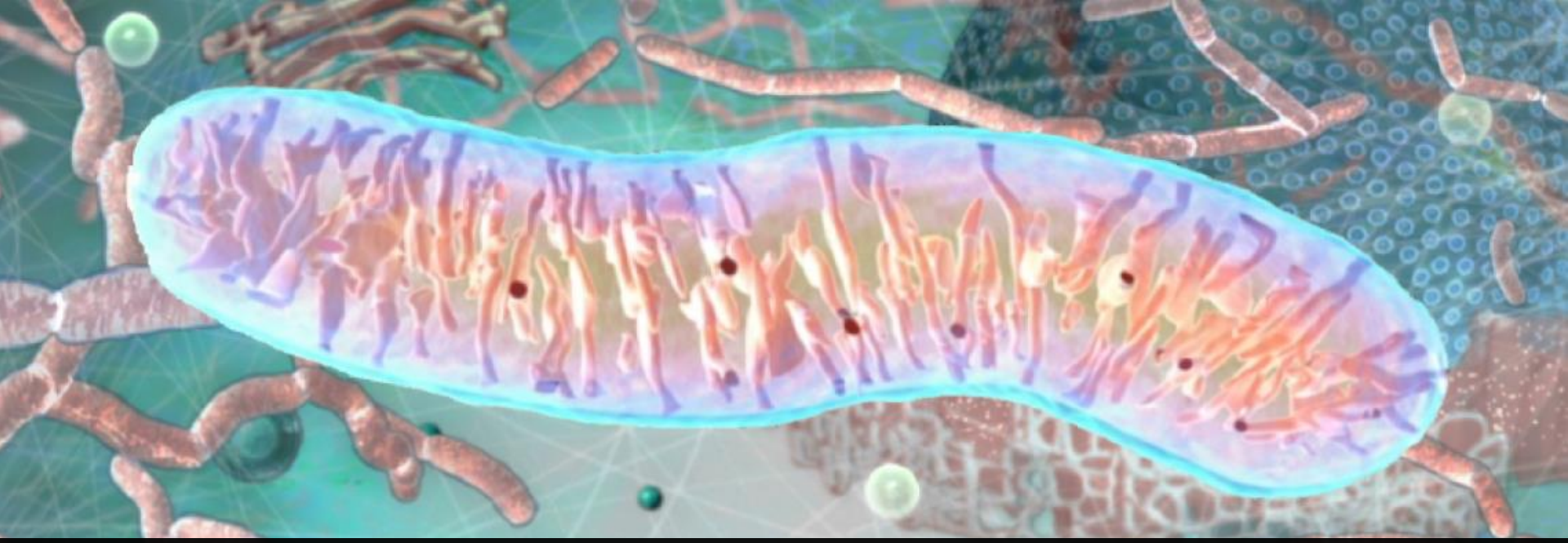


The enzymatic basis of energy-generation

Lecture 1: How does life conserve energy?

Dr Chris Greening

Lecturer / Group Leader

Monash University

May 4 2016

What is the course about?

- Focuses on life harnesses energy from organic, inorganic, and light sources. About the membrane-based energy-converting mechanisms NOT the metabolic pathways.

- Quite dense content and some challenging concepts. Light chemistry-wise, but rich organic and inorganic chem discussed. A basic knowledge of protein biochemistry will be helpful.

How is the course examined?

- One half of the exam will be short questions based. Will cover all of the lecture material. Will test how well you understand concepts NOT how many details you can remember.

- The second half of the exam will be an essay on a pre-set question (revealed in Lecture 2). For this, you will be expected to read the literature to get top marks.

Lecture course overview

Lecture 1: How does life conserve energy?

- Oxidative phosphorylation
- Electron transport chains
- ATP synthase: a molecular rotor

Lecture 2: Respiration of organic compounds

- Complex I: a respiratory supercomplex
- Complex III: bifurcating electrons
- Complex IV: reducing O_2
- Electron transport chain plasticity

Lecture 3: Respiration of inorganic compounds

- Prokaryotic versatility
- Nitrification and denitrification
- Aerobic H_2 respiration
- Anaerobic H_2 respiration

Lecture 4: Photosynthesis light reactions

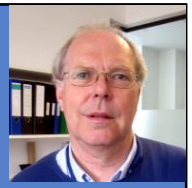
- Principles of photosynthesis
- Photosynthetic electron transport chains
- Redox-independent energy-conservation

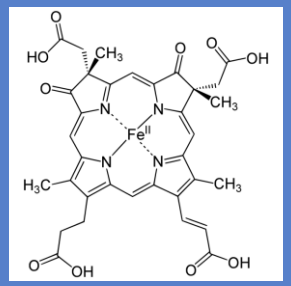
And who am I?

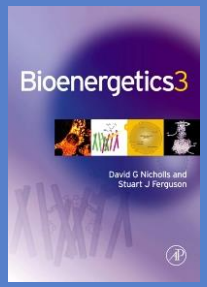
2006 - 2010

BSc Hons(I)

University of Oxford







2013 – 2014

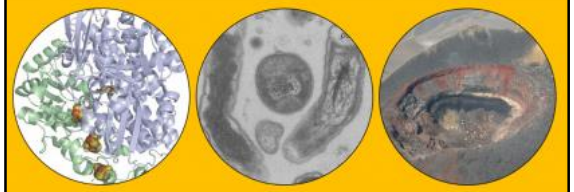
Lecturer & Postdoc

University of Otago

2016 –

Group Leader

Monash University

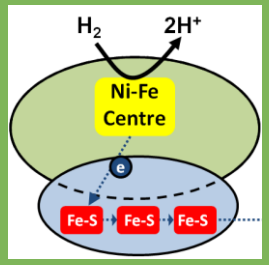


ENZYMES ⟷ CELLS ⟷ ECOSYSTEMS

2010 – 2013

Doctorate

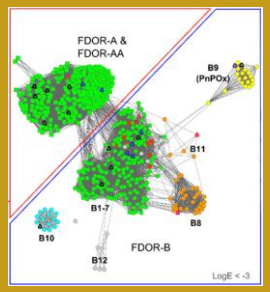
University of Otago



2014 – 2016

Research Fellow

CSIRO & ANU



Lecture 1: How does life conserve energy?

I. Oxidative phosphorylation

II. Electron transport chains

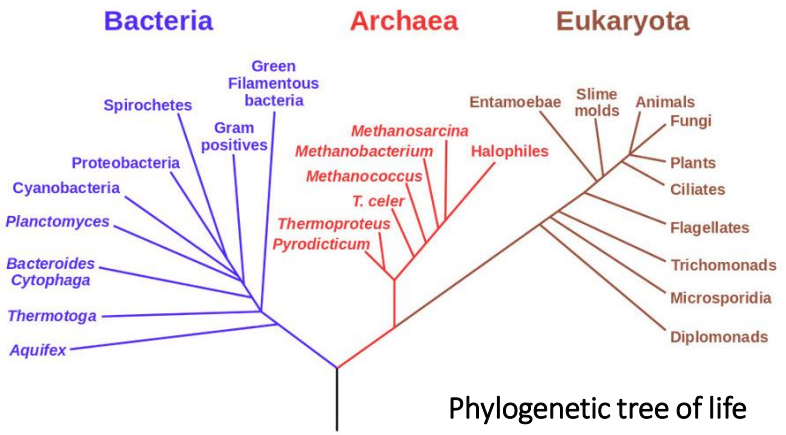
III. ATP synthase

Three energy sources for life

- Energy is required to sustain life, e.g. for growth/reproduction, maintaining organisation, macromolecule synthesis. Three main energy sources:

Energy source	Term	Organisms	Discussed
Organic compounds (e.g. glucose)	Heterotrophy	Animals, bacteria	Lecture 2
Inorganic compounds (e.g. H_2)	Lithotrophy	Bacteria, archaea	Lecture 3
Light (photons)	Phototrophy	Plants, bacteria, archaea	Lecture 4

- There is variety in energy generation mechanisms between the three domains of life (bacteria, archaea, eukaryotes).



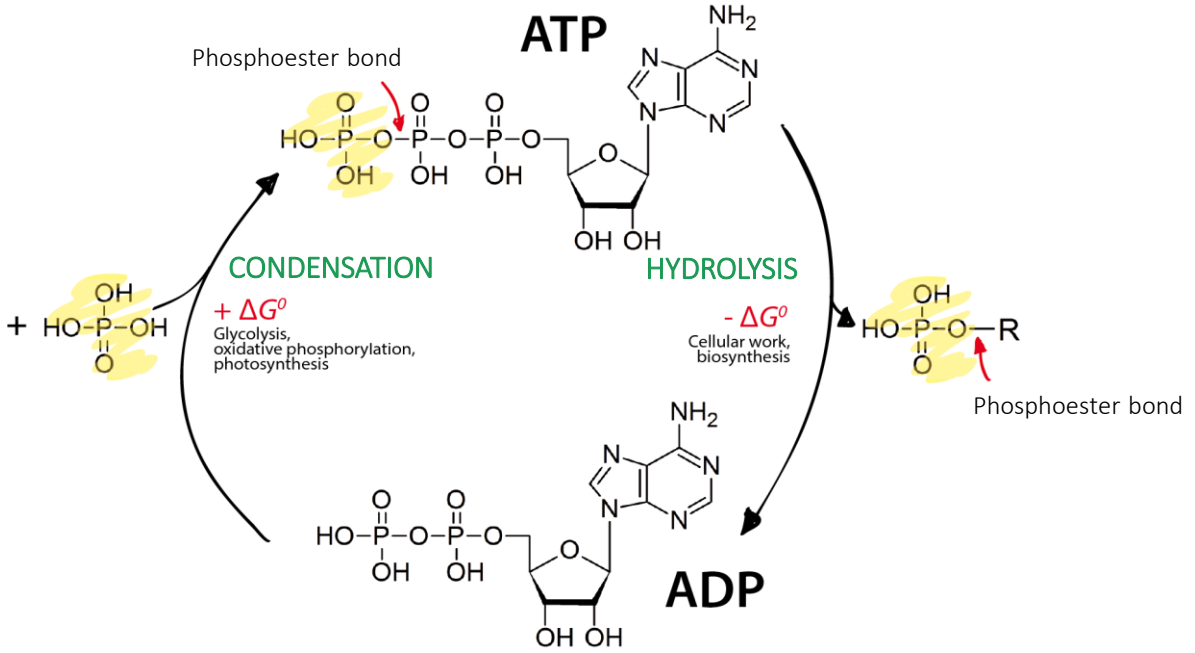
Phylogenetic tree of life

ATP is the dominant energy currency of the cell

- ATP (adenosine triphosphate) serves as the main energy store of the cell.
  - The energy from heterotrophy, lithotrophy, and phototrophy is used to synthesize ATP.
  - This energy is released through hydrolysis and powers most internal cellular processes.

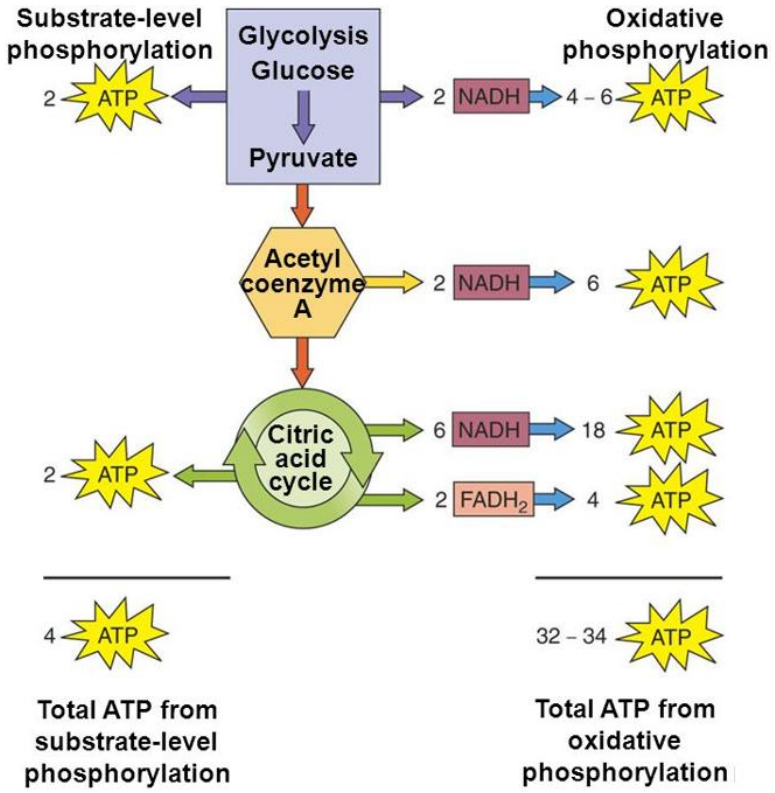
$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + P_i^{2-} + H^+$$

$\Delta G^{\circ\prime} = -31$ kJ mol^{-1}, $\Delta G_{cytosol} = -57$ kJ mol^{-1}



Two mechanisms to generate ATP

Process	ATP synthesis mechanism	Mechanism	ATP yield
SLP: Substrate-level phosphorylation	Direct transfer of phosphoryl groups to ADP generates ATP	Phosphoryl transfer from reactive glycolytic intermediates (phosphoenolpyruvate, bisphosphoglycerate)	Low
OXPHOS: Oxidative phosphorylation	Proton gradients across cellular membranes drive ATP synthesis	Proton gradients created by oxidation of coenzymes (e.g. NADH, $FADH_2$) through electron transport chains	High



ATP-generating pathways during the complete oxidation of a molecule of glucose. Not necessary to know exact pathways for this course.

Respiration and photosynthesis use OXPHOS

- OXPHOS enables organisms to maximise their ATP generation during respiration and photosynthesis. SLP is the only mode of ATP generation during fermentation.

Process	Energy-conversion mechanism	ATP source
Fermentation (heterotrophy)	Incomplete oxidation of organic compounds through glycolysis resulting in endproduct formation	SLP
Respiration (heterotrophy)	Complete oxidation of organic compounds through glycolysis, TCA cycle, and electron transport chains	OXPHOS, SLP
Respiration (lithotrophy)	Complete oxidation of inorganic compounds through electron transport chains	OXPHOS
Photosynthesis (phototrophy)	Light-induced oxidation of water through electron transport chains	OXPHOS

OXPHOS described in Mitchell's postulates

- Until 1961, it was thought that SLP was the dominant mechanism of energy-generation. Peter Mitchell introduced the concept of OXPHOS (Mitchell, 1961, Nature). Three postulates:

  1) An intact energy-converting membrane required to maintain a proton gradient
  2) Electron transport through primary proton pumps generates a proton gradient
  3) Downhill proton movement through a secondary proton pump drives ATP synthesis

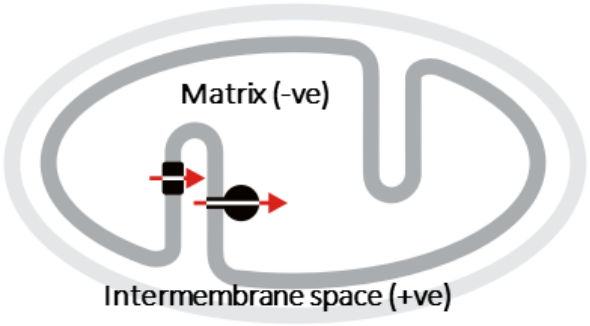
- Very controversial as SLP thought to be major energy-generation mechanism. By 1978, his three postulates were proven and Mitchell awarded Nobel Prize.

Nobel Laureate Peter D. Mitchell (1920-1992)

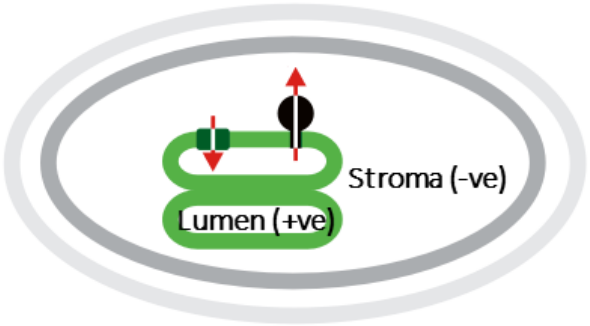
Energy-converting membranes

- OXPHOS indeed depends on the generation and maintenance of a proton gradient across biological membranes. Three main energy-converting membranes:

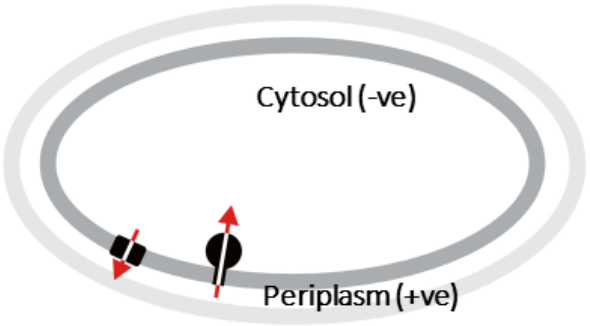
Mitochondrial inner membrane:



Chloroplast thylakoid membrane:



Bacterial/archaeal cell membrane:



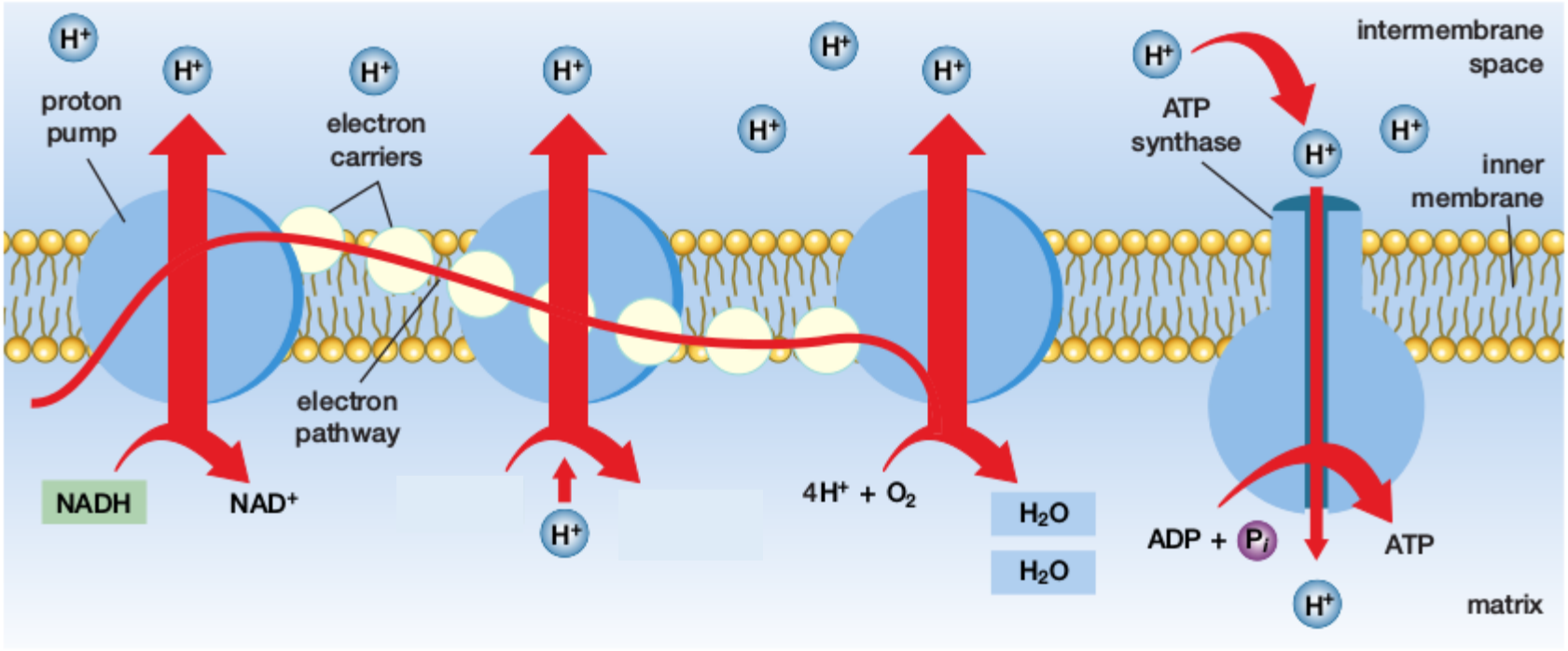
-ve side = N phase
+ve side = P phase

- Evolutionarily related through endosymbiosis: mitochondria derived from heterotrophic bacterium (alphaproteobacteria), chloroplasts from phototrophic bacterium (cyanobacteria)

Membranes contain pairs of proton pumps

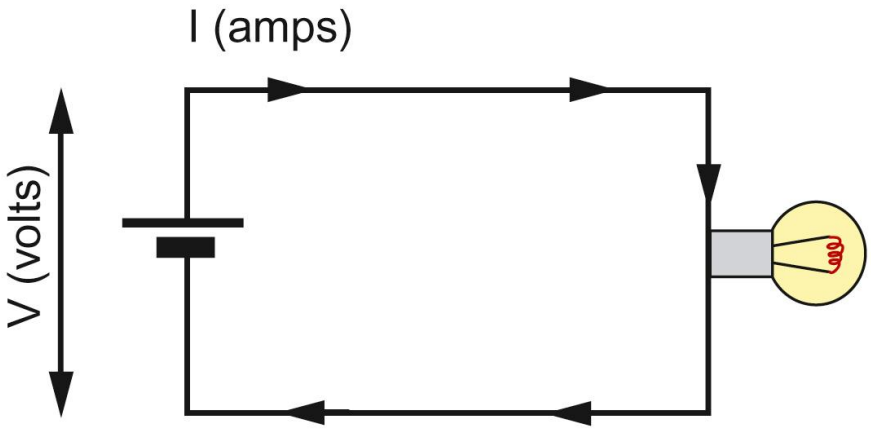
- Primary pump (gradient formation): Uses energy released from electron transfers to pump protons across membrane. Multiple pumps responsible that form electron transport chains.

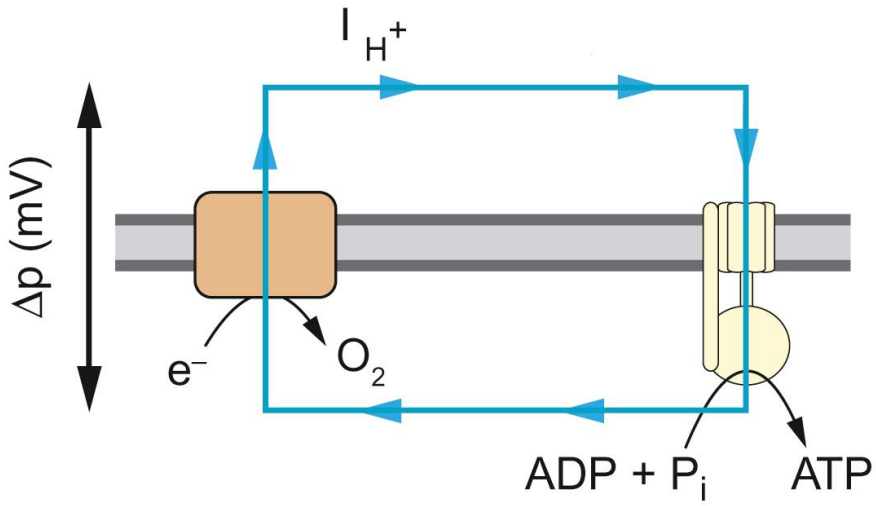
- Secondary pump (gradient utilisation): Uses potential energy of the proton gradient to drive ATP synthesis. One conserved enzyme responsible: ATP synthase.



A proton circuit

- Proton circuits are analogous to electrical circuits:
  - Primary pumps (like batteries) generates voltage
  - Voltage generated depends on current (proton flux) and resistance (permeability)
  - ATP synthase (like lightbulb) performs useful work
  - Uncouplers (like a piece of wire) can short-circuit the system





Quantifying proton-motive force

- Proton-motive force (Δp) describes the potential energy (in millivolts) generated by proton pumping across energy-transducing membranes. Formed by two components:
  - pH gradient (ΔpH): diffusion force caused by proton concentration gradient
  - Membrane potential (ΔΨ): electrostatic force caused by electrical potential gradient

$$\Delta p = \Delta\Psi + \frac{2.3RT}{F}\Delta\text{pH} \approx \Delta\Psi - 61\Delta\text{pH}$$

Differential contributions of ΔΨ and ΔpH

- Mitochondrial membranes have a low capacitance (approx. 1 μF cm^{-1}) hence they can store a high voltage per amount of charge moved. Hence ΔΨ is the dominant contributor to Δ*p*. In chloroplasts, the weight of these contributions is reversed and ΔpH is dominant.

Term	Mitochondria	Chloroplasts
ΔΨ	150 mV	75 mV
ΔpH	-0.5 units	-3 units
Δ*p*	= 150 – 61(0.5) = 182 mV	= 75 – 61(-3) = 258 mV

Lecture 1: How does life conserve energy?

I. Oxidative phosphorylation

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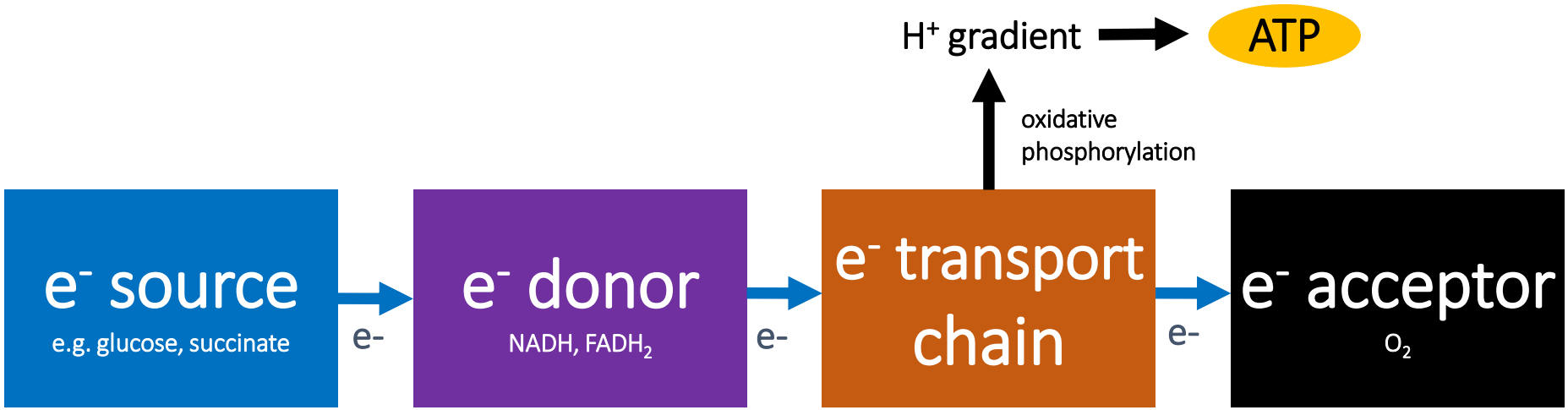
Electron transport chains generate Δ*p*

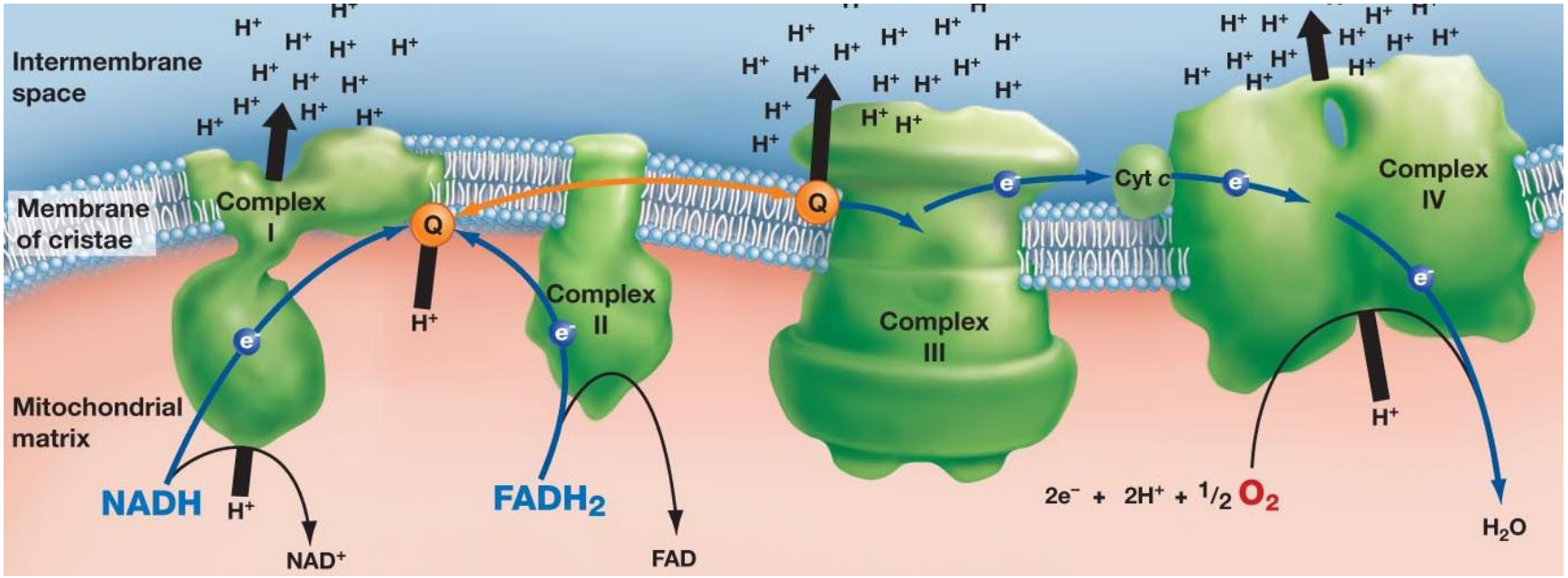
- Electron transport chains (ETCs) in energy-converting membranes couples electron transfer from donor to acceptor to the pumping of protons across the membrane. ETCs differ between mitochondria, chloroplasts, and bacteria/archaea.

- In mitochondria, the e^- donors are cofactors (NADH, $FADH_2$) reduced *via* oxidation of organic compounds (glycolysis, TCA cycle). Electron acceptor is always O_2 derived from breathing air.



Architecture of the mitochondrial ETC

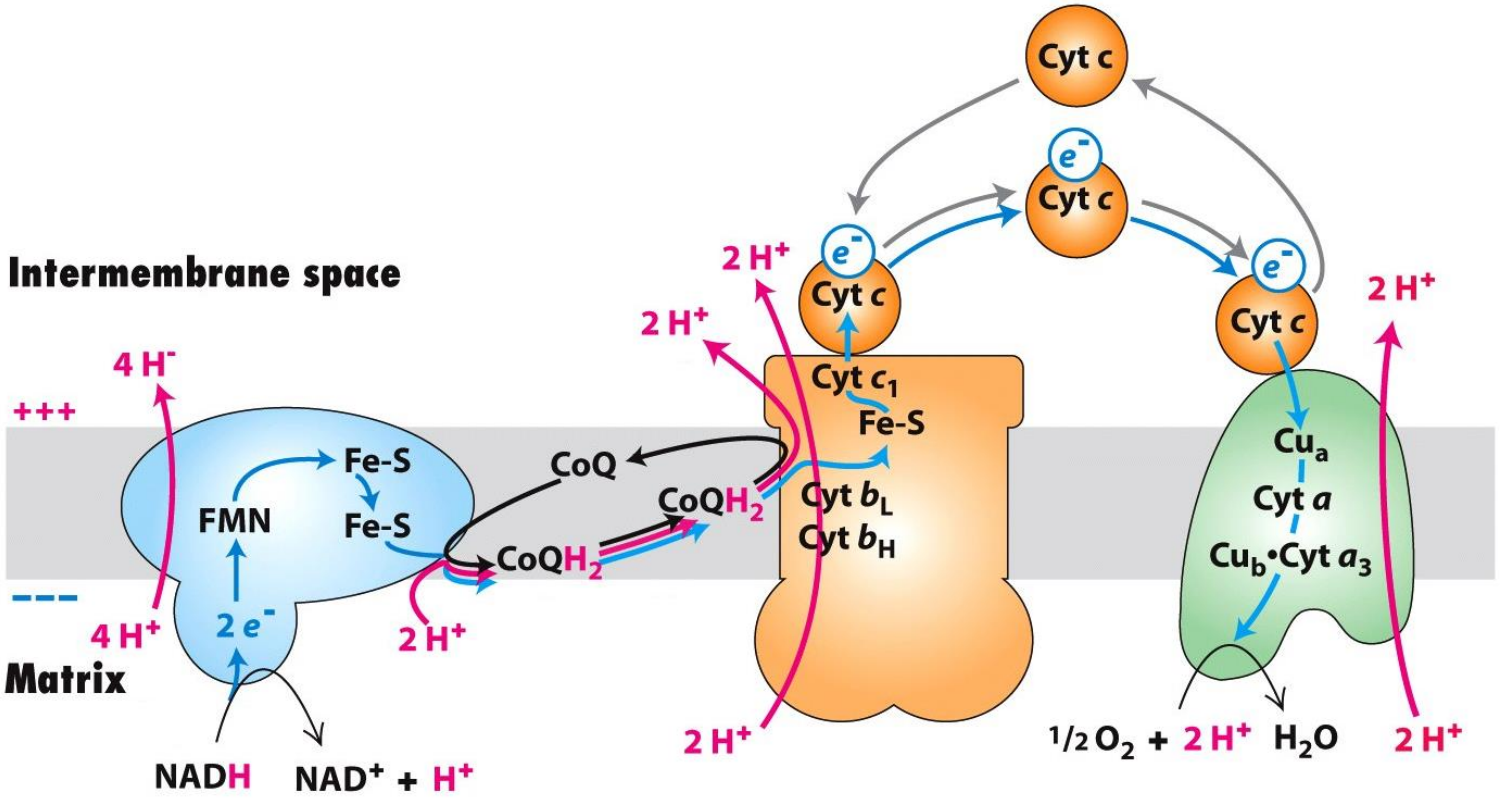
- In mitochondria, the ETC is a linear chain composed of five main components:
  - **Three transmembrane protein complexes:** Complex I, III, and IV sequentially couple electron transfer from donor (NADH) to acceptor (O_2) to proton translocation.
  - **Two membrane-diffusible carriers:** The lipid ubiquinone (UQ) and the heme-containing protein cytochrome *c* transfer electrons between complexes.



- Arranged in linear chain: NADH → Complex I → UQ → Complex III → Cyt *c* → Complex IV → O_2. Electrons also enter at UQ *via* three non-translocating flavoproteins including complex II.

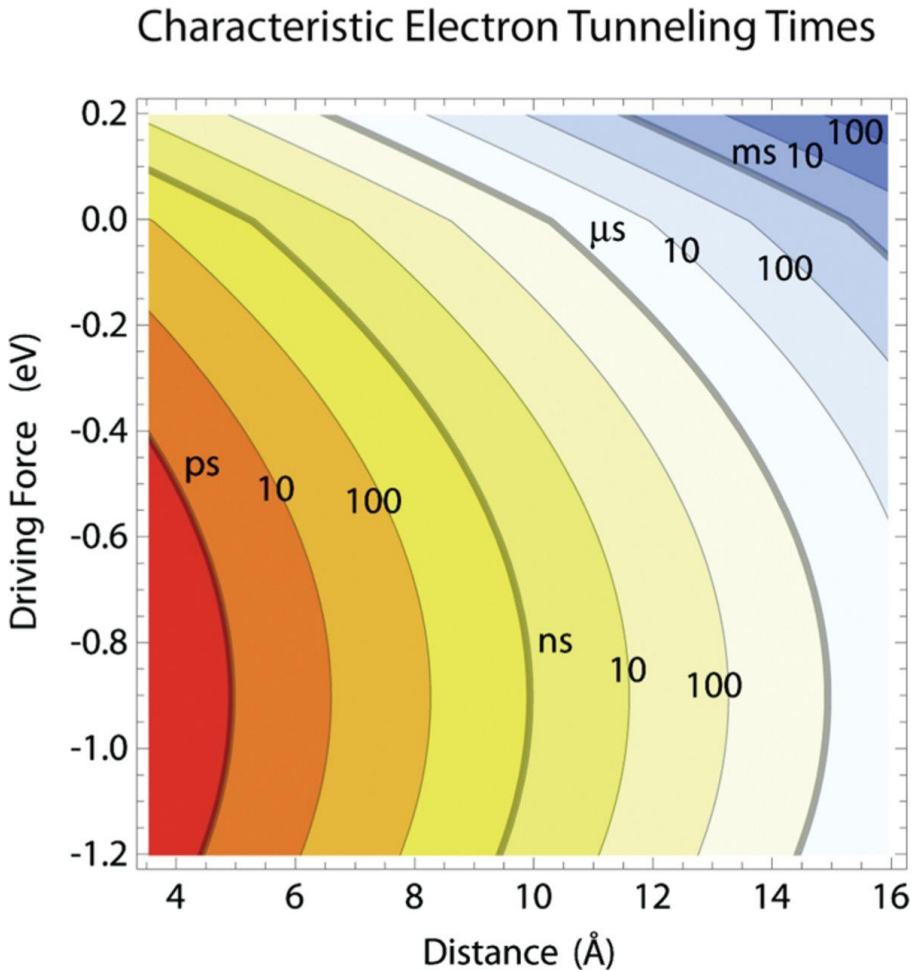
ETC complexes contain multiple redox centres

- Electrons are sequentially transferred through multiple redox centres bound (covalently or noncovalently) bound to ETC complexes, i.e. flavins, cytochromes, FeS clusters, and Cu.



Engineering principles of electron transfer

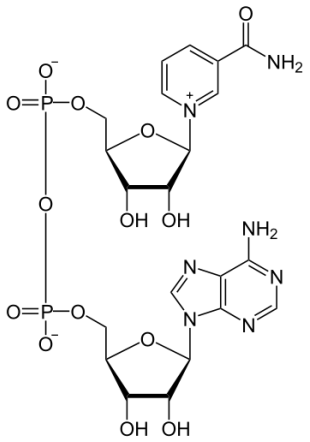
- Redox centres a maximum of 14 Å apart. This enables rapid transfer of e^- between centres through quantum mechanical tunnelling. Wavefunction of an e^- held in an energy well on donor shows there is a low but finite probability that e^- will be found at a potential acceptor.



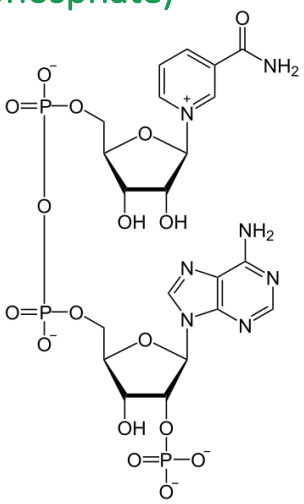
Page et al., Nature, 1999

Electron carriers in energy-generation

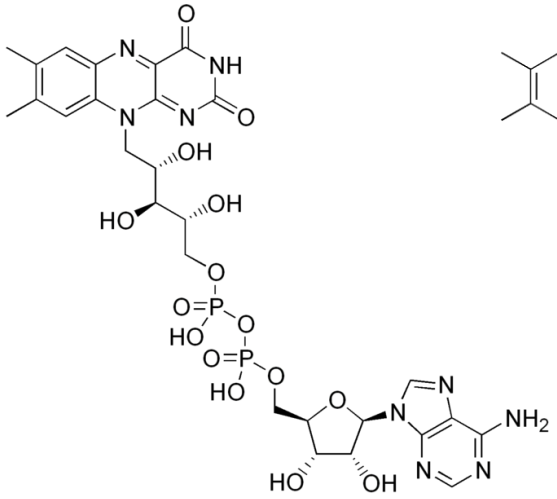
NAD (nicotinamide adenine dinucleotide)



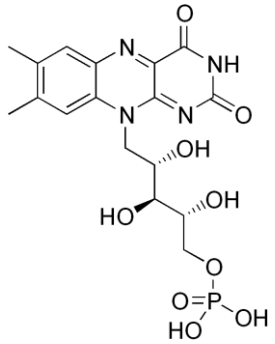
NADP (nicotinamide adenine dinucleotide phosphate)



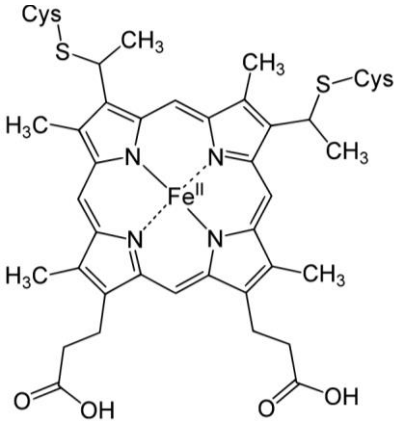
FAD (flavin adenine dinucleotide)



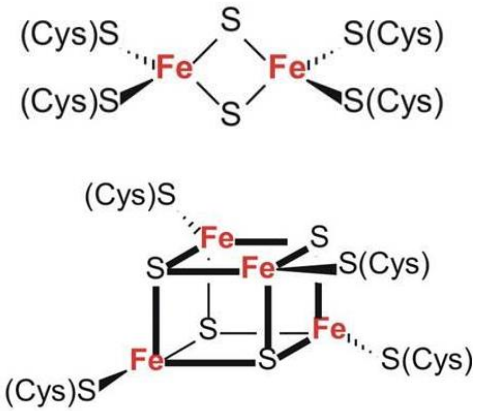
FMN (flavin mononucleotide)



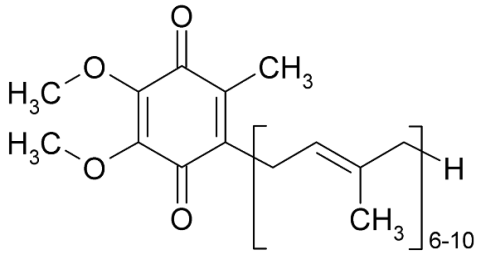
Heme (cytochromes)



Iron-sulfur clusters

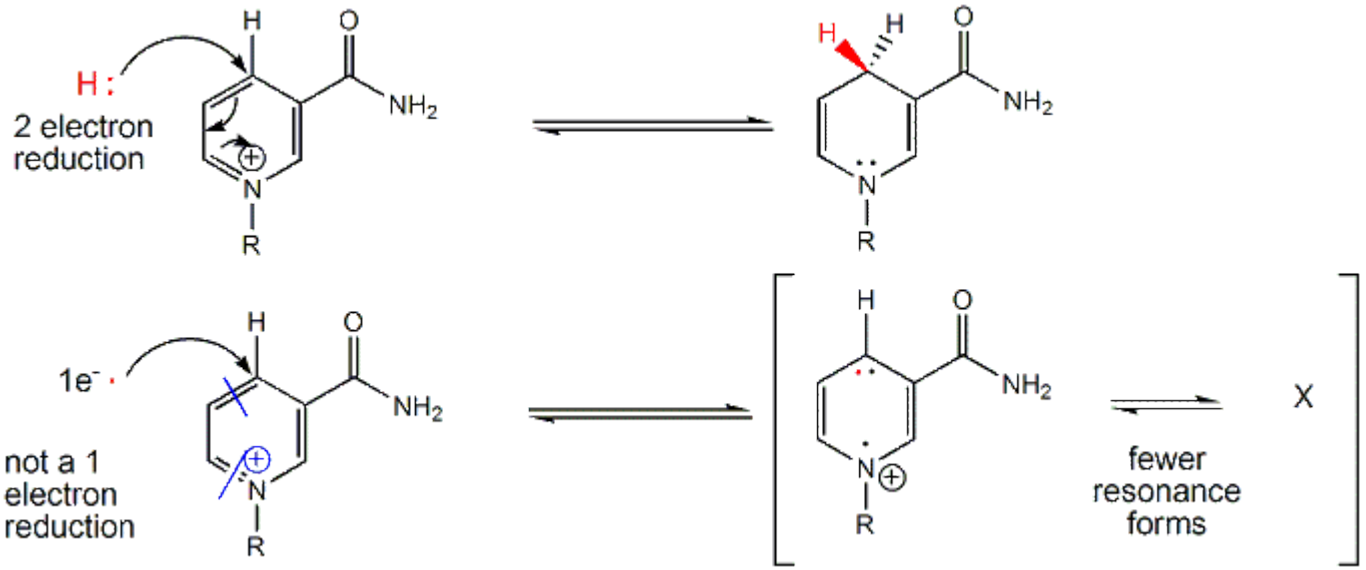


Ubiquinone



NAD, NADP are water-soluble electron carriers

- Nicotinamides (NAD, NADP) are obligate two-electron carriers derived from niacin (vitamin B_3). They accept or donate hydride groups ($2e^- + 1H^+$). NAD and NADP have same chemistry, but different cellular roles; enzymes discriminate them *via* the phosphate group of NADP.

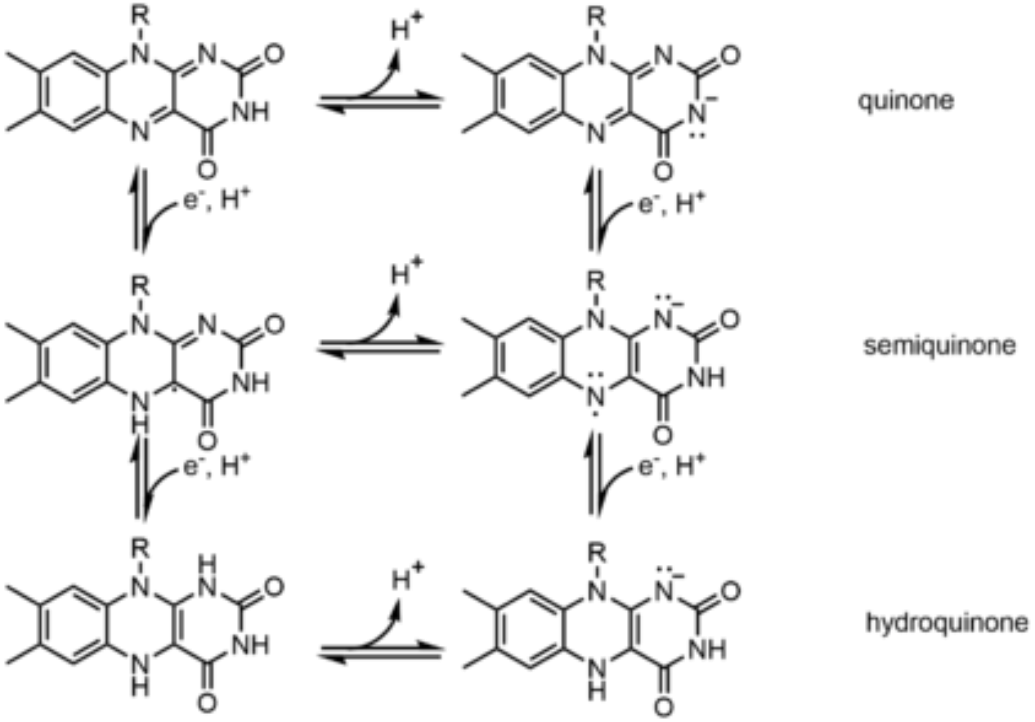


- NAD^+ is the oxidant in most catabolic reactions (favourable due to high $[NAD^+]/[NADH]$ ratio). NADPH is the reductant in most anabolic processes (driven by high $[NADPH]/[NADP^+]$ ratio). NADH serves as the major e donor in respiratory chain.

FMN, FAD are major prosthetic groups

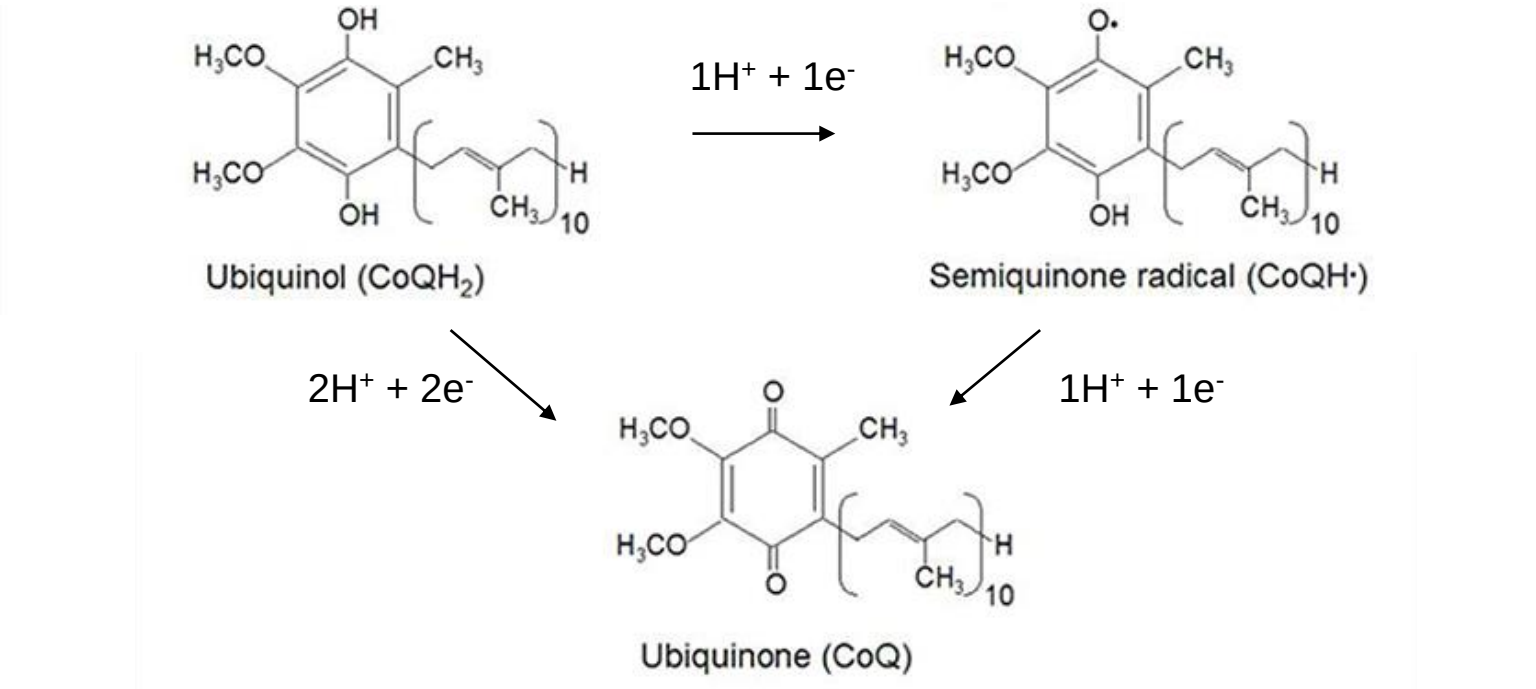
- Flavins (FAD, FMN): versatile one- and two-electron carriers derived from riboflavin (vitamin B_2). Bound as a prosthetic group to flavoproteins due to their high oxygen-sensitivity. Act as gates between $1e^-$ and $2e^-$ reactions. Semiquinone radical form resonance-stabilised.

- Unlike NADH, $FADH_2$ is not a direct donor to ETC. It is a prosthetic group in the three non-translocating complexes that donate electrons to the respiratory chain.



Quinones are lipid-soluble electron carriers

- Quinones are electron shuttles that diffuse within energy-converting membranes. They contain a redox-active quinone headgroup and multiple hydrophobic isoprene units. Like flavins, they are stable in three forms (oxidised, semiquinone, reduced):



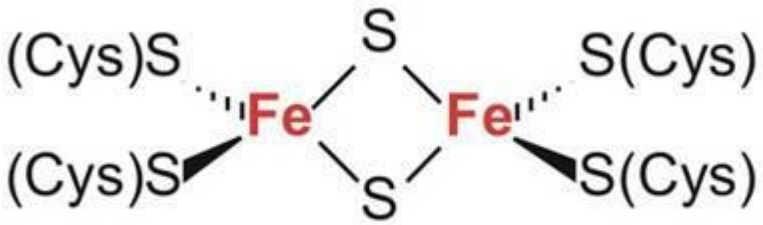
- Three major quinones in energy-conversion: ubiquinone (mitochondria, proteobacteria), menaquinone (bacteria, archaea), and plastoquinone (chloroplasts, cyanobacteria).

Metal centres also facilitate respiration

- The mitochondrial ETC depends on two transition metals. Fe serves as a $1e^-$ carrier in iron-sulfur clusters and cytochromes. Cu serves primarily as an O_2-activating site in complex IV.

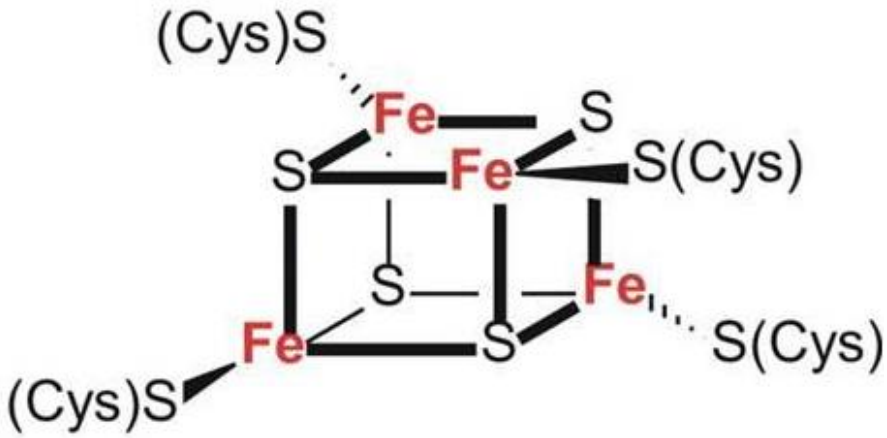
- Iron-sulfur clusters are redox-active crystals of sulfur and iron atoms. Numerous forms found, the main ones being [2Fe2S] and [4Fe4S]. Almost always undergo $1e^-$ chemistry.

[2Fe2S] clusters:



$(Fe^{III})_2 + 1e^- \rightarrow (Fe^{III})_1(Fe^{II})_1$

[4Fe4S] clusters:

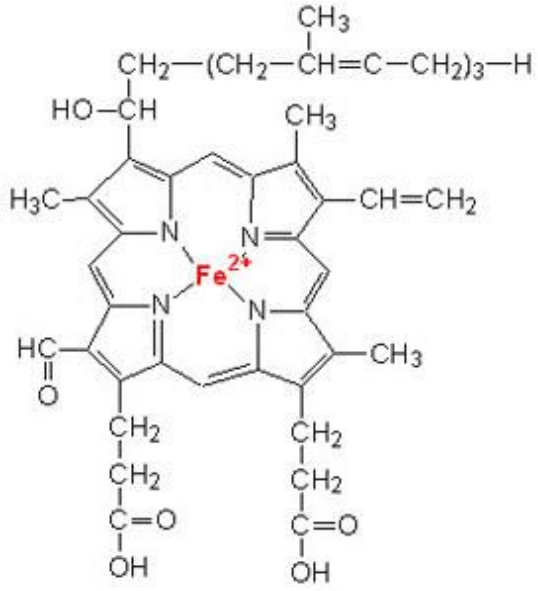


$(Fe^{III})_3(Fe^{II})_1 + 1e^- \rightarrow (Fe^{III})_2(Fe^{II})_2$

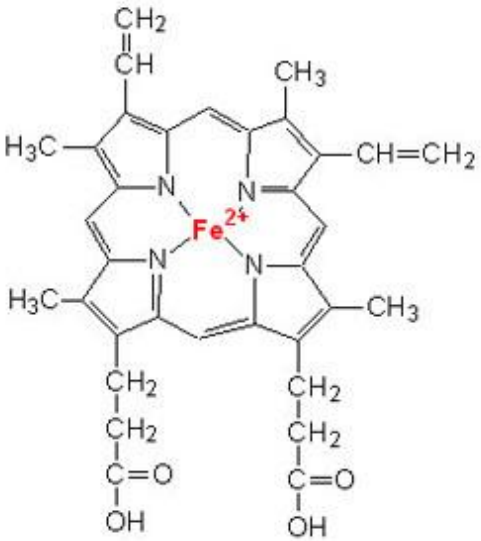
Cytochromes are electrochemically versatile

- The other major $1e^-$ carriers are cytochromes. Comprise heme centre (i.e. iron ligated with porphyrin ring) bound to a protein scaffold. The iron undergoes Fe^{III} to Fe^{II} transitions.

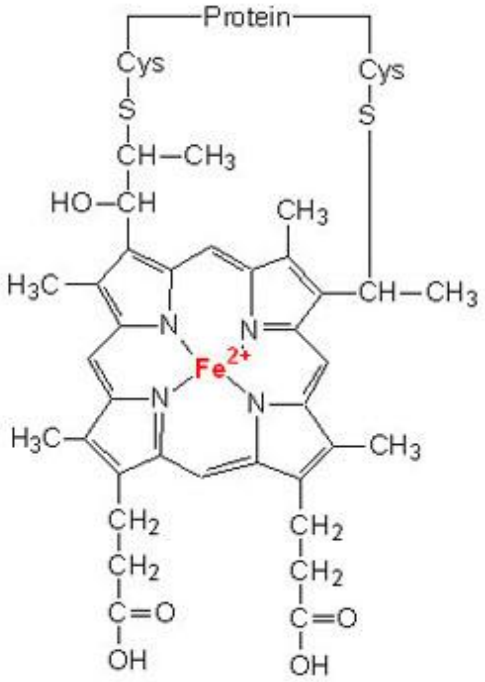
- Seven spectroscopically- and functionally-distinct cytochromes in ETC (*a*, a_3, *b*, b_L, b_H, *c*, c_1). They include the diffusible electron carrier cytochrome *c* and redox centres embedded in complexes II, III, and IV. Differ due to their porphyrin structure and axial protein ligands.



Heme *a*



Heme *b*



Heme *c*

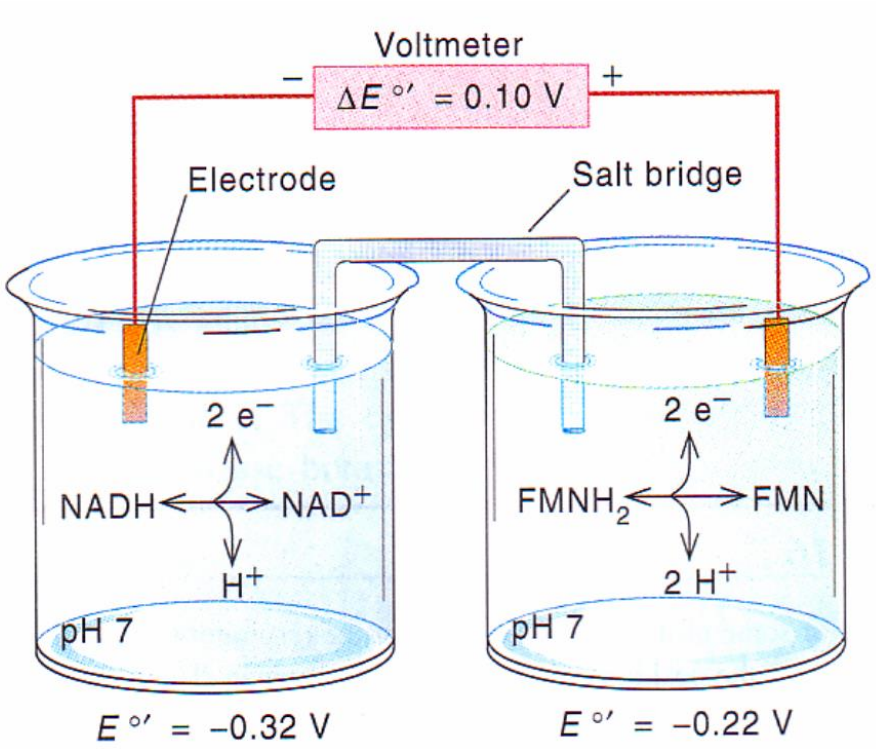
Summary of electron carriers

Cofactor	Capacity	ETC function
NAD nicotinamide adenine dinucleotide	$NAD^+ + 2e^- + H^+ \rightarrow NADH$	Primary electron donor in respiration (NADH)
NADP nicotinamide adenine dinucleotide phosphate	$NADP^+ + 2e^- + H^+ \rightarrow NADPH$	Terminal electron acceptor in photosynthesis (NADP)
FAD flavin adenine dinucleotide	$FAD + 1e^- + H^+ \rightarrow FADH\bullet$ $FAD + 2e^- + 2H^+ \rightarrow FADH_2$	Prosthetic group in complex II
FMN flavin mononucleotide	$FMN + 1e^- + H^+ \rightarrow FMNH\bullet$ $FMN + 2e^- + 2H^+ \rightarrow FMNH_2$	Prosthetic group in complex I
Ubiquinone	$UQ + 1e^- + H^+ \rightarrow UQH\bullet$ $UQ + 2e^- + 2H^+ \rightarrow UQH_2$	Electron carrier between complexes I/II and III
Cytochrome *c*	Cyt*c*-$Fe^{III} + 1e^- \rightarrow$ Cyt*c*-Fe^{II}	Electron carrier between complexes III and IV
Other cytochromes	Cyt-$Fe^{III} + 1e^- \rightarrow$ Cyt-Fe^{II}	Prosthetic groups in complexes II, III, and IV
[2Fe2S] dinuclear iron-sulfur cluster	$(Fe^{III})_2 + 1e^- \rightarrow (Fe^{III})_1(Fe^{II})_1$	Prosthetic groups in complexes I, II, and III
[4Fe4S] tetranuclear iron-sulfur cluster	$(Fe^{III})_3(Fe^{II})_1 + 1e^- \rightarrow (Fe^{III})_2(Fe^{II})_2$	Prosthetic groups in complexes I and II
Cu_A and Cu_B dinuclear and mononuclear copper centres	$Cu^{II} + 1e^- \rightarrow Cu^{I}$	Prosthetic groups in complex IV

Generating Δ*p* from electron transfer

- Energy is yielded during electron transfer from an electronegative donor to an electropositive acceptor. This energy can be transduced into generation of Δ*p*.

- As with other chemical reactions, the energetics of biological electron transfers can be calculated by measuring redox potentials in half-cells against standard hydrogen electrode.



Standard redox potentials of ETCs

Compound	Half-equation	Standard redox potential ($E^{o'}$)
NADH / NADPH	$NAD^+ + H^+ + 2e^- \rightarrow NADH$ $NADP^+ + H^+ + 2e^- \rightarrow NADPH$	-0.32 V
FAD / FMN	$FAD + 2H^+ + 2e^- \rightarrow FADH_2$ $FMN + 2H^+ + 2e^- \rightarrow FMNH_2$	-0.21 V (free)
Succinate	$Fumarate + 2H^+ + 2e^- \rightarrow Succinate$	+0.03 V
Ubiquinone	$UQ + 2H^+ + 2e^- \rightarrow UQH_2$	+0.06 V
Cytochrome *c*	$Cyt\,c\text{-}Fe^{III} + e^- \rightarrow Cyt\,c\text{-}Fe^{II}$	+0.22 V
O_2	$\frac{1}{2} O_2 + 2H^+ + 2e^- \rightarrow H_2O$	+0.82 V

- There are extensive variations in the redox potentials of the iron-sulfur cluster (-0.400 to 0 V) and cytochrome (0 V to +0.400 V) prosthetic groups within the ETC. Modulated by their amino acid ligands. Not necessary to know the details for this course.

Three simple equations central to bioenergetics

- The standard redox potential of a reaction can be calculated from the two half-reactions:

$$\Delta E^{o\prime} = E^{o\prime}_{\text{acceptor}} - E^{o\prime}_{\text{donor}}$$

- The actual redox potential of a redox reaction can be calculated by the Nernst equation based on specified temperature and concentrations or ratios of products over reactants.

$$E = \Delta E^{o\prime} - \frac{RT}{nF} ln \frac{[\text{Products}]}{[\text{Reactants}]}$$

$E^{o\prime}$ is the standard redox potential, R is the universal gas constant (8.3×10^{-3} kJ K^{-1} mol^{-1}), T is temperature (in K), n is number of moles of electrons transferred, F is the Faraday constant (96 kJ $Vmol^{-1}$).

- The (standard) free energy change can be determined from the (standard) redox potential:

$$\Delta G = -nF\Delta E \qquad \Delta G^{o\prime} = -nF\Delta E^{o\prime}$$

ΔG is free energy change in kJ, $\Delta G^{o\prime}$ is standard free energy change in kJ, ΔE is redox potential in mV, $\Delta E^{o\prime}$ is standard redox potential in mV, n is number of moles of electrons transferred, F is the Faraday constant (96 kJ $Vmol^{-1}$).

Example: energetics of complex I

- NADH:ubiquinone oxidoreductase (Complex I) catalyses the reaction:

$$NADH + UQ + H^+ \rightarrow NAD^+ + UQH_2$$

- $\Delta E^{o\prime}$ can be calculated from the standard redox potentials of the half-reactions:

$NAD^+ + 2\ H^+ + 2\ e^- \rightarrow NADH + H^+$ $\quad E^{o\prime} = -0.32$ V

$UQ + 2\ H^+ + 2\ e^- \rightarrow UQH_2$ $\quad E^{o\prime} = +0.06$ V

$\Delta E^{o\prime} = E^{o\prime}_{acceptor} - E^{o\prime}_{donor} = +0.06 - (-0.32)$ $\quad = +0.38$ V

$\Delta G^{o\prime} = -nF\Delta E^{o\prime} = -2 \times 96.5 \times 0.38$ $\quad = -73$ kJ mol^{-1}

- ΔE can be calculated with Nernst equation. In mitochondrial preparation, assume T = 298 K, $[NAD^+]/[NADH] \approx 10$, $[UQ]/[UQH_2] \approx 20$.

$$\Delta E = \Delta E^{o\prime} - \frac{RT}{nF} ln \frac{[NAD^+][UQH_2]}{[NADH][UQ]} : +0.38 - \frac{8.3 \times 10^{-3} \times 298}{2 \times 96} ln\,(10 \times 20) = 0.38 - 0.0128 \times 5$$

$= +0.32$ V

$\Delta G = -nF\Delta E = -2 \times 96.5 \times 0.32 = -61$ kJ mol^{-1}

Potentials reflects efficiency of proton pumping

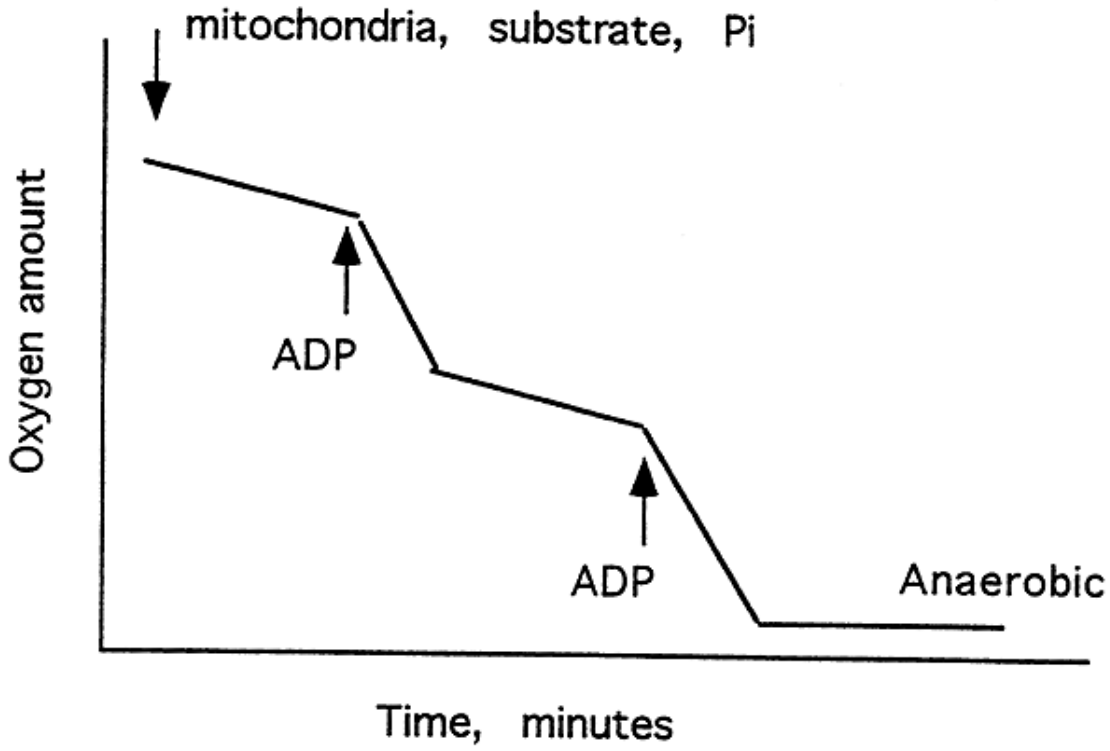
- The energy released from electron transfer is the main factor that determines how many protons can be pumped. High energy release for complex I and IV so four protons pumped for every two electrons transferred. Low energy release in complex II so no protons pumped.

Enzyme	Half-equations	$E^{o\prime}$	$\Delta E^{o\prime}$	$\Delta G^{o\prime}$	Efficiency
Complex I	$NAD^+ + H^+ + 2e^- \rightarrow NADH$ $UQ + 2H^+ + 2e^- \rightarrow UQH_2$	-0.32 V +0.06 V	+0.38 V	-73 kJ mol^{-1}	$4H^+/2e^-$
Complex II	Fumarate + $2H^+ + 2e^- \rightarrow$ Succinate $UQ + 2H^+ + 2e^- \rightarrow UQH_2$	+0.03 V +0.06 V	+0.03 V	-5.6 kJ mol^{-1}	$0H^+/2e^-$
Complex III	$UQ + 2H^+ + 2e^- \rightarrow UQH_2$ Cyt*c*-$Fe^{III} + e^- \rightarrow$ Cyt*c*-Fe^{II}	+0.06 V +0.22 V	+0.16 V	-30 kJ mol^{-1}	$2H^+/2e^-$
Complex IV	Cyt*c*-$Fe^{III} + e^- \rightarrow$ Cyt*c*-Fe^{II} $½\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	+0.22 V +0.82 V	+0.60 V	-58 kJ mol^{-1}	$4H^+/2e^-$

- Enzymology important. Some simple NADH:ubiquinone oxidoreductases catalyse NADH oxidation but lack enzymatic mechanism to couple this to proton pumping ($0H^+/2e^-$).

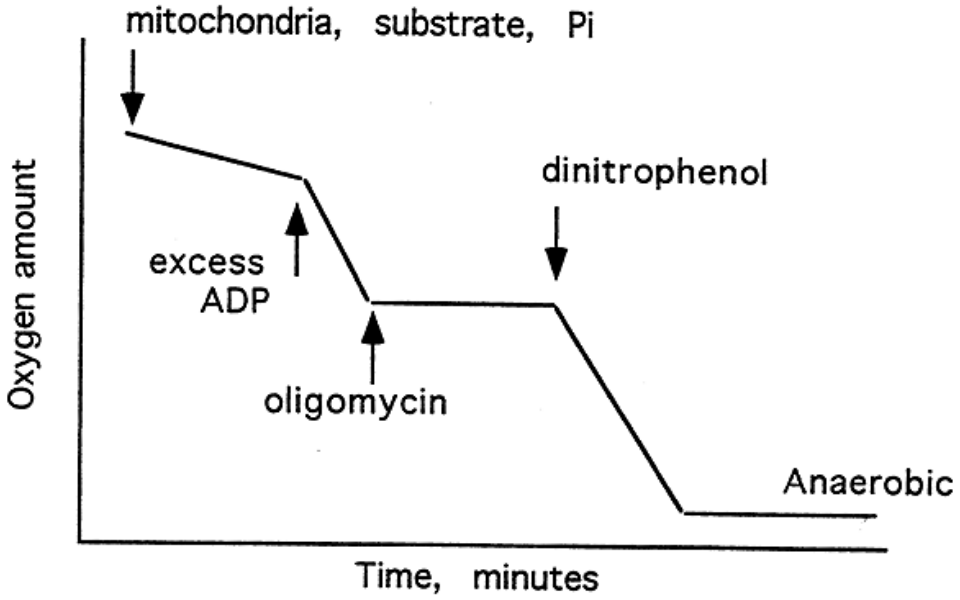
Oxygen electrode evidence for OXPHOS

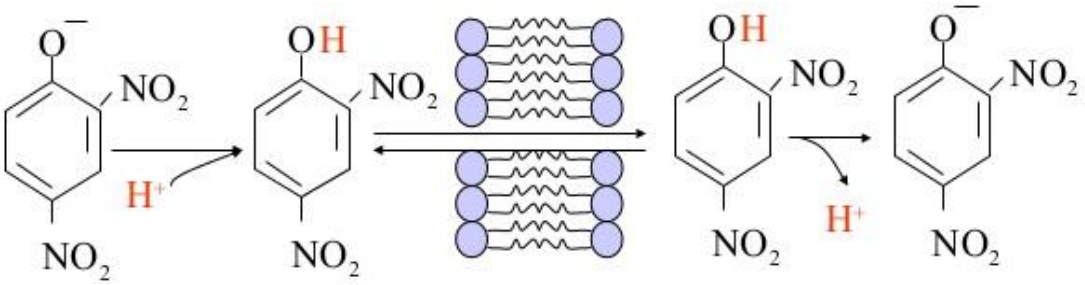
- Mitochondrial preparations synthesize ATP when sufficient substrate, ADP, and O_2 are available. ATP synthase inhibitor oligomycin inhibits ATP synthesis.



Uncoupler evidence for OXPHOS

- Uncoupler dinitrophenol stimulates O_2 consumption independent of ATP synthesis. Dinitrophenol is a weak acid: protonates at P side of membrane and deprotonates at N side. By delocalising negative charge, it can freely diffuse through lipid membrane.





Lecture 1: How does life conserve energy?

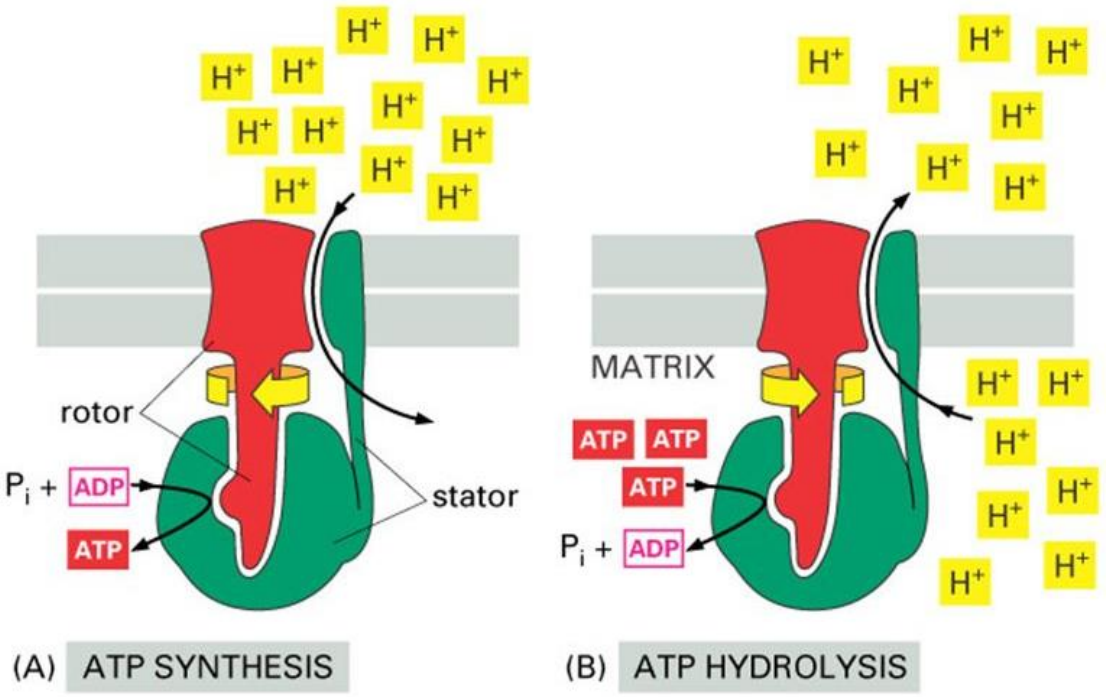
I. Oxidative phosphorylation

II. Electron transport chains

III. ATP synthase

ATP synthase is a reversible coupling device

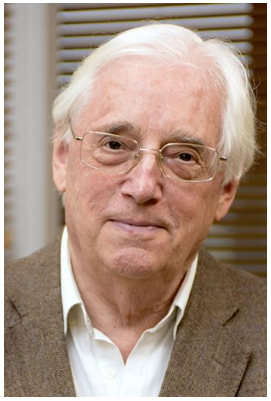
- The electron transport chains that generate Δp differ between mitochondria, chloroplasts, and bacteria. However, the ATP synthases that utilise this Δp are highly conserved.

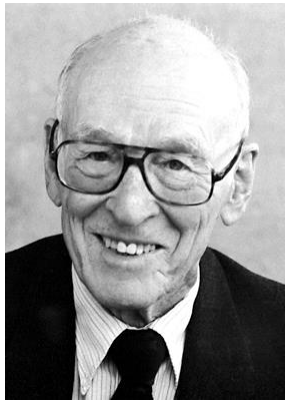


- ATP synthases generally couple downhill proton translocation to ATP synthesis. Also act in reverse to create Δp by ATP hydrolysis (e.g. in fermentative bacteria). Direction determined respective magnitudes of Δp and ΔG for ATP synthesis (as modulated by ATP/ADP ratios).

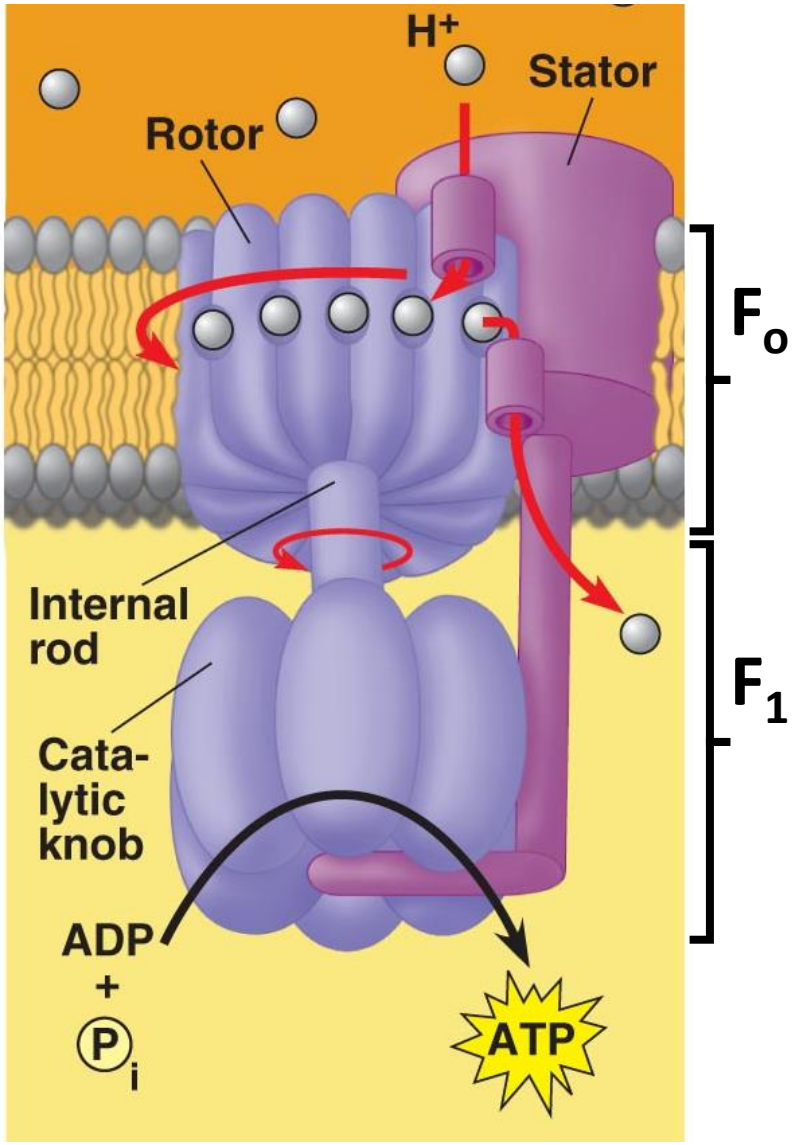
ATP synthase is a molecular motor

- Two interlocked subcomplexes, the soluble F_1 and membrane-bound F_o.
- Subcomplexes separable with 8 M urea. F_1 hydrolyses ATP, F_o membranes leaky to protons (blockable with oligomycin). Both required together to couple Δp utilisation to ATP synthesis.
- Coupling mechanism same as a motor. F_o rotation drives F_1 stator to synthesize ATP.





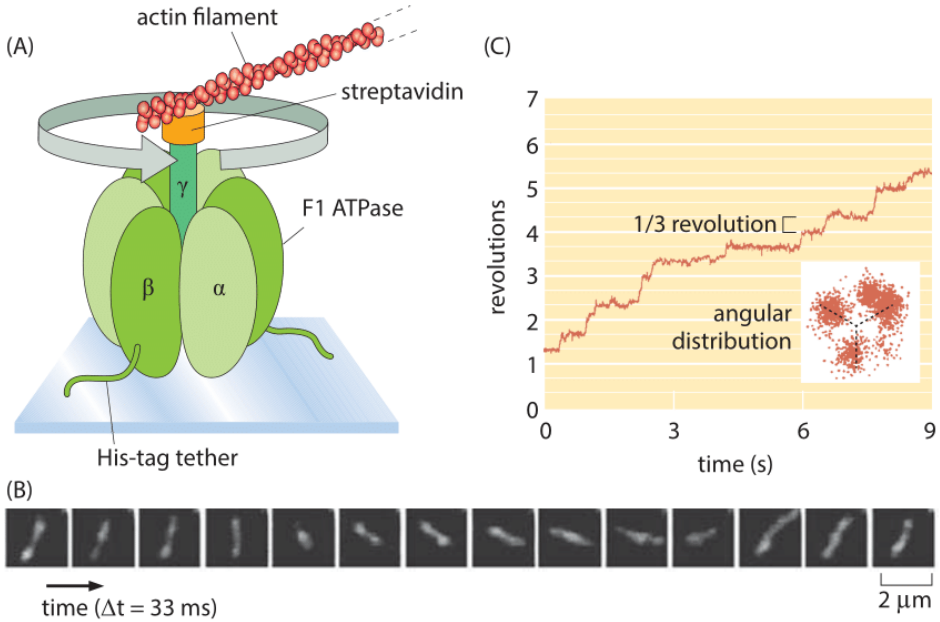
Nobel Laureates John E. Walker (1941 -), Paul D. Boyer (1918 -)



Visualising a rotary mechanism

Experiment 1:

Fluorescein-labelled actin filament attached to central stalk of immobilised ATP synthase and visualised by fluorescence microscopy.



Noji et al, Nature, 1997

Showed sustained anticlockwise movement. 120° steps implying one step per ATP hydrolysed. However, friction of actin filament slowed rotation.

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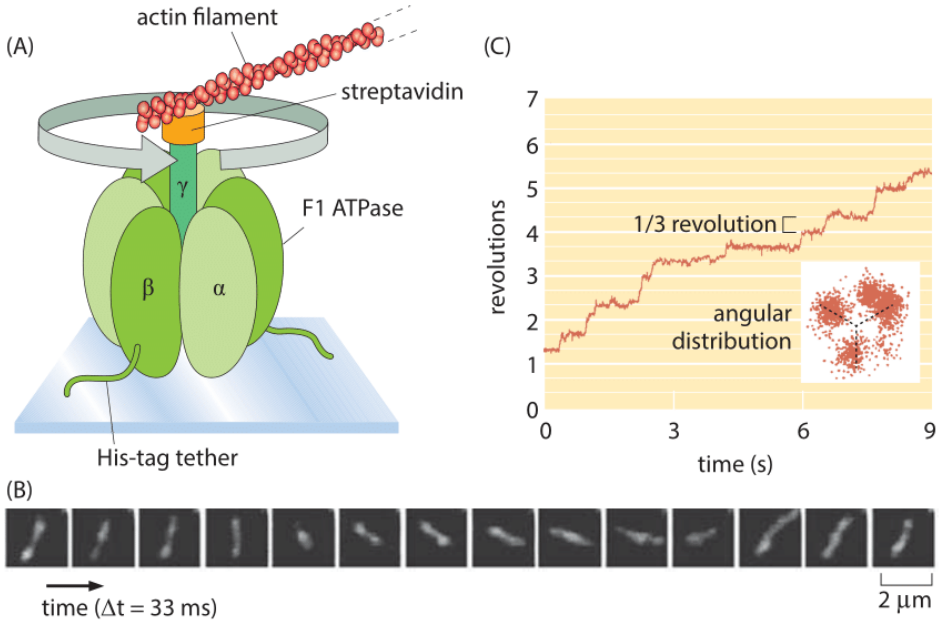
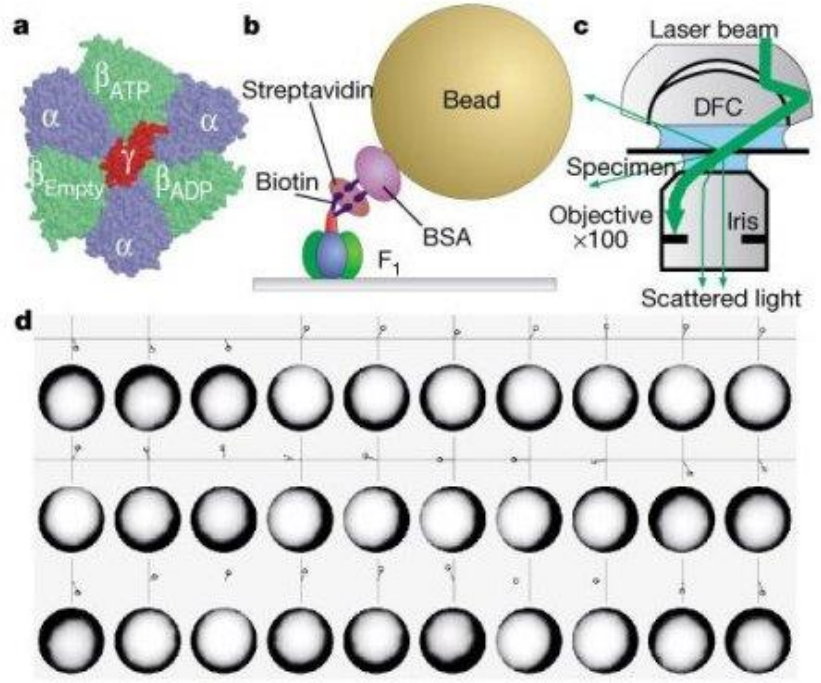


Noji et al, Nature, 1997

Showed sustained anticlockwise movement. 120° steps implying one step per ATP hydrolysed. However, friction of actin filament slowed rotation.

Experiment 2:

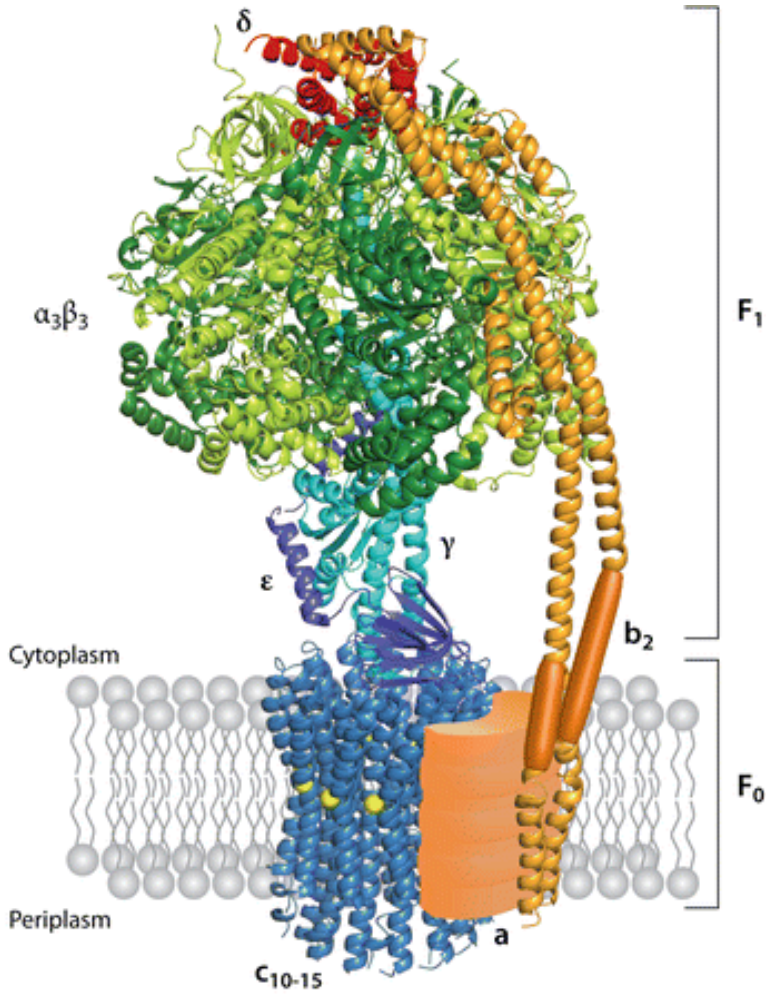
Actin filament replaced by 40 nm gold bead and visualised by laser light scattering. Resulted in less friction and higher resolution.



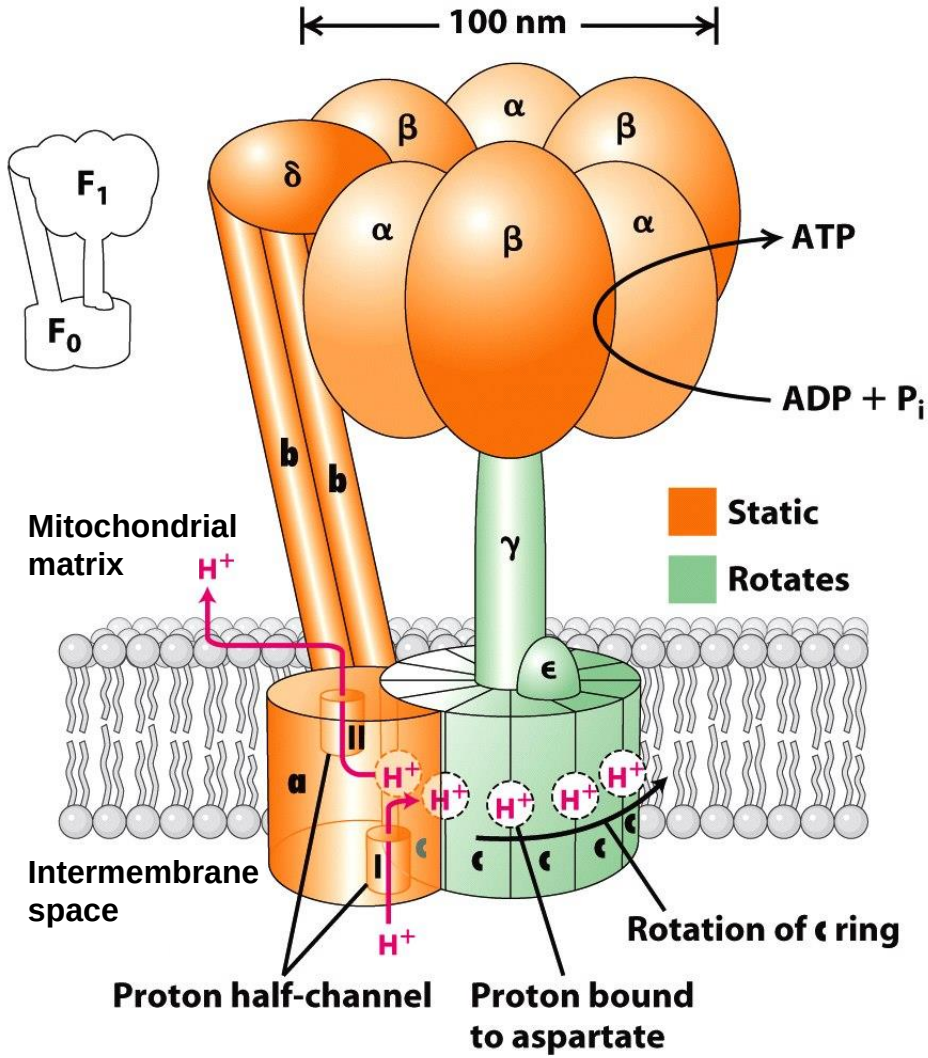
Yasuda et al, Nature, 2001

At low [ATP], motor rotates in 120° steps. At high [ATP], rotation continuous and saturates with Michaelis-Menten kinetics at 7800 rpm.

Crystal structure of ATP synthase



Stock et al, Science, 2000

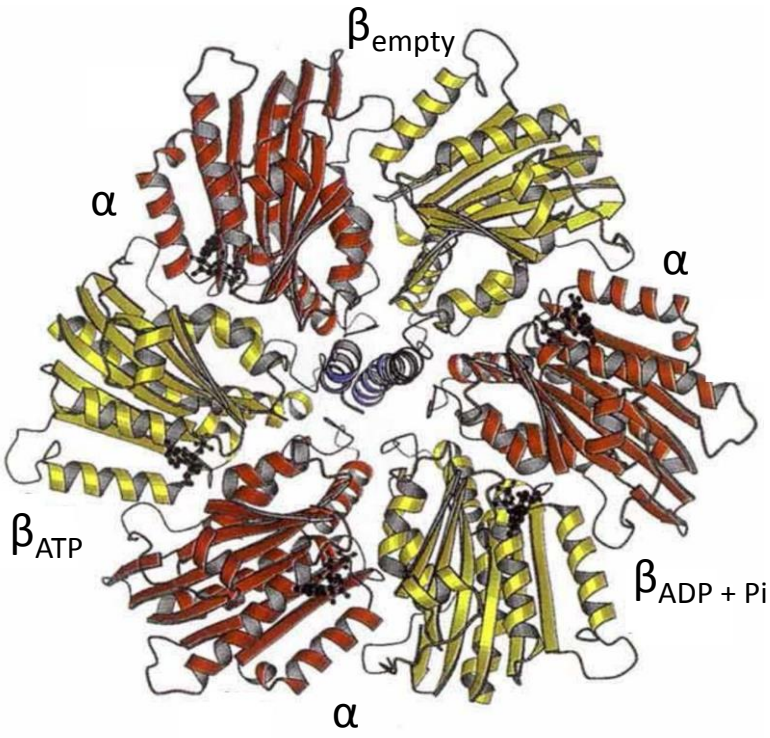


F_1 stoichiometry: $\alpha_3\beta_3\gamma\delta\varepsilon$

F_o stoichiometry: $ab_2c_{8\text{-}15}$

Focusing in on the F_1 domain

- F_1 core formed by alternating α and β subunits ($\alpha_3\beta_3$) each with mixed αβ fold, three-domain structure. ATP synthesis occurs at β-subunit. Binds ATP and ADP *via* conserved P-loop motif. F_1 structure shows each site differentially occupied and has different P-loop conformations.

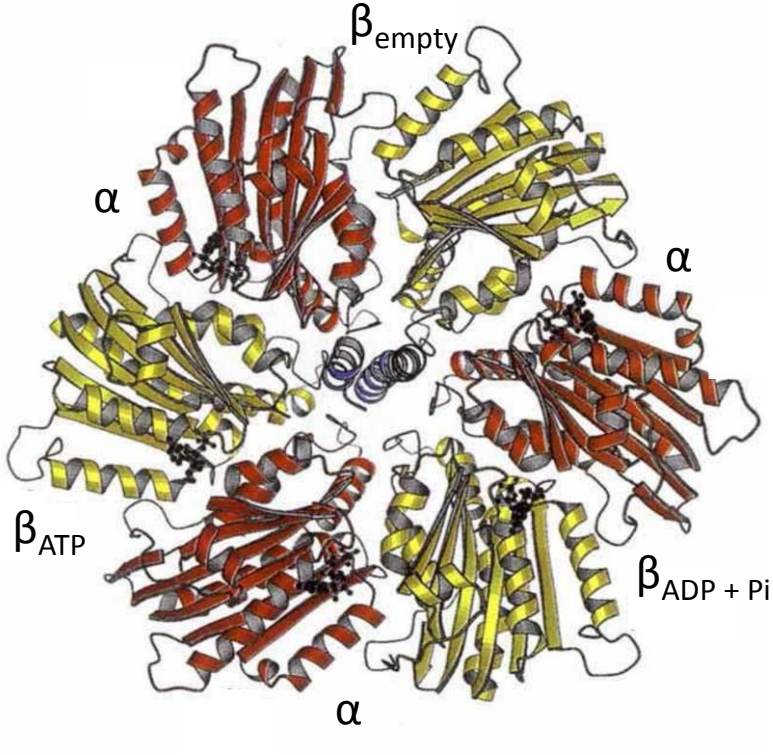


Abrahams et al, Nature, 1994

Focusing in on the F_1 domain

- Stalk formed by the γ subunit. Contains a kinked α-helix that is asymmetric with the $\alpha_3\beta_3$ central axis and hence interacts differentially with each β-subunit. Main interaction is between a short helical region of γ and the DELSEED motif of β.

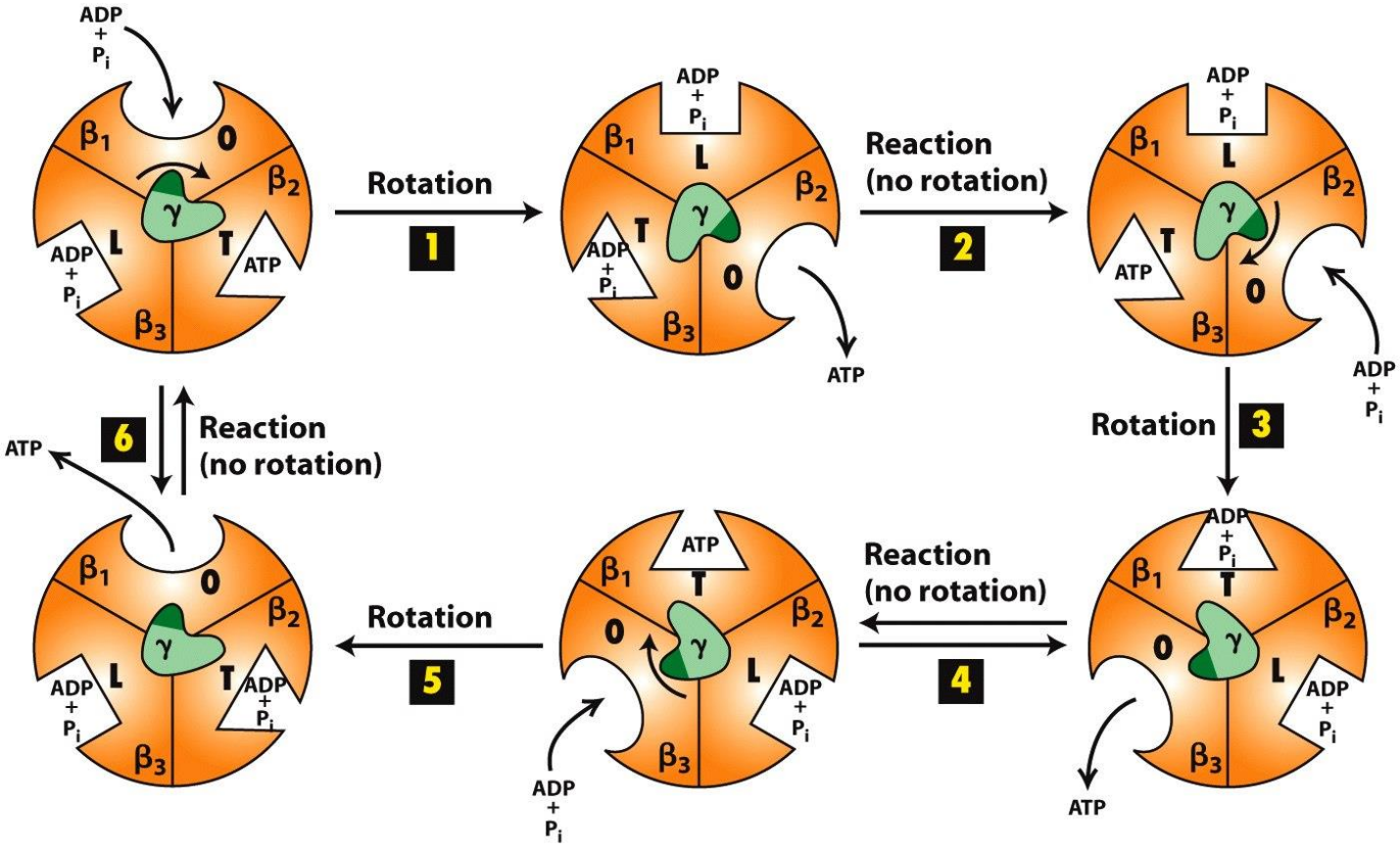
- Each β subunit undergoes conformational and affinity changes based on position of the rotating γ subunit. DELSEED interaction may induce changes in environment around P-loop that increase affinities for ADP and ATP. Strong evidence of interactions and cooperativity between β-subunits.



Abrahams et al, Nature, 1994

Visualising the reaction mechanism

- For a β-subunit to synthesize one ATP, F_o must rotate 360° in three 120° steps. Differential γ-β subunit interactions necessary for the β-subunit to switch between three conformations:
  - Loose (L): Active site loosely binds ADP + P_i. Exergonic process independent of Δ*p*.
  - Tight (T): Substrates tightly bound, ATP is formed. Driven by β-β and β-γ interactions.
  - Open (O): ATP is released. Endergonic process dependent on Δ*p*. P-loop hinges outwards.



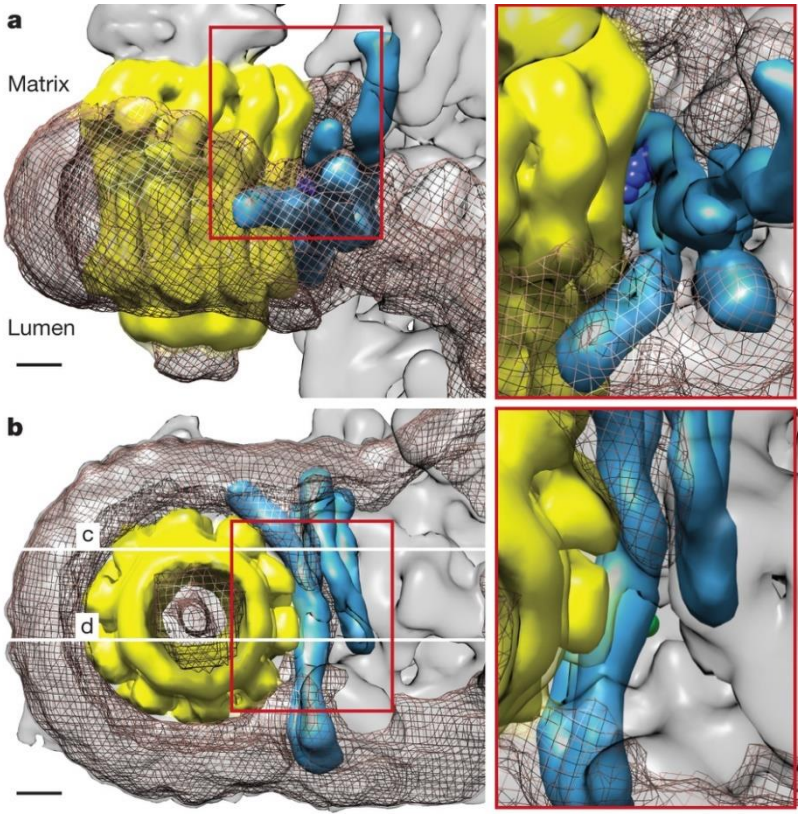
F_o is a transmembrane proton pump

- Isolated rings of *c* subunits have been crystallised from multiple organisms. Each subunit contains two transmembrane helices (N-terminal inside, C-terminal outside) separated by a loop on the N side of the membrane.

- *a* subunit has been structurally characterised in low resolution by cryo-EM. Contains four long, horizontal membrane-intrinsic α-helices at a right angle to *c* subunit helices.

- Interaction of *a* subunit and *c* ring is responsible for proton pumping.

Cryo-EM showing c ring (yellow) and its interaction with the a subunit (blue):

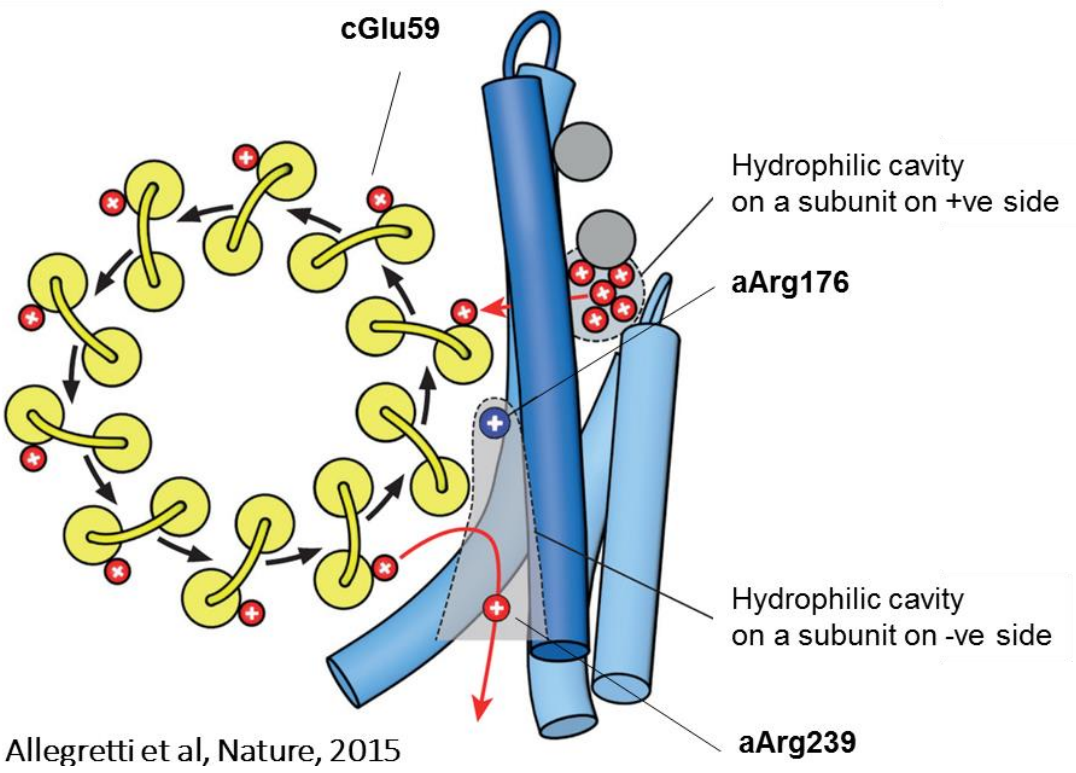


Three-step mechanism for proton pumping

1. Protons (red) enter *via* the P phase cavity on the *a* subunit and protonate cGlu59. cGlu59 has low pK_a of approximately 2 due to electrostatic interaction with aArg176.

2. Protonation releases attraction between cGlu59 and aArg176 causing *c* ring to move by one. pK_a of cGlu59 increases to 4 due to disruption of interaction, but proton obscured from release.

3. When *c* ring has almost completely rotated, cGlu59 reaches the N phase cavity of the *a* subunit and the proton is released.



Allegretti et al, Nature, 2015

Variable H^+/ATP ratios for proton pumping

- The 360° rotation of a *c* ring with *n* subunits requires *n* protons. A complete rotation drives the completion of the catalytic cycle of the three β subunits of F_1 and hence the synthesis of 3 ATP. Hence, *n*/3 protons are required to synthesize 1 ATP.

- For reasons not understood, the number of *c* subunits differs. In turn, the number of protons required to ATP synthesis and hence the efficiency of Δ*p* utilisation differs:

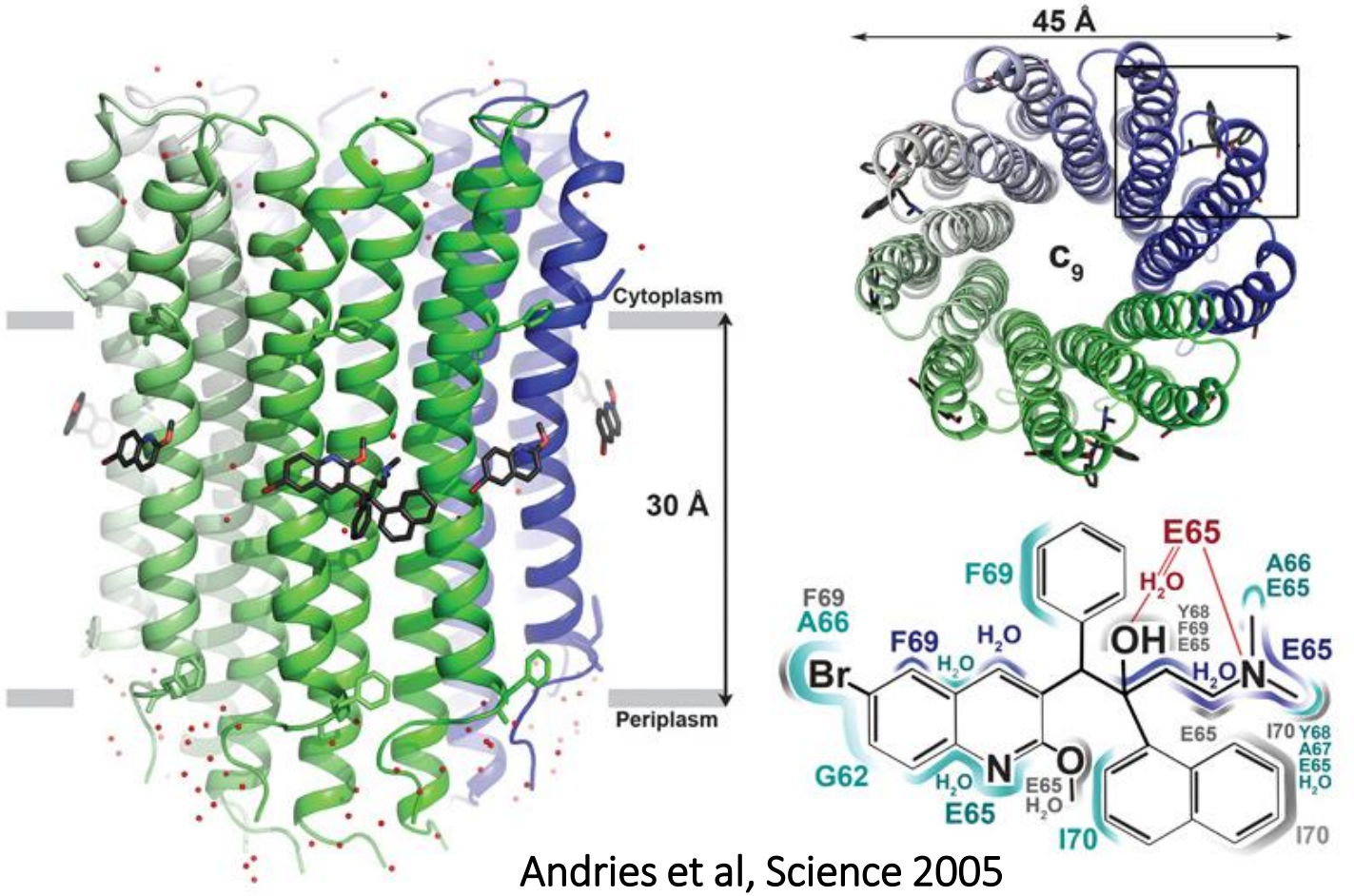
System	Number of *c* ring subunits	Number of protons pumped per ATP made
Mammalian mitochondria	8	8/3 (2.67)
Yeast mitochondria	10	10/3 (3.33)
Plant chloroplasts	14	14/3 (4.67)
Bacterial membranes	11 to 15	11/3 (3.66) to 15/3 (5)

Time for a movie

https://www.youtube.com/watch?v=GM9buhWJjlA

ATP synthase is a clinically-approved drug target

- Bedaquiline, the first new antituberculosis drug to be clinically approved in 50 years, is a specific inhibitor of ATP synthase. It specifically binds the *c*-ring of *Mycobacterium tuberculosis* F_o and occludes the proton-binding Glu residue thus preventing rotation.

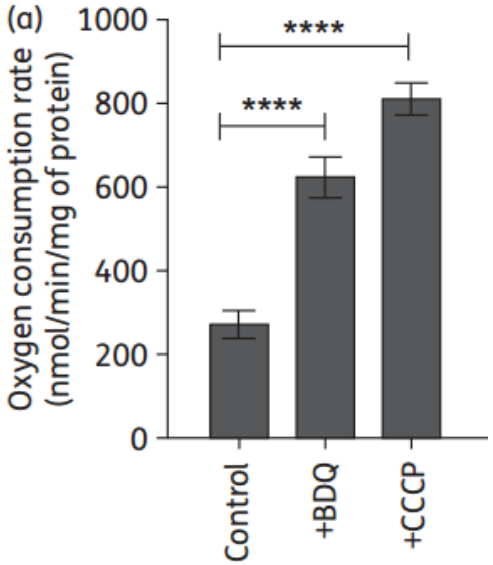


Andries et al, Science 2005

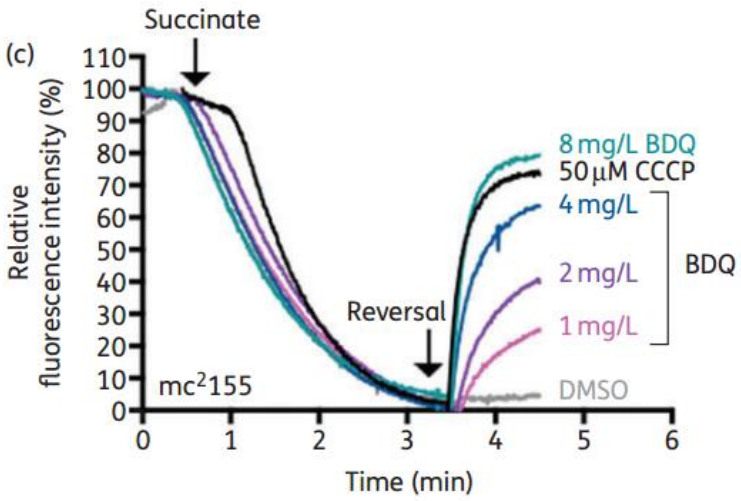
Bedaquiline acts an uncoupler

- Recent work shows that bedaquiline behaves in equivalent manner to the uncoupler CCCP. It collapses pH gradient in a manner dependent on ATP synthase binding. May lead to proton leak at *a*-*c* subunit interface causing futile proton cycling uncoupled from ATP synthesis.

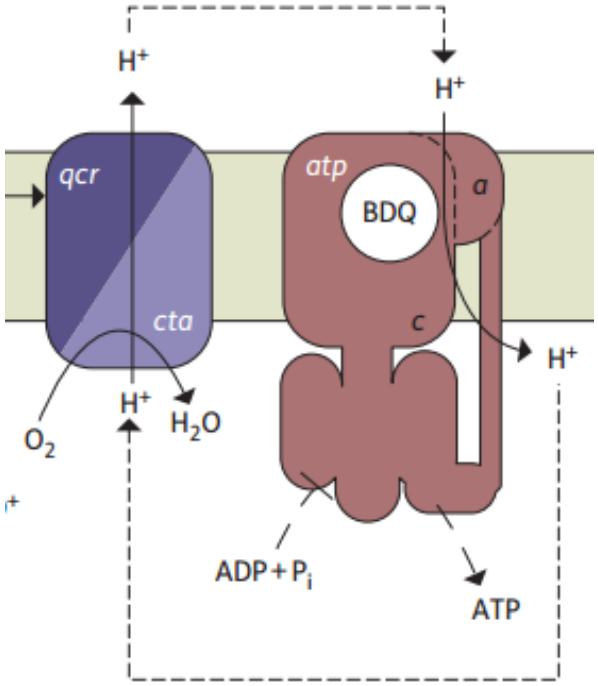
Bedaquiline stimulates O_2 consumption:



Bedaquiline stimulates collapse of pH gradient:



Bedaquiline causes futile proton cycling:



Hards et al, JAC 2015

Lecture summary

- Life conserves energy by generating and utilising proton-motive force (Δp) over energy-transducing membranes. Δp is sum of membrane potential (Ψ) and pH gradient (ΔpH).

- Electron transport chains generate Δp by coupling exergonic electron transfers to proton translocation. Contain transmembrane proton-pumping complexes connected by e^- carriers.

- Nicotinamides, flavins, quinones, iron-sulfur clusters, and cytochromes are the major e^- carriers in energy-conservation. Differ in their redox potentials and stoichiometry.

- ATP synthase uses Δp to create ATP. Proton flow through transmembrane F_o causes rotation of *c* ring and γ stalk. Causes conformational changes in F_1 driving ATP synthesis and release.

Recommended reading

Recommended reading:

Nicholls DG & Ferguson SJ (2015). Bioenergetics 4. Elsevier Press.

Comprehensive, up-to-date textbook on bioenergetics.

Walker JE (2013). The ATP synthase: the understood, the uncertain and the unknown. BST 41, 1-16.

Concise review covering the structure and function of ATP synthase.

Supplementary reading:

Allegretti M et al. (2015). Horizontal membrane-intrinsic α-helices in the stator *a*-subunit of an F-type ATP synthase. Nature 521, 237-240.

All available for download at greeninglab.com