalk to rotate as well.

By rotating, the gamma subunit stimulates the release of ATP from the alpha beta hexameter through the binding-change mechanism.

the presence of the b and delta subunits connects the a subunit and the alpha beta hexameter that need to remain stationary while the rest of the complex rotates.

The ATP synthase

https://www.youtube.com/watch?v=kXpzp4RDGJI


Outer membrane
H+
H+
H+
Cyt c
I
Q
III
IV
ATP
Synthase
NADH
NAD+ + H+
H2O
O2
ATP
ADP
+
Pi
H+
Matrix
Citric
acid
cycle
Succinate
Fumarate
H2O
O2
Inner membrane
II
III
IV
Q
I
H+
H+
Intermembrane space

Take home messages

Cellular respiration that requires oxygen is known as **aerobic respiration**. It involves 4 key processes: glycolysis, pyruvate decarboxylation, the TCA cycle and the oxidative phosphorylation that occurs on the electron transport chain.

Glycolysis

Glycolysis occurs in the cytosol it breaks down glucose into two pyruvate molecules with the production of 2 NADH and 2 ATP molecules. *It does not require oxygen*

Pyruvate decarboxylation

Pyruvate (in the presence of oxygen) is transported into the mitochondrial matrix and transformed int acetyl-CoA with the production of 2 NADH molecules and one of CO2. (2X)

TCA cycle

Acetyl-CoA enters a cycle of 8 reactions that leads to the production of 2 CO2, 3 NADH, 1 FADH2, and 1 GTP molecule. (2X)

The oxidative phosphorylation

In **oxidative phosphorylation** the electrons stored in NADH and FADH2 are used to produce ATP in the mitochondria. A single NADH molecule produced in the mitochondrial matrix yields 3 ATPs while a single cytosolic NADH or FADH2 yields 2 ATPs.

A net total of **36 ATP** molecules are produced by **1 glucose** molecule