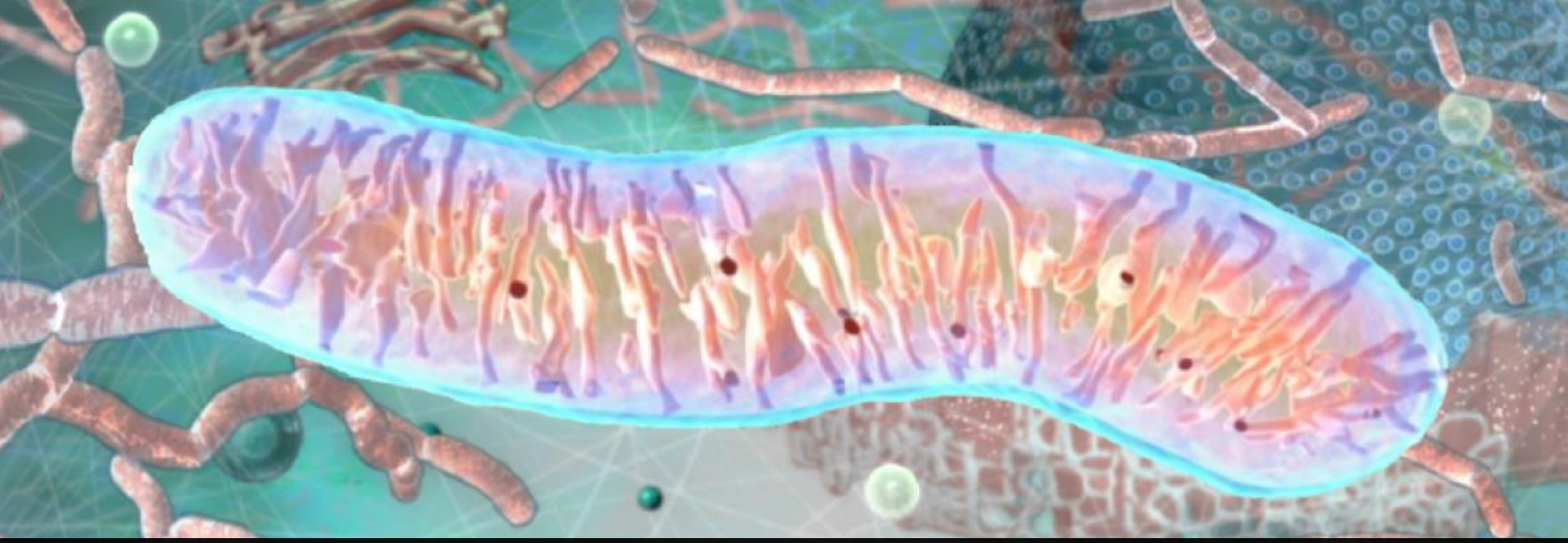

The enzymatic basis of energy-generation

Lecture 3: Respiration of inorganic compounds

Dr Chris Greening

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May 6 2016

Lecture 3: Respiration of inorganic compounds

I. Prokaryotic versatility

II. Nitrification / denitrification

III. Aerobic H_2 respiration

IV. Anaerobic H_2 respiration

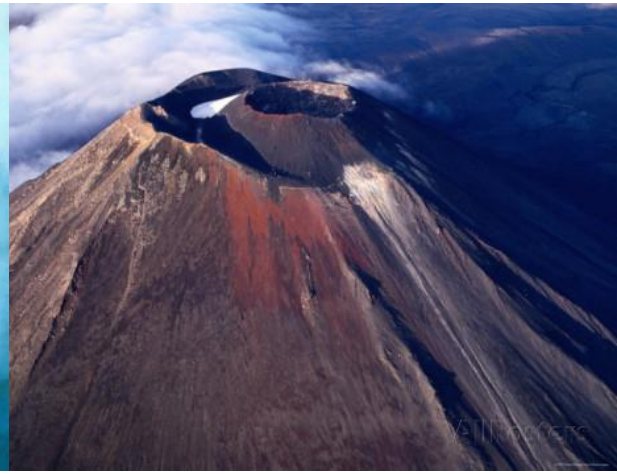
Prokaryotes inhabit every environment

- Prokaryotes (bacteria, archaea) are present in large cell numbers in every environment on earth: from animal guts to hot springs to deep-sea sediments. They can flourish in such environments because of their metabolic flexibility. Three I'm currently studying:

Robinson Ridge
(Antarctic desert)

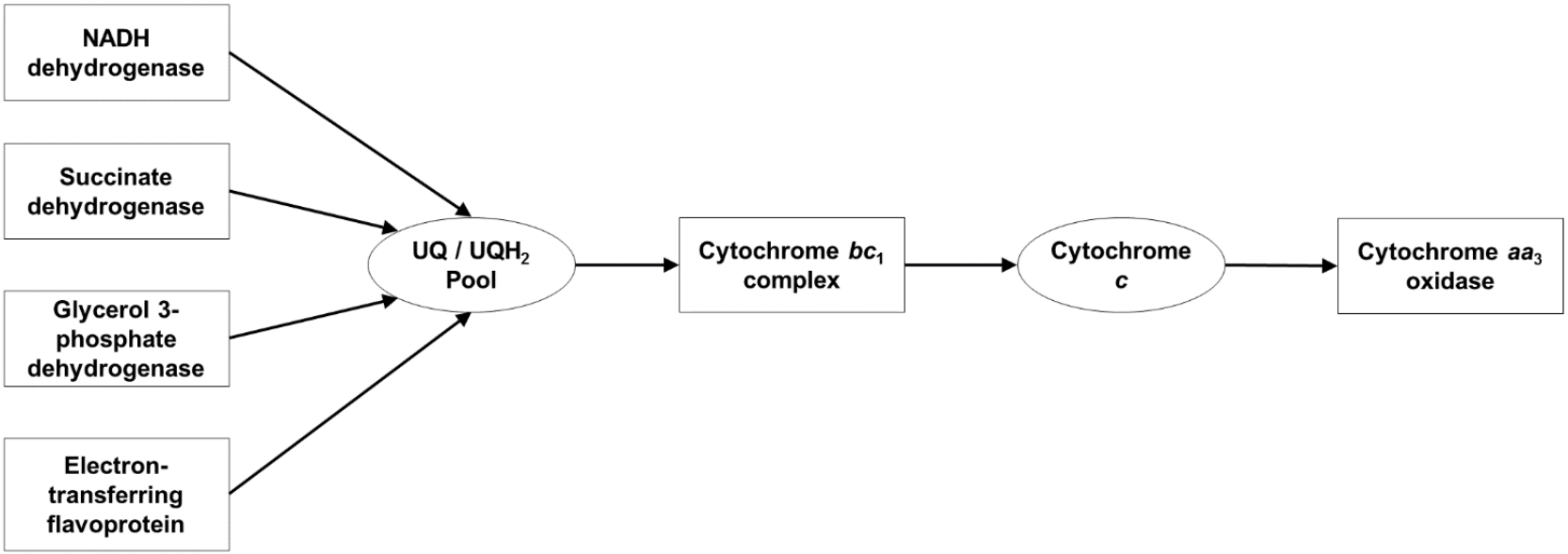
Mariner Trench
(deep-sea vents)

Mount Ngauruhoe
(volcanic crater)



Mitochondria are efficient but inflexible

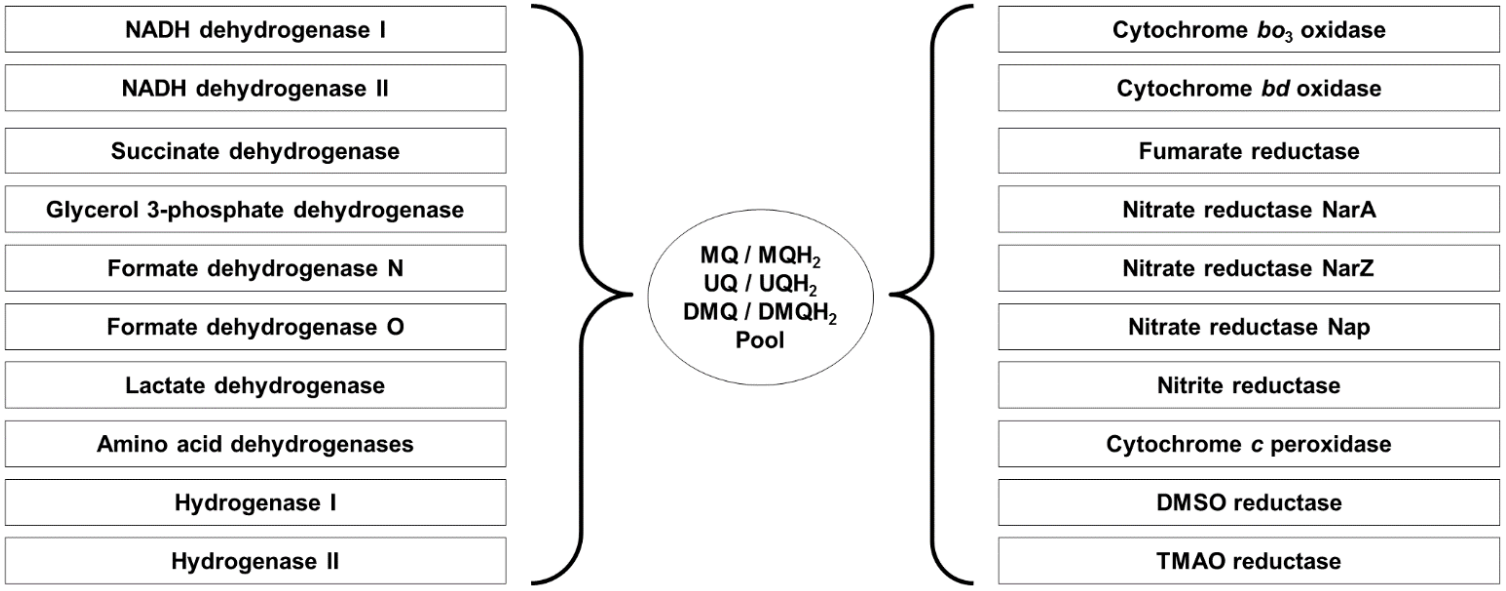
- Mitochondria have very limited flexibility. Their e^- donors are all derived from organic carbon compounds and their sole e^- acceptor is O_2. Energy is transduced through highly efficient but inflexible linear electron transport chains.



- In many ecosystems, the available electron donors (organic carbon sources), acceptors (O_2), and physical conditions are insufficient to sustain animal life.

Prokaryotes are highly metabolically diverse

- Microorganisms can prosper in almost all ecosystems due to their respiratory flexibility:
  - They can substitute organic e^- donors for inorganic e^- donors (e.g. H_2)
  - They can substitute O_2 for anaerobic e^- acceptors (e.g. NO_3^{2-})
  - Employ branched electron transport chains that can use multiple donors and acceptors



- In addition to respiration, most microorganisms can sustain energy-conservation by fermentation (substrate-level phosphorylation) in the absence of exogenous e^- acceptors. Many organisms also capture light and/or fix inorganic carbon.

Alternative e^- sources and sinks

Redox couple	$E^{o'}$
CO_2 / CO	-0.492 V
CO_2 / Formate	-0.432 V
$2H^+$ / H_2	-0.414 V
CO_2 / CH_4	-0.239 V
SO_4^{2-} / H_2S	-0.218 V
CoM-S-S-CoB / CoM-SH + CoB-SH	-0.140 V
Fumarate / Succinate	+0.030 V
NO_2^- / NH_3	+0.340 V
NO_3^- / NO_2^-	+0.431 V
Fe^{3+} / Fe^{2+}	+0.770 V

Alternative e^- sources and sinks

Redox couple	$E^{o'}$	Primary dehydrogenase
CO_2 / CO	-0.492 V	Carbon monoxide dehydrogenase
CO_2 / Formate	-0.432 V	Formate dehydrogenase
$2H^+$ / H_2	-0.414 V	Hydrogenotrophic hydrogenase
CO_2 / CH_4	-0.239 V	Methane monooxygenase
SO_4^{2-} / H_2S	-0.218 V	Various inc. sulfide oxidoreductase
CoM-S-S-CoB / CoM-SH + CoB-SH	-0.140 V	N/A
Fumarate / Succinate	+0.030 V	Succinate dehydrogenase
NO_2^- / NH_3	+0.340 V	Ammonia monooxygenase
NO_3^- / NO_2^-	+0.431 V	Nitrite oxidoreductase
Fe^{3+} / Fe^{2+}	+0.770 V	Iron oxidase

Alternative e^- sources and sinks

Redox couple	$E^{o'}$	Primary dehydrogenase	Terminal reductase
CO_2 / CO	-0.492 V	Carbon monoxide dehydrogenase	N/A
CO_2 / Formate	-0.432 V	Formate dehydrogenase	N/A
$2H^+$ / H_2	-0.414 V	Hydrogenotrophic hydrogenase	Hydrogenogenic hydrogenase
CO_2 / CH_4	-0.239 V	Methane monooxygenase	Methanogenesis pathways
SO_4^{2-} / H_2S	-0.218 V	Various inc. sulfide oxidoreductase	Various inc. sulfite reductase
CoM-S-S-CoB / CoM-SH + CoB-SH	-0.140 V	N/A	Heterodisulfide reductase
Fumarate / Succinate	+0.030 V	Succinate dehydrogenase	Fumarate reductase
NO_2^- / NH_3	+0.340 V	Ammonia monooxygenase	Nitrite reductase
NO_3^- / NO_2^-	+0.431 V	Nitrite oxidoreductase	Nitrate reductase
Fe^{3+} / Fe^{2+}	+0.770 V	Iron oxidase	Iron reductase

Directionality depends on environment

- Microorganisms can effectively mix-and-match the electron donors and acceptors they use depending on what is available in the environment.

Term	e^- donors	e^- acceptor
Aerobic organotrophy	Organic: sugars, amino acids, formate, methane, acetylene, lignin, TNT, etc.	O_2
Aerobic lithotrophy	Inorganic: H_2, CO, NH_3, NO_2^-, Fe^{2+}, etc.	O_2
Anaerobic organotrophy	Organic: sugars, amino acids, formate, methane, etc.	NO_3^-, NO_2^-, SO_4^{2-}, Fe^{3+}, CO_2, fumarate, H^+, etc.
Anaerobic lithotrophy	Inorganic: H_2, CO, H_2S, etc.	NO_3^-, NO_2^-, SO_4^{2-}, Fe^{3+}, CO_2, etc.

- In oxic environments, a wide range of compounds can be used as fuel sources for aerobic respiration (e.g. NH_3, H_2, H_2S, CH_4). In anoxic environments, the same compounds can be produced as electron sinks during anaerobic respiration. All down to simple energetics.

If there's a negative ΔG...

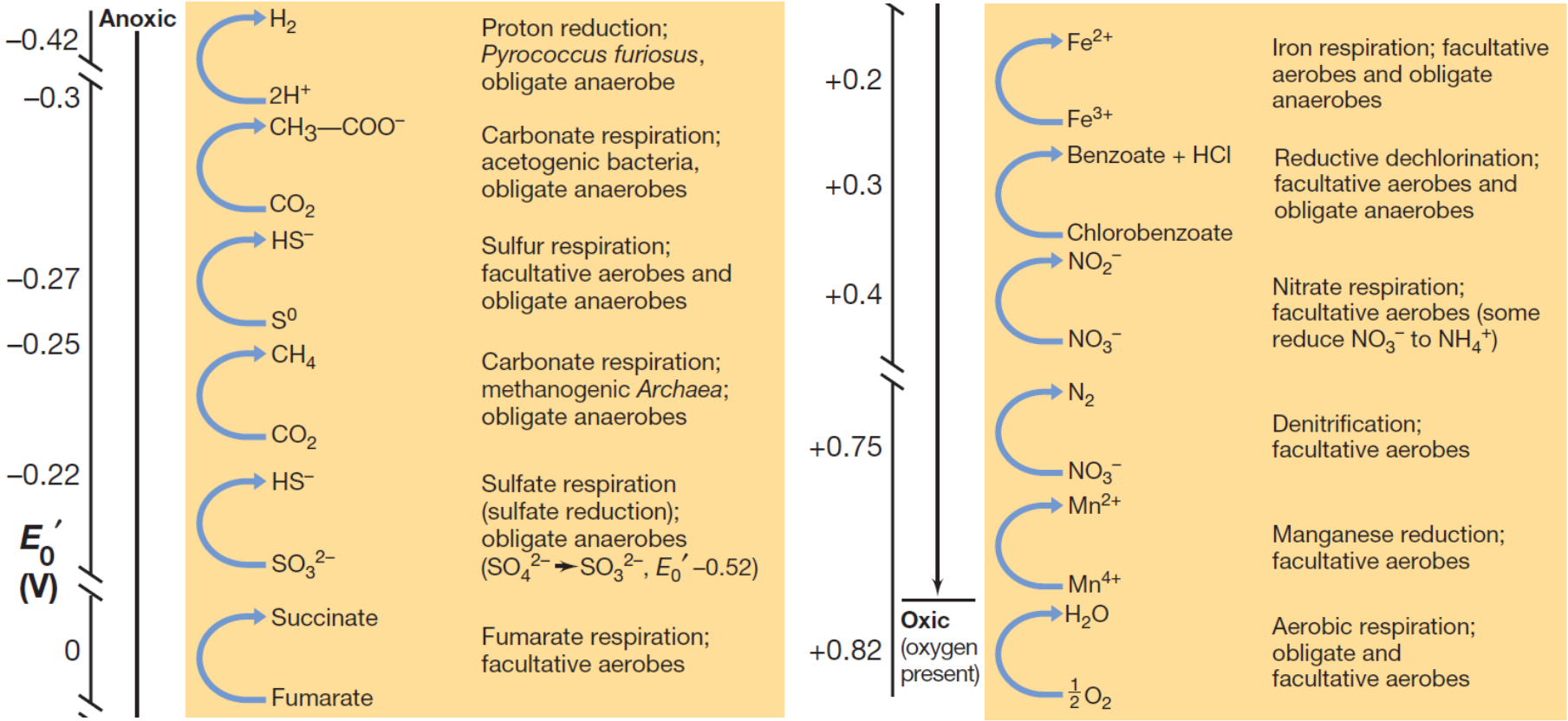
- A golden rule in microbial energetics is that, if an e^- donor and an e^- acceptor are available for a thermodynamically-favourable reaction to occur, some organism will be able to mediate it. This is even the case when the free energy released is very low. Some examples:

Process	Half-equations	$E^{o\prime}$	$\Delta E^{o\prime}$	$\Delta G^{o\prime}$
Aerobic organotrophy	$NAD^+ + H^+ + 2e^- \rightarrow NADH$ $½\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	-0.320 V +0.816 V	+1.136 V	-219 kJ mol^{-1}
Aerobic lithotrophy	$Fe^{3+} + 1e^- \rightarrow Fe^{2+}$ $½\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$	+0.770 V +0.816 V	+0.046 V	-8.8 kJ mol^{-1}
Anaerobic organotrophy	$CO_2 + H^+ + 2e^- \rightarrow$ Formate $2H^+ + 2e^- \rightarrow H_2$	-0.432 V -0.414 V	+0.018 V	-3.5 kJ mol^{-1}
Anaerobic lithotrophy	$2H^+ + 2e^- \rightarrow H_2$ $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	-0.414 V +0.431 V	+0.845 V	-163 kJ mol^{-1}

- The approaches used greatly vary both within and between organisms. Some prokaryotes have specialist metabolism that enables them to dominate certain niches, whereas others are highly versatile and can adapt to a wide range of environments.

The redox tower of e^- acceptor utilisation

- In environments where there is more than one e^- acceptor available (e.g. O_2, NO_3^-), the highest potential acceptor (O_2) will be used over the others (NO_3^-). The lowest potential e^- acceptors (i.e. protons) are only used in the most energy-poor environments.



Main factors shaping e^- acceptor utilisation

Regulation:

Metabolically flexible organisms (e.g. facultative aerobes such as *E. coli*) sense e^- acceptor availability. If multiple acceptors are available, they upregulate the reductases of high-energy acceptors (e.g. cytochrome *c* oxidase) and downregulate the others (e.g. nitrate reductase).

Competition:

Metabolically inflexible organisms reliant on low-potential e^- acceptors (e.g. obligate anaerobes such as sulfate-reducers) are outcompeted in energy-rich environments. Their ETCs yield less energy per organic molecule oxidised (lower $H^+/2e^-$ ratios) than e.g. *E. coli*. They in turn grow much slower.

Lecture 3: Respiration of inorganic compounds

I. Prokaryotic versatility

II. Nitrification / denitrification

III. Aerobic H_2 respiration

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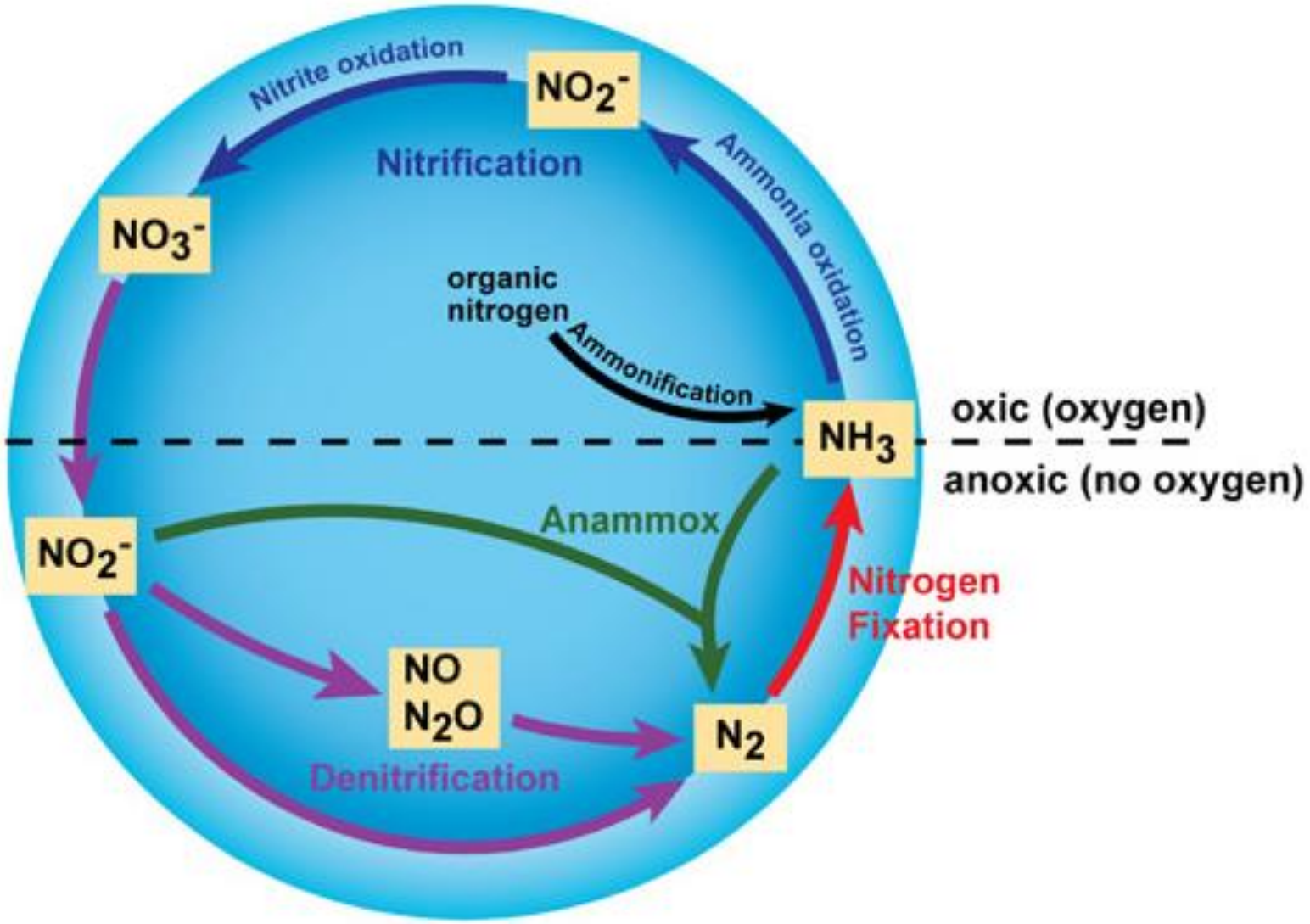
Nitrogen cycle



Nitrification: N compounds as e^- donors

- Nitrification is a two-step process which oxidizes ammonia and nitrite as fuel sources. Due to high potential of the e^- donors, O_2 is required as an e^- acceptor.

$2\ NH_3 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 2\ H^+$ $\quad \Delta E^{o\prime} = +0.816 - +0.340 = + 0.476\ V$

$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$ $\quad \Delta E^{o\prime} = +0.816 - +0.431 = + 0.385\ V$

- Despite the biogeochemical significance of nitrification, only a few organisms can mediate the process. Those that do are specialist lithotrophs that grow on few other fuel sources.

- Classically thought different organisms living in symbiosis mediate NH_3 oxidation (e.g. *Nitrososphaera*) and nitrite oxidation (e.g. *Nitrobacter*). However, complete nitrifiers (e.g. *Nitrospira*) were recently identified through high-throughput sequencing (Nature 2015).

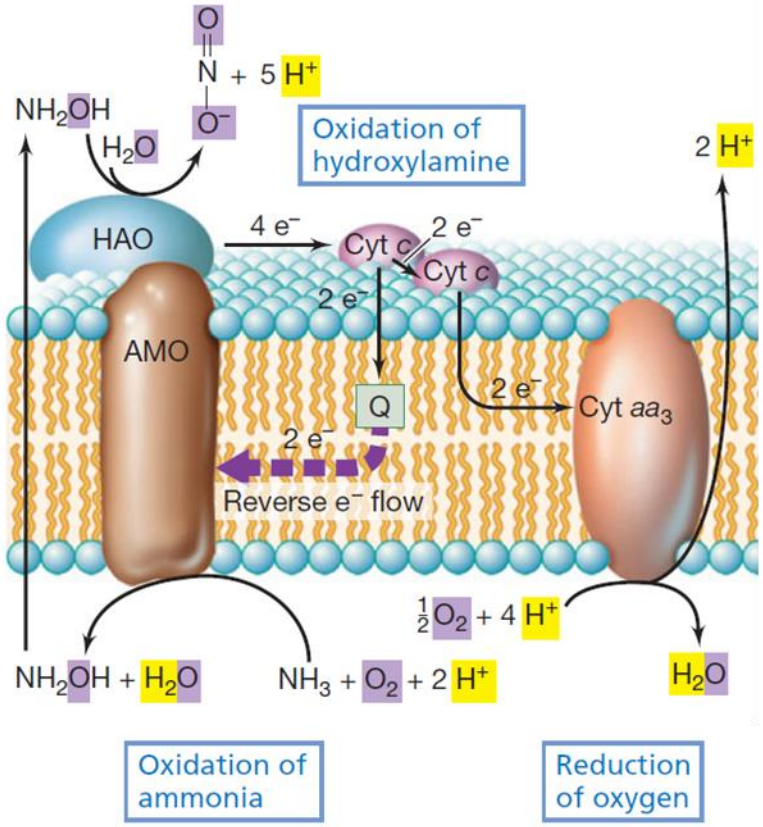
Sergei Winogradsky (1856 – 1953):
Discoverer of lithotrophy and nitrification

Electron transport chains in nitrification

- Specialised primary dehydrogenases, ammonia monooxygenase and nitrite oxidoreductase (structures not solved), input electrons into ETC. Electrons transferred *via* cytochrome *c* to proton-translocating cytochrome *c* oxidase. Δp drives F_1F_o ATP synthase.

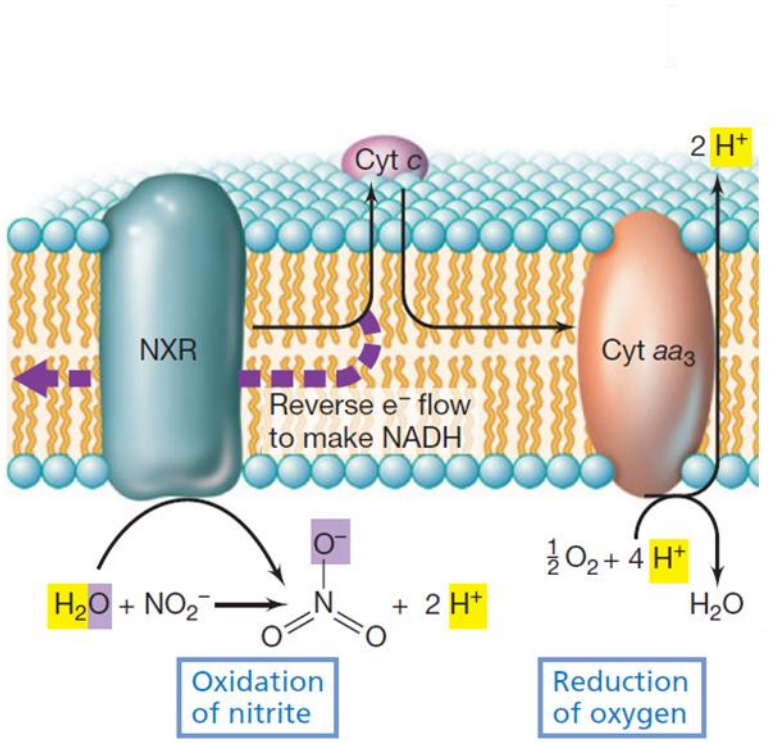
Ammonia oxidation

e.g. Nitrososphaera, Nitrospira



Nitrite oxidation

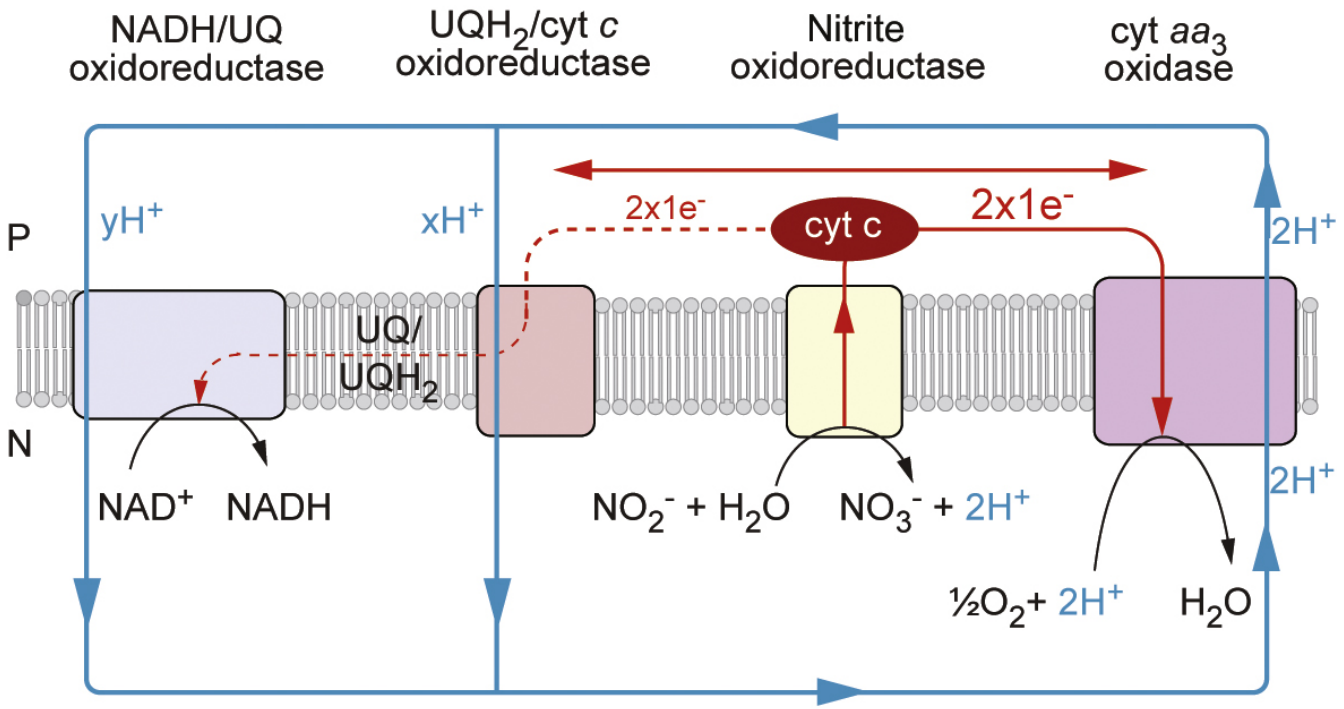
e.g. Nitrobacter, Nitrospira



Reversed electron flow in nitrification

- All organisms generate reductant (e.g. NADH) to sustain biosynthetic processes. Oxidation of organic compounds, H_2, and CO can be favourably coupled to NAD^+ reduction. However, it is thermodynamically impossible to couple NH_3 and NO_2^- oxidation to NAD^+ reduction.

- Reversed electron flow is the solution. Δp can be consumed to drive a reversed endergonic e^- transfer pathway: nitrite → cytochrome *c* → Complex III → UQ → Complex I → NAD^+.



Denitrification is a form of anaerobic respiration

- Denitrification is a form of anaerobic respiration. Nitrogen oxides (i.e. nitrate, nitrite) are reduced to either N_2 or NH_3 depending on the organism through a series of enzymatic steps. Electrons can be derived from various from organic or inorganic sources.

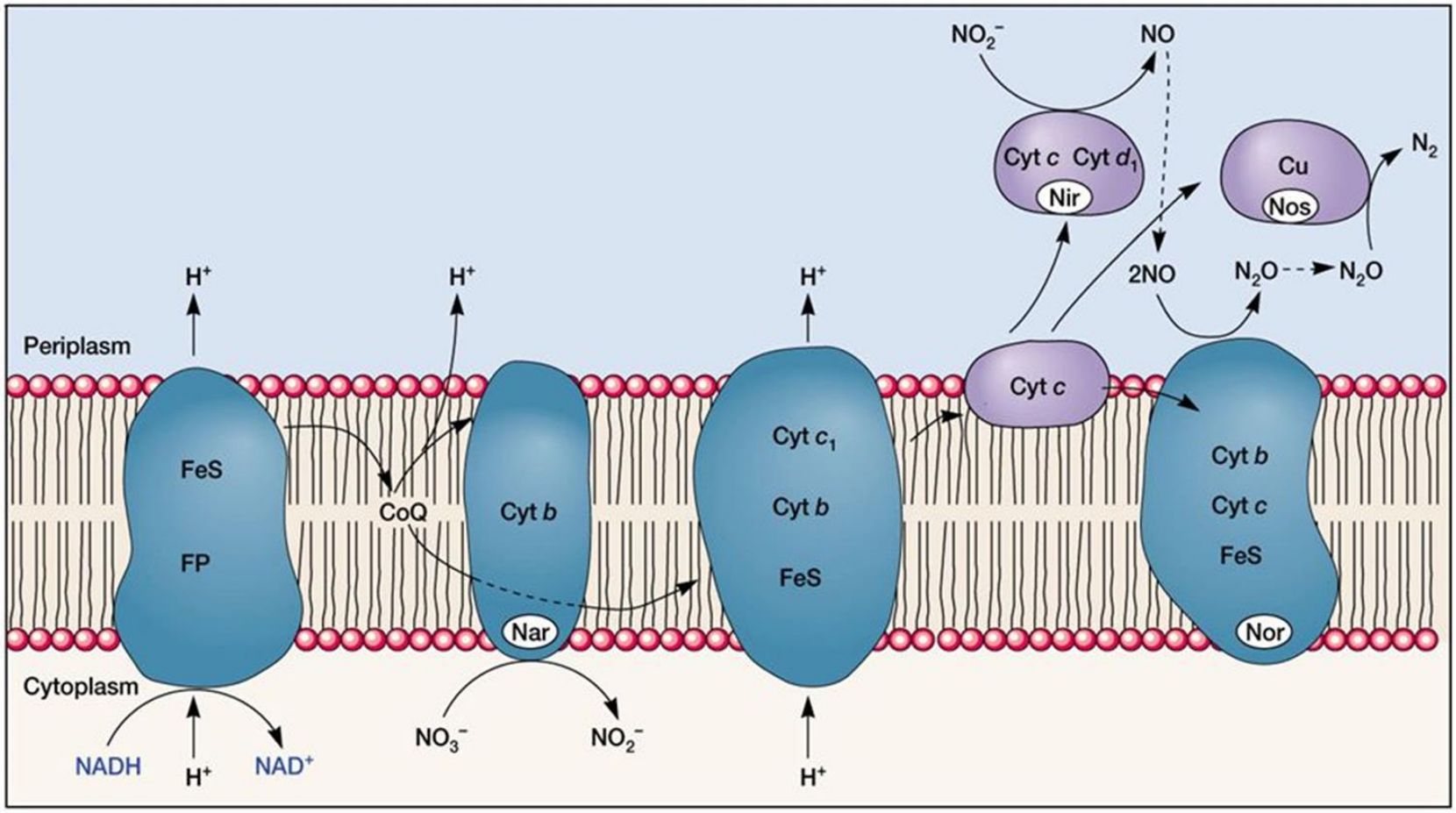
$NADH + NO_3^- + H^+ \rightarrow NAD^+ + NO_2^- + H_2O$ $\quad \Delta E^{o\prime} = +0.431 - -0.340 = + 0.771$ V

$3\ NADH + NO_2^- + 4\ H^+ \rightarrow 3\ NAD^+ + NH_3 + 2\ H_2O$ $\quad \Delta E^{o\prime} = +0.340 - -0.320 = + 0.680$ V

- Whereas nitrification is mediated by a few specialist organisms, denitrification is performed by a wide range of facultative denitrifiers. This is because nitrate is a dependable e^- acceptor for anaerobic respiration: it is a highly electropositive and is available in most ecosystems.

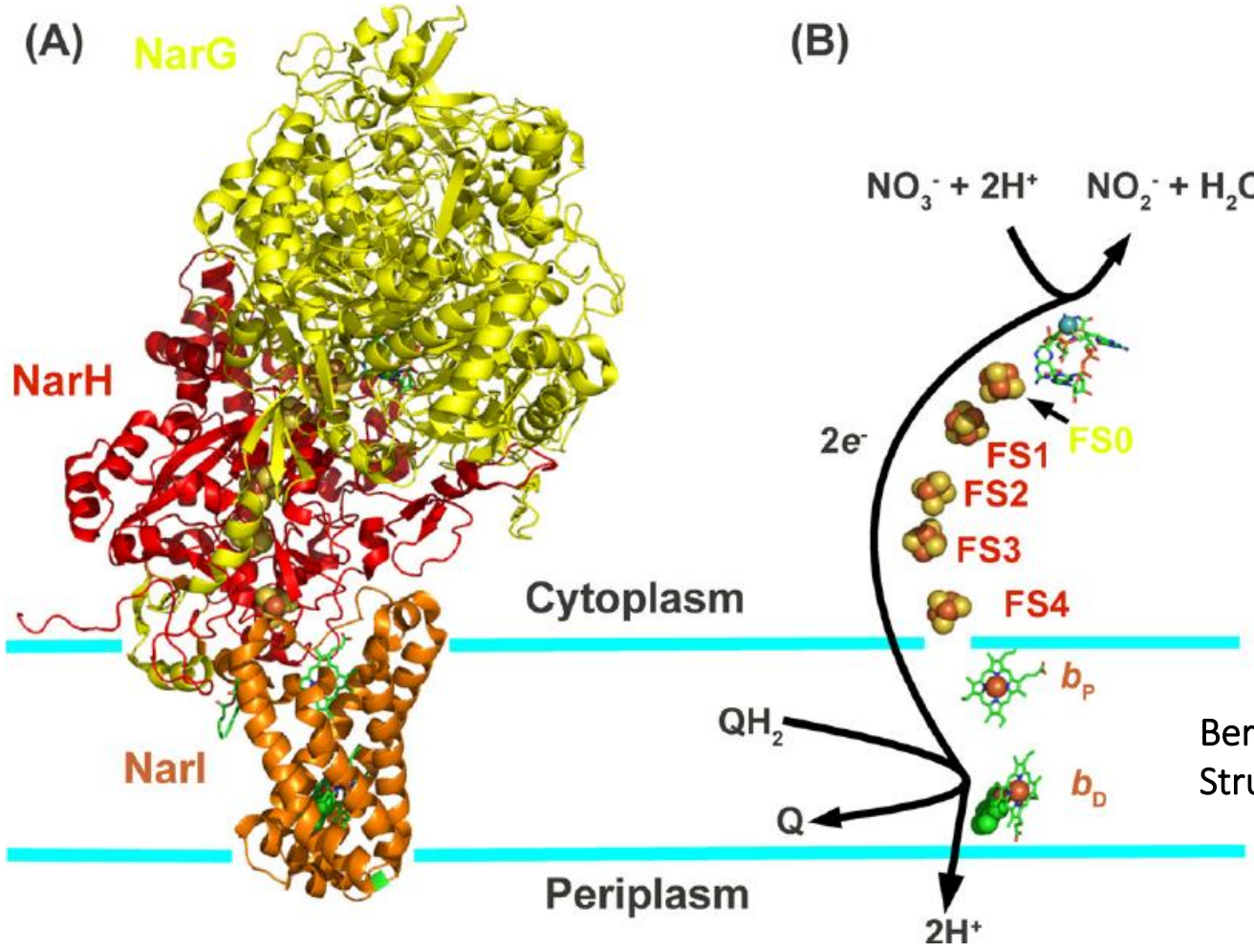
Electron transport chains in denitrification

- In *Paracoccus denitrificans*, NO_3^- is sequentially reduced by dedicated complexes to NO_2^- (Nar), NO(Nir), N_2O (Nor), and N_2 (Nir). Electrons transferred to reductases via mitochondria-like electron transport chain: NADH → Complex I → UQ → Complex III → cyt *c* → reductase.



Nitrate reductase is a molybdoenzyme

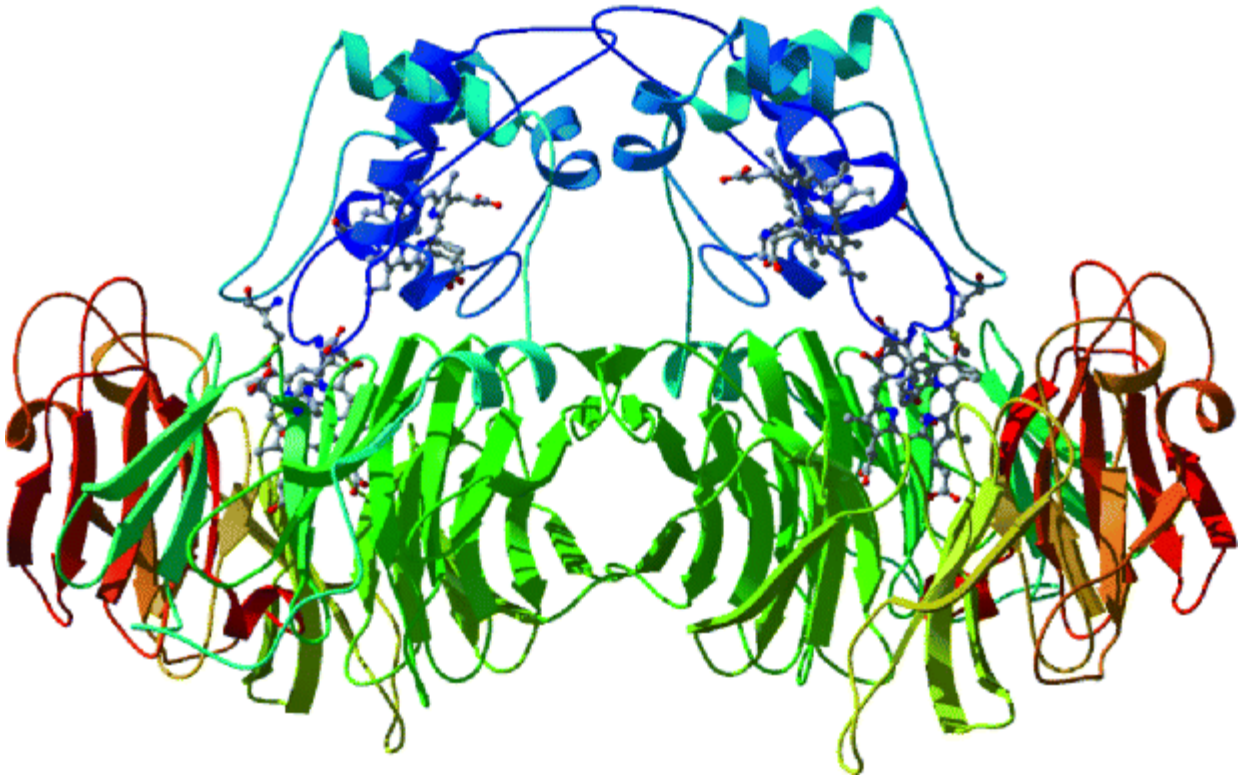
- Nitrate is bound and reduced by a specialised molybdenum-containing organometallic cofactor (Mo-MGD). Electrons are funnelled from ubiquinone *via* two *b*-type hemes and five iron-sulfur clusters to active site. Protons are translocated by redox-loop mechanism.

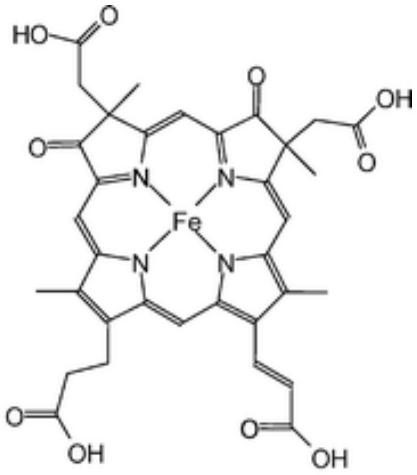


Bertero et al., Nature Struct Mol Biol, 2012

Nitrite reductase contains a unique heme

- The active site of nitrite reductase contains a unique heme, heme d_1, that is more electron-withdrawing than standard heme. It is electrochemically well-adapted to bind NO_2^- and release NO. Also contains a cytochrome *c* domain that receives electrons from ETC.





Williams et al., Nature 1997

Hierarchical regulatory control

High O_2 (+0.82 V, $10H^+/2e^-$)
Aerobic respiration

Low O_2 (+0.82 V, $6H^+/2e^-$)
Microaerobic respiration

High Nitrate (+0.42 V, $6H^+/2e^-$)
Nitrate respiration

High Fumarate (+0.03 V, 6H+/$2e^-$)
Fumarate respiration

No Respiratory Acceptors (SLP)
Fermentation

Regulation of denitrification

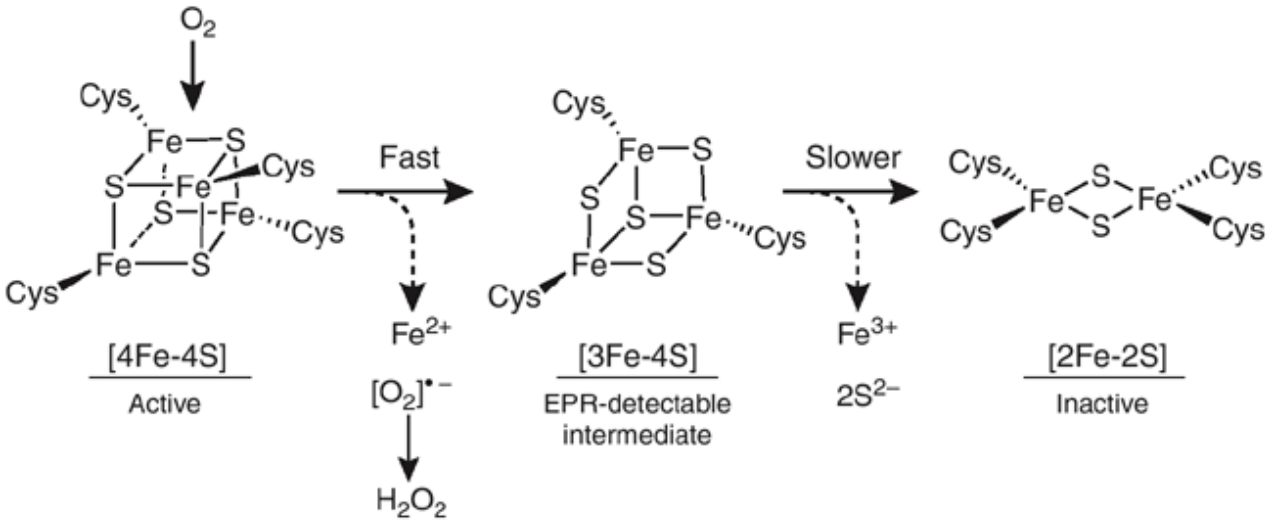
- Denitrification is less energetically efficient than aerobic respiration in terms of both ΔG released and $H^+/2e^-$ ratios. Hence, denitrifying organisms only express nitrate reductase and nitrite reductase when O_2 is absent. Two main sensory mechanisms.

ArcB: redox-sensing repressor

ArcB is a membrane protein that senses Q/QH_2 ratio. In anoxic conditions, quinol accumulation leads to reduction of the disulfide bond of ArcB. Reduced ArcB activates transcription factor ArcA. Leads to downregulation of cyt *c* oxidase.

Fnr: oxygen-sensing activator

Fnr is a cytosolic FeS protein that senses O_2. In oxic conditions, oxygen causes destruction of the iron-sulfur cluster and deactivation of Fnr. In anoxic conditions, intact Fnr binds DNA and activates transcription of nitrate reductase.



Lecture 3: Respiration of inorganic compounds

I. Prokaryotic versatility

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H_2 is a desirable energy reservoir

- Hydrogenases are metalloenzymes that catalyze the reversible heterolytic cleavage of H_2:

$$H_2 \rightleftharpoons [H^+ + H^-]^{\ddagger} \rightleftharpoons 2H^+ + 2e^-$$

- H_2 has the highest energy density of any molecule and is rapidly diffusible. As a result, a majority of microorganisms have evolved the capacity to metabolise this compound (Greening et al, ISME 2016). Organisms metabolise H_2 through three main processes:

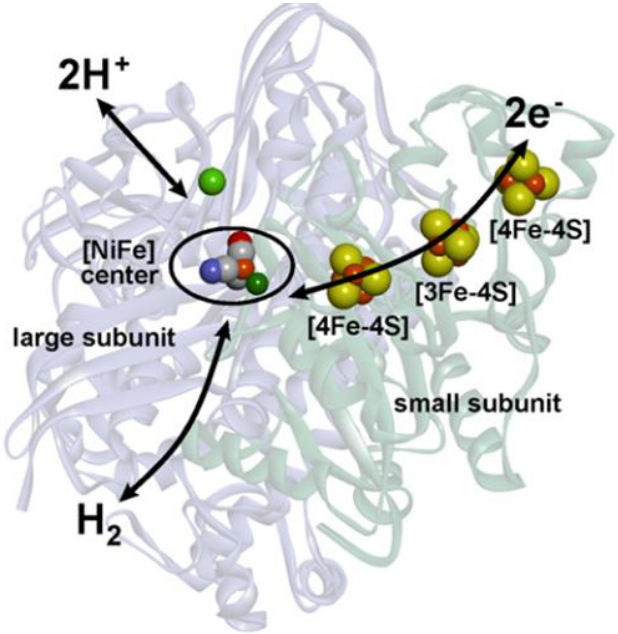
1. Hydrogenotrophic respiration: H_2 is the low-potential e^- donor in diverse respiratory processes
2. Hydrogenogenic respiration: H_2 is the endproduct of anaerobic proton respiration
3. Hydrogenogenic fermentation: H_2 is a dissipatable endproduct in many fermentation processes

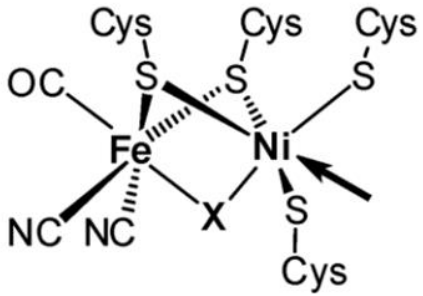
Marjory Stephenson (1885 – 1948):
Discoverer of H_2 metabolism

Convergent evolution of two metalloenzymes

[NiFe]-hydrogenases

Mainly involved in respiration processes
Include O_2 sensitive and tolerant variants
Present in a wide variety of bacteria and archaea





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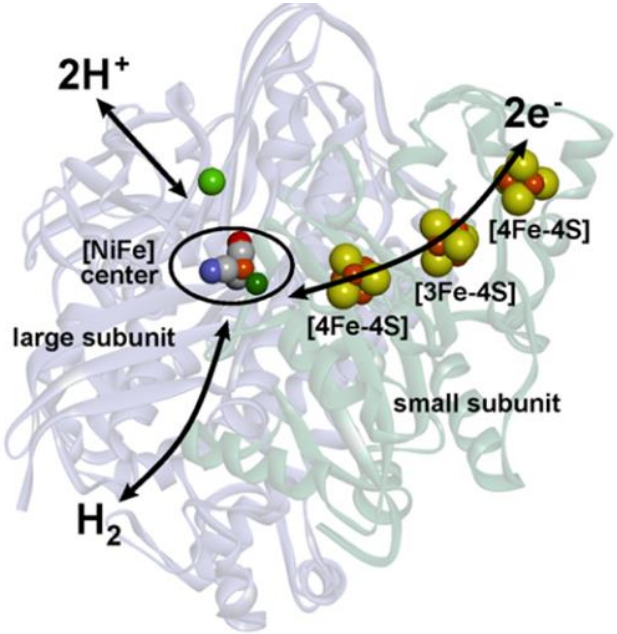
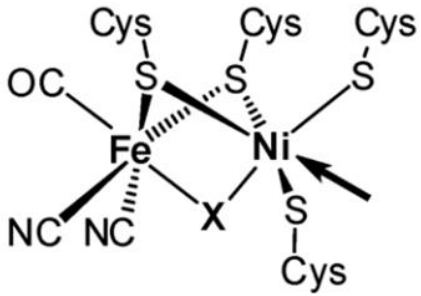
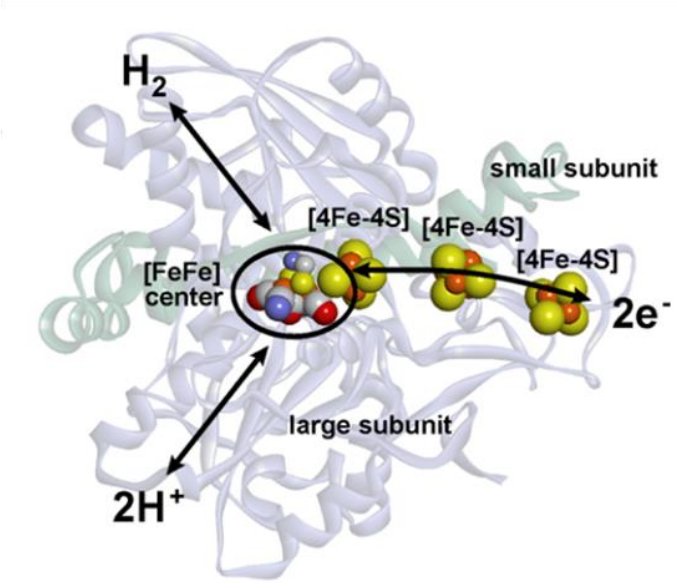
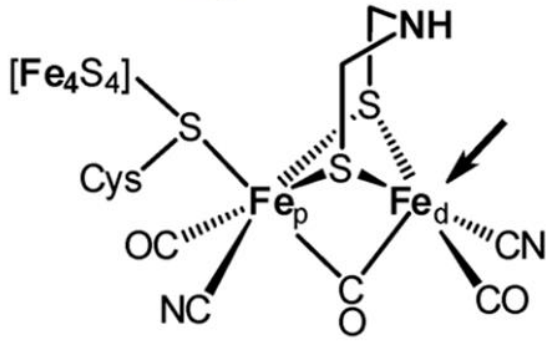




[FeFe]-hydrogenases

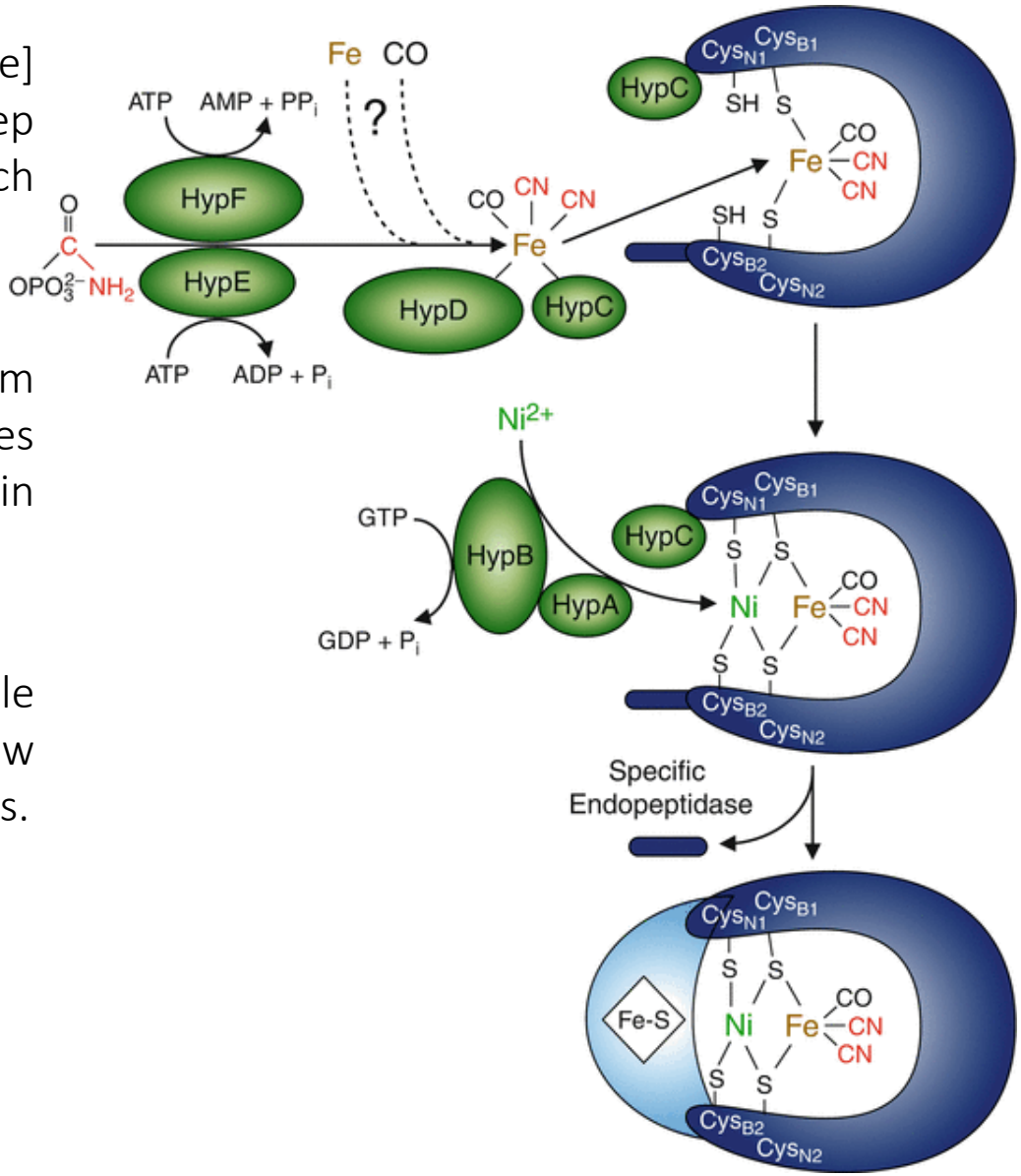
Mainly involved in fermentation processes
Faster-acting but irreversibly destroyed by O_2
Restricted to anaerobic bacteria and eukaryotes





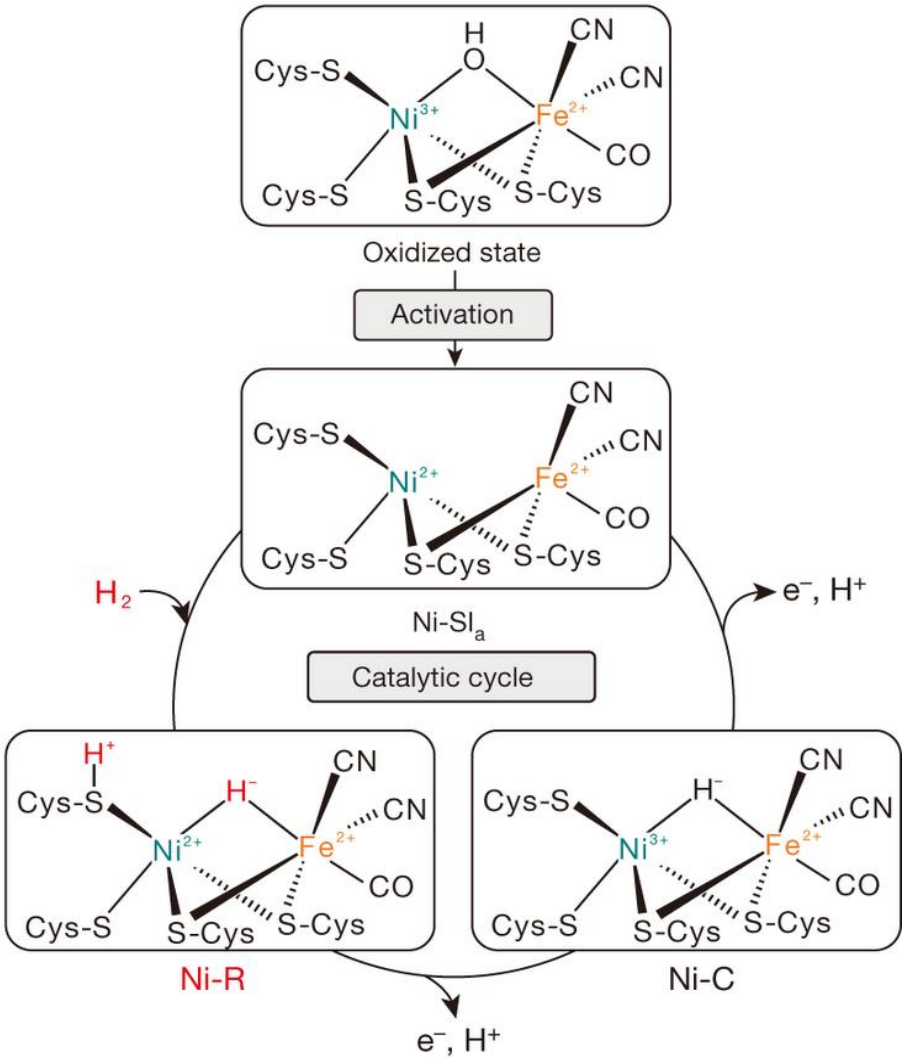
Hydrogenase synthesis is complicated

- Note that [NiFe] and [FeFe] hydrogenases are matured in multistep mechanisms involving rich organometallic and radical chemistry.
- CN and CO ligands are synthesized from organic precursors. Specific chaperones mediate metal cofactor insertion, protein folding, and complex assembly.
- Cutting-edge field subject of multiple Nature and Science papers in last few years. Not necessary to know any details.



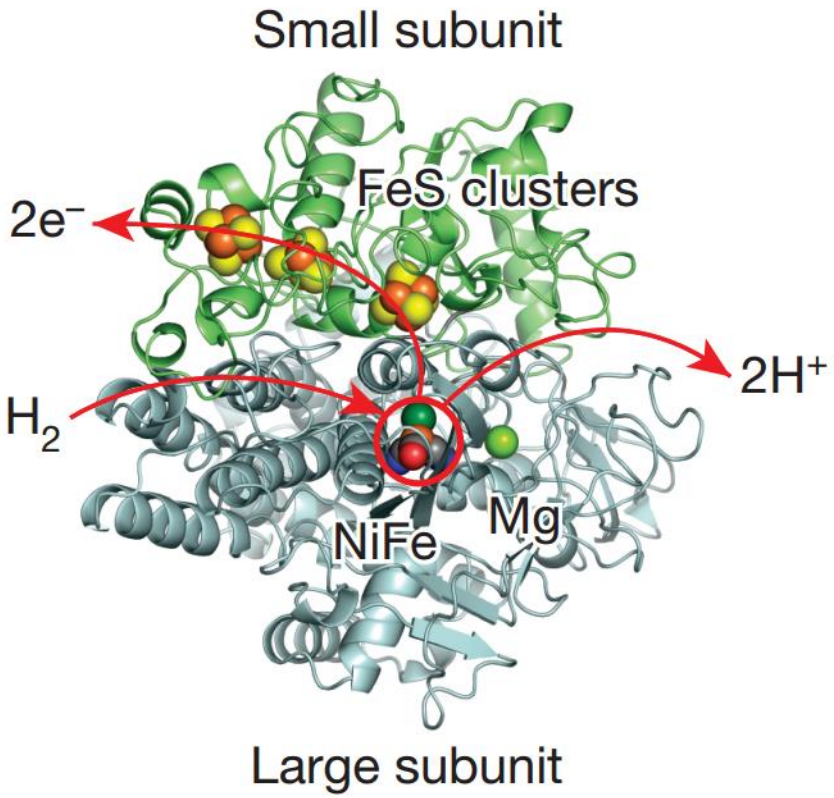
Mechanism of [NiFe]-hydrogenase

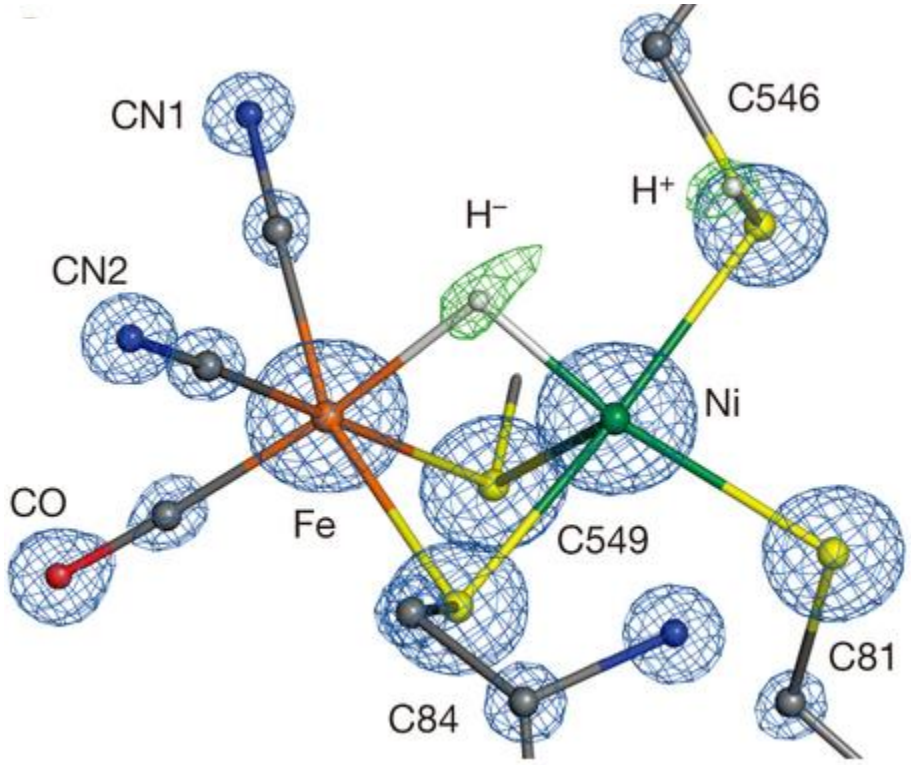
- Following hydrogenase reaction mechanism has been proposed based on extensive EPR and FTIR spectroscopy, protein film voltammetry, and X-ray crystallography studies.



H_2 heterolysis observed at subatomic resolution

- 0.89 Å resolution structure of anoxically-isolated [NiFe]-hydrogenase from the sulfate-reducing *Desulfovibrio vulgaris* confirmed Ni-R structure. A hydride ion (H^-) bridges the Ni and Fe atoms. A proton (H^+) attaches to the thiol of one of the Ni-ligating cysteine residues.

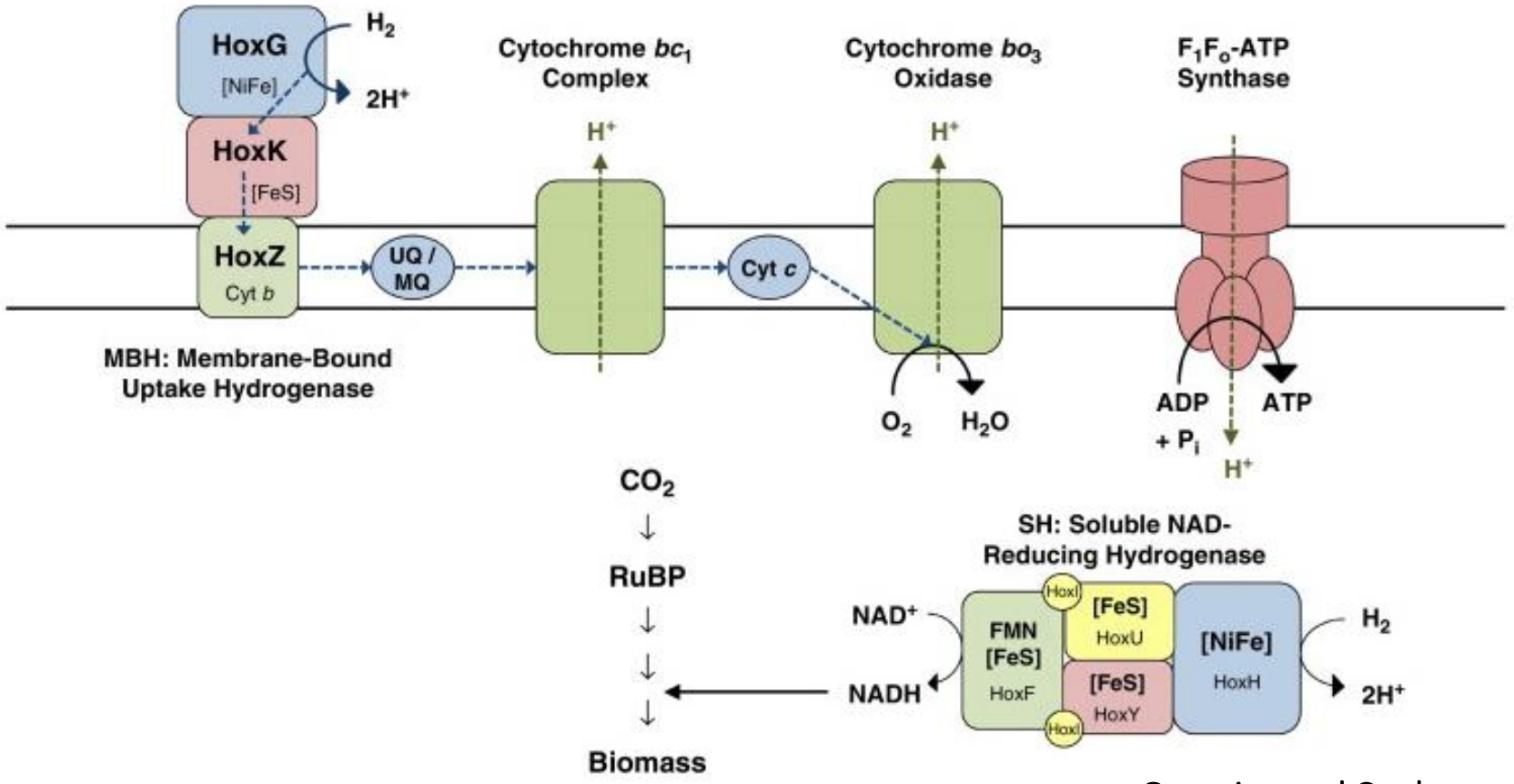




Ogata et al., Nature 2015

Hydrogenotrophic aerobic respiration

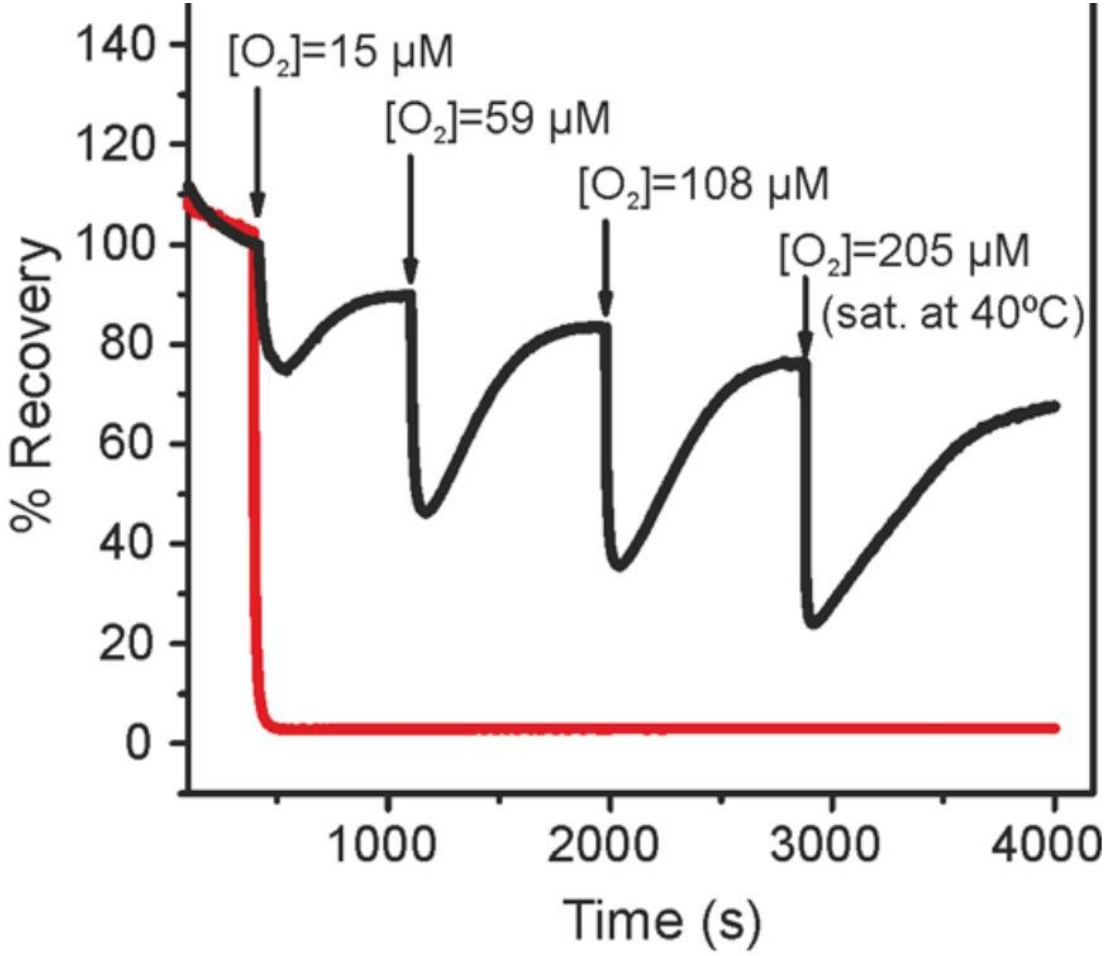
- Aerobic soil bacteria such as *Ralstonia eutropha* can grow using H_2 as the sole e^- donor, O_2 as the sole e^- acceptor, and CO_2 as the carbon source. This depends on two hydrogenases: one that inputs electrons into ETC, another that generates NADH for CO_2 fixation.



Greening and Cook,
Curr Opin Microbiol 2014

An oxygen-tolerant [NiFe]-hydrogenase

- Oxygen binds the [NiFe] centre of most hydrogenases leading to irreversible competitive inhibition. However, amperometric measurements show that *R. eutropha* membrane-bound hydrogenase rapidly reactivated following inactivation with O_2.

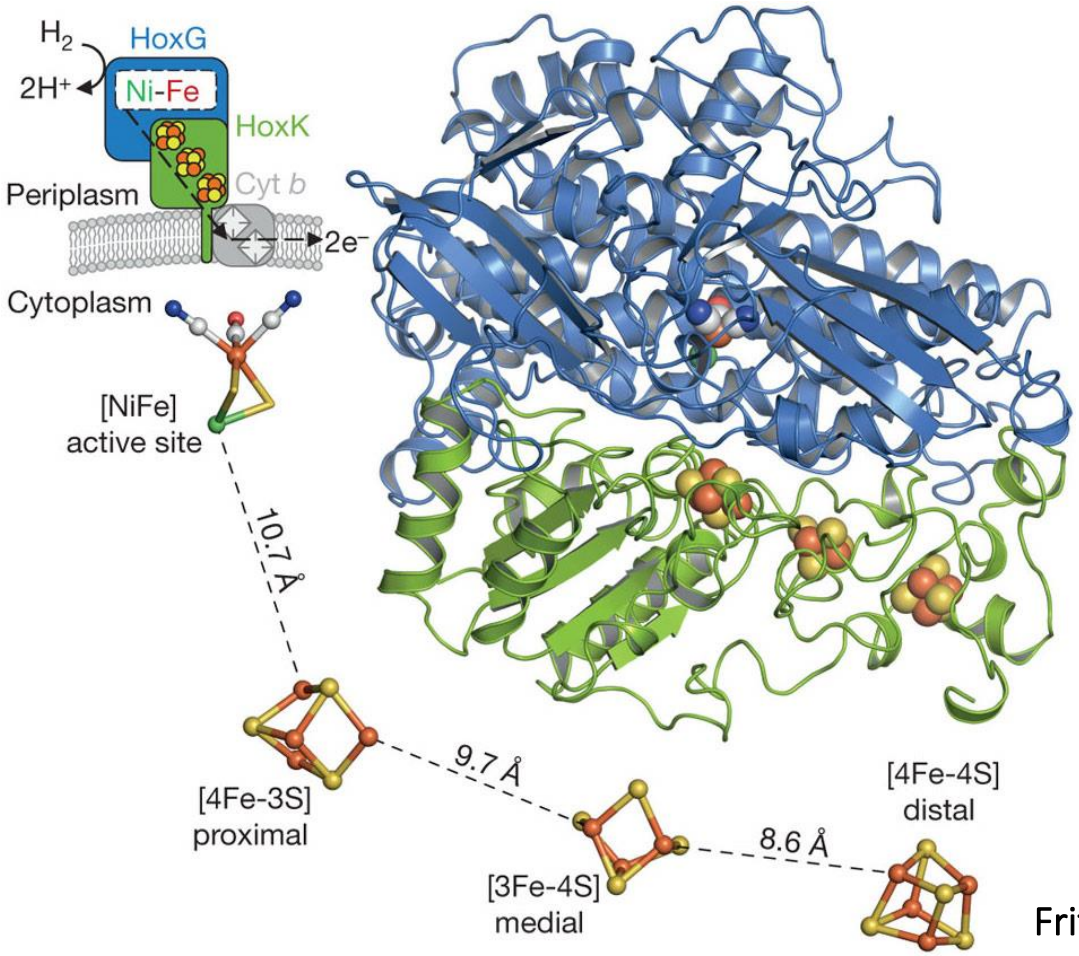


Red = Oxygen-sensitive MBH (e.g. *Desulfovibrio vulgaris*)

Black = Oxygen-tolerant MBH (e.g. *Ralstonia eutropha*)

Structural basis of oxygen-tolerance

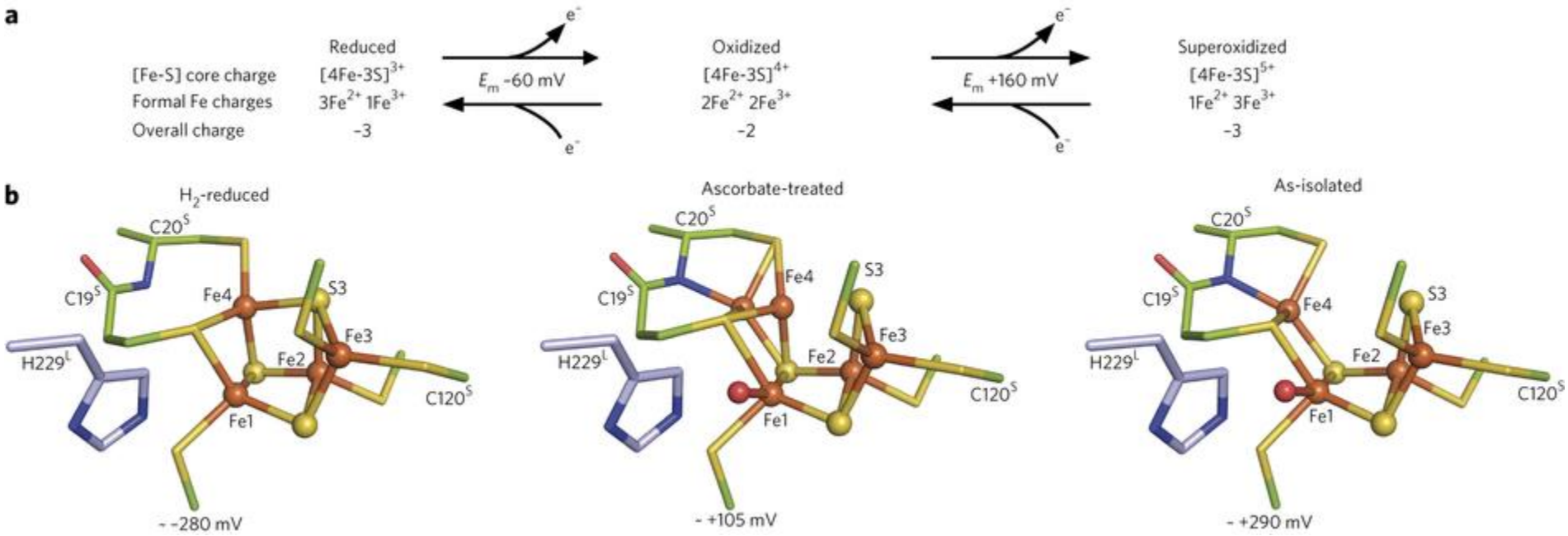
- Structure of *R. eutropha* membrane-bound hydrogenase very similar to that of *D. vulgaris*. However, the small subunit contains a unique 6Cys[4Fe3S] cluster proximal to active site.



Fritsch et al., Nature 2011

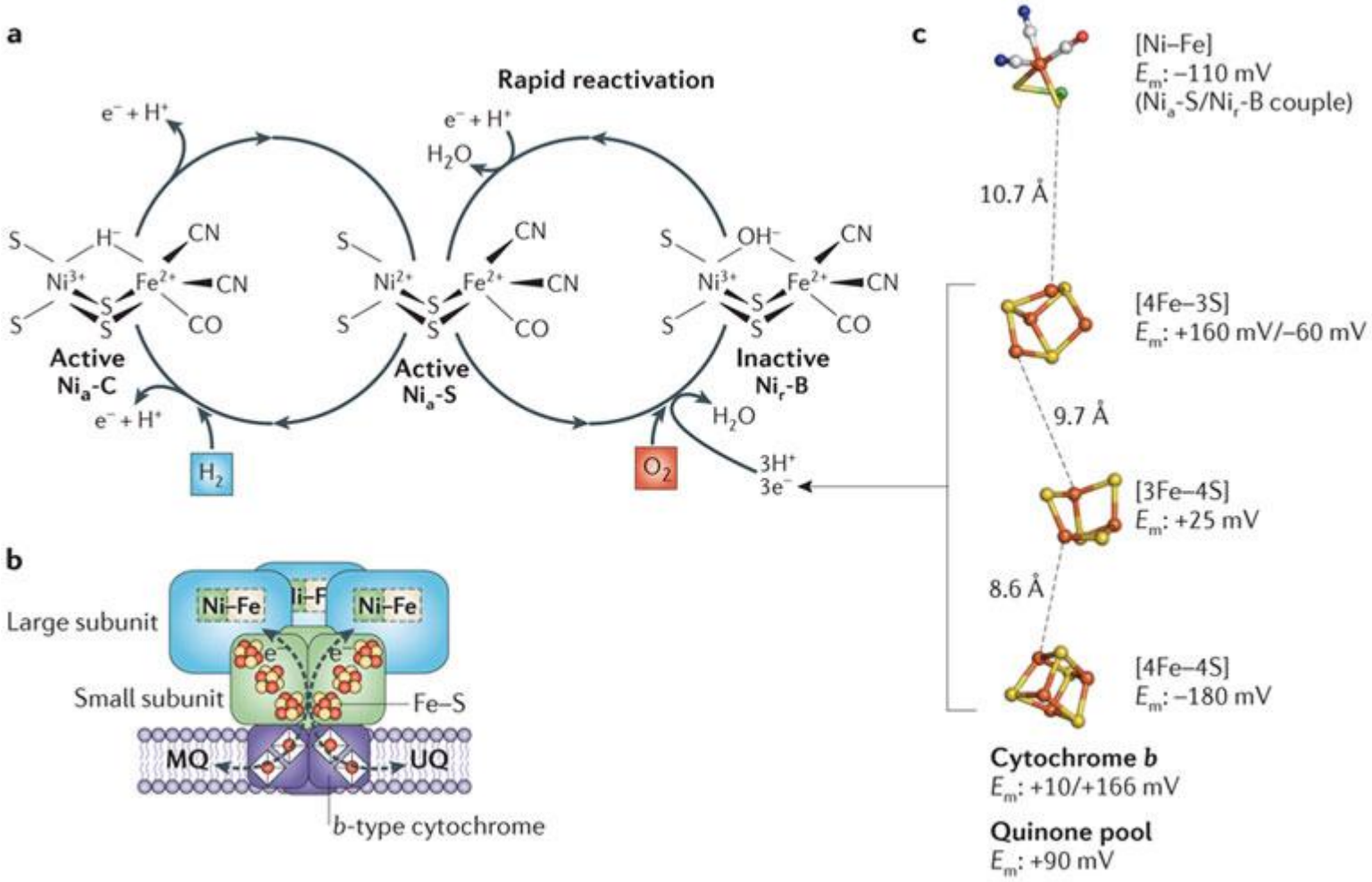
[4Fe3S] cluster can undergo 2e⁻ chemistry

- Nearly all FeS clusters can only undergo 1e⁻ chemistry. However, the [4Fe3S] cluster is stable following 1e⁻ and 2e⁻ oxidations due to extensive structural rearrangements.



A dual H_2 oxidase and O_2 reductase

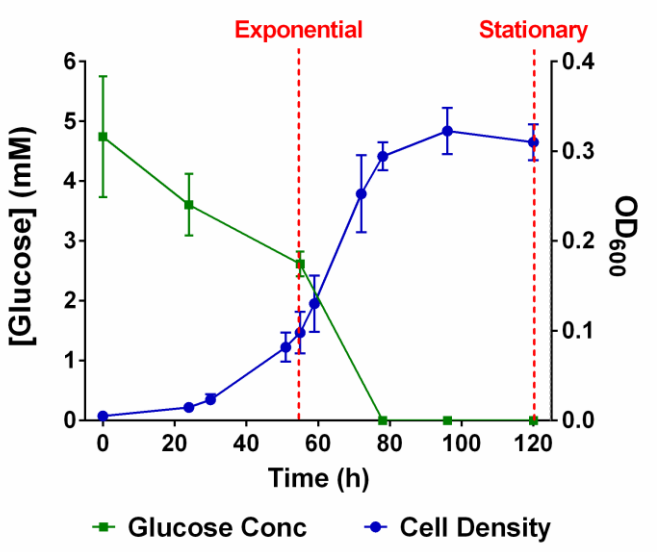
- In its H_2-bound form, *R. eutropha* oxidizes H_2 and transfers the two electrons one-by-one via FeS clusters to ETC. In O_2-bound form, four electrons are transferred via FeS clusters and the O_2 is rapidly reduced to H_2O. Only possible through the $2e^-$ chemistry of the [4Fe3S] cluster.



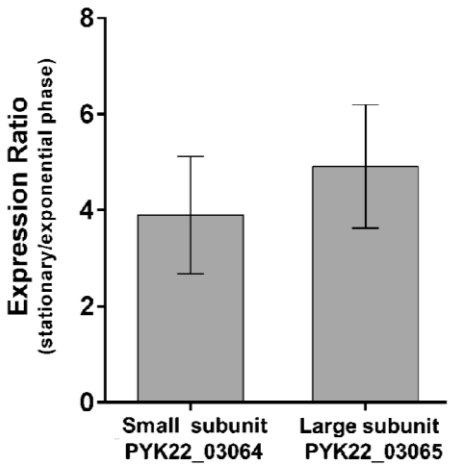
High-affinity oxygen-tolerant hydrogenases

- My work has shown that a majority of soil bacteria encode a novel class of oxygen-tolerant hydrogenase with a nanomolar affinity for H_2. These enzymes are upregulated when organic carbon sources are depleted and enhance survival by scavenging H_2 from the atmosphere.

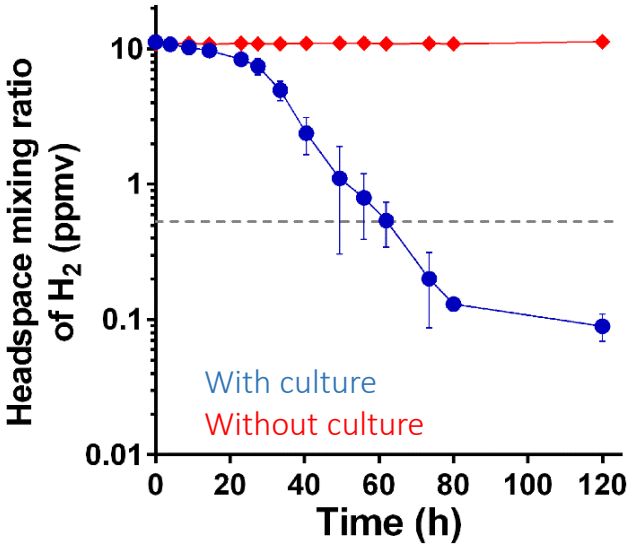
Glucose depletion during growth:



Hydrogenase upregulation during persistence:



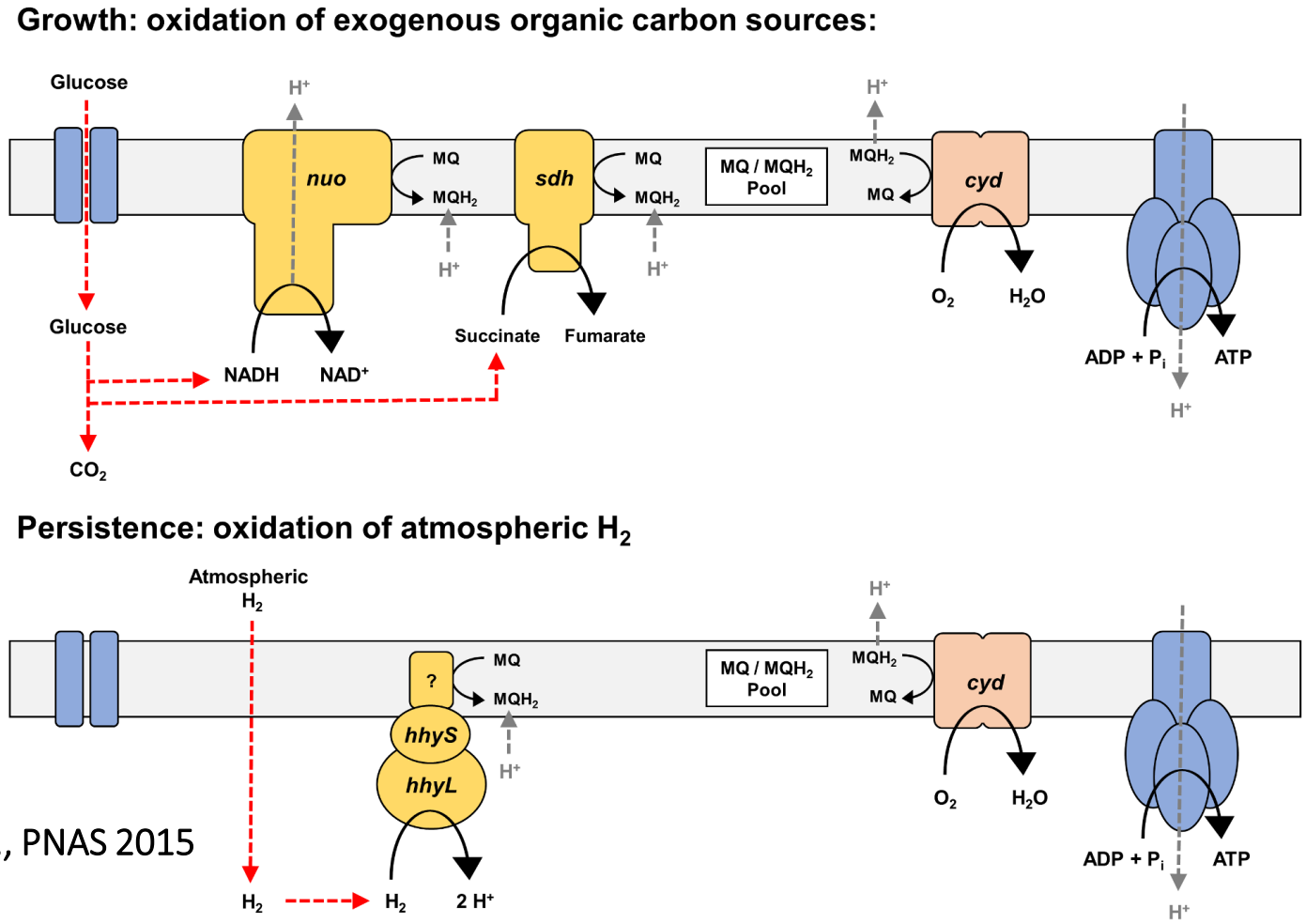
Hydrogen scavenging during persistence:



Greening et al., PNAS 2014
Greening et al., PNAS 2015

A minimalistic strategy for long-term survival

- While atmospheric H_2 is insufficient to sustain growth, consumption of this ubiquitous, diffusible trace gas provides the maintenance energy needed for microorganisms to survive chemically and physically challenging soil conditions.



Greening et al., PNAS 2015

H_2 as a source of primary production

Phototrophy

Energy sources: Sunlight
Carbon sources: CO_2
Primary producers: Phototrophs (e.g. plants, cyanobacteria)
Ecosystems supported: Nearly all ecosystems directly or indirectly

Geothermal chemotrophy

Energy sources: H_2, H_2S, S_0 derived from tectonic activity
Carbon sources: CO_2, CH_4
Primary producers: Obligate anaerobes (e.g. methanogens)
Ecosystems supported: Hydrothermal vents, aquifers
Chapelle et al, Nature 2002; Kelley et al, Science 2005

Atmospheric chemotrophy

Energy sources: H_2, CO, CH_4
Carbon sources: CO_2, CO, CH_4
Primary producers: Obligate aerobes (e.g. actinobacteria)
Ecosystems supported: Hyperarid deserts
Our latest work soon to be published...

Lecture 3: Respiration of inorganic compounds

I. Prokaryotic versatility

II. Nitrification / denitrification

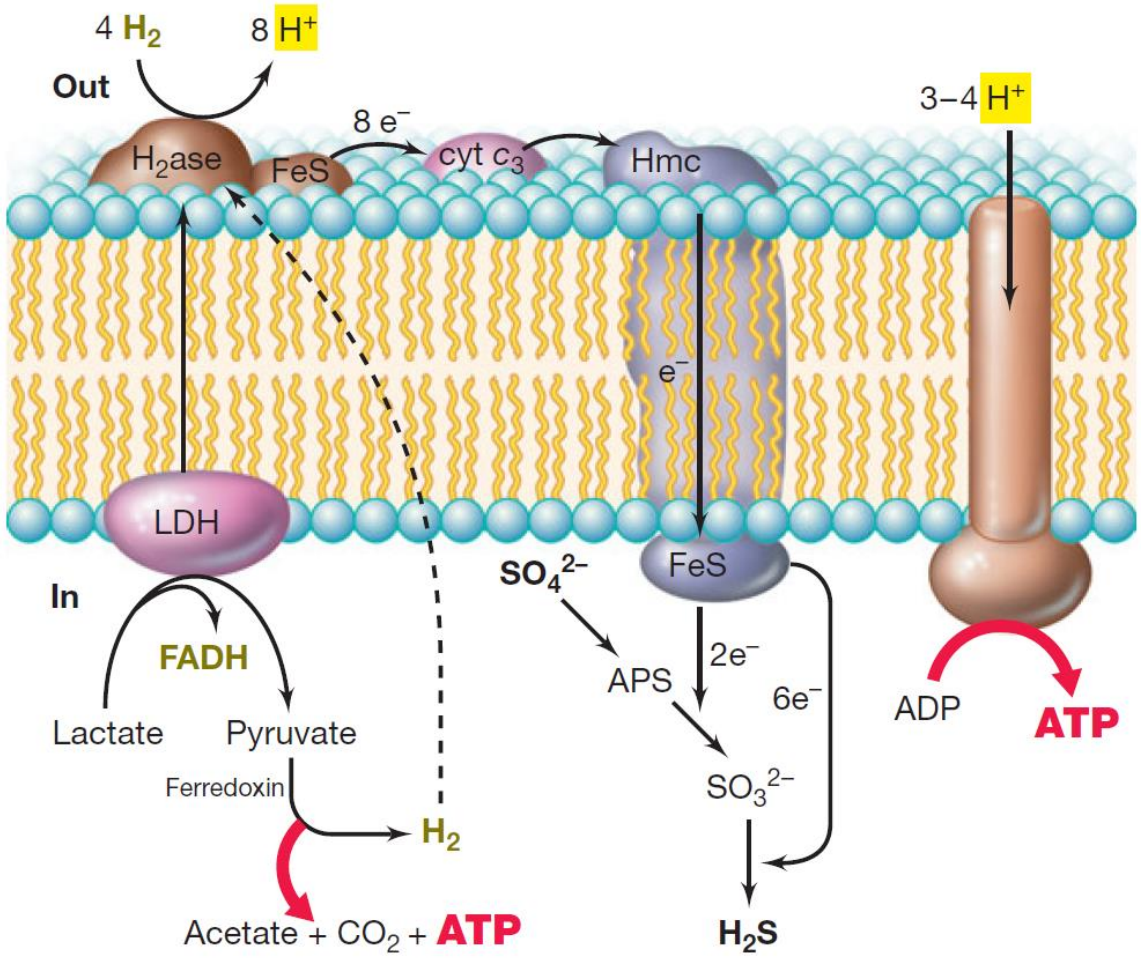
III. Aerobic H_2 respiration

IV. Anaerobic H_2 respiration

Hydrogenotrophic anaerobic respiration

- As H_2 is such a low-potential donor, its oxidation can also be coupled to the reduction various anaerobic e^- acceptors, including sulfate (below), nitrate, fumarate, and CO_2.

- Membrane-bound [NiFe]-hydrogenases input electrons into electron transport chain *via* a *b*-type cytochrome. These are subsequently transferred to a quinone carrier to a terminal reductase. Δp is generated through charge displacement.



Methanogenesis is a hydrogenotrophic process

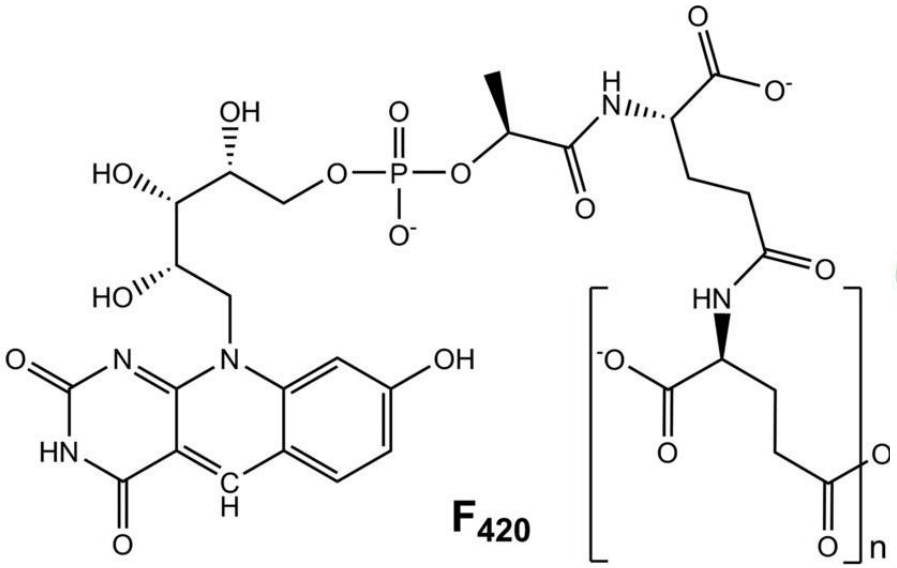
- Methanogens are anaerobic archaea that produce the potent greenhouse gas methane as an endproduct of their metabolism. Full methanogenesis pathways are complicated and outside scope of this course, but some points still worth touching on.

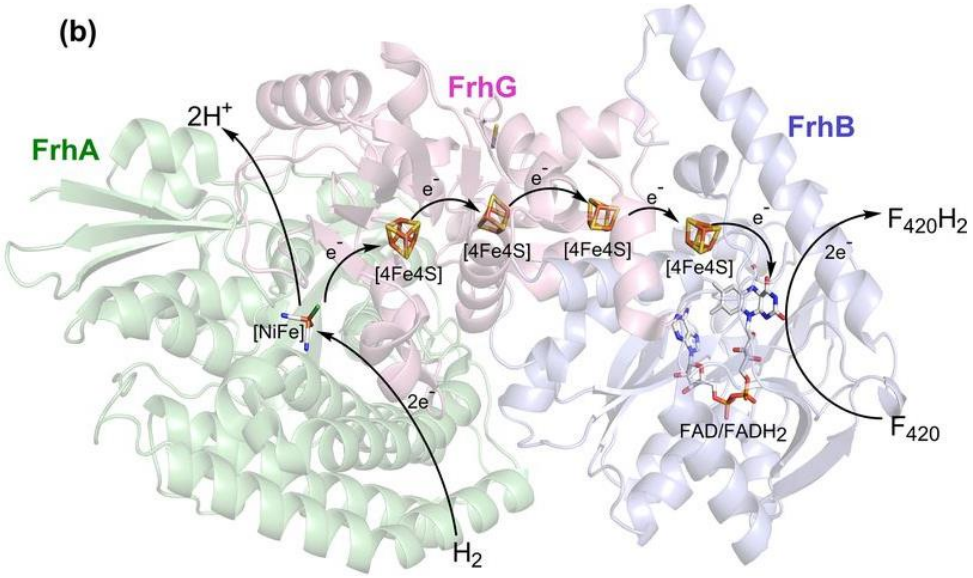
- H_2 provides the reductant for these organisms to reduce CO_2. Two main [NiFe]-hydrogenases:
  - F_{420}-reducing hydrogenase (Frh): Reduces the central catabolic cofactor F_{420}
  - Electron-bifurcating hydrogenase (Mvh): Simultaneously reduces ferredoxin and heterodisulfide

Rudolf Thauer (1939 -): Pioneer in methanogenesis and anaerobic metabolism

F_{420} is a central catabolic cofactor

- F_{420} is a special microbial redox cofactor. It is structurally similar to FAD, but functionally akin NAD. It is low-potential (-0.34 V) cofactor and a obligate $2e^-$ carrier.



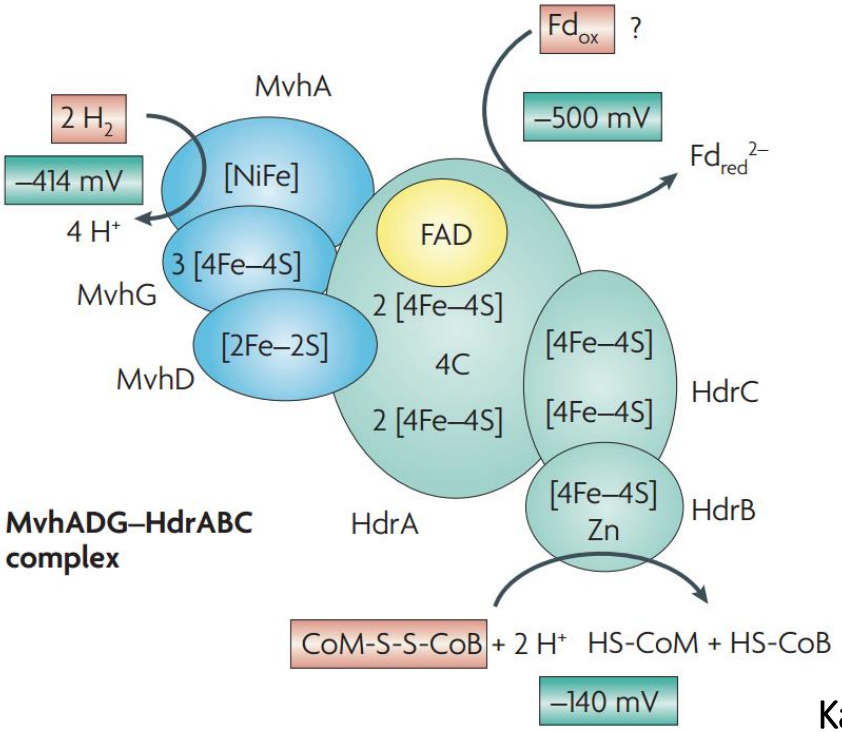


Greening et al., MMBR 2016

- F_{420} is reduced by the cytosolic hydrogenase Frh. $F_{420}H_2$ is used to progressively reduced CO_2. In some methanogens, $F_{420}H_2$ can also be respired using a proton-translocating ancestor to Complex I. This depends on the use of heterodisulfide as an e^- acceptor.

Ferredoxin is reduced by electron-bifurcation

- Ferredoxins are low-potential iron-sulfur clusters. They serve a central role in methanogenesis as the electron donors to several H^+- and Na^+-translocating primary pumps.

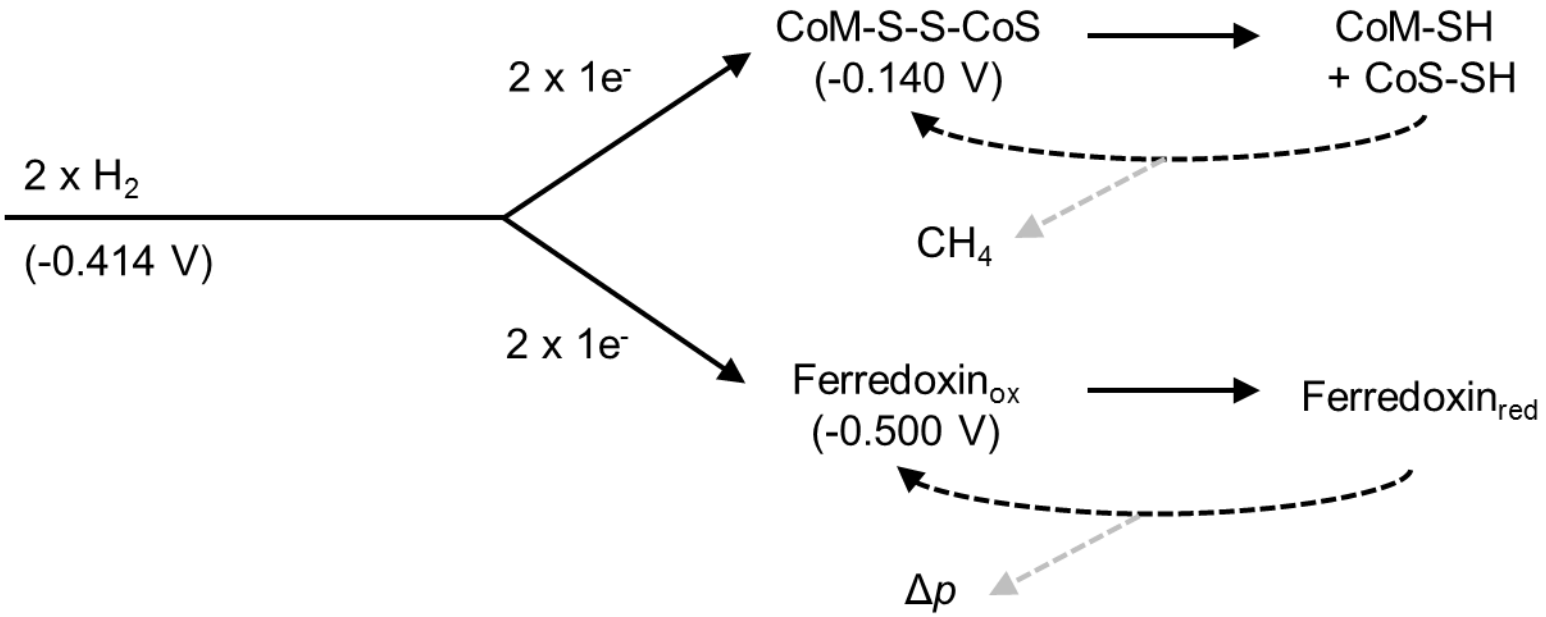


Kaster et al., PNAS 2011

- As ferredoxin has a lower redox potential than H_2, it can only be reduced through electron-bifurcation. In this process, the electrons from H_2 (-0.41 V) are simultaneously passed to the higher-potential heterodisulfide (-0.14 V) and lower-potential ferredoxin (-0.50 mV).

Electron-bifurcation in bacterial metabolism

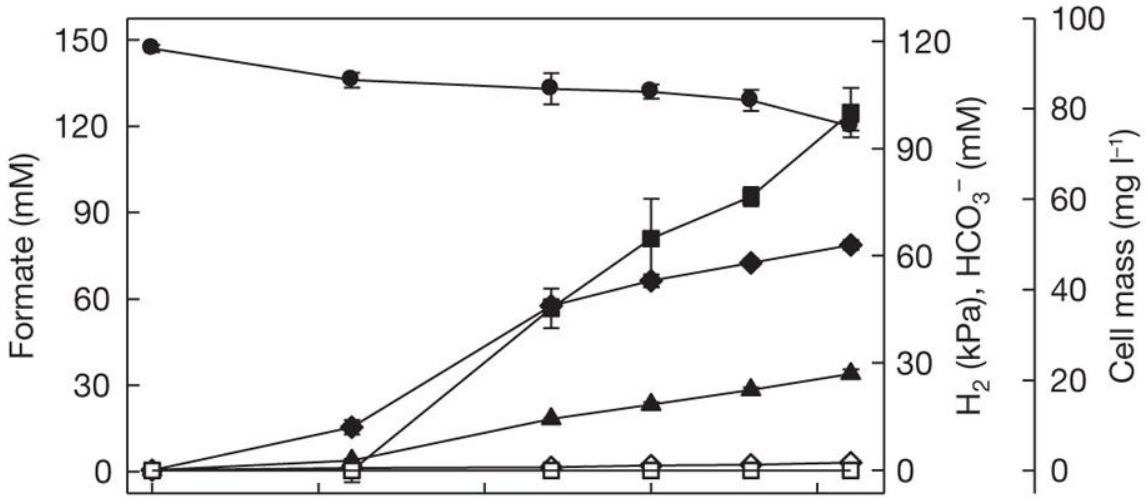
- In electron-bifurcation, electrons from a $2e^-$ donor (e.g. H_2) are simultaneously passed to higher-potential (e.g. heterodisulfide, NAD) and lower-potential (ferredoxin) donors. This makes the endergonic reduction of ferredoxin thermodynamically favourable.



- Once thought to be a quirk of Complex III. However, work led by Thauer over last five years has shown electron-bifurcation is a dominant mechanism of energy-conservation in anaerobic bacteria, including in methanogenesis, acetogenesis, and fermentation.

Formate-coupled proton respiration

- It was recently discovered that the deep-sea thermophilic archaeon *Thermococcus onnurineus* can grow by respiring formate (-0.432 V) as the sole e^- donor and protons (-0.414 V) as the sole e^- acceptor resulting in H_2 evolution.



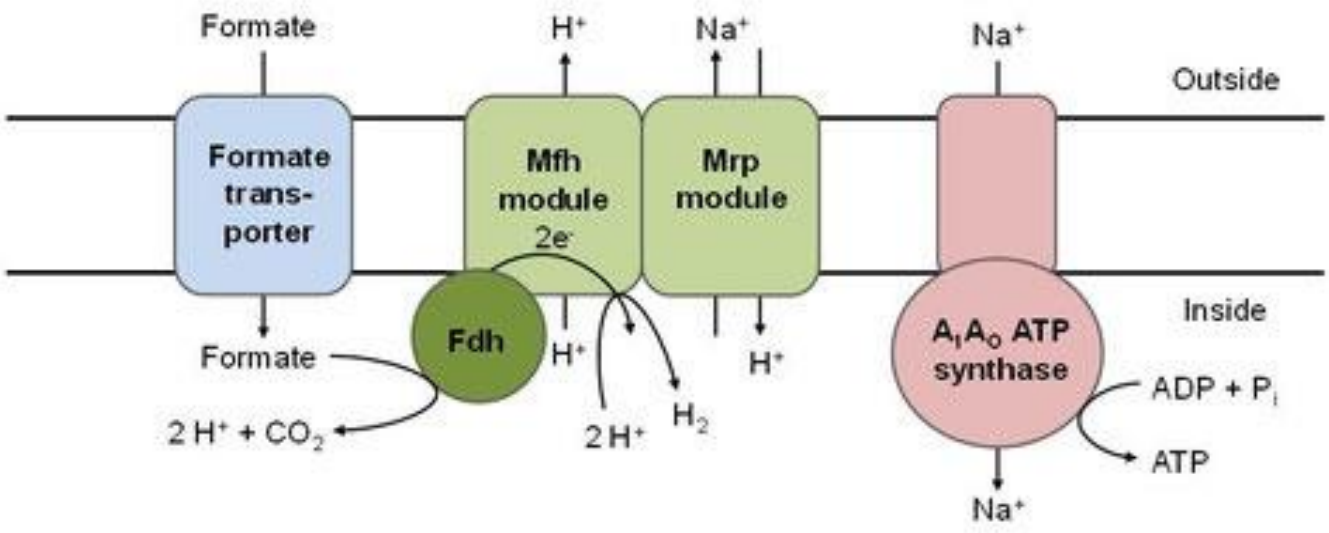
Kim et al, 2010

Changes in cell mass (squares), formate (circles), H_2 (diamonds) and CO_2 (triangles) during growth of *Thermococcus onnurineus*

- While formate-coupled H_2 production is central in fermentation, it wasn't thought to support respiration as energy change between donor and acceptor is so low (+0.018 V, -3.5 kJ mol^{-1}). Only worthwhile for those organisms living in the most deprived environments.

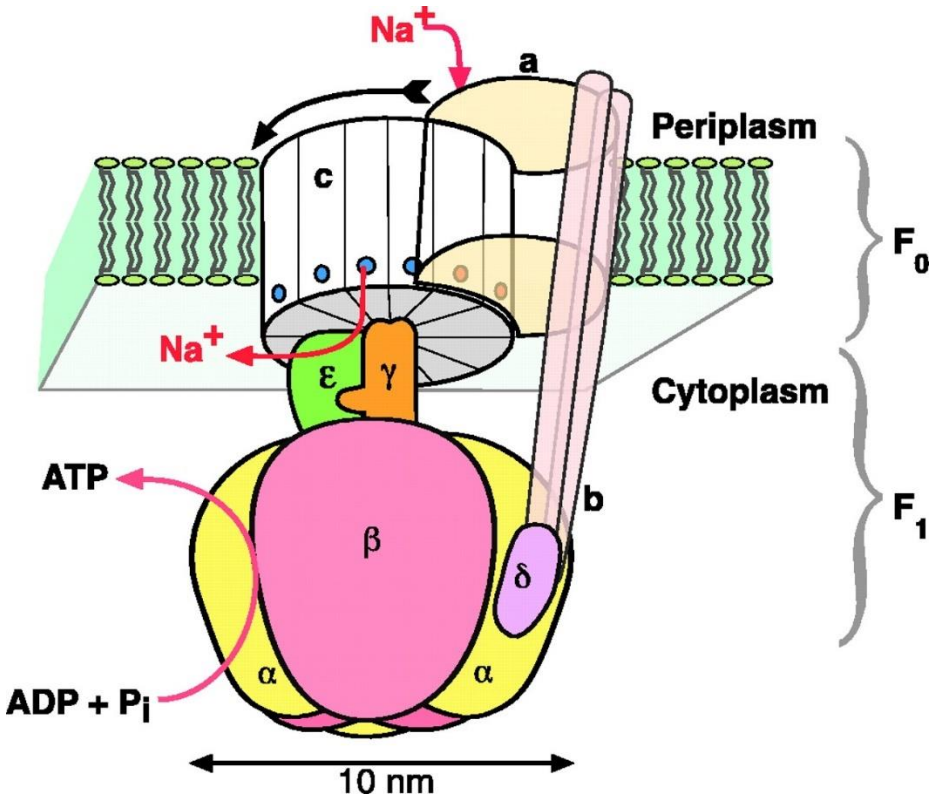
Respiratory minimalism

- *Thermococcus onnurineus* is able to conserve energy of formate-proton couple with a highly efficient complex that serves as minimalistic respiratory chain. Three main components:
  - Fdh module: oxidises the e^- donor formate to CO_2
  - Mfh module: oxidises the e^- acceptor H^+ to H_2
  - Mrp module: uses conformational changes of e^- transfer to generate electrochemical gradient



Sodium-motive force

- Many anaerobes, including *T. onnurineus*, use Na^+ instead of H^+ as the coupling ion to drive ATP synthesis. Like Δp, sodium-motive force ($\Delta\mu Na^+$) is a transmembrane electrochemical gradient that is the sum of the membrane potential ($\Delta\Psi$) and [Na^+] gradient (ΔpNa^+).



- The primary pumps used to generate $\Delta\mu Na^+$ differ from those of standard respiration. The ATP synthase used is also modified so that the *c* ring translocates Na^+ instead of H^+.

Lecture summary

- Microorganisms are highly flexible in their metabolism, with capacities to oxidise organic and inorganic fuel sources, respire aerobically and anaerobically, and ferment persistently.

- Respiratory flexibility allows microbes to prosper in every environment. Primary production in deep-sea vents and hyperarid deserts is driven by microbial chemotrophy.

- Nitrification is a specialist metabolism in which ammonia and nitrite are aerobically oxidised. The reverse pathway denitrification is a widespread mechanism of anaerobic respiration.

- Hydrogenases are metalloenzymes that catalyse the reversible heterolysis of H_2. While all hydrogenases are inactivated by O_2, some can reactivate using a unique FeS cluster.

- Anaerobic respiration is made possible by a range of unusual adaptations, including electron-bifurcation, minimalistic respiratory chains, and use of sodium-motive force.

Recommended reading

Recommended reading:

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

Comprehensive, up-to-date textbook on bioenergetics.

Schwartz E, Fritsch J, Friedrich B (2013). H_2-metabolizing prokaryotes. In The Prokaryotes.

An excellent review on the biochemistry and microbiology of H_2 metabolism.

All available for download at greeninglab.com

Essay Question

Describe how transmembrane complexes use redox reactions to generate electrochemical gradients