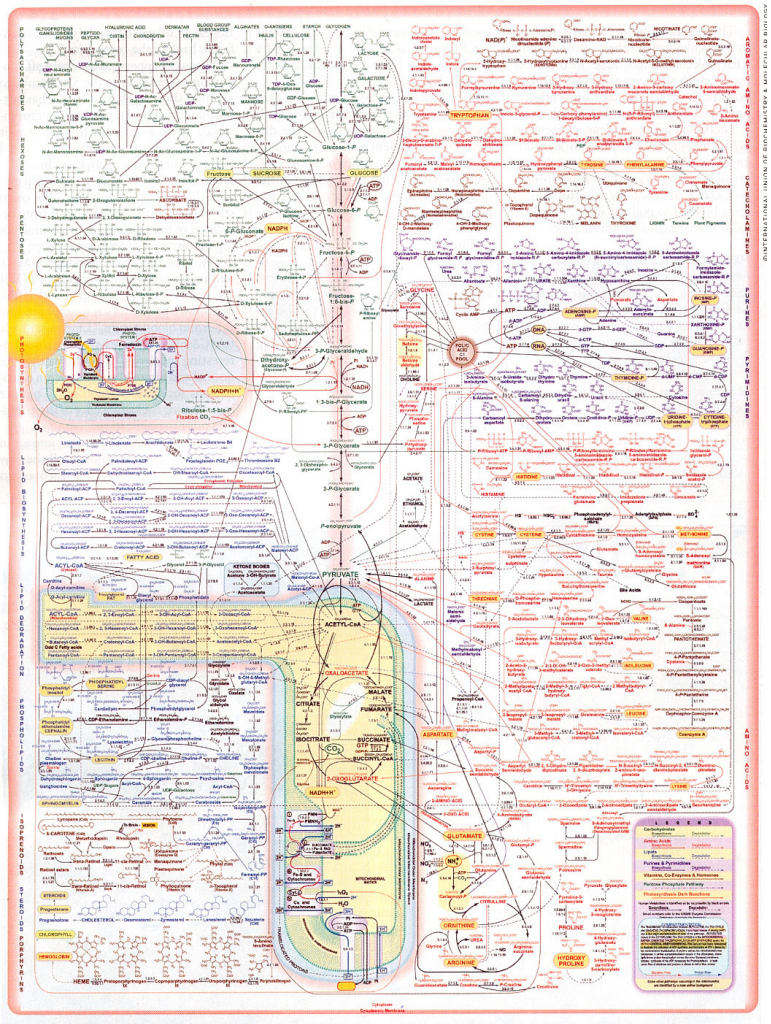
Driving natural systems: Chemical energy production and use

Metabolism

- Metabolism: the sum of the physical and chemical processes in an organism by which its material substance is produced, maintained, and destroyed *[anabolism]*, and by which energy is made available *[catabolism]*
- Metabolism allows organisms to control biomass and energy
- A set of chemical transformations, often enzyme-catalysed
- A complicated network: simplified through tools like flux balance analysis
- Metabolism provides the energy for inference and control
- Metabolism is itself controlled and regulated: metabolic control analysis, active regulation



Free energy

- Energy that can be harnessed to perform work
- At constant temperature and pressure (which we shall assume), the appropriate expression is the Gibbs free energy $G(p, T)$

$$G(p, T) = U + pV - TS$$

- Internal energy U, pressure p, volume V, temperature T, entropy S
- Change in free energy, where α_j, X_j are a feature and associated potential that influence our system (e.g. N, μ: copy number and chemical potential of chemical species):

$$dG = Vdp - SdT + \sum_i \mu_i dN_i - \sum_j X_j d\alpha_j$$

- We'll consider chemical reactions, which take place at fixed p and T
- Particle numbers N_i may be changed by reactions, and we also have to include the pair $\{\Delta\Psi, q\}$ for charge across a membrane potential
- Our Gibbs free energy change

$$dG = \sum_i \mu_i dN_i - F\Delta\Psi dq$$

Energy from chemical reactions

- Reversible chemical reaction with S_i substrates, P_i products, and stoichiometry given by a_i, b_i:

$$a_1 S_1 + a_2 S_2 + ... \rightleftharpoons b_1 P_1 + b_2 P_2 + ...$$

- Change in free energy

$$dG = \sum_i \mu_i dN_i - F \Delta\Psi dq$$

- Chemical potential μ is a measure of the 'concentration gradient' in a system

$$\mu_i \sim \mu_i^0 + RT \ln c_i$$

- For above reaction:

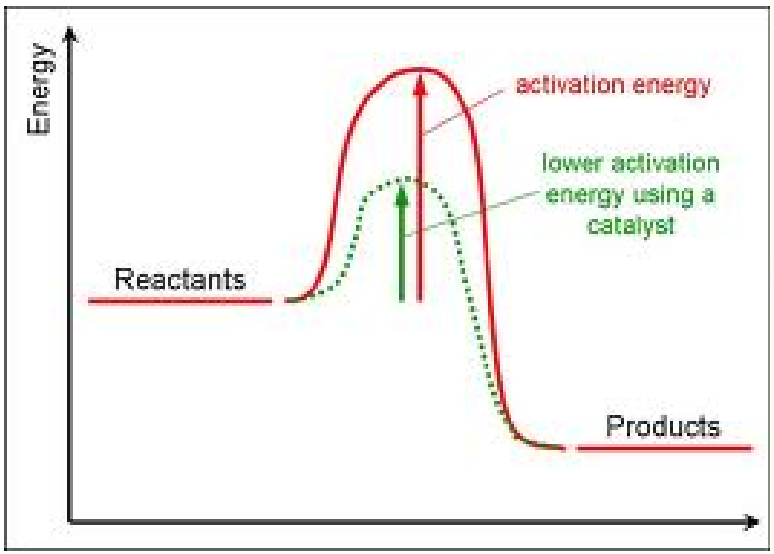
$$\begin{aligned} \mu_{S_i} dN_{S_i} &= (\mu_{S_i}^0 + RT \ln[S_i]) \times (-a_i) \\ \mu_{P_i} dN_{P_i} &= (\mu_{P_i}^0 + RT \ln[P_i]) \times b_i \\ \sum_i \mu_i dN_i &= C(\mathbf{a}, \mathbf{b}, \mu^{\mathbf{0}}) + RT \sum_j \ln[P_j]^{b_j} - RT \sum_j \ln[S_j]^{a_j} \end{aligned}$$

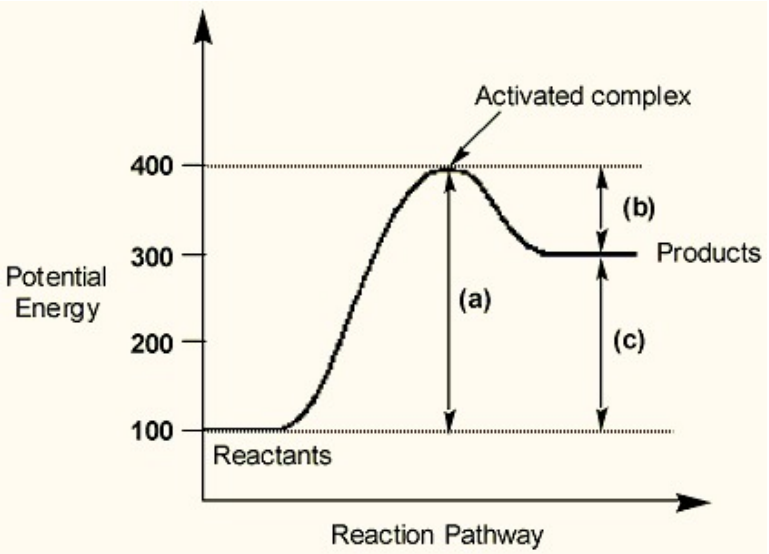
- Broadly, if Z is total charge carried through a potential $\Delta\Psi$ ($\Delta\Psi_{mito} \simeq -160$ mV):

$$\Delta G = \Delta G^0 + RT \ln \left(\frac{\prod_i [P_i]^{b_i}}{\prod_i [S_i]^{a_i}} \right) - ZF\Delta\Psi$$

Activation energies

- $\Delta G = \Delta G^0 + RT \ln \left(\frac{\prod_i [P_i]^{b_i}}{\prod_i [S_i]^{a_i}} \right) - ZF\Delta\Psi$
- Reactions with a negative ΔG net release energy and are sometimes described as 'happening spontaneously'
- There is still an activation energy / geometric contraints to overcome (though this is sometimes possible to do thermally)
- ΔG doesn't tell us how fast a reaction will progress





Rates of chemical reactions

- Chemical reaction

$$a_1 S_1 + a_2 S_2 + ... \rightleftharpoons b_1 P_1 + b_2 P_2 + ...$$

- Law of mass action: reaction rate $\nu \propto$ collision probability $\propto$ reactant concentration $[X_i]$

$$\nu = \nu_+ - \nu_- = k_+ \prod_i [S_i]^{a_i} - k_- \prod_j [P_j]^{b_j}$$

- Equilibrium constant is calculated when $\nu_+ = \nu_-$

$$k_{eq} = \frac{k_+}{k_-} = \frac{\prod [P_j^{eq}]^{b_j}}{\prod [S_i^{eq}]^{a_i}}$$

- At equilibrium, in absence of charge coupling:

$$0 = \Delta G = \Delta G^0 + RT \ln \left(\frac{\prod_i [P_i]^{b_i}}{\prod_i [S_i]^{a_i}} \right)$$

- So

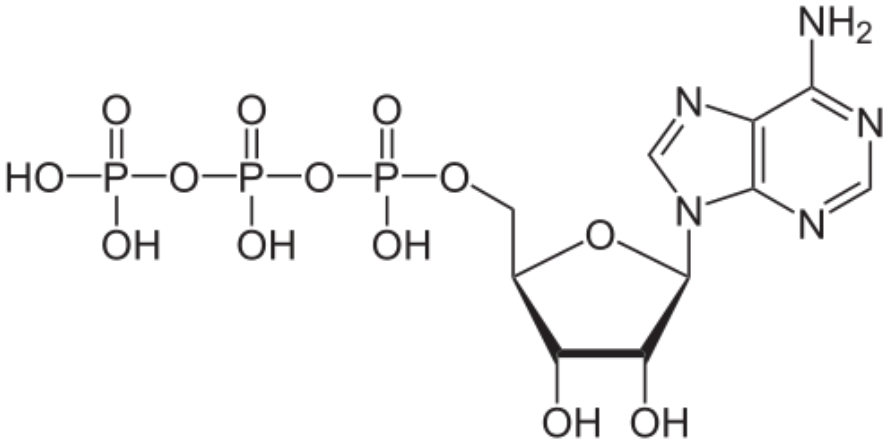
$$\Delta G^0 = -RT \ln k_{eq}$$

- Negative $\Delta G^0 \rightarrow k_+ > k_- \rightarrow$ forward reaction

ATP (adenosine triphosphate) as a cellular fuel source

- ATP is used to provide energy for most energy-demanding cellular processes
- Neurotransmitter synthesis: provides the energy for inference
- Gene expression and regulation: provides the energy for control
- Muscle contraction: motion
- Active transport across membranes
- How do organisms synthesise and obtain energy from ATP?

ATP (adenosine triphosphate) as a cellular fuel source



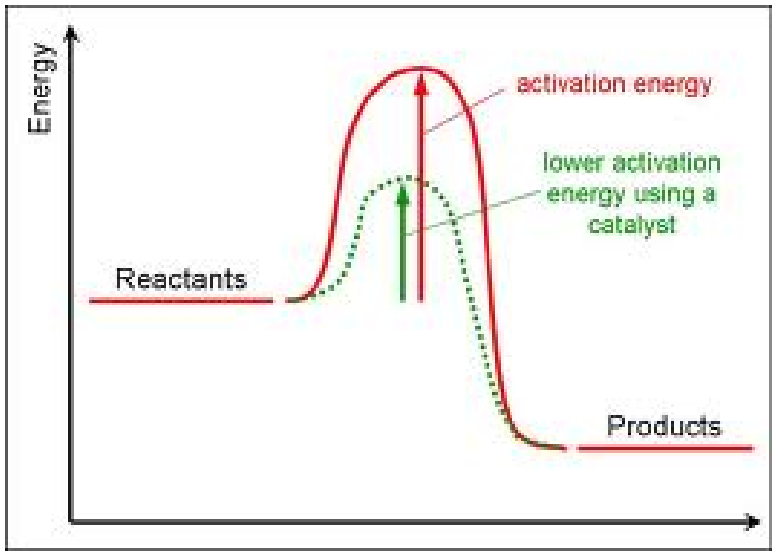
- Two phosphate bonds which may be hydrolysed, releasing energy (ATP $\leftrightarrow$ ADP $\leftrightarrow$ AMP)
- Cells maintain ATP/ADP ratio out of equilibrium
- $ATP + H_2O \rightarrow ADP + P_i\,;\ \Delta G^0 = -30.5$ kJ mol^{-1}
- Under physiological conditions and typical cellular ATP/ADP ratio, $\Delta G \simeq -(40 - 60)$ kJ mol^{-1}
- 'Energy charge' sometimes used for energetic status

$$\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

- The human body contains 0.2 mol ATP. We require the hydrolysis of 100-150 mol ATP per day (50-75 kg). Each ATP molecule is recycled 500-750 times per day.

ATP production

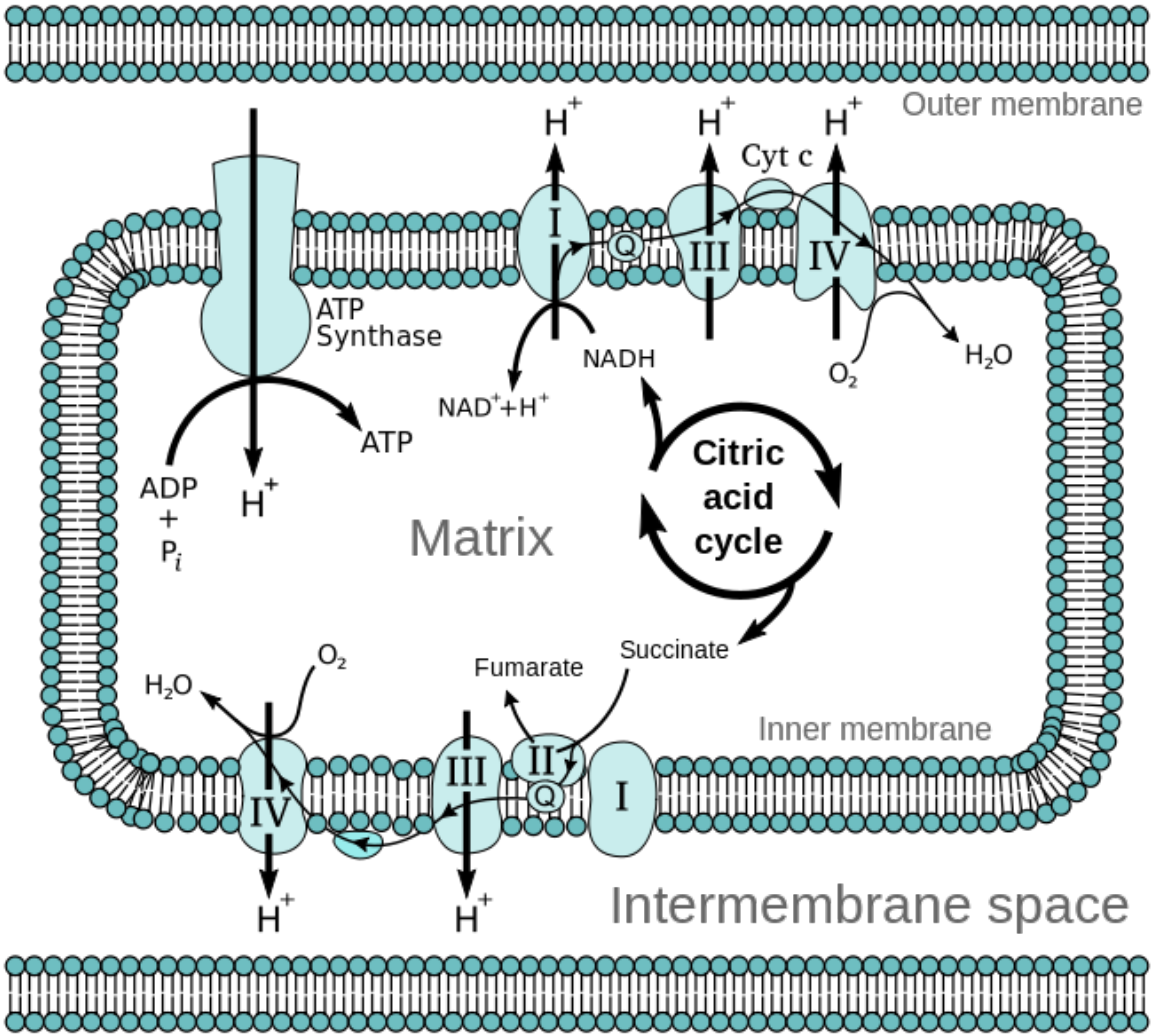
- $ATP + H_2O \rightarrow ADP + P_i$; $\Delta G^0 = -30.5$ kJ mol^{-1}
- Under physiological conditions and typical cellular ATP/ADP ratio, $\Delta G \simeq -(40 - 60)$ kJ mol^{-1}
- But this is net energy release – ATP rarely breaks down on its own (it would be a poor energy currency if it did)
- Proteins that harness ATP are usually ATPases – i.e. enzymes that catalyse the hydrolysis of ATP (hence overcoming the activation energy)



Some other players in bioenergetics

- NADH: an electron donor used to transfer high-energy e^- ($NADH + H^+ + \frac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O$: $\Delta G^0 = -220$ kJ mol^{-1} – one NADH used to synthesis several ATP)
- Means of producing ATP (and NADH):
  - Glycolysis: energy production without oxygen (glucose $\rightarrow$ 2 pyruvate + 2 ATP + NADH + H^+)
  - Krebs cycle / citric acid cycle / TCA cycle: a circular set of reactions that takes in 'fuel' once per cycle and feeds oxidative respiration (we'll look at this in the practical)
  - Oxidative phosphorylation: energetic e^- power proton pumps, setting up a harnessable electrochemical gradient
  - Fermentation (glucose $\rightarrow$ lactic acid)
  - Photosynthesis (photons, proton pumps)
  - Replenishment with nucleoside diphosphate kinases (GTP $\rightarrow$ GDP)

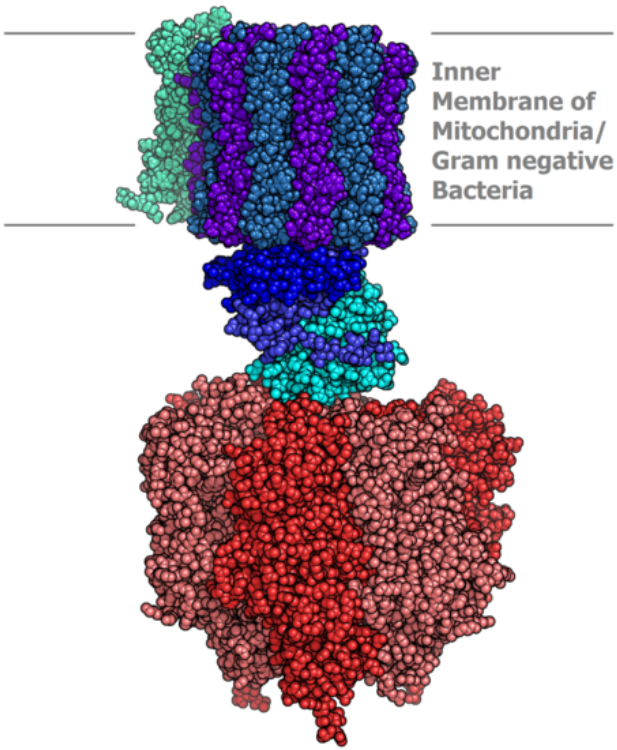
Oxidative phosphorylation



Oxidative phosphorylation

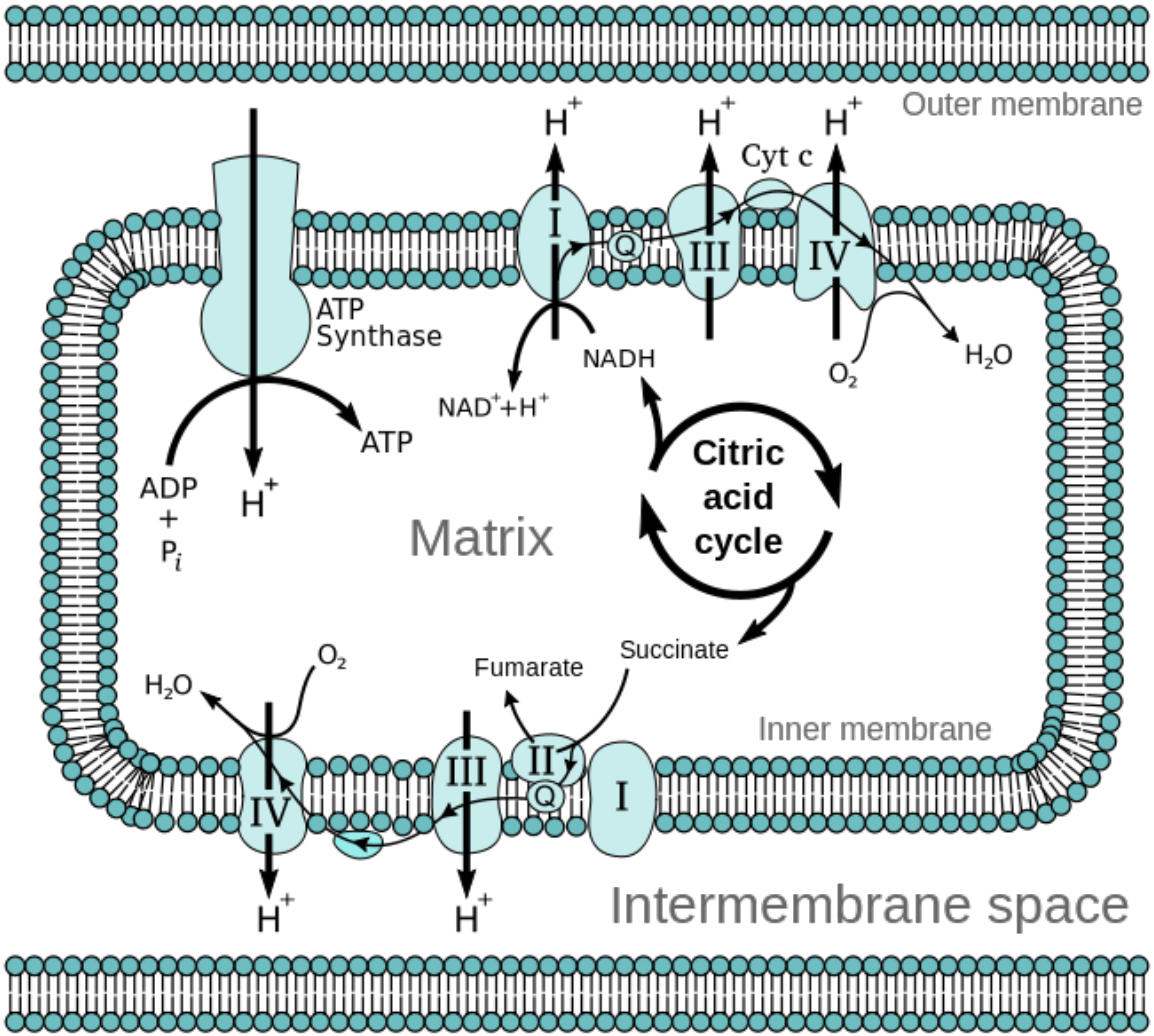
- (Biochemical detail is not examinable)
- Krebs cycle produces NADH and succinate
- A series of complexes pump protons through the inner mitochondrial membrane
- Complex I: $NADH + H^+ \rightarrow NAD^+$, pumps 4 protons, reduces coenzyme Q
- Complex II: succinate $\rightarrow$ fumarate, reduces coenzyme Q
- Complex III: Oxidises coenzyme Q, reduces cytochrome C, pumps 4 protons
- Complex IV: Reduces cytochrome C, pumps 2 protons
- Electrochemical potential (charge separation + chemical gradient) set up across membrane

Oxidative phosphorylation



- Complex V: energetic protons flow back into matrix
- F_1 subunit in membrane; F_o subunit in the matrix
- (Amazing structure best understood by viewing animation)
- Structure and function: John E. Walker, 1997 Nobel Prize in Chemistry

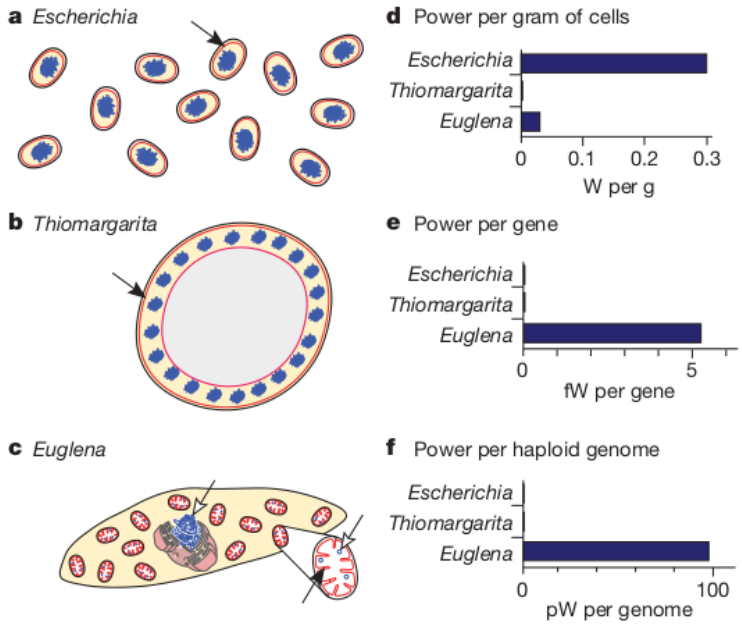
Oxidative phosphorylation



Energy and life

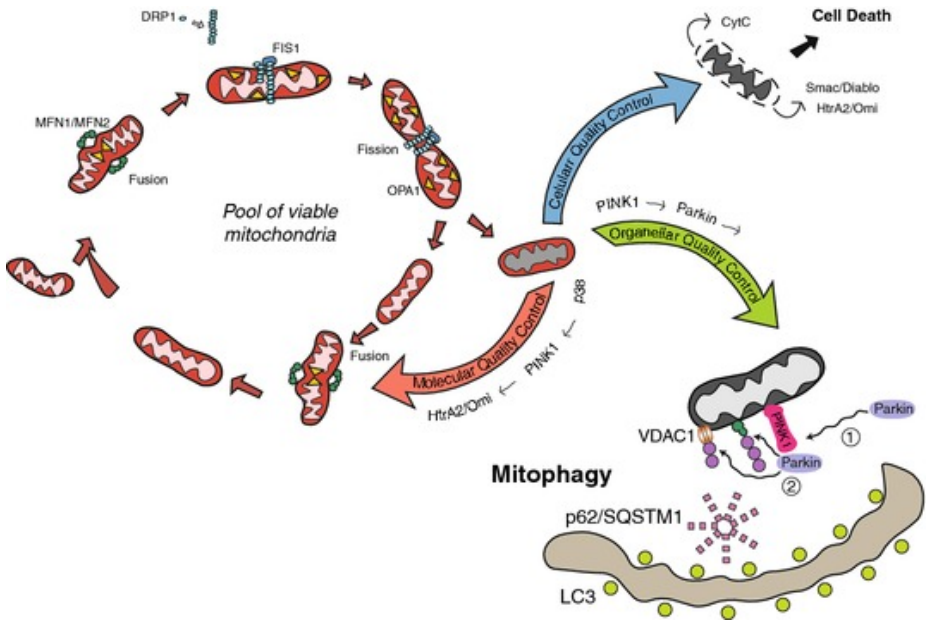
- Nick Lane: energy per gene expressed is key factor in evolution of complex life
- Local mitochondrial genomes: local control of mitochondria
- Alternative 1: non-local genome, power sources not individually addressable
- Alternative 2: many full genomes localised to power generation: huge amount of nucleic acid
- Mitochondria: small, individually addressable genomes localised to power generation

- '... being large and having masses of DNA is not enough to attain complexity: cells need to control energy coupling across a wide area of membranes using small, high copy, bioenergetically specialized genomes like mtDNA'
- Express 2×10^5 more genes with no energy penalty



Mitochondrial bioenergetic control

- How can mitochondria be individually addressed by control?
- 'Quality control': individual mitochondrial performance is sensed (membrane potential $\Delta\Psi$ and others)?
- Good mitochondria are allowed to fuse into a network and remain safe from degradation
- Bad mitochondria remain fragmented and, if they don't recover, are targeted for authophagy and recycling
- An exercise: how does this map to the types of control we have considered?



Control on mitochondrial respiration

- Cells with ‘good’ and ‘bad’ mitochondria show little difference in respiration rate
- There are pronounced physiological differences but compensatory mechanisms exist to control respiration (this is modern and debated research)
- Bad mitochondria produce more ROS (damaging ‘exhaust’) production than good mitochondria
- Cells producing more ROS have more mtDNA
- $\rightarrow$ Cells with bad mitochondria produce more mitochondria to retain overall respiratory capacity
- An exercise: how does this map to the types of control we have considered?

Modelling systems of chemical reactions

- We will look at several ways of modelling the chemical processes that drive natural systems
- Now: ODE modelling – physical models for the species concentrations and physical properties of bioenergetic systems (particularly oxidative phosphorylation and the mitochondrion)
- Next: FBA (flux balance analysis) – coarse-grained representation of large networks of metabolic components with constraints and optimisations
- Then: MCA (metabolic control analysis) – analysis of how fluxes and concentrations in metabolic networks respond to perturbations in network properties

A simple example of ODE modelling

- Adenylate kinases swap high-energy phosphates between adenosine frames in the inter-membrance space of the mitochondrion

$$2ADP \rightleftharpoons ATP + AMP$$

- Rate of this reaction

$$\begin{aligned} \nu &= \nu_+ - \nu_- = k_+ \prod [S_i]^{m_i} - k_- \prod [P_j]^{m_j} \\ &= k_+ [ADP]^2 - k_- [AMP][ATP] \\ &\equiv X_{AK} \left(K_{AK} [ADP]^2 - [AMP][ATP] \right) \end{aligned}$$

- X_{AK} is the activity; K_{AK} the reaction parameter
- For example, $\frac{d[ATP]}{dt} = \nu / V$

An electrochemical example

- Complex I uses energy from NADH electrons to pump protons out of the mitochondrial matrix

$$H^+ + NADH + Q \rightleftharpoons NAD^+ + QH_2 + 4\Delta H^+$$

- $4\Delta H^+$ represents four protons pumped across the membrane
- These protons need to work against an electrochemical gradient: $\Delta G_H = RT \ln\left(\frac{[H^+]_{out}}{[H^+]_{in}}\right) - F\Delta\Psi$
- Reaction rate $\propto$ collision probability
- We also have dependence on 'proton flux probability' represented by the Boltzmann factor $e^{-\Delta G_H/RT}/Z$
- (Model) combination:

$$\begin{aligned} \text{reaction rate } \nu &\propto \text{(collision probability)} \times \text{(proton flux probability)} \\ &\propto \text{(concentrations)} \times \text{(Boltzmann factor)} \end{aligned}$$

$$\begin{aligned} \nu &= k'_+[H^+][NADH][Q] - k'_-[NAD^+][QH_2]e^{4\Delta G_H/RT} \\ &\equiv X'_{CI}\left(K'_{CI}[H^+][NADH][Q] - [NAD^+][QH_2]e^{4\Delta G_H/RT}\right) \end{aligned}$$

Systems of chemical ODEs

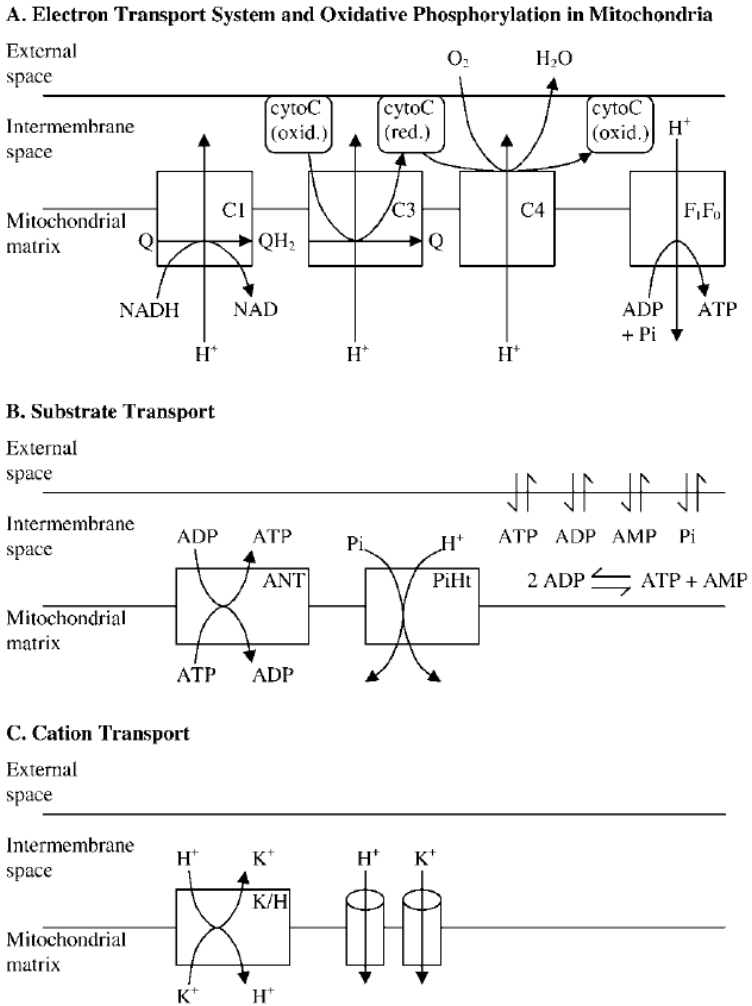


Figure 1. Illustration of the Components Included in the Model of Mitochondrial Oxidative Phosphorylation

$$J_{C1} = X_{C1}\left(e^{-(\Delta G_{o,\,C1}+4\Delta G_H - RT\ln([H^+]_x/10^{-7}) - RT\ln([Q]/[QH_2]))/RT}\right.$$
$$\left.[NADH]_x - [NAD]_x\right)$$

$$J_{PiHt} = X_{PiHt}\left(\frac{[H_2PO_4^-]_i[H^+]_x - [H_2PO_4^-]_x[H^+]_e}{[H_2PO_4^-]_i + k_{PiHt}}\right),$$

$$J_{Hle} = X_{Hle}\Delta\Psi\left(\frac{[H^+]_e e^{+F\,\Delta\Psi/RT} - [H^+]_x}{e^{+F\,\Delta\Psi/RT} - 1}\right).$$

.....

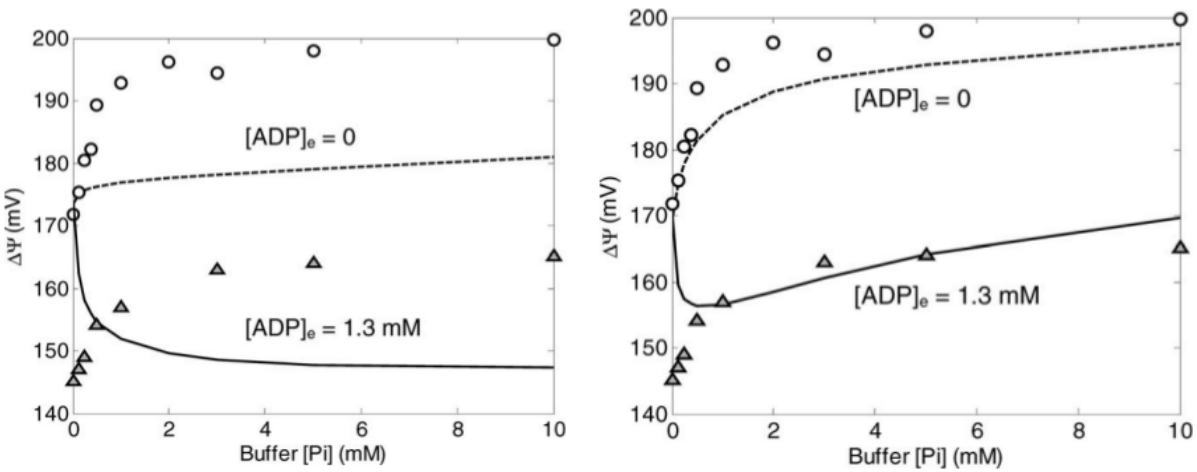
$$\begin{aligned}
d[H^+]_x/dt &= \frac{1}{r_{buff}}(+J_{DH} - (4+1)J_{C1} - (4-2)J_{C3} \\
&\quad - (2+2)J_{C4} + (n_A - 1)J_{F1} \\
&\quad + 2J_{PiHt} + J_{Hle} - J_{KH})/V_x \\
d[K^+]_x/dt &= (+J_{KH} + J_K)/V_x \\
d[Mg^{2+}]_x/dt &= (-J_{MgATPx} - J_{MgADPx})/V_x \\
d[NADH]_x/dt &= (+J_{DH} - J_{C1})/V_x \\
d[QH_2]_x/dt &= (+J_{C1} - J_{C3})/V_x \\
d[cytC(red)^{2+}]_x/dt &= (+2J_{C3} - 2J_{C4})/V_x \\
d[ATP]_x/dt &= (+J_{F_1F_0} - J_{ANT})/V_x \\
d[mATP]_x/dt &= (+J_{MgATPx})/V_x \\
d[mADP]_x/dt &= (+J_{MgADPx})/V_x \\
d[Pi]_x/dt &= (-J_{F_1F_0} + J_{PiHt})/V_x \\
d[ATP]_i/dt &= (+J_{ATPt} + J_{ANT} + J_{AKi})/V_i \\
d[ADP]_i/dt &= (+J_{ADPt} - J_{ANT} - 2J_{AKi})/V_i \\
d[AMP]_i/dt &= (+J_{AMPt} + J_{AKi})/V_i \\
d[mATP]_i/dt &= (+J_{MgATPi})/V_i \\
d[mADP]_i/dt &= (+J_{MgADPi})/V_i \\
d[Pi]_i/dt &= (+J_{Pit} - J_{PiHt})/V_i \\
d\Delta\Psi/dt &= (4J_{C1} + 2J_{C3} + 4J_{C4} - n_A J_{F1} \\
&\quad - J_{ANT} - J_{Hle} - J_K)/C_{IM}
\end{aligned}$$

Systems of chemical ODEs elucidate biochemical control

- Simulation of physiological ODE model allows us to determine that phosphate control acts on Complex III flux:

$$J_{C3} = X_{C3} \left(e^{-(\Delta G_{o,\,C3} + 4\Delta G_H + 2RT\ln([H^+]_x/10^{-7}) - 2F\Delta\Psi + RT\ln([Q]/[QH_2]))/2RT} \cdot [\mathrm{cytC(ox)}^{3+}] - [\mathrm{cytC(red)}^{2+}] \right), \quad (7)$$

$$J_{C3} = X_{C3} \left(\frac{1 + [\mathrm{Pi}]_x/k_{\mathrm{Pi},3}}{1 + [\mathrm{Pi}]_x/k_{\mathrm{Pi},4}} \right) \cdot \left(e^{-(\Delta G_{o,\,C3} + 4\Delta G_H - 2F\Delta\Psi + RT\ln([Q]/[QH_2]))/2RT} \cdot [\mathrm{cytC(ox)}^{3+}] - [\mathrm{cytC(red)}^{2+}] \right), \quad (24)$$



- Can't match data without $\propto (1 + [P_i]/k_1)/(1 + [P_i]/k_2)$ term
- We will explore other features of this model in the practical.