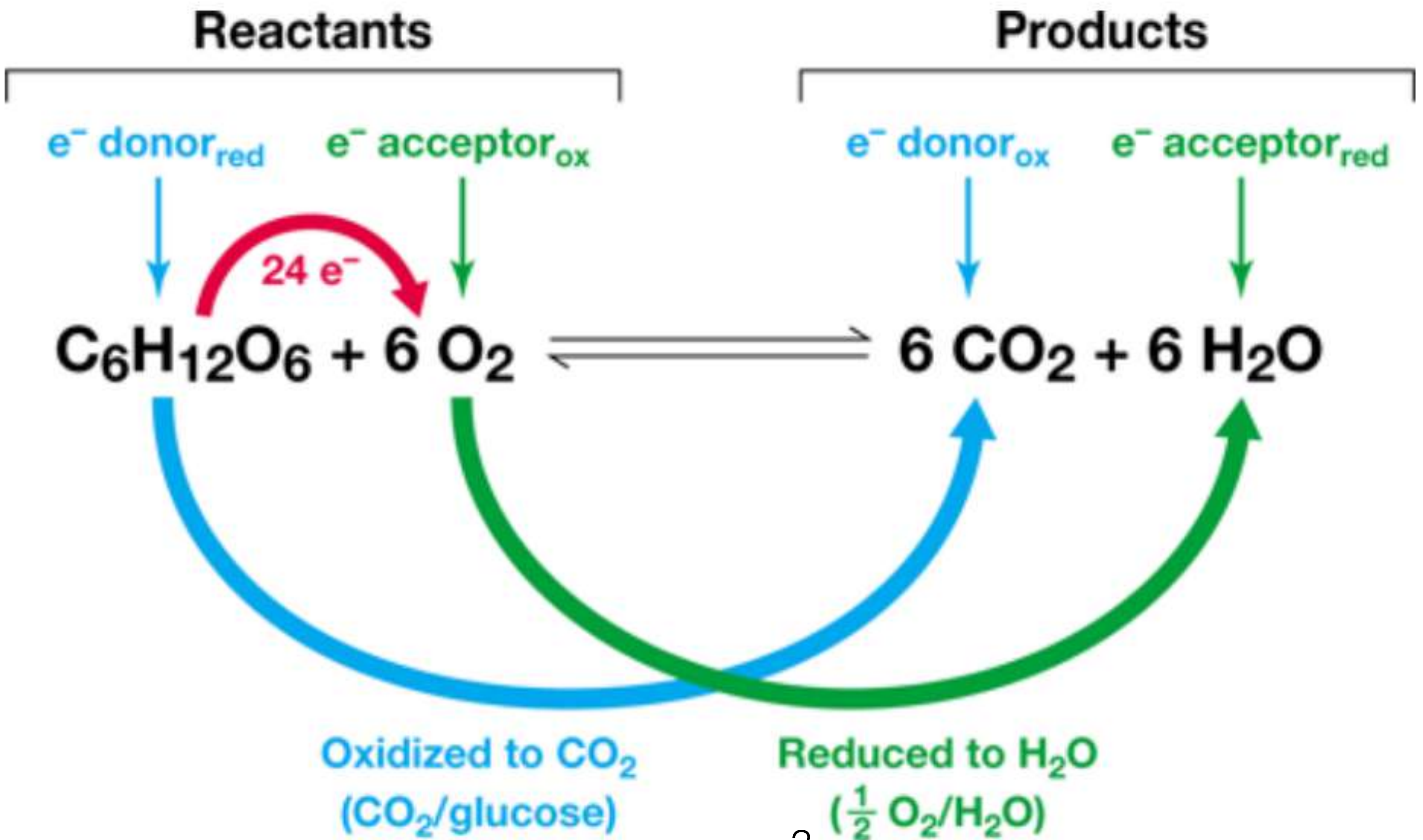
L03b

Recap L03

Fundamentals in Metabolisms

- Conserve energy in ATP
- Reactions are not performed in single-step —> consecutive reactions in different part of the cells
- Transfer e-/ need of soluble e- carriers: NAD+/NADH, FAD+/FADH2, NADP+/NADPH



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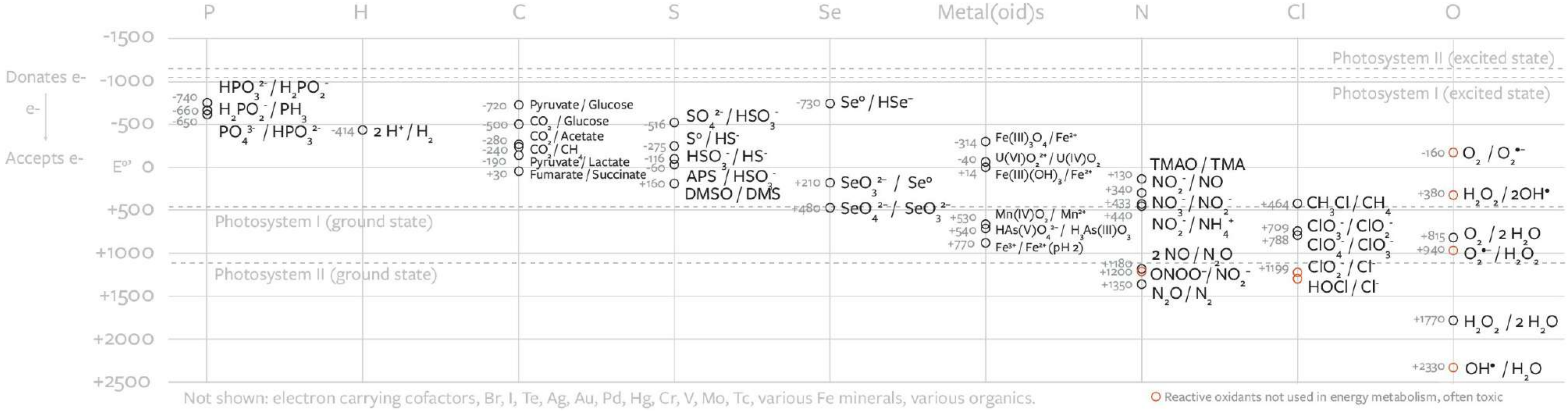
Microbial Redox couples

Redox couples and potentials (mV) for elements common in biology at pH 7 and temperature 25 C *

Redox potential indicates the propensity for a compound to transfer electrons to another compound. A more-negative redox potential means a compound is more likely to donate electrons (e-).
All of life gets its energy by capturing the change in potential energy from the transfer of electrons from the reducing compound to the oxidizing compound.



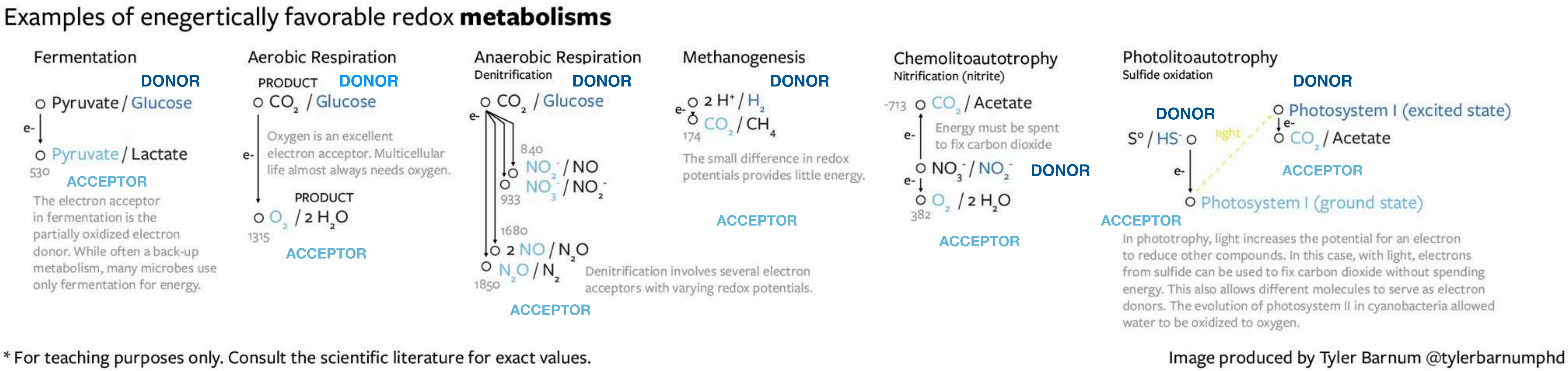
- **Across periodic table**

- **P, H, C, S, Se, Fe, U, Mn, As, N, Cl, O**

* For teaching purposes only. Consult the scientific literature for exact values.

Image produced by Tyler Barnum @tylerbarnumphd

Microbial Redox couples structure the metabolism



Examples of enegertically favorable redox **metabolisms**

The electron acceptor in fermentation is the partially oxidized electron donor. While often a back-up metabolism, many microbes use only fermentation for energy.

Oxygen is an excellent electron acceptor. Multicellular life almost always needs oxygen.

Denitrification involves several electron acceptors with varying redox potentials.

The small difference in redox potentials provides little energy.

Energy must be spent to fix carbon dioxide

In phototrophy, light increases the potential for an electron to reduce other compounds. In this case, with light, electrons from sulfide can be used to fix carbon dioxide without spending energy. This also allows different molecules to serve as electron donors. The evolution of photosystem II in cyanobacteria allowed water to be oxidized to oxygen.

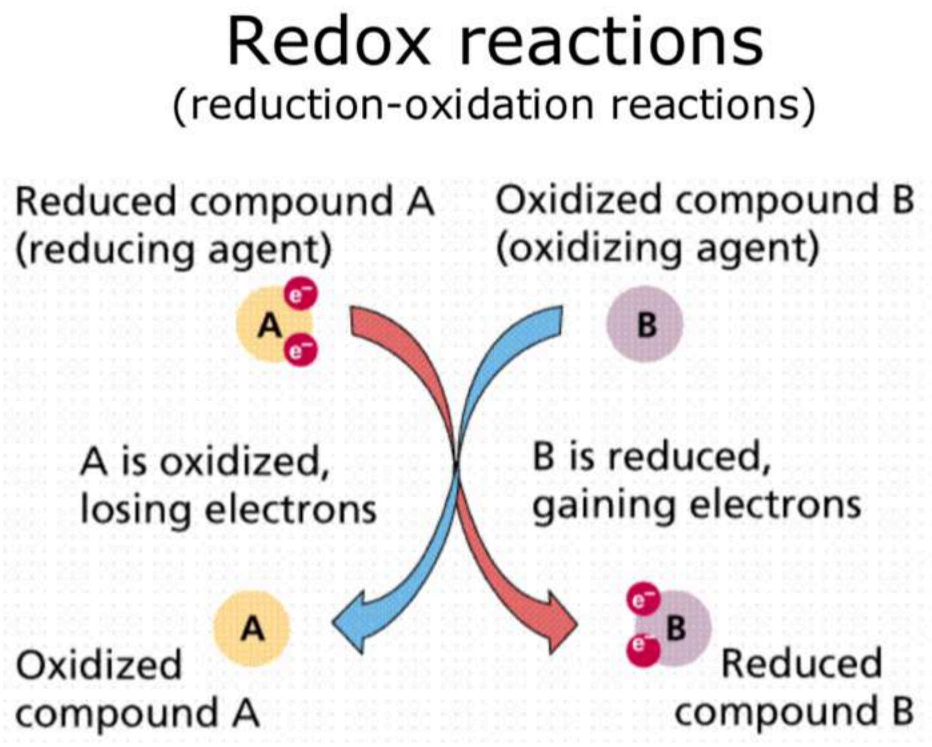
* For teaching purposes only. Consult the scientific literature for exact values.

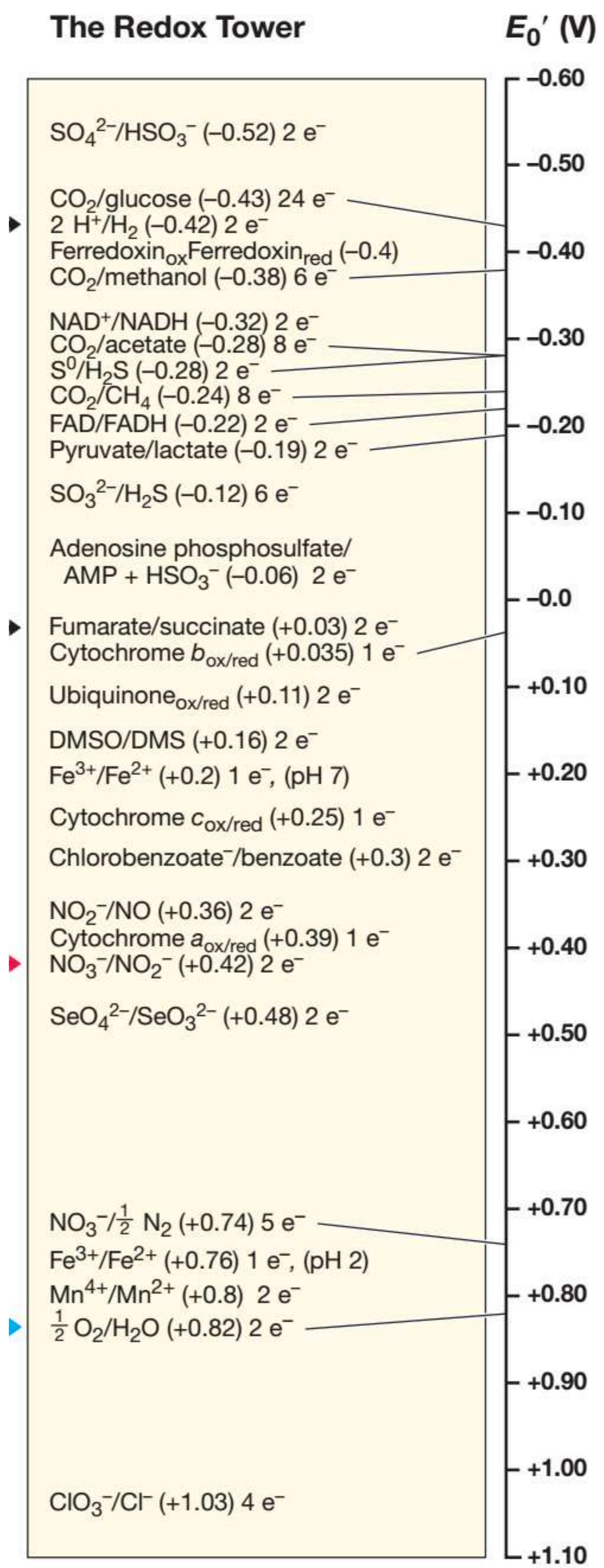
Image produced by Tyler Barnum @tylerbarnumphd

The Redox Tower

- Redox couples are arranged from the **strongest e- donors at the top (E_0'<0)** to the strongest e- acceptors at the bottom (E_0'>0)

- **The larger the difference in reduction potential between electron donor and electron acceptor, the more free energy is released** (ΔG_0' can be computed via Nernst equation from reduction potential)





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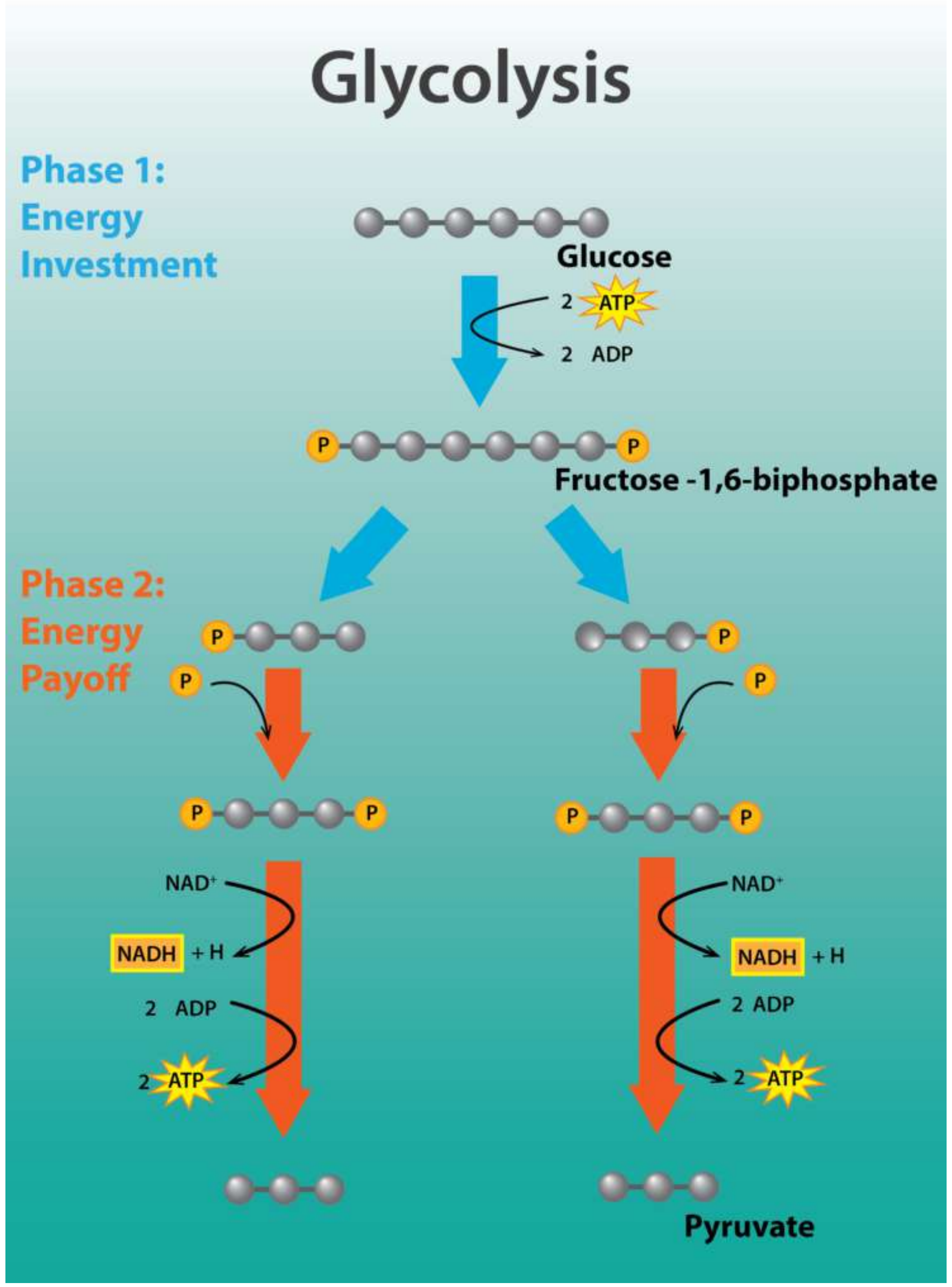
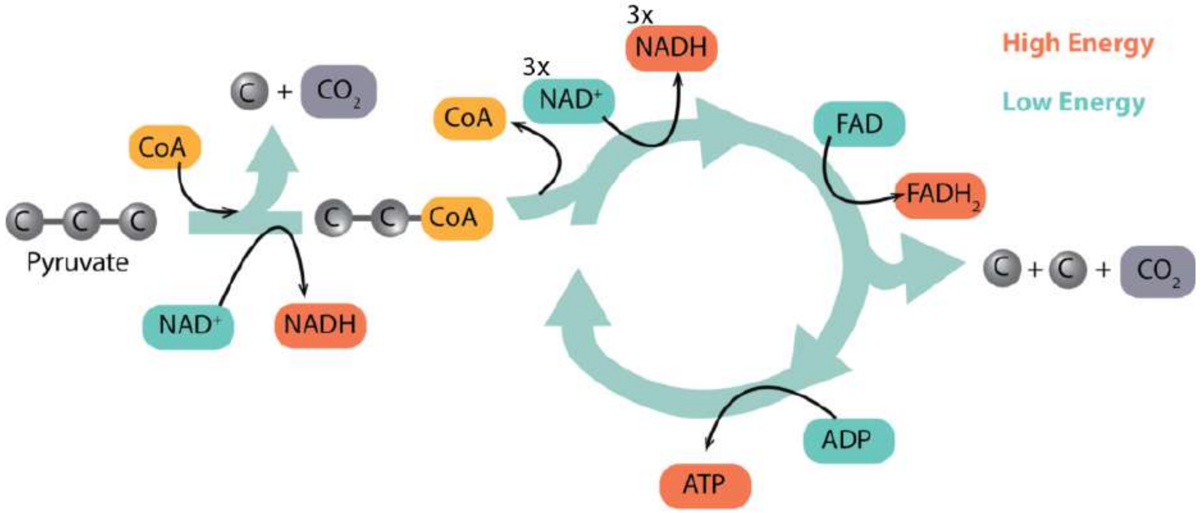
Central metabolism



https://open.oregonstate.education/generalmicrobiology/chapter/chemoorganotrophy/



Tricarboxylic Acid Cycle (TSA)

Citric acid cycle (CAC)

Krebs cycle

- **Creates a little pool of ATP**
- **Creates reducing power —> ETC anaerobic and aerobic respiration**

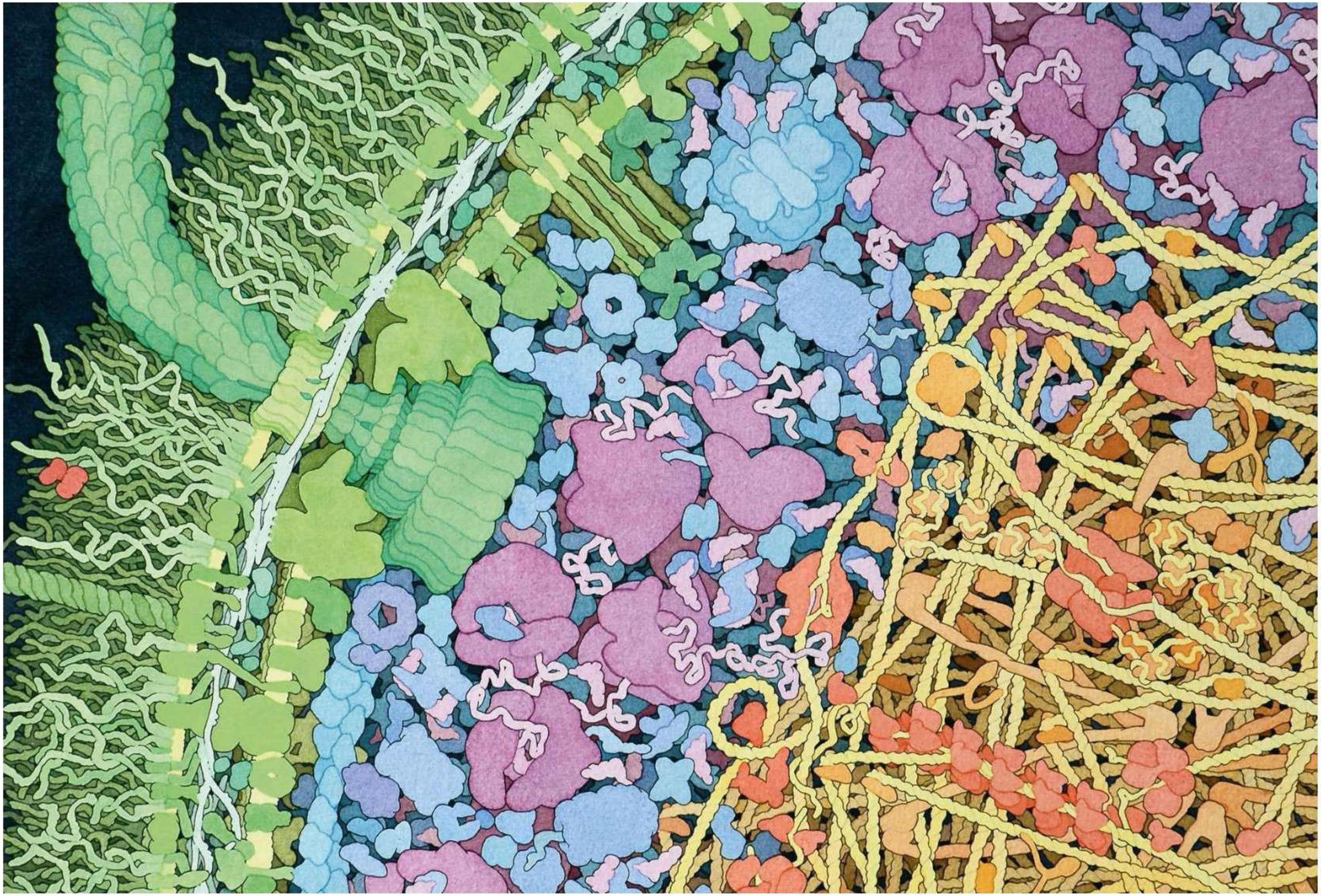
Substrate-Level-Phosphorylation

- Glycolysis can generate ATP in the absence of oxygen: anaerobic metabolism
- Glycolysis and citric acid cycle (CAC) result from substrate-level phosphorylation (SLP)
- SLP is distinct from oxidative phosphorylation that occurs in ETC
- Substrate-level phosphorylation refers to the formation of ATP from ADP and a phosphorylated intermediate, rather than from ADP and inorganic phosphate, Pi, as is done in oxidative phosphorylation (ET)

FOCUS

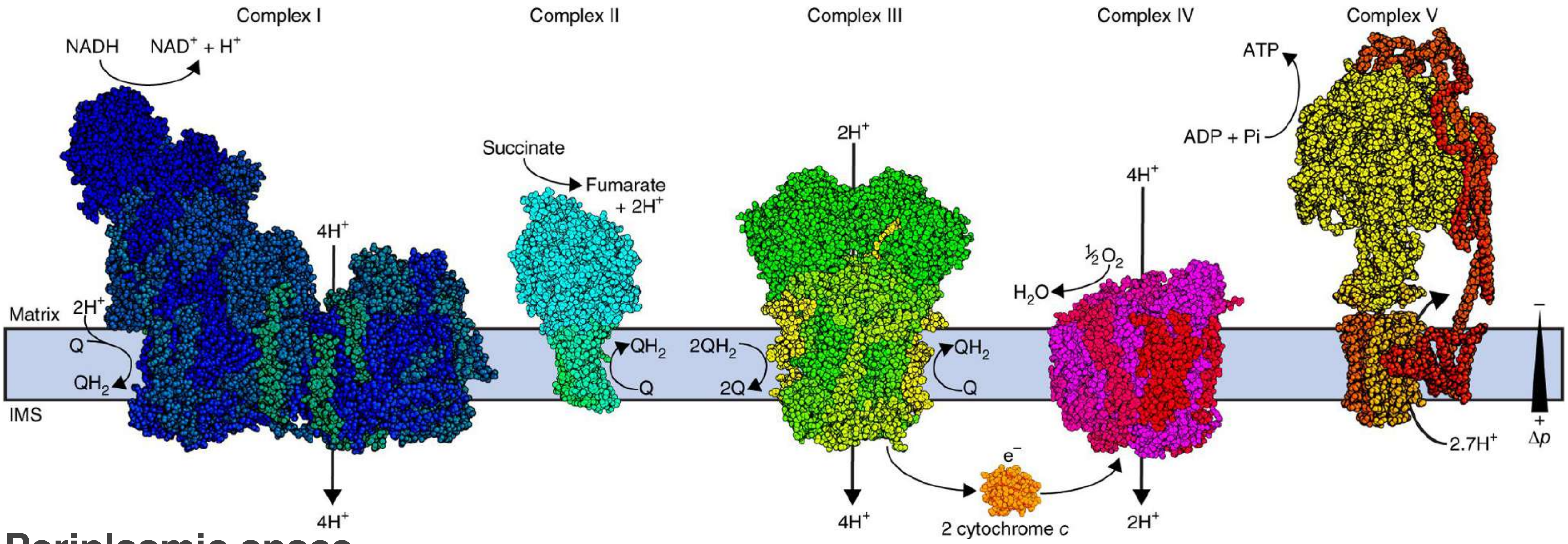
- Electron transport chain (ETC)
- Fermentation
- Aerobic/Anaerobic respiration
- Oxygenic and Anoxygenic Photosynthesis
- Chemolithotrophy

David S. Goodsell



Electron transport chain (ETC), I

Cytoplasm



Periplasmic space

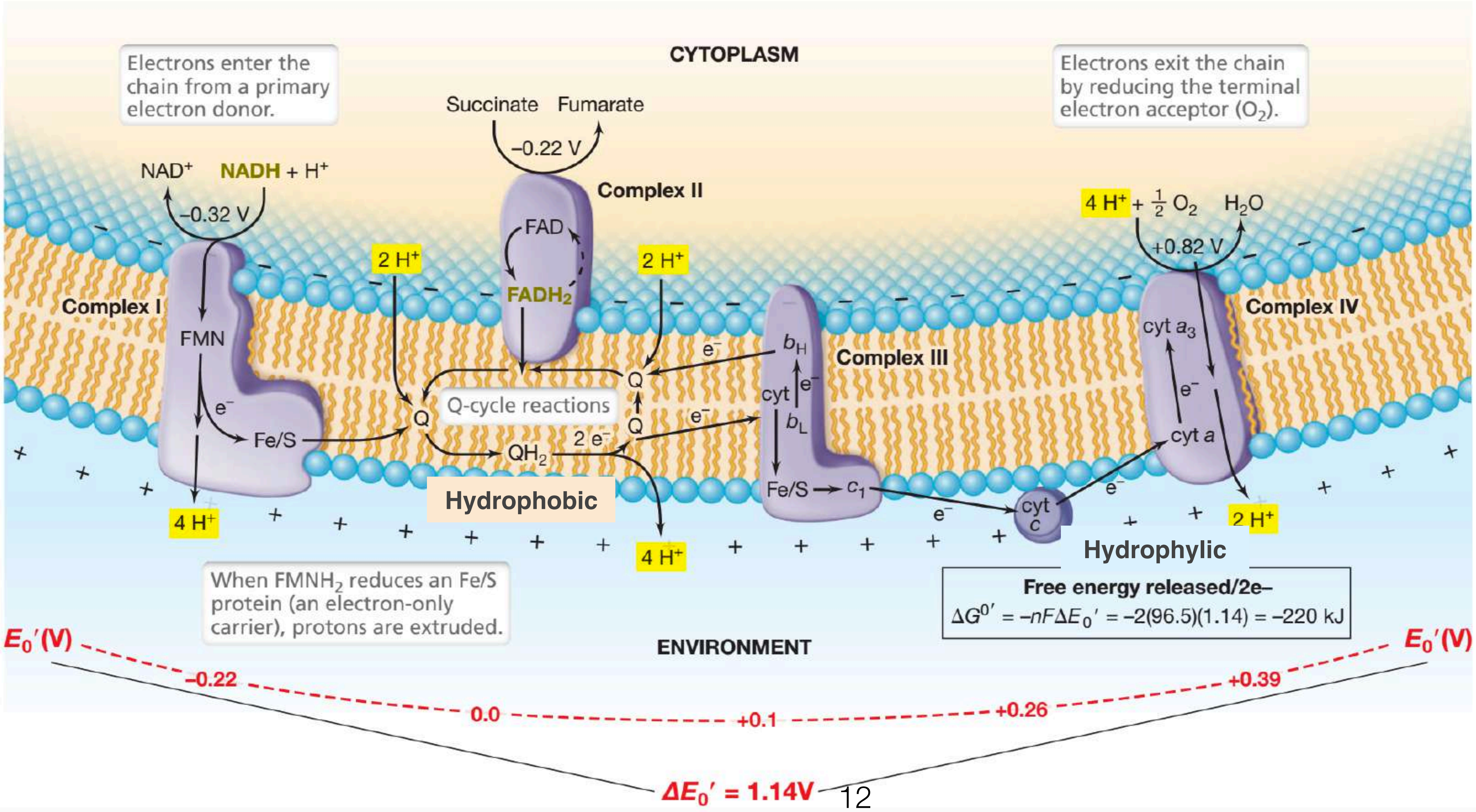
- In the membrane
- Intimate interaction between proteins (dehydrogenase, flavoproteins, iron-sulfur proteins) and diffusible molecules (quinons and cytochromes)
- Electrons are swapped
- Protons are pumped outside the cell (cytoplasm —> periplasmic space)

Electron transport chain, II

- A set of membrane-bound electron carriers (4) organized from **high to low redox** potentials —> **spontaneous** flow of electrons to the **terminal electron acceptor**

- The **membrane carriers are not structurally linked** so they can **diffuse** laterally in the membrane and collide with one another to promote the rapid exchange of electrons

- *Escherichia coli* uses lipophilic organic molecules called **quinones to electronically link a dehydrogenase enzyme complex to a specific terminal reductase**



Electrons enter the chain from a primary electron donor.

Electrons exit the chain by reducing the terminal electron acceptor (O_2).

When $FMNH_2$ reduces an Fe/S protein (an electron-only carrier), protons are extruded.

Free energy released/2e–

$\Delta G^{0\prime} = -nF\Delta E_0{}' = -2(96.5)(1.14) = -220$ kJ

Madigan et al. 2018

Electron transport chain, III

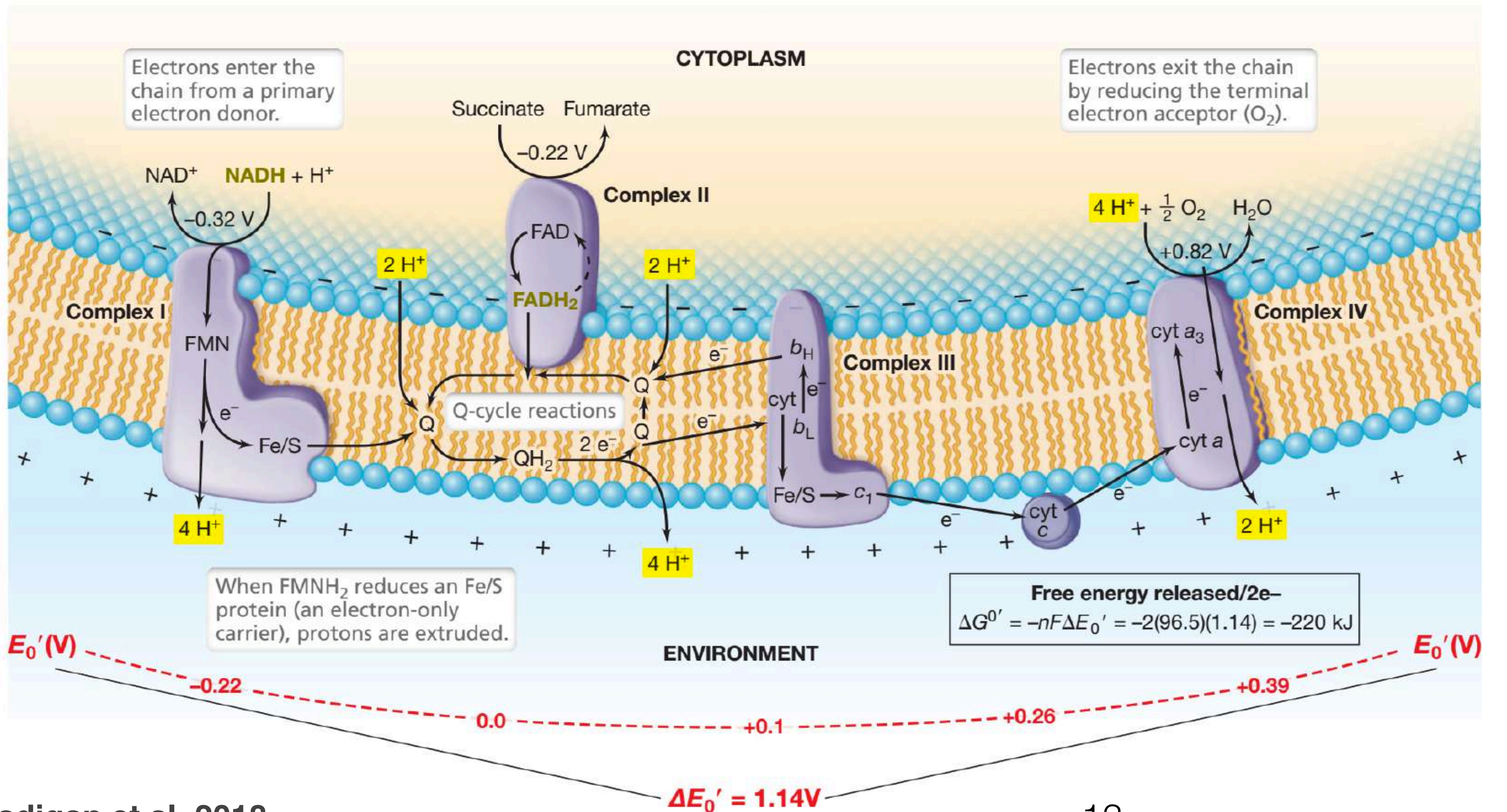
General features:

(1) **Carriers** are arranged in order of **increasingly more positive E_0'** (reduction potential)

(2) **Alternation of electron-only and electron-plus-proton carriers** in the chain

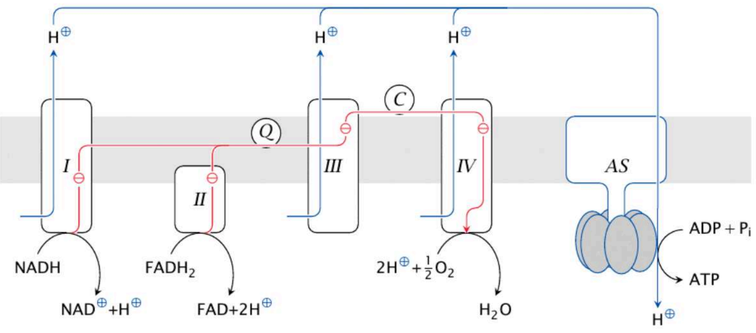
(3) Net result is **reduction of terminal electron acceptor** (such as O_2) + **generation proton motive force** (PMF, thanks to harnessing e^- flow)

(4) ATP production by PMF (ATP synthesis is driven by an ion gradient through the activity of ATP synthase)



Environment

H^+ flow



e^- flow

Cytoplasm

Madigan et al. 2018

Electron transport chain, III

General features:

(1) **Carriers** are arranged in order of **increasingly more positive E_0'** (reduction potential)

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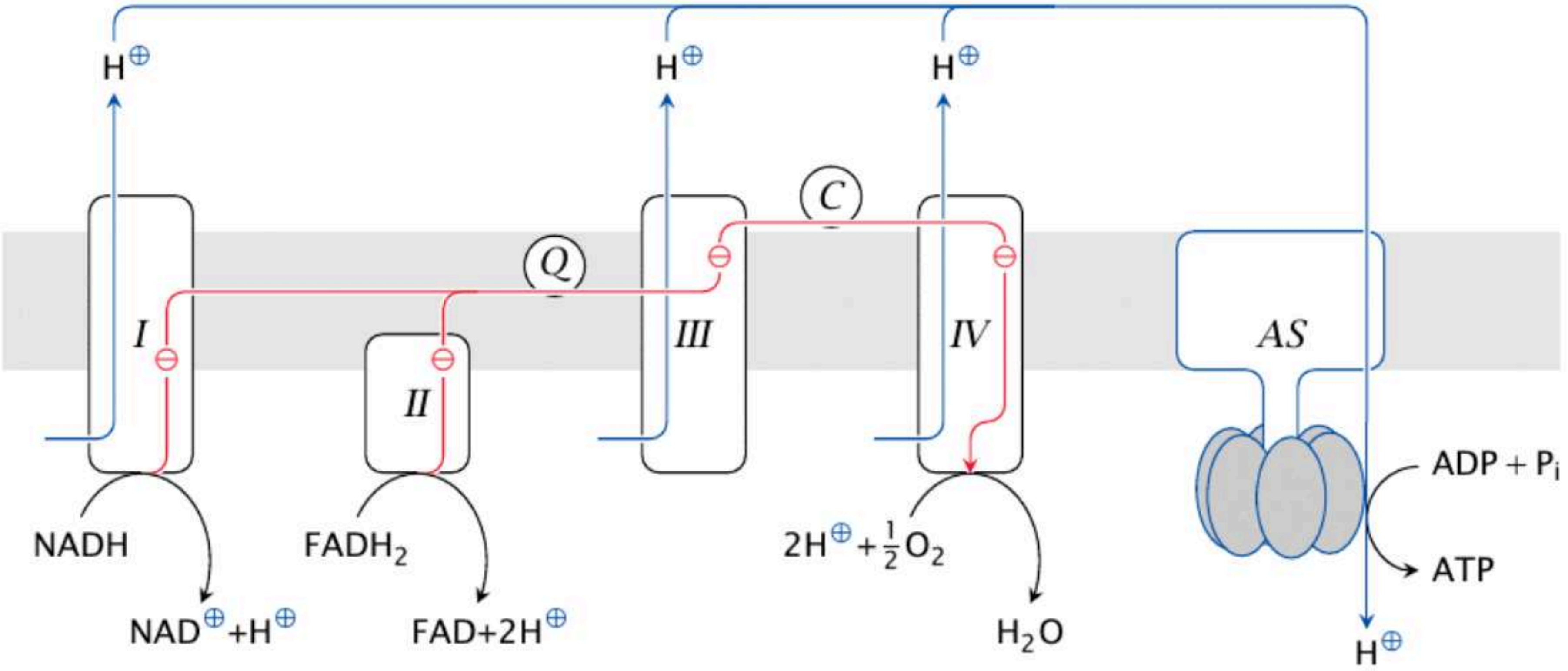

H^+ flow

e^- flow

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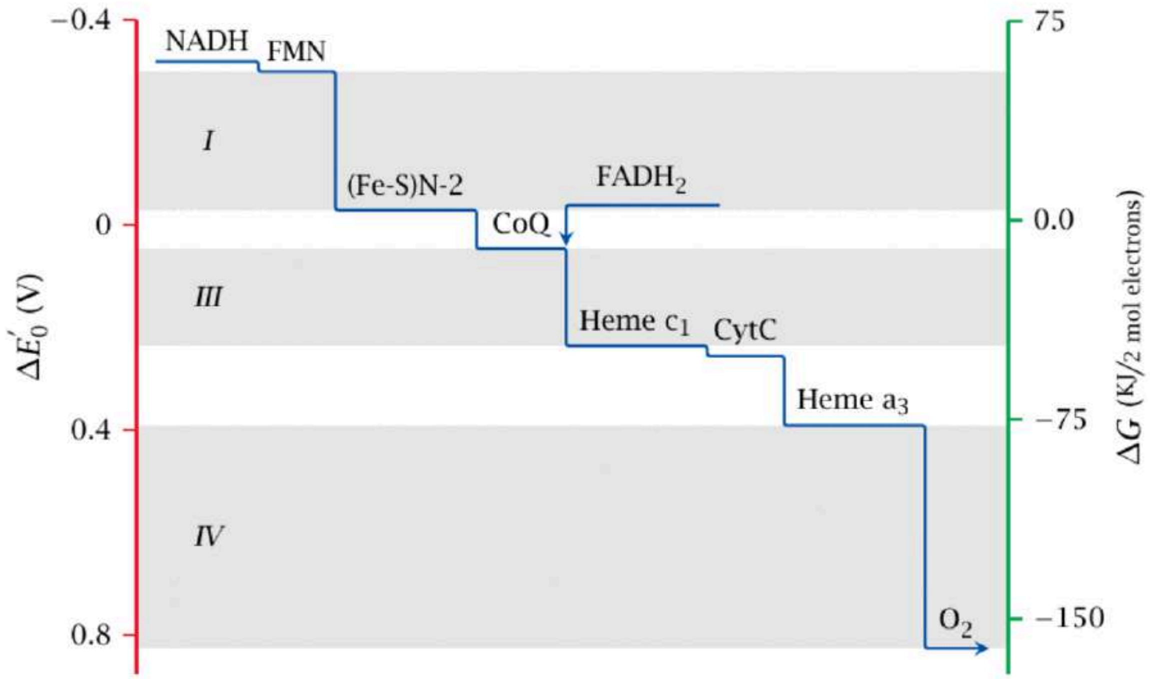
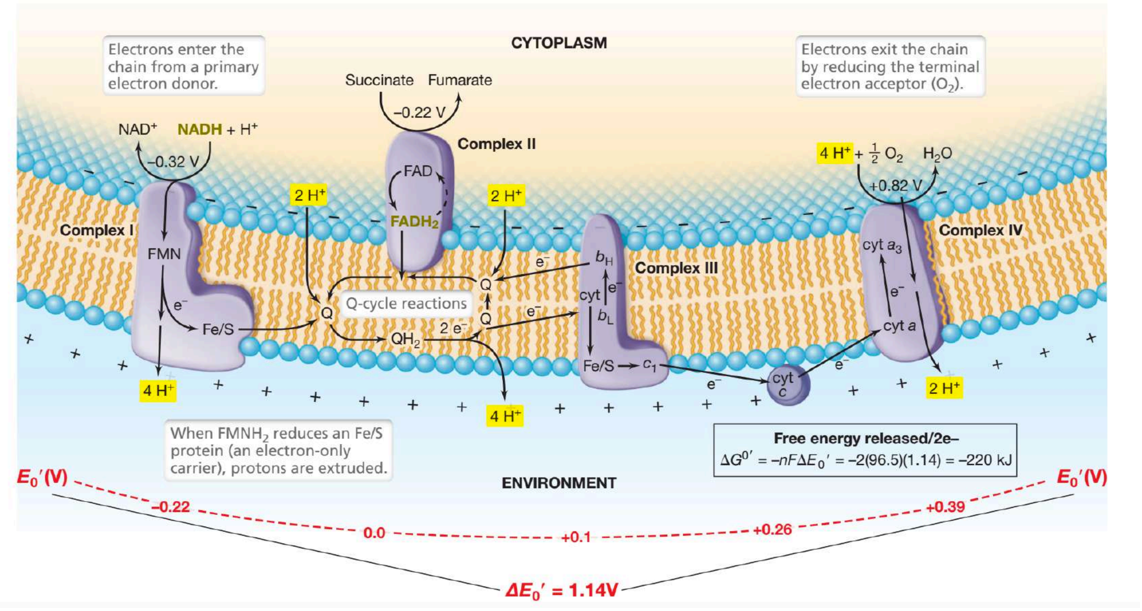
Structural orientation for ATP production

Redox potentials and free energies in the respiratory chain



http://watcut.uwaterloo.ca/webnotes/Metabolism/RespiratoryChain.html



Electrons enter the chain from a primary electron donor.

Electrons exit the chain by reducing the terminal electron acceptor (O_2).

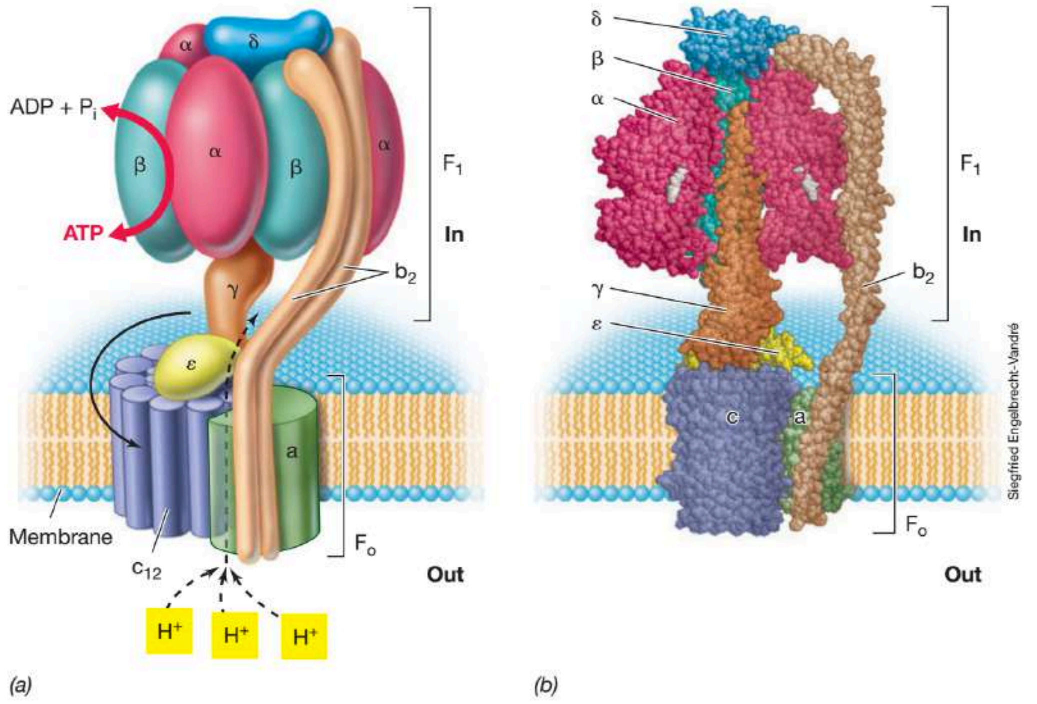
When $FMNH_2$ reduces an Fe/S protein (an electron-only carrier), protons are extruded.

Free energy released/2e−
$\Delta G^{0\prime} = -nF\Delta E_0' = -2(96.5)(1.14) = -220$ kJ

- Spontaneous flow of electrons (E_0')
- H^+ are separated from e^- across membrane (spatial localization ETC)
- Inner and outer surfaces of the membrane differ in charge, pH, and electrochemical potential
- Electrochemical potential is proton motive force (PMF) and energizes the membrane, much like a battery
- Only three of the four mentioned electron carriers are capable of transporting protons from the matrix to the intermembrane space: I, III, and IV

Madigan et al. 2018

ATP production



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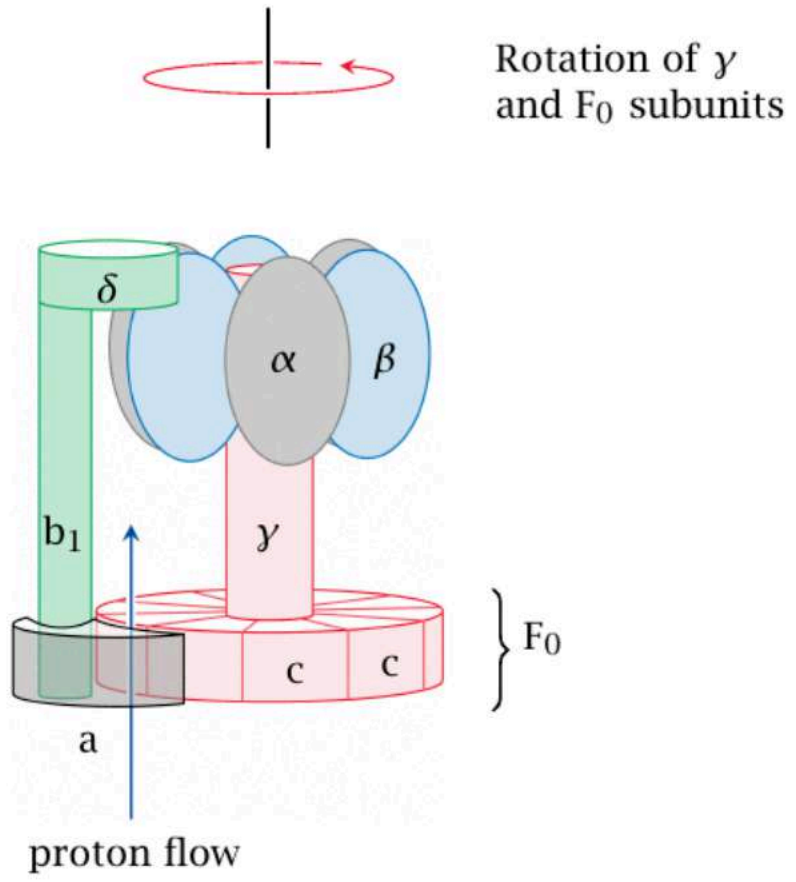
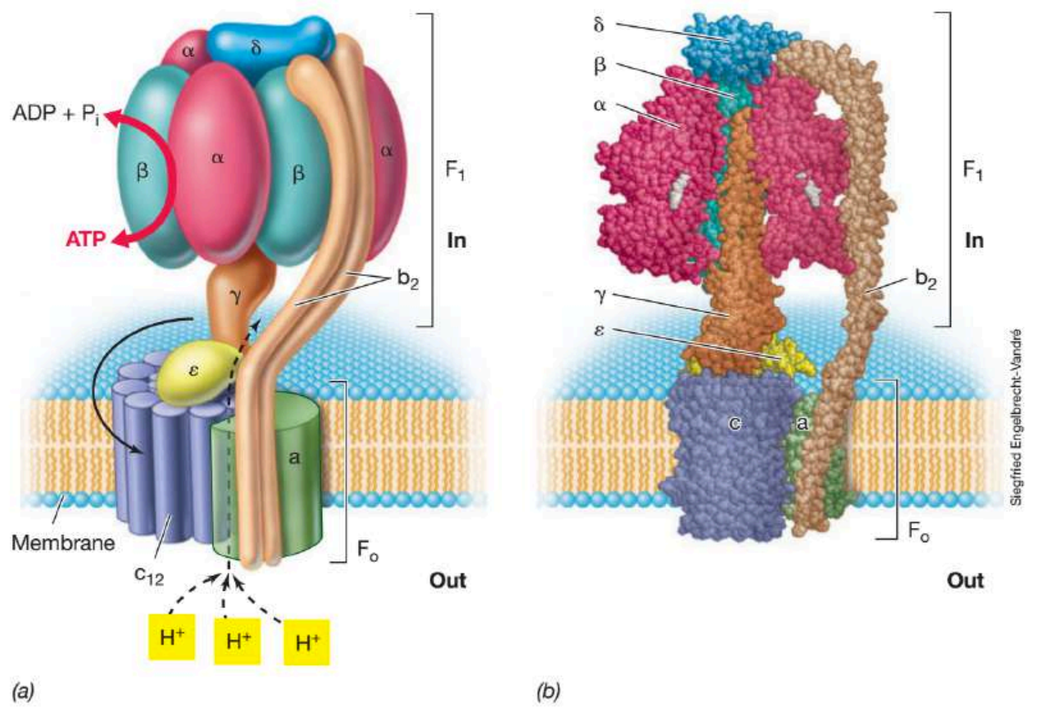


http://watcut.uwaterloo.ca/webnotes/Metabolism/RespiratoryChain.html

- H^+ gradient that drives phosphorolation of ADP to ATP as well as several other important transport systems (nutrient transport, flagellar rotation, and other energy-requiring reactions)
- 3 H^+ —> ATP (Noguchi et al., 2004): F1 is the catalytic complex responsible for the interconversion of ADP + Pi and ATP. Fo, the rotor, is integrated in the membrane

ATP production



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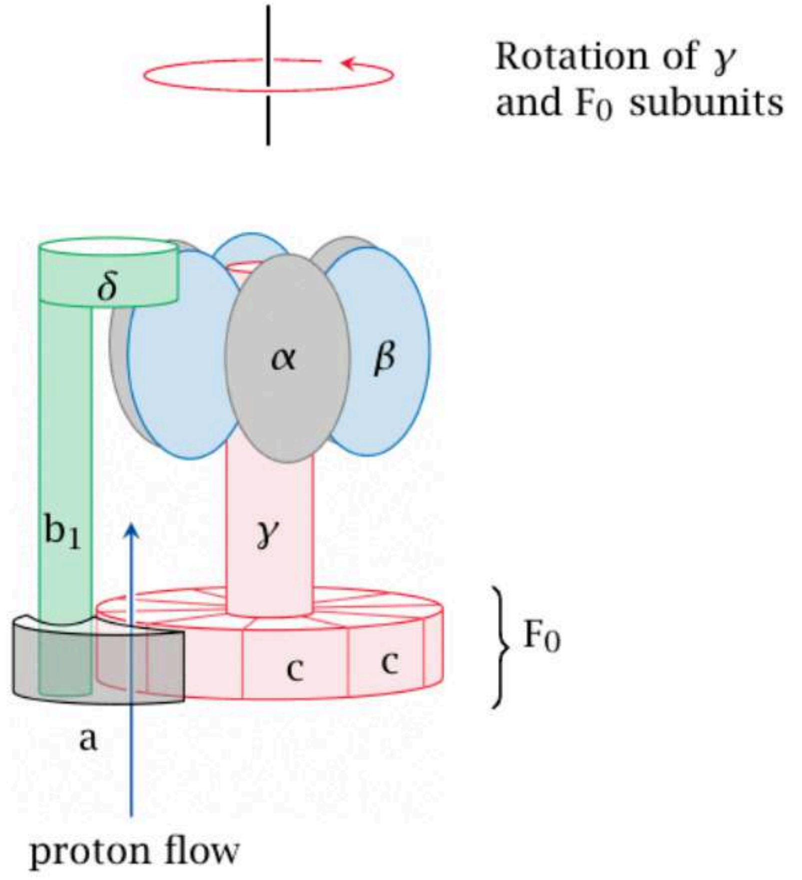
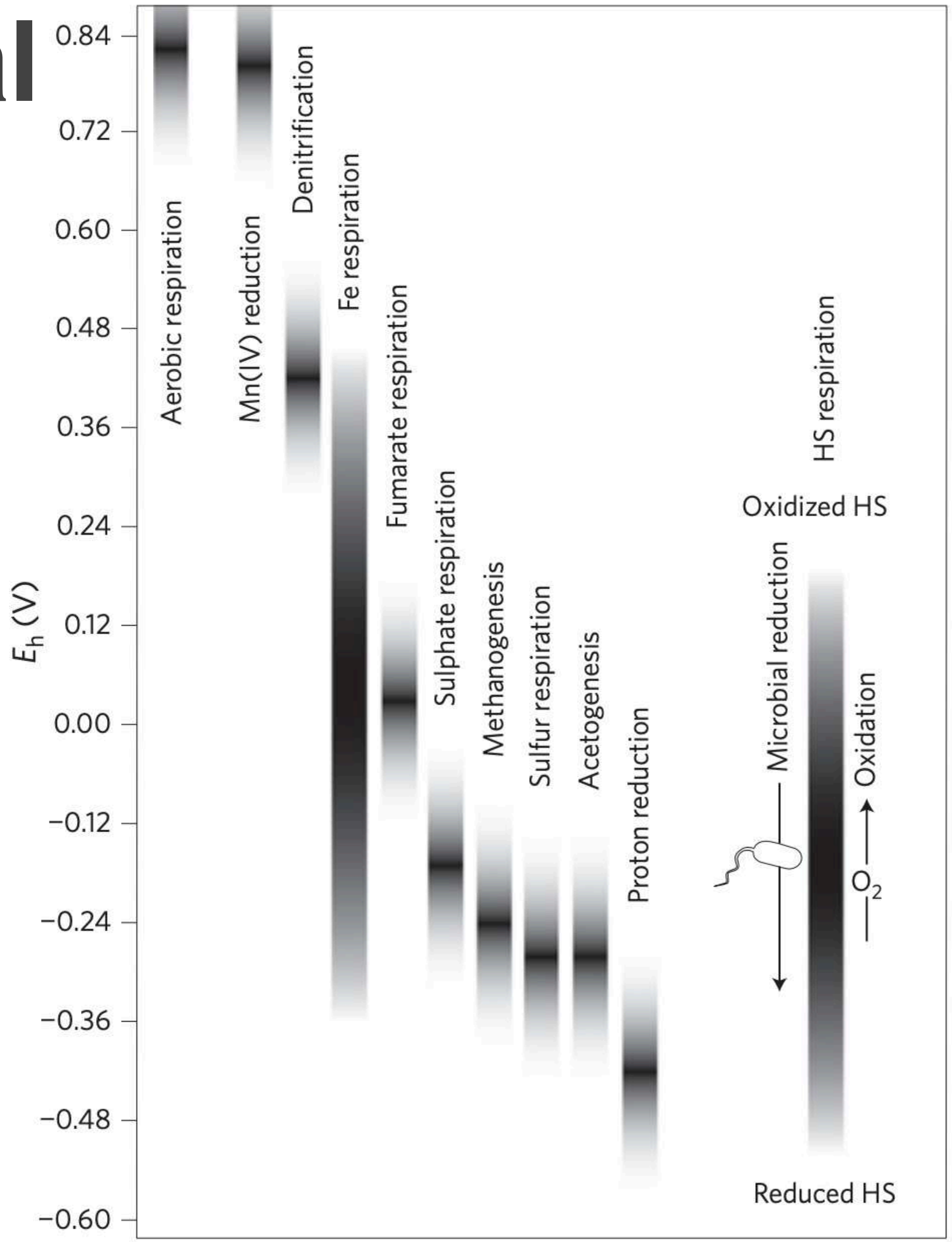


http://watcut.uwaterloo.ca/webnotes/Metabolism/RespiratoryChain.html

- In analogy to how dissipation of the pmf applies torque that rotates the bacterial flagellum, the pmf also creates torque in the large membrane protein complex that synthesizes ATP
- This complex is called ATP synthase (ATPase)
- The activity of ATPase is driven by the pmf, and the formation of ATP from respiratory electron flow is called oxidative phosphorylation (contrast this with substrate-level phosphorylation in fermentation)

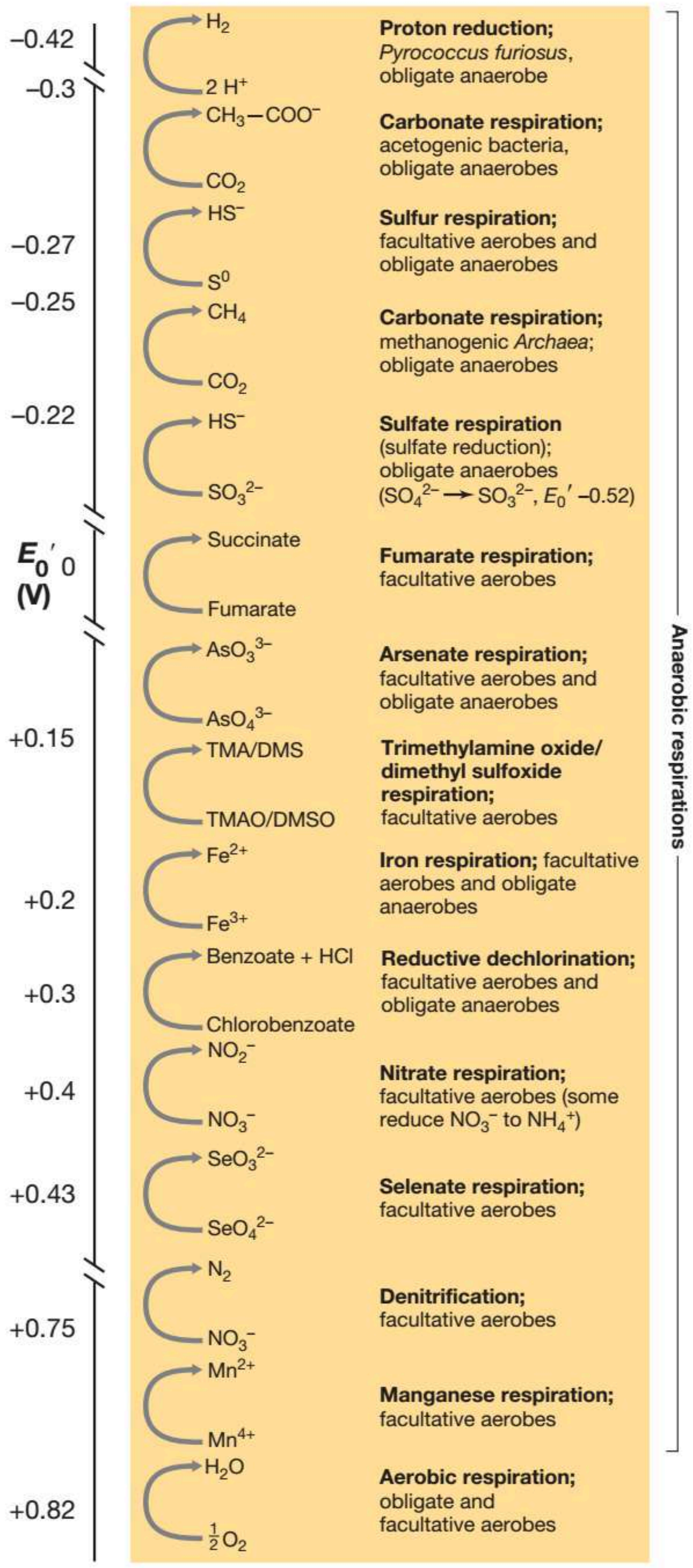
Reduction potential ranges of microbial respiration

- The achievable energy yield of ETC depends on the difference in electrical potential between electron donor and acceptor
- Microbes able to respire in multiple ways will always choose available acceptors with the **biggest potential difference** to the donor (e.g., *E. coli* O2 > NO3-> fumarate)

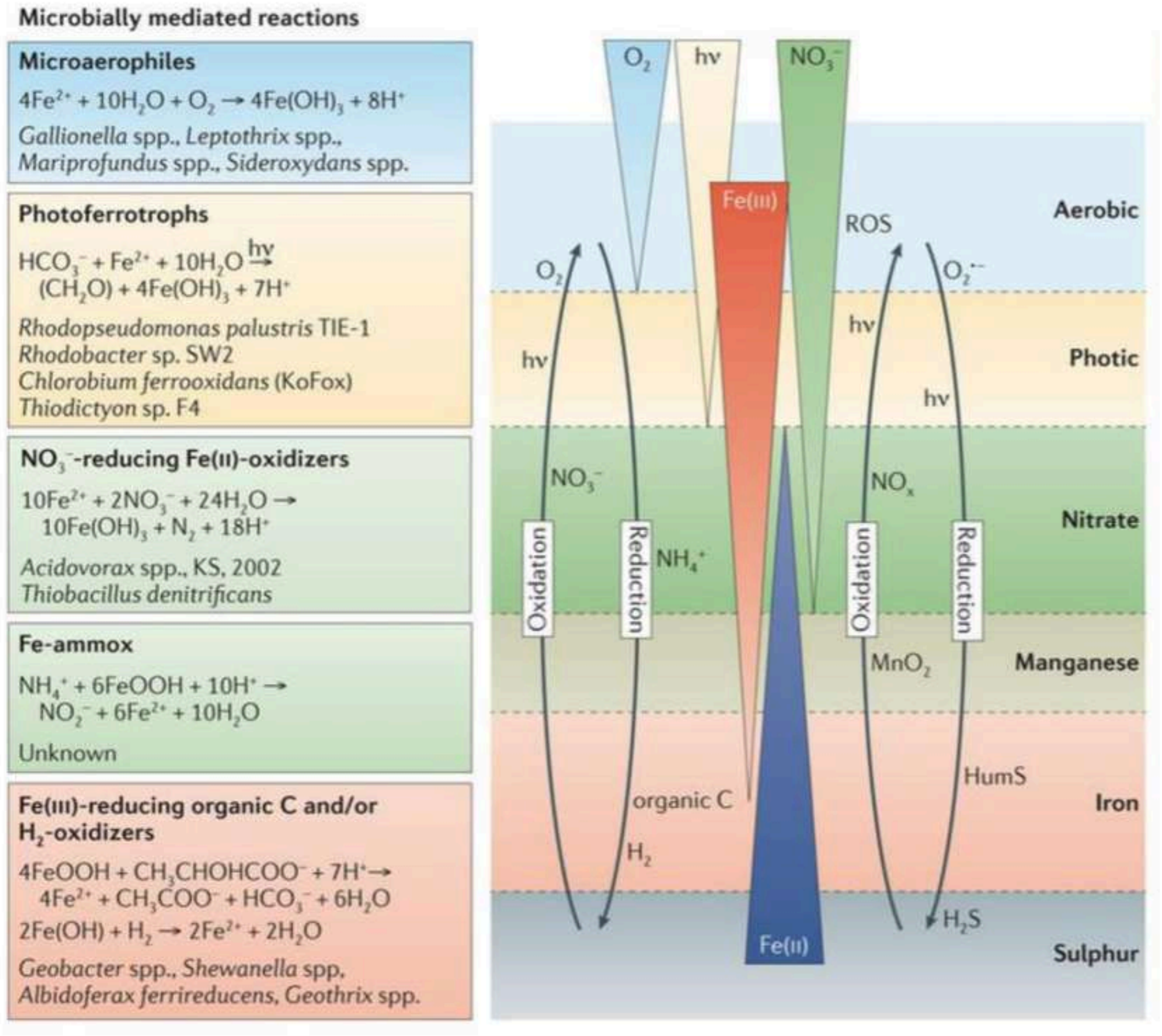


Klüpfel et al., 2014

Anaerobic respiration

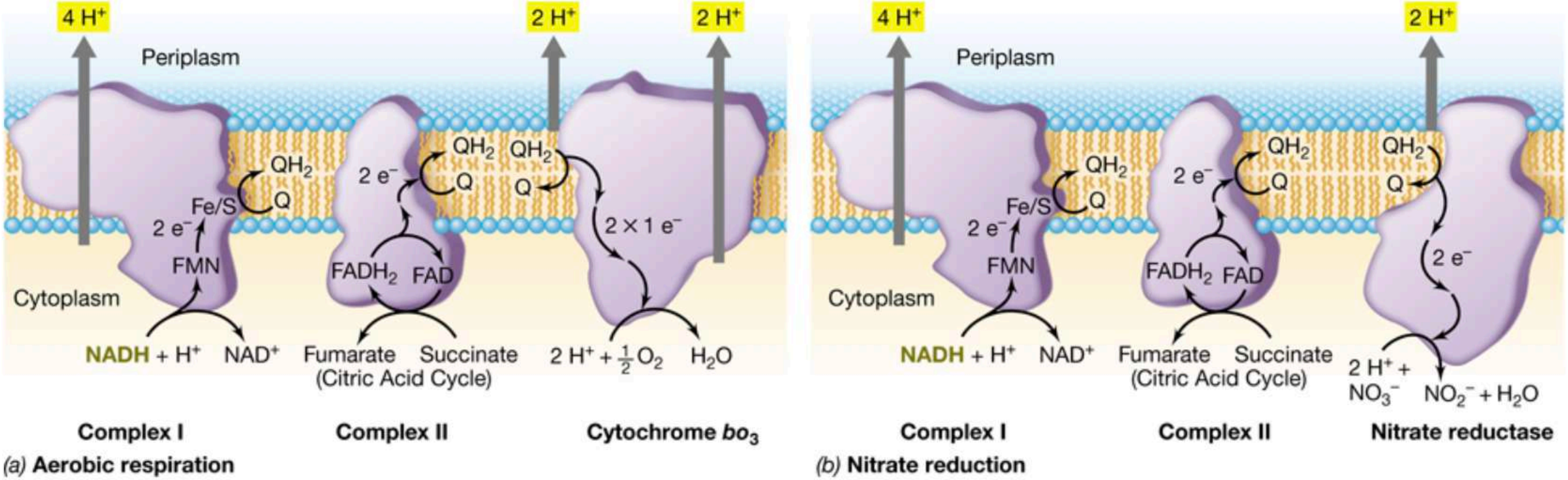


Madigan et al. 2018



Melton et al., 2014

Figure 3.23 **Respiration and nitrate-based anaerobic respiration in *Escherichia coli*.**



The basic organization of the *E. coli* respiratory chain (Figure 3.23) is Complex I followed by Complex II followed by *quinones* (for example, ubiquinone) and a *terminal reductase* that contains *b*-type and *o*-type cytochromes (in contrast to the *a*-type cytochromes of Complex IV). Depending on environmental conditions *E. coli* can swap out these components, employing alternative quinones (for example, ubiquinone or menaquinone), or employing alternative dehydrogenases or terminal reductases (*E. coli* has at least five sets of each). Note, however, that Complex III is missing from *E. coli* (Figure 3.23); this has energetic consequences.

Figure 3.21 Energetics in fermentation and aerobic respiration.

Lactic acid fermentation

Glycolysis

Glucose → 2 pyruvate → 2 lactate

$2\ NAD^+$ → **2 NADH**

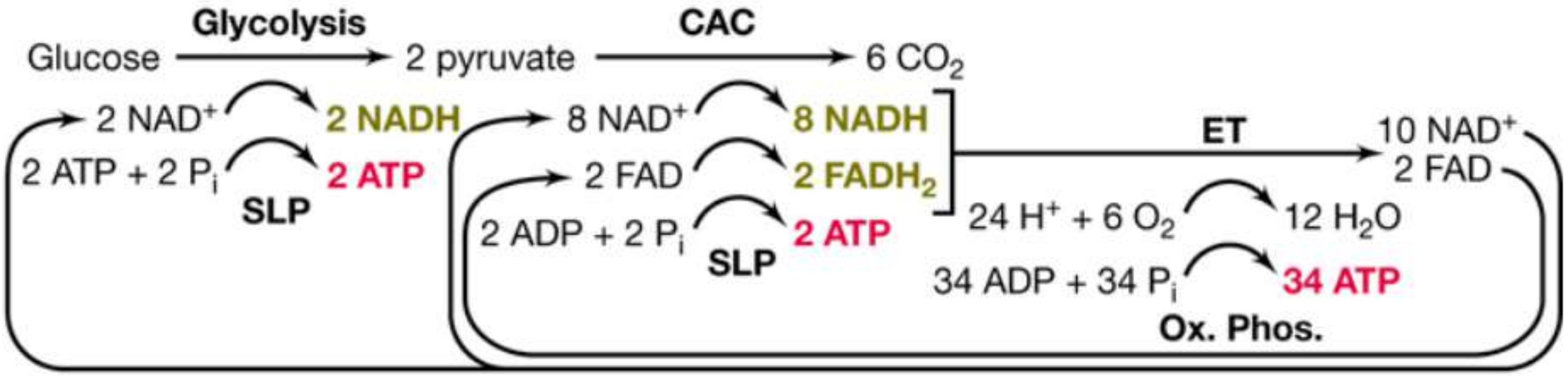
2 NADH → $2\ NAD^+$

$2\ ATP + 2\ P_i$ → **2 ATP**

SLP

Net: Glucose + 2 ADP + $2\ P_i$ ⟶ 2 lactate + **2 ATP**

Aerobic respiration



Glycolysis

Glucose → 2 pyruvate

CAC

2 pyruvate → $6\ CO_2$

$2\ NAD^+$ → **2 NADH**

$2\ ATP + 2\ P_i$ → **2 ATP**

SLP

$8\ NAD^+$ → **8 NADH**

2 FAD → **$2\ FADH_2$**

$2\ ADP + 2\ P_i$ → **2 ATP**

SLP

ET

$10\ NAD^+$

2 FAD

$24\ H^+ + 6\ O_2$ → $12\ H_2O$

$34\ ADP + 34\ P_i$ → **34 ATP**

Ox. Phos.

Net: Glucose + $6\ O_2$ + 38 ADP + $38\ P_i$ ⟶ $6\ CO_2 + 6\ H_2O$ + **38 ATP**

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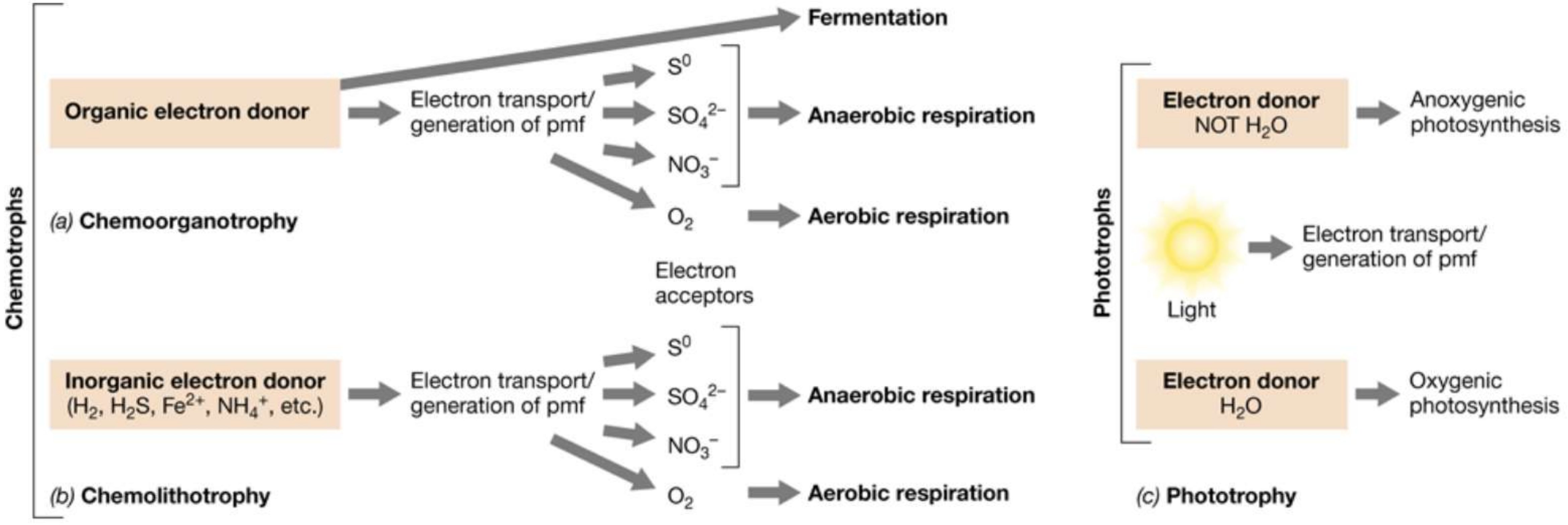
Figure 3.22 **Metabolic diversity and its relationship to oxygen.**



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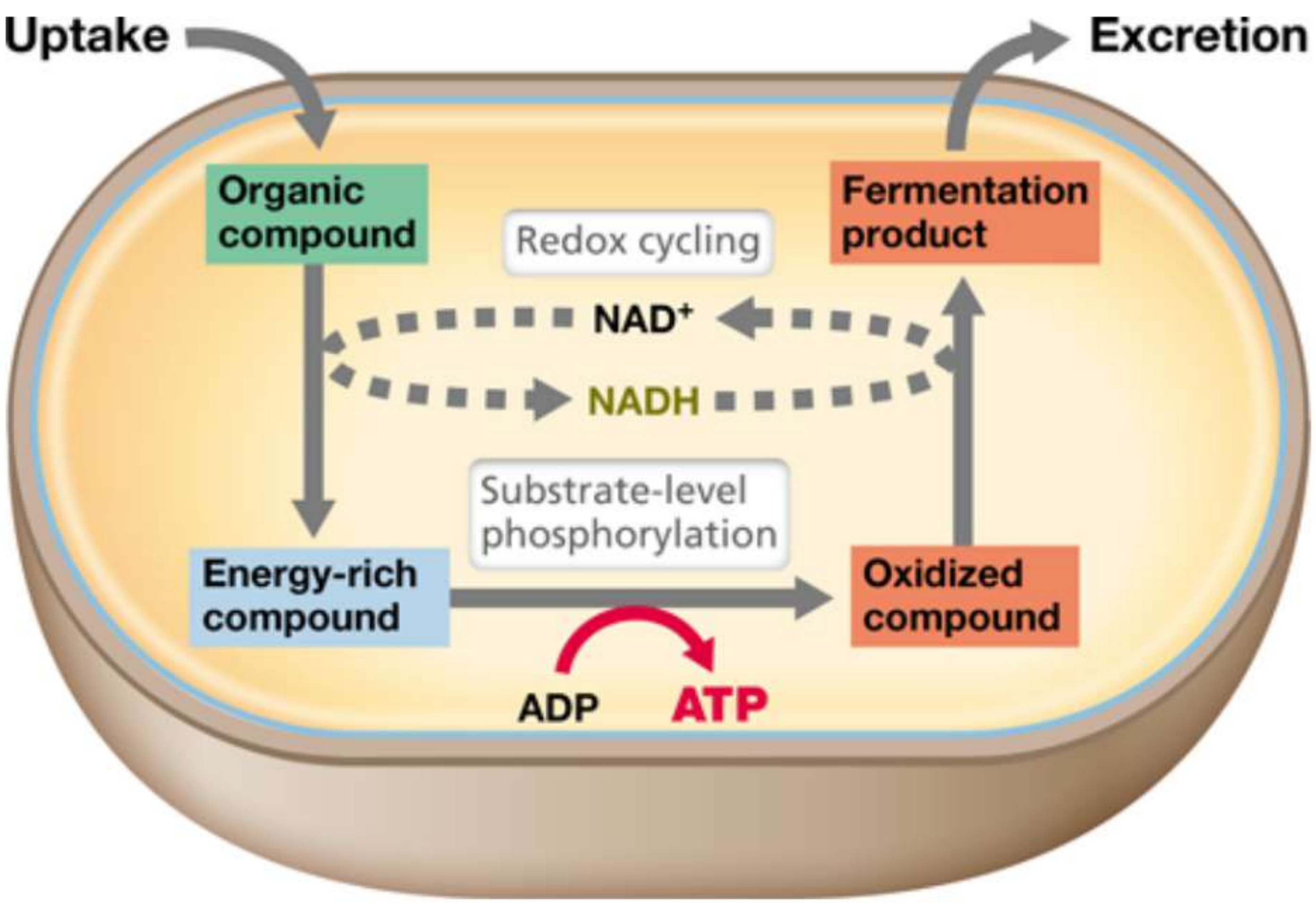
Fermentation

Figure 3.14 **The essentials of fermentation.**



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- Both organic compounds accept and donate e-
- No need to external e- acceptor to achieve balance

- An organic compound is oxidized
- e- are recycled back to one of the oxidized organic products <u>because an external e- acceptor is lacking</u>
- Product is exceed from the cell and ATP is produced by substrate-level phosphorylation

Fermentation, II

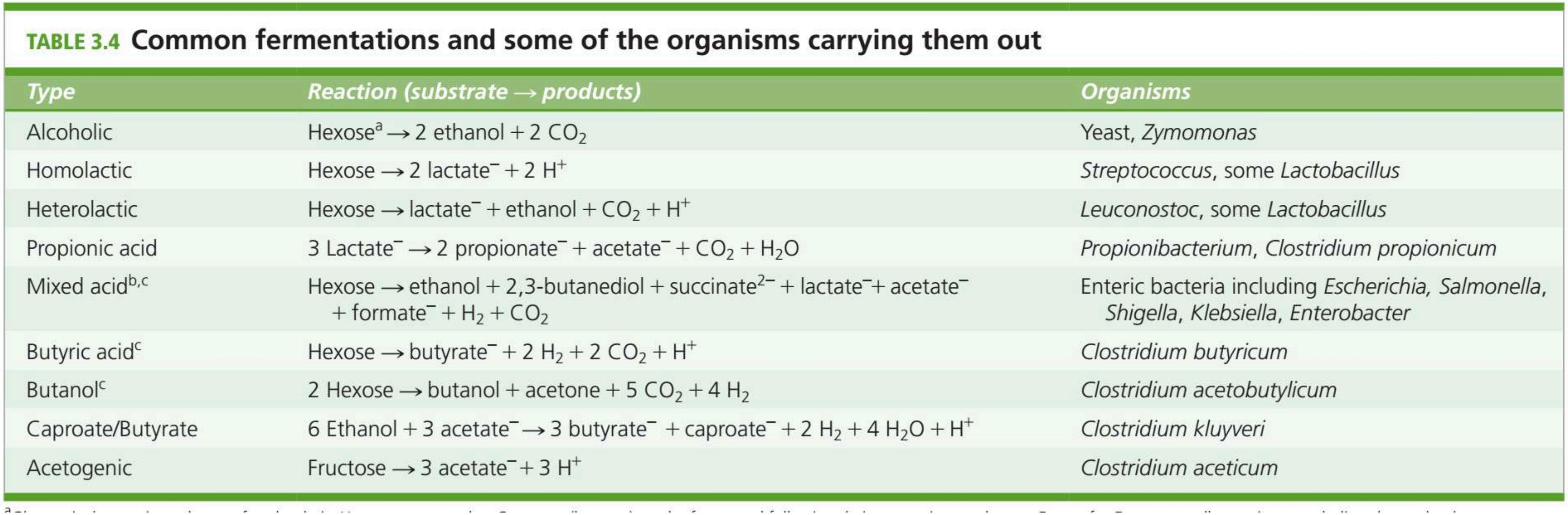


TABLE 3.4 Common fermentations and some of the organisms carrying them out

Type	*Reaction (substrate → products)*	*Organisms*
Alcoholic	Hexose[a] → 2 ethanol + 2 CO_2	Yeast, *Zymomonas*
Homolactic	Hexose → 2 $lactate^-$ + 2 H^+	*Streptococcus*, some *Lactobacillus*
Heterolactic	Hexose → $lactate^-$ + ethanol + CO_2 + H^+	*Leuconostoc*, some *Lactobacillus*
Propionic acid	3 $Lactate^-$ → 2 $propionate^-$ + $acetate^-$ + CO_2 + H_2O	*Propionibacterium*, *Clostridium propionicum*
Mixed acid[b,c]	Hexose → ethanol + 2,3-butanediol + $succinate^{2-}$ + $lactate^-$ + $acetate^-$ + $formate^-$ + H_2 + CO_2	Enteric bacteria including *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*
Butyric acid[c]	Hexose → $butyrate^-$ + 2 H_2 + 2 CO_2 + H^+	*Clostridium butyricum*
Butanol[c]	2 Hexose → butanol + acetone + 5 CO_2 + 4 H_2	*Clostridium acetobutylicum*
Caproate/Butyrate	6 Ethanol + 3 $acetate^-$ → 3 $butyrate^-$ + $caproate^-$ + 2 H_2 + 4 H_2O + H^+	*Clostridium kluyveri*
Acetogenic	Fructose → 3 $acetate^-$ + 3 H^+	*Clostridium aceticum*

- Not all compounds are inherently fermentable, but sugars (e.g. glucose, other hexoses, most disaccharides, other relatively small sugars) —are fermentable

- Polysaccharides (e.g. cellulose, starch, chitin) are also fermentable by bacteria that produce enzymes that attack these large molecules and produce sugars from them if the latter are not glucose, they must first be converted to glucose before they enter glycolysis

- 2 net ATP molecules in glycolysis

- More ATP synthesis by substrate-level phosphorylation if fatty acid because the fatty acid is formed from its coenzyme-A precursor (energy-rich molecules)

Fermentation/*Respiration*

- **Fermentation is a form of anaerobic catabolism in which organic compounds both donate electrons and accept electrons, and redox balance is achieved without the need for external electron acceptors**

- **ATP is made from these energy-rich compounds by substrate-level phosphorylation, a process whereby the energy-rich phosphate bond on the organic compound is transferred directly to ADP to form ATP**

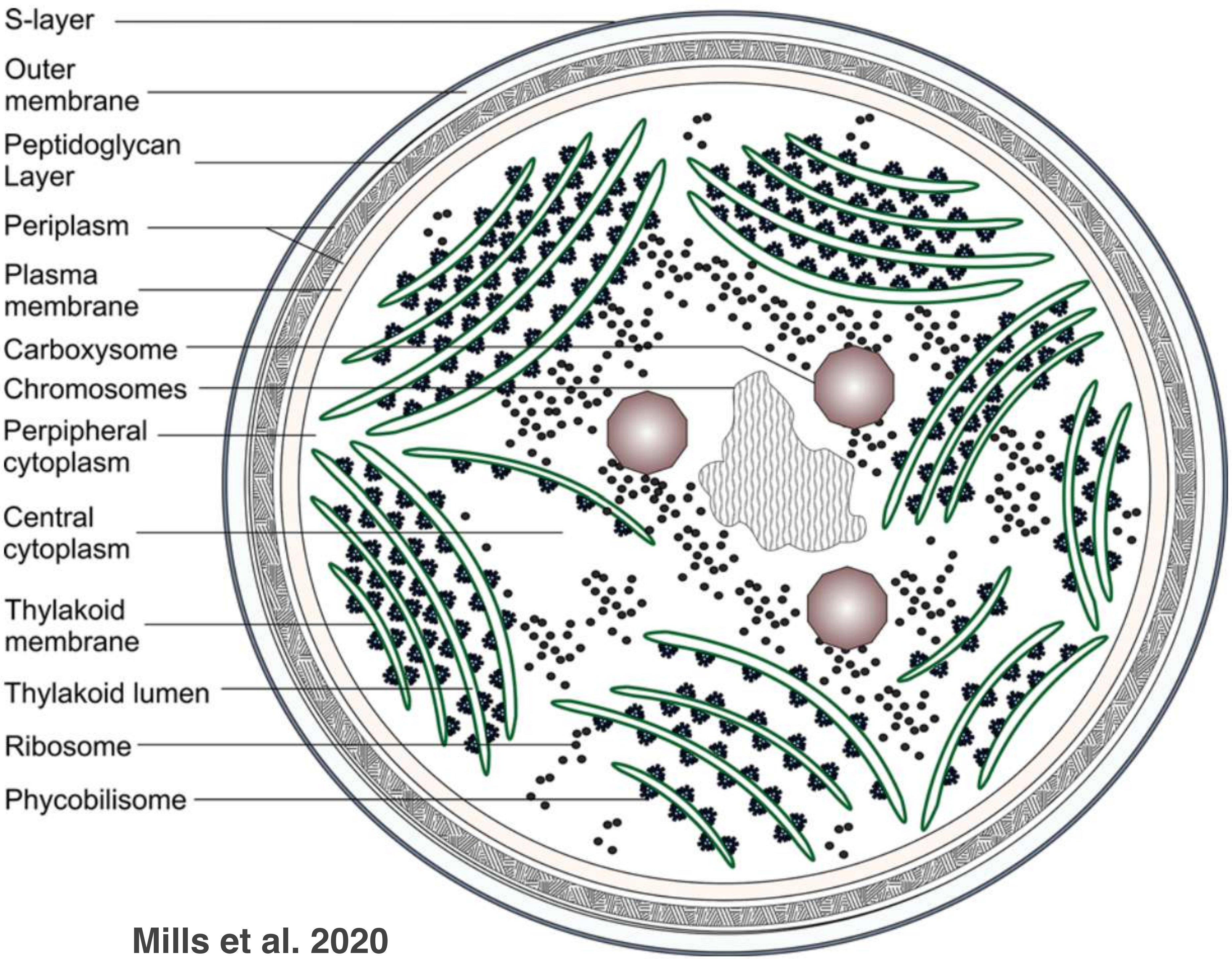
- **Glucose fermentation into alcoholic or lactic acid: 2 ATP**

- *Respiration is a form of aerobic or anaerobic catabolism in which an organic or inorganic electron donor is oxidized with* O_2 *(in aerobic respiration) or some other compounds (in anaerobic respiration) functioning as electron acceptors*

- *ATP is made by PMF*

- *Glucose aerobic respiration into* CO_2*: 38 ATP*

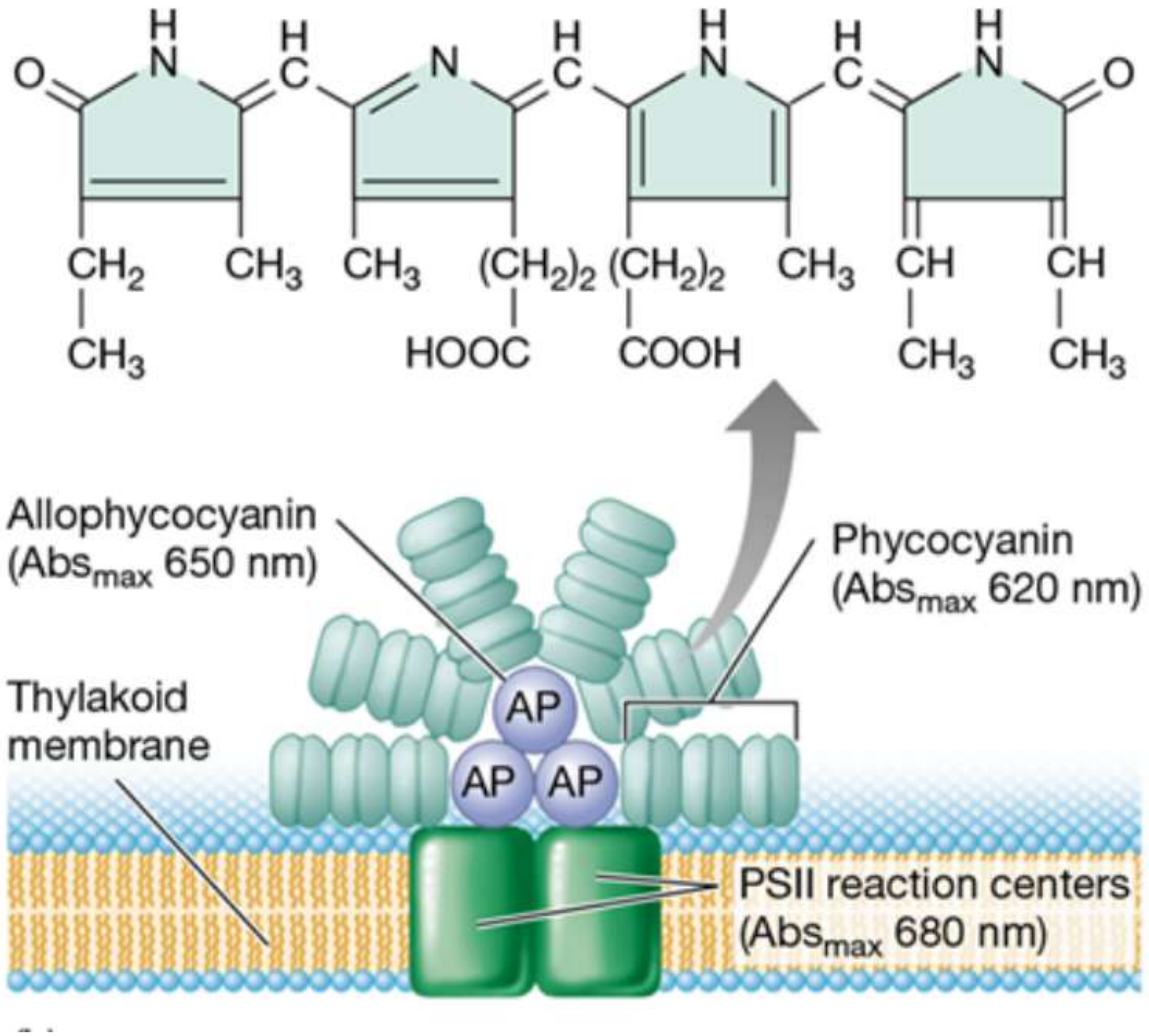
Photo Synthesis: Calvin–Benson–Bassham

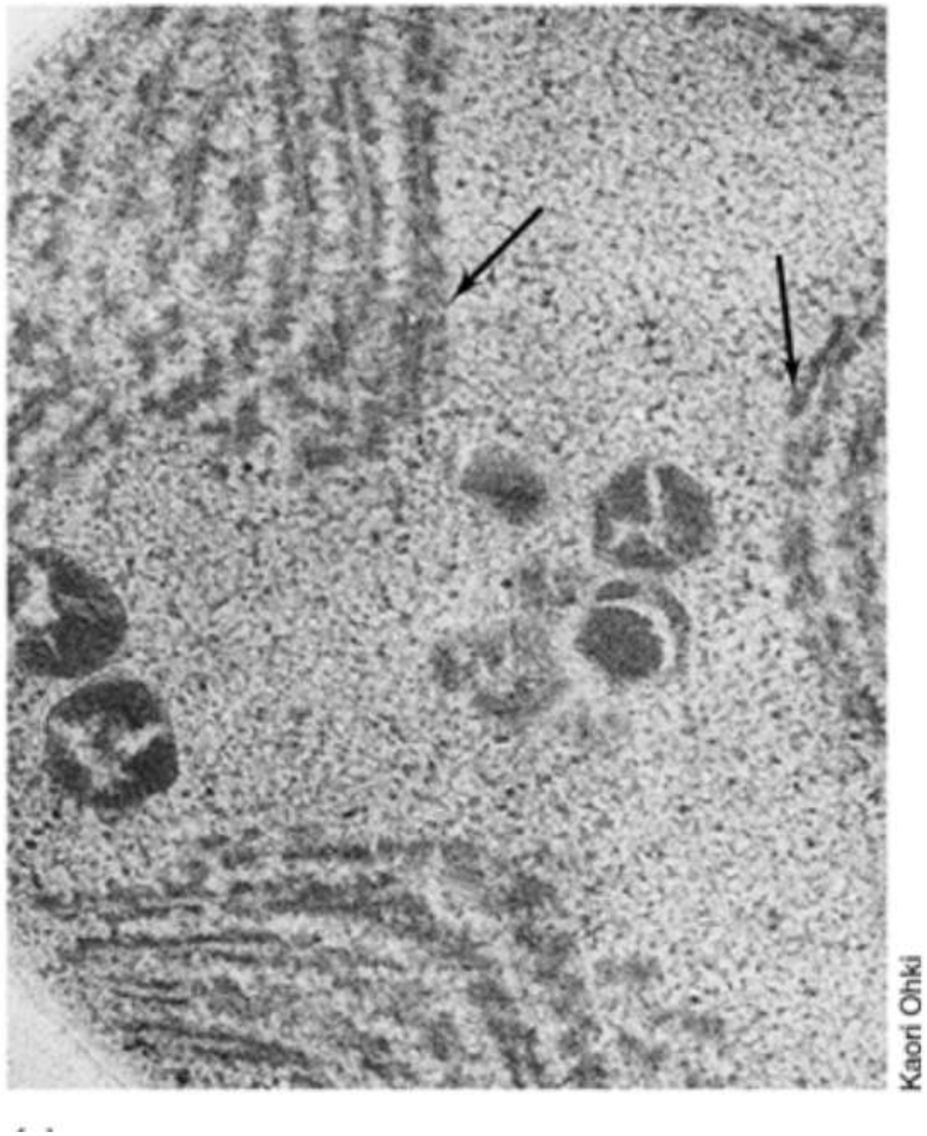


Mills et al. 2020

- Carboxysomes are made of polyhedral protein shells about 80 - 140 nm in diameter
- Concentrate carbon dioxide to overcome the inefficiency of RuBisCo (ribulose bisphosphate carboxylase/oxygenase)
- RuBisCO predominant enzyme in carbon fixation and the rate limiting enzyme in the Calvin-Benson-Bassham cycle

Oxygenic photosynthesis





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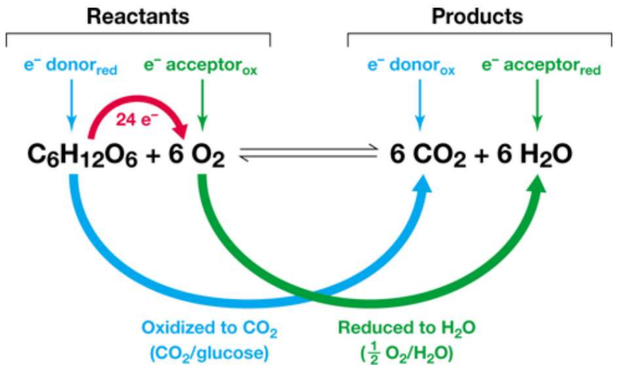
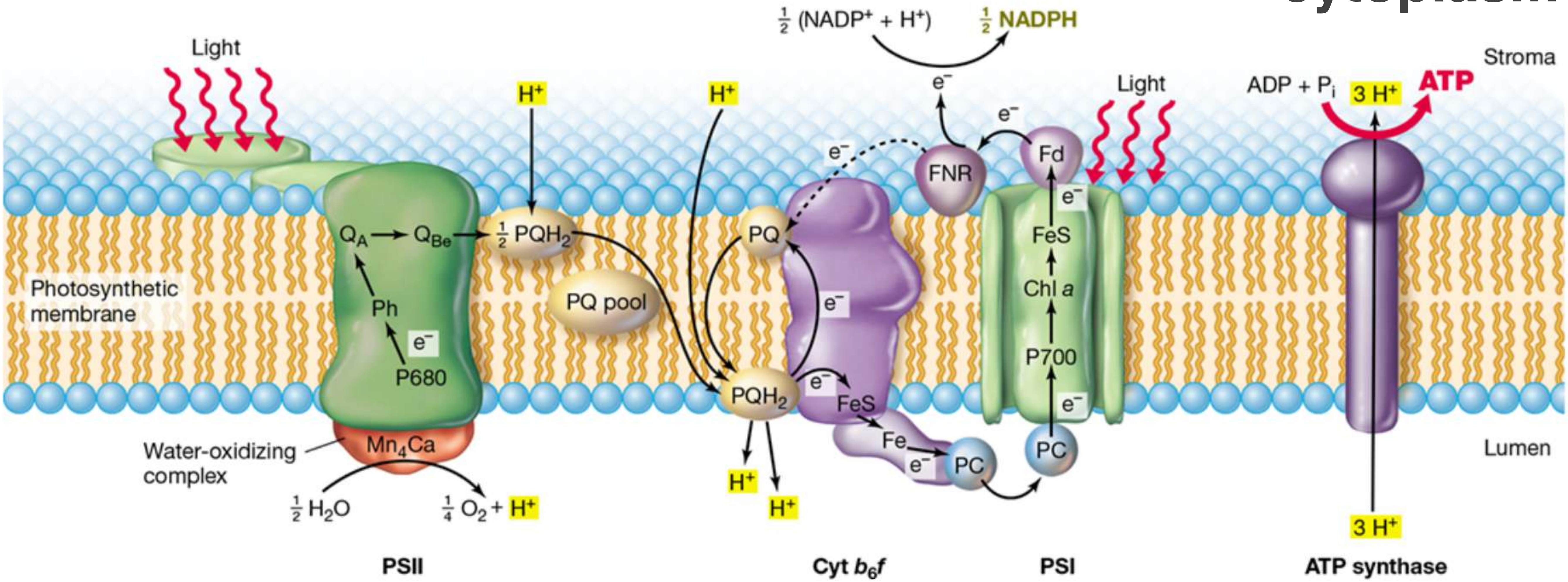
- Physical location within the cell (Cyanobacteria)
- Bilayer w. proteins and complex that capture light, phycobilisome

Photo



cytoplasm



- Splitting of H2O
- Generation H+ motive force
- Generation of NADPH —> **C fixation (from CO2)** via Calvin–Benson–Bassham cycle
- ATP production

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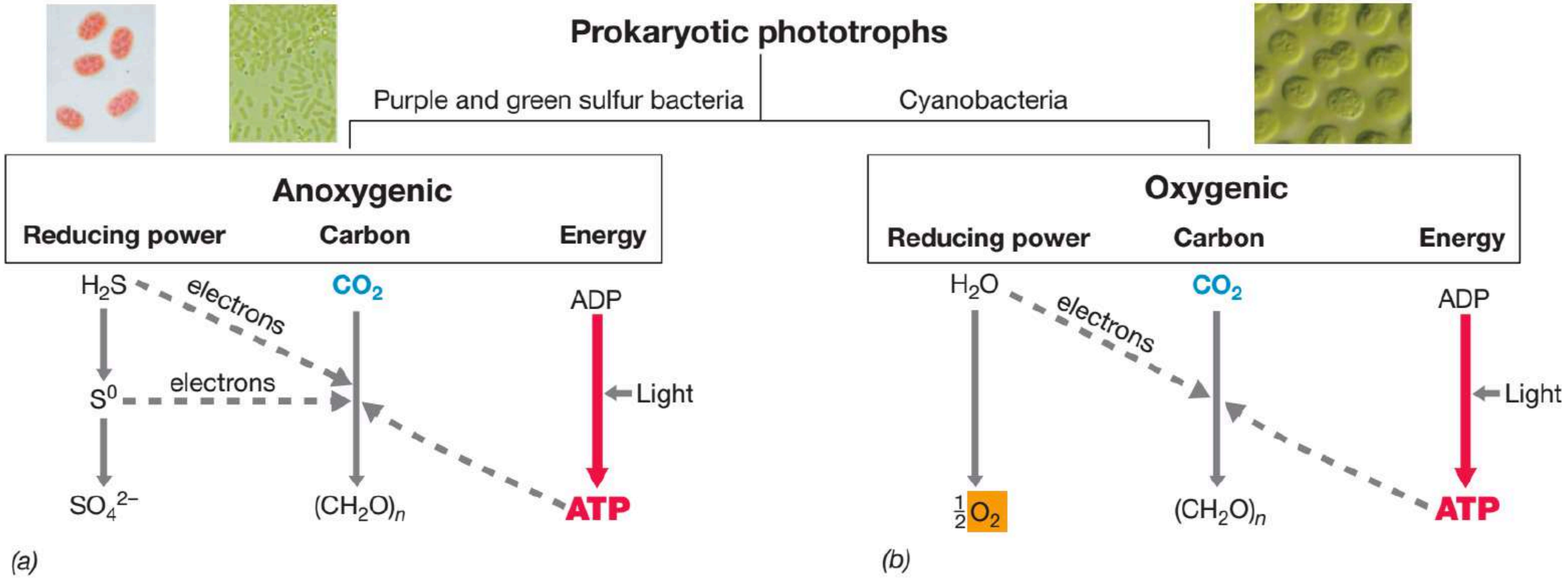
Light driven processes



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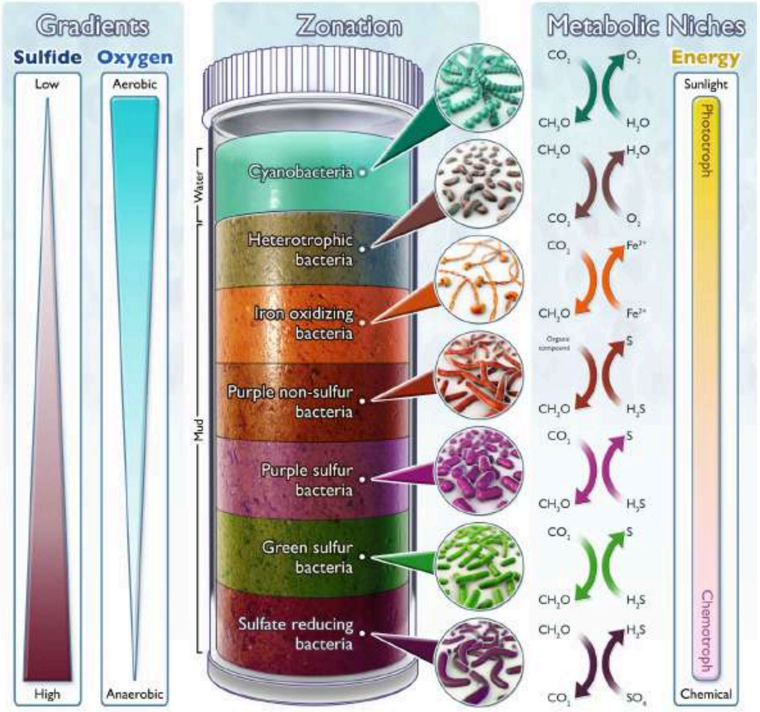
Winogradsky columns



From HHMI

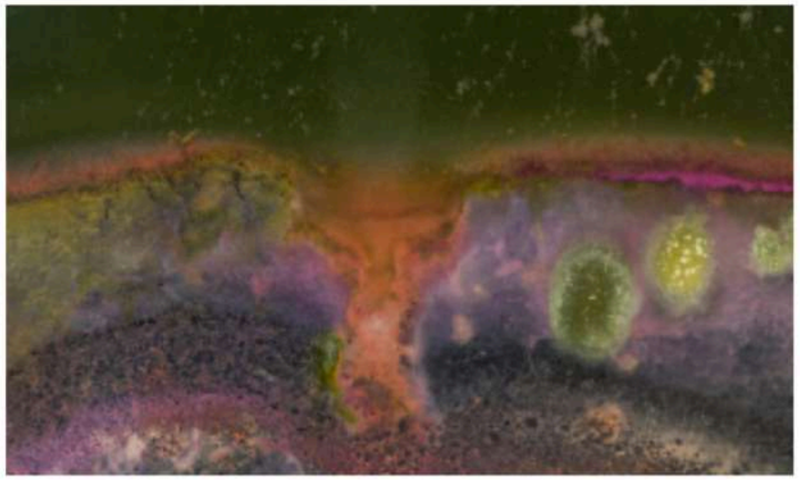
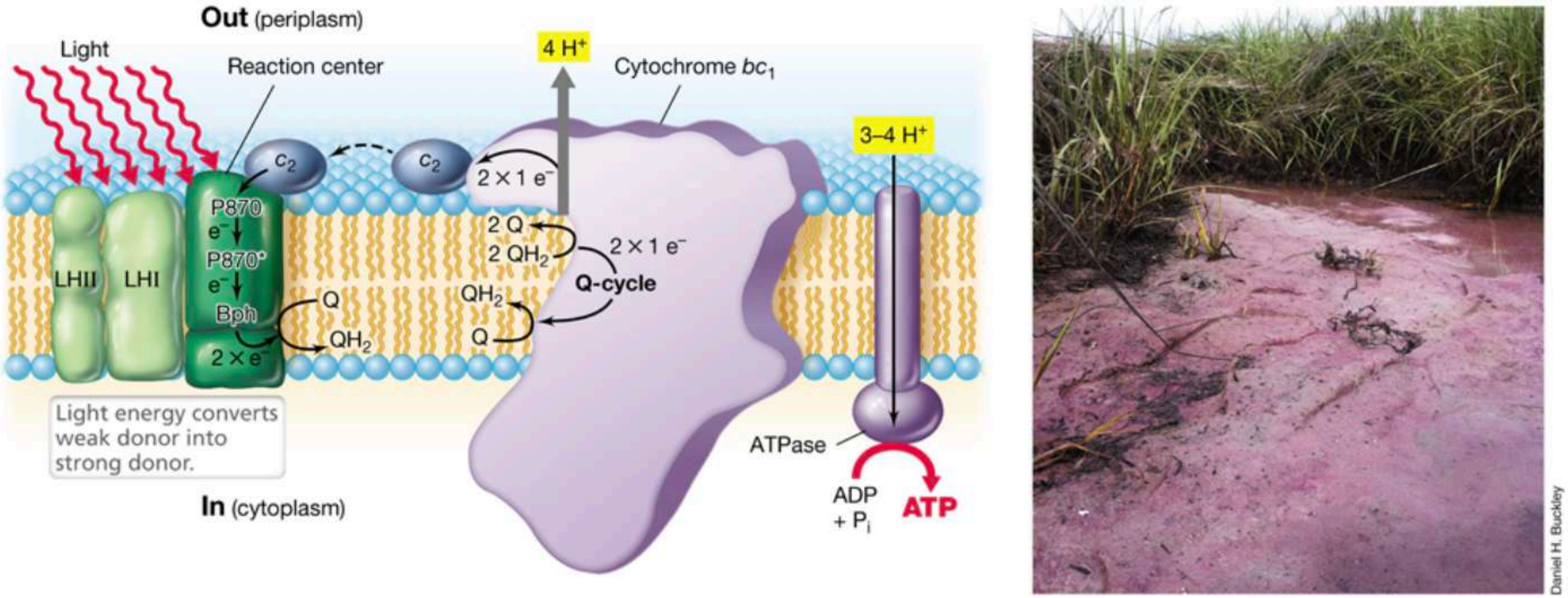


Figure 3. The upper sediment interface on day 15. Aerobic cyanobacteria and algae (upper aqueous phase), yellow-orange microaerophilic iron-oxidizing bacteria, and anaerobic green and purple photosynthetic bacteria develop into layered communities.

https://schaechter.asmblog.org/schaechter/2018/08/how-to-build-a-giant-winogradsky-column.html

Figure 3.25 Electron flow in anoxygenic photosynthesis in a purple bacterium.



Daniel H. Buckley

(a) **Electron transport in purple bacteria**

(b) **Bloom of purple bacteria in a salt marsh**

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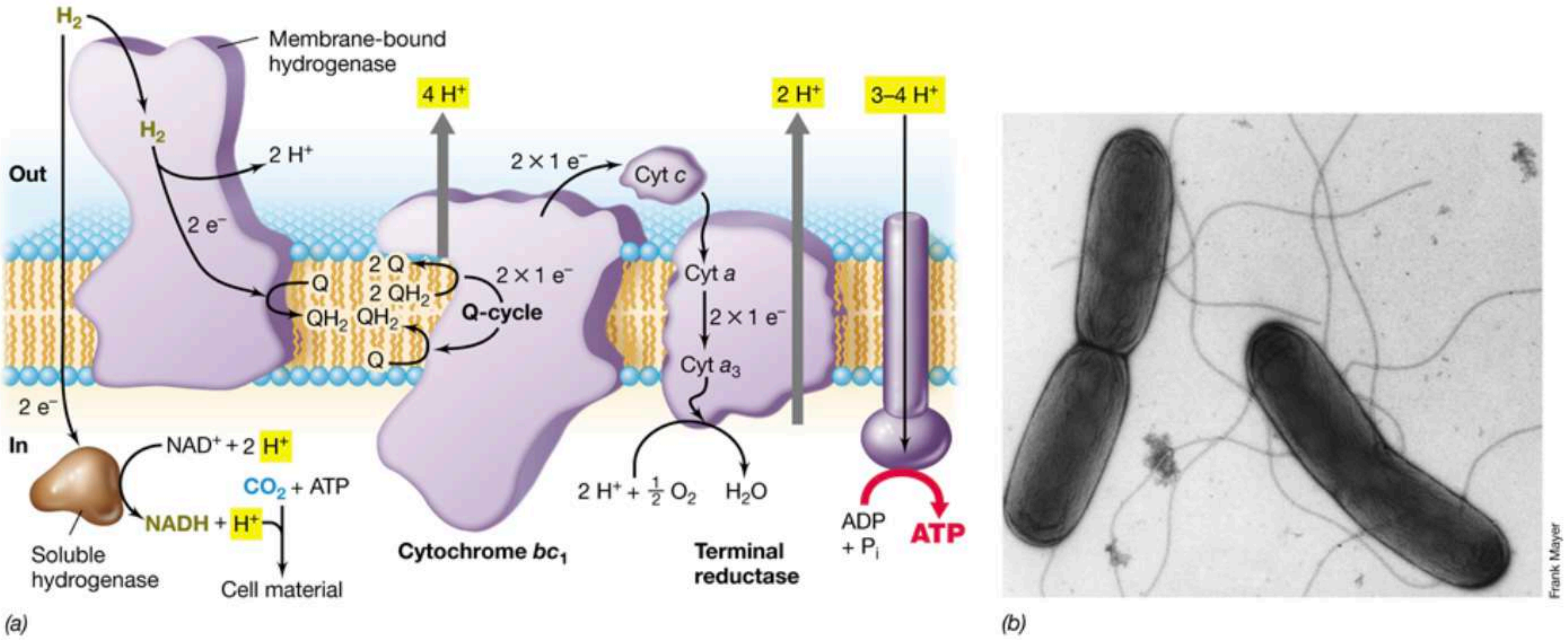
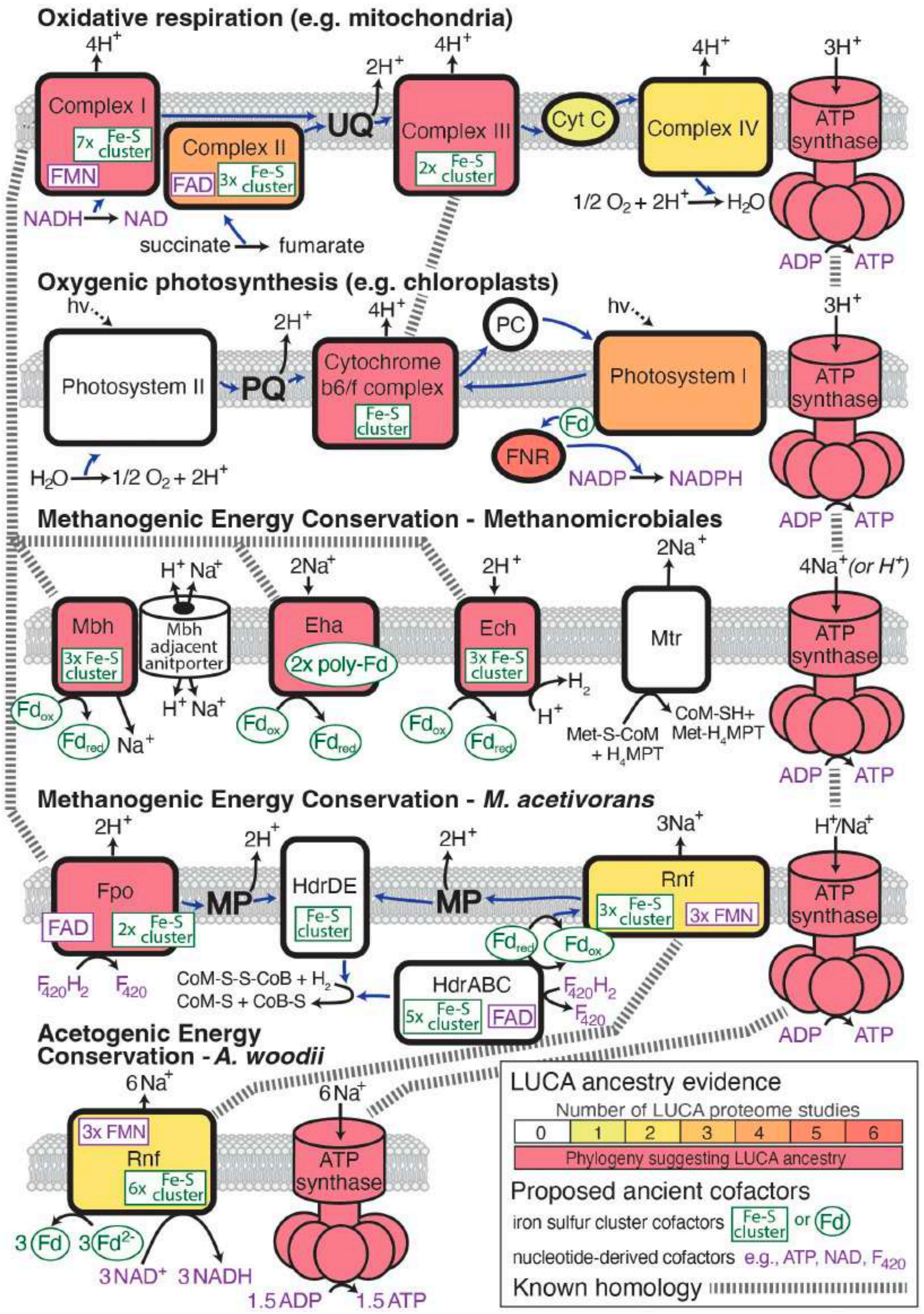
Figure 3.24 Energy conservation in *Ralstonia eutropha*, an autotrophic chemolithotroph that oxidizes H_2.



(a) In *R. eutropha*, two hydrogenases are present; the membrane-bound hydrogenase participates in electron transport, whereas the soluble hydrogenase makes NADH for the Calvin cycle (see Section 3.12). Cyt, cytochrome; Q, quinone. For details of the Q-cycle, see Figure 3.19. *(b)* Transmission electron micrograph of negatively stained cells of *R. eutropha*. A cell is about 0.6 µm in diameter and contains several flagella.

Rewiring of exhibiting membrane-associated micromachies



Goldman et al. 2023

Electron transport chains as a window into the earliest stages of evolution

Signatures of early evolution across different types of chemiosmotic energy conservation.

Electron flow is shown as blue arrows.

Likely ancestry from the LUCA is reflected by either direct phylogenetic evidence or the number of different LUCA proteome studies (out of eight total) that predict a component of the complex to be descended from the LUCA.

Protein cofactors that are potential relics of prebiotic mineral catalysis or ribozyme catalysts are highlighted in green and purple, respectively.

Homology across different ETC components is indicated by a dashed line.

Electron carrier proteins that are components of ETC complexes such as cytochrome B are not shown.

Energy generating metabolic pathways

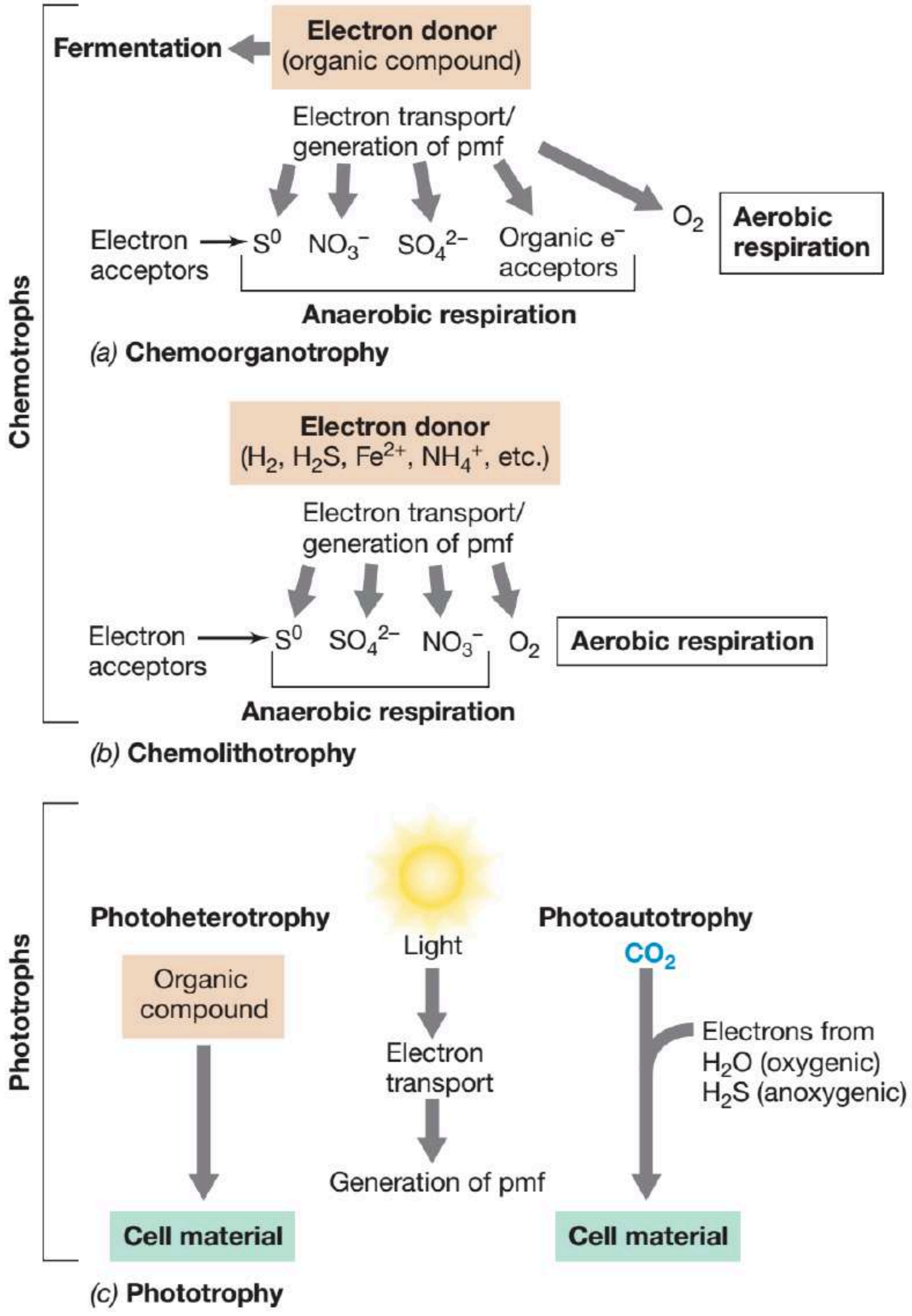
•Oxygenic Photosynthesis
ATP and NADPH are made in large amounts
Produces oxygen as a bi-product during splitting of water for reducing power

•Anoxygenic Photosynthesis
ATP made in large amounts
Reduction of NADP does not involve water; hence no oxygen produced

•Aerobic Respiration
ATP and NADH are made in abundance
Requires oxygen

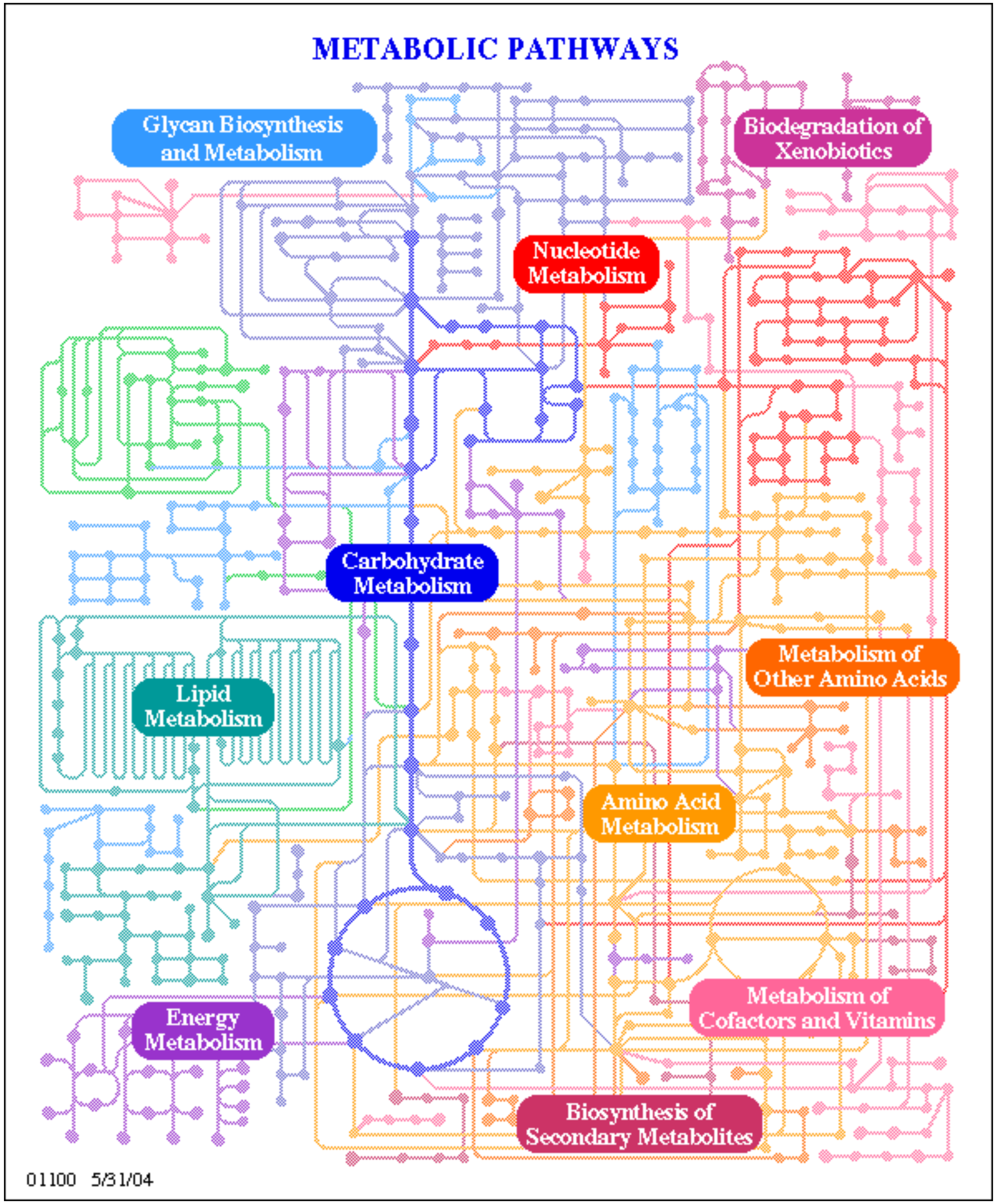
•Anaerobic Respiration
Lower ATP yield than aerobic respiration; NAD easily reduced
Requires electron acceptor other than oxygen

Fermentation
Little ATP, no net NAD reduction, MOST SIMPLE SYSTEM



Madigan et al. 2018

Integrative approach, I

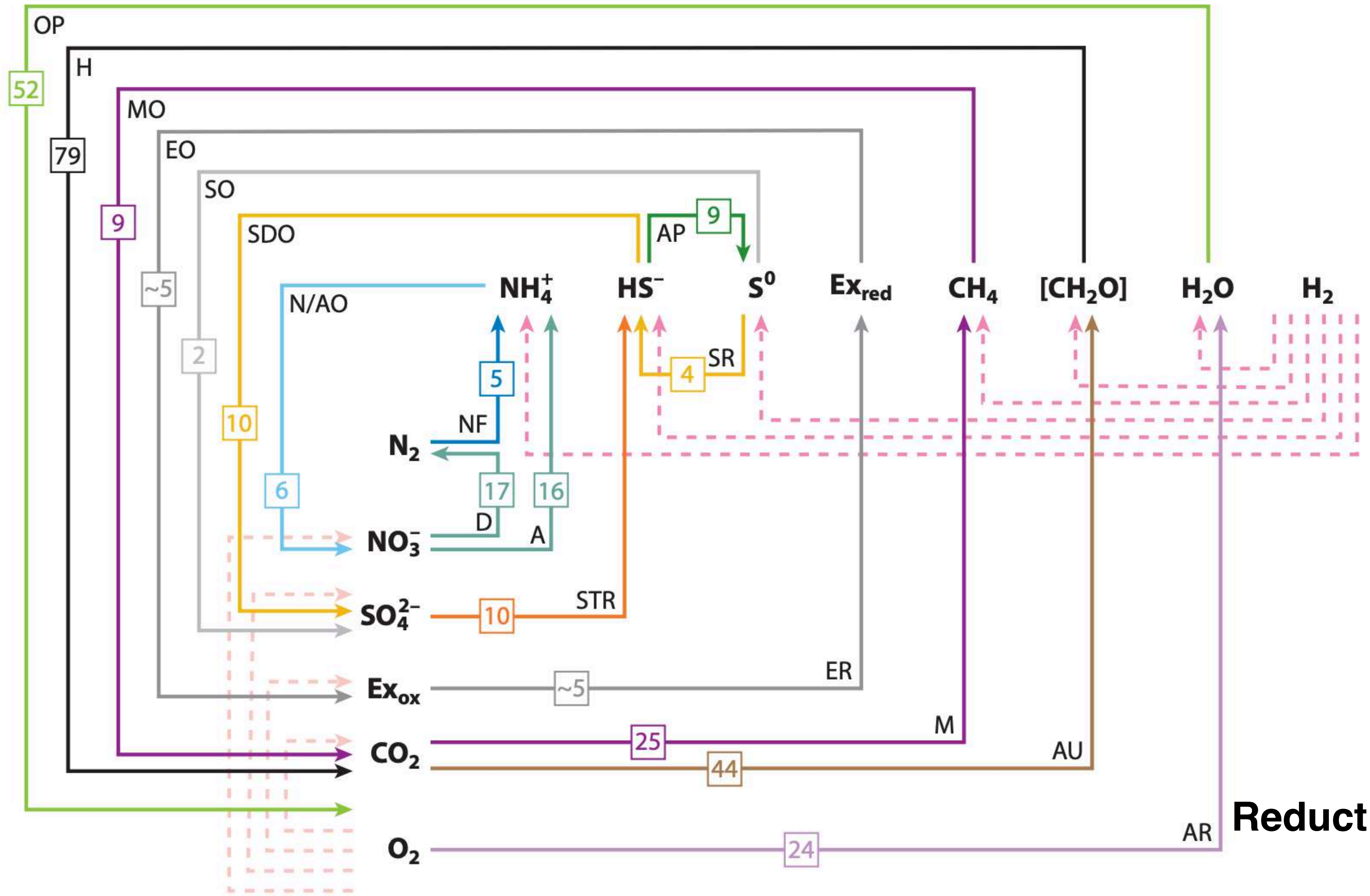


https://www.genome.jp/

Metabolic pathways evolved to utilize available substrates produced as end products of other types of microbial metabolism, either by modification of existing metabolic pathways or by using established ones in reverse

Integrative approach, II

Oxidative reactions

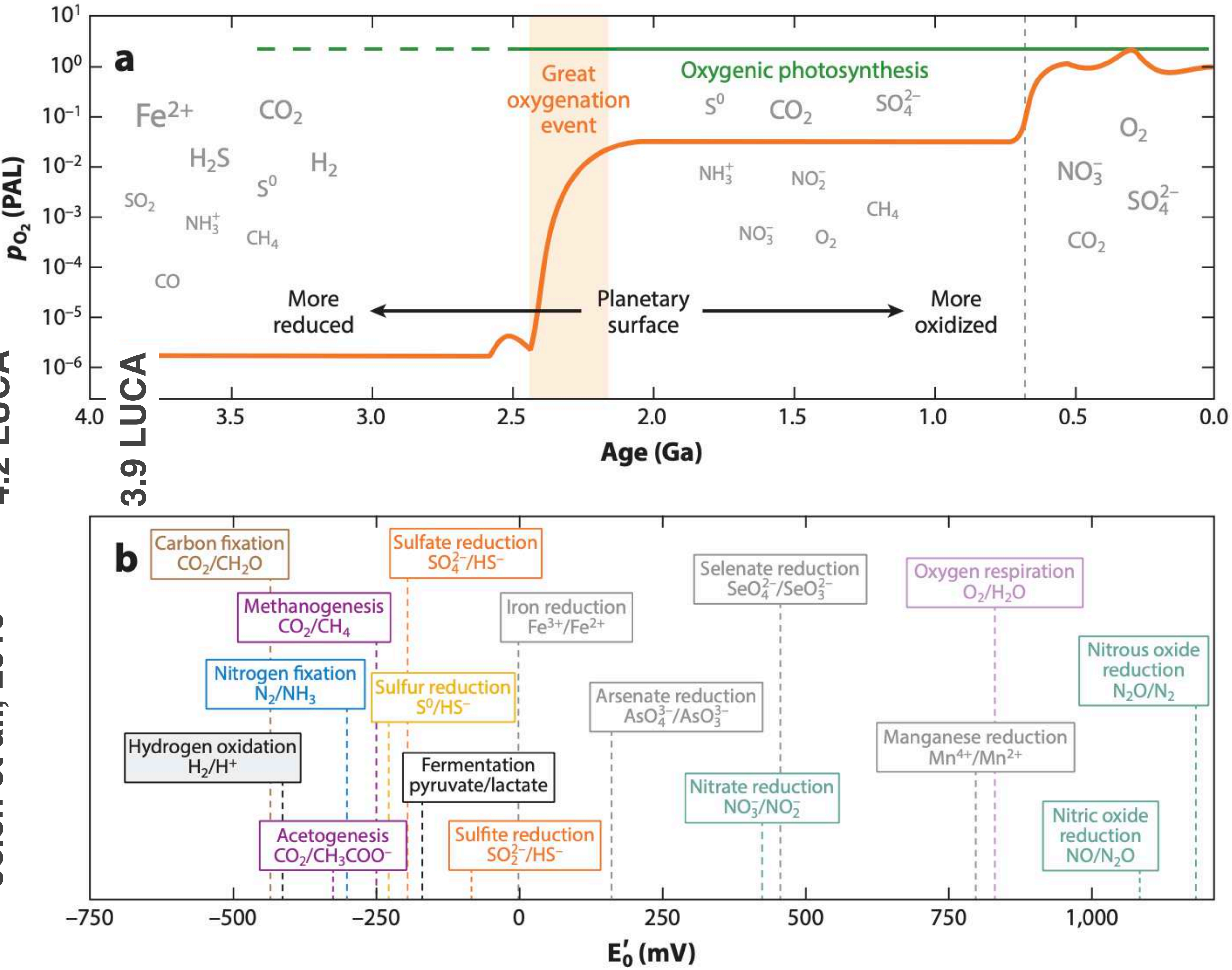


Reductive reactions

Jelen et al. 2016

A, ammonification; AP, anoxygenic photosynthesis; AR, aerobic respiration; AU, autotrophy; D, denitrification; Exox, other elements oxidation; Exred, other elements reduction; H, heterotrophy; M, methanogenesis; MO, methane oxidation/methanotrophy; N/AO, nitrification/ammonia oxidation; NF, nitrogen fixation; OP, oxygenic photosynthesis; SDO, sulfide oxidation; SO, sulfur oxidation; SR, sulfur reduction; STR, sulfate reduction

Coevolution of geosphere and biosphere through time as depicted by change in planetary redox state, availability of redox couples



4.2 LUCA

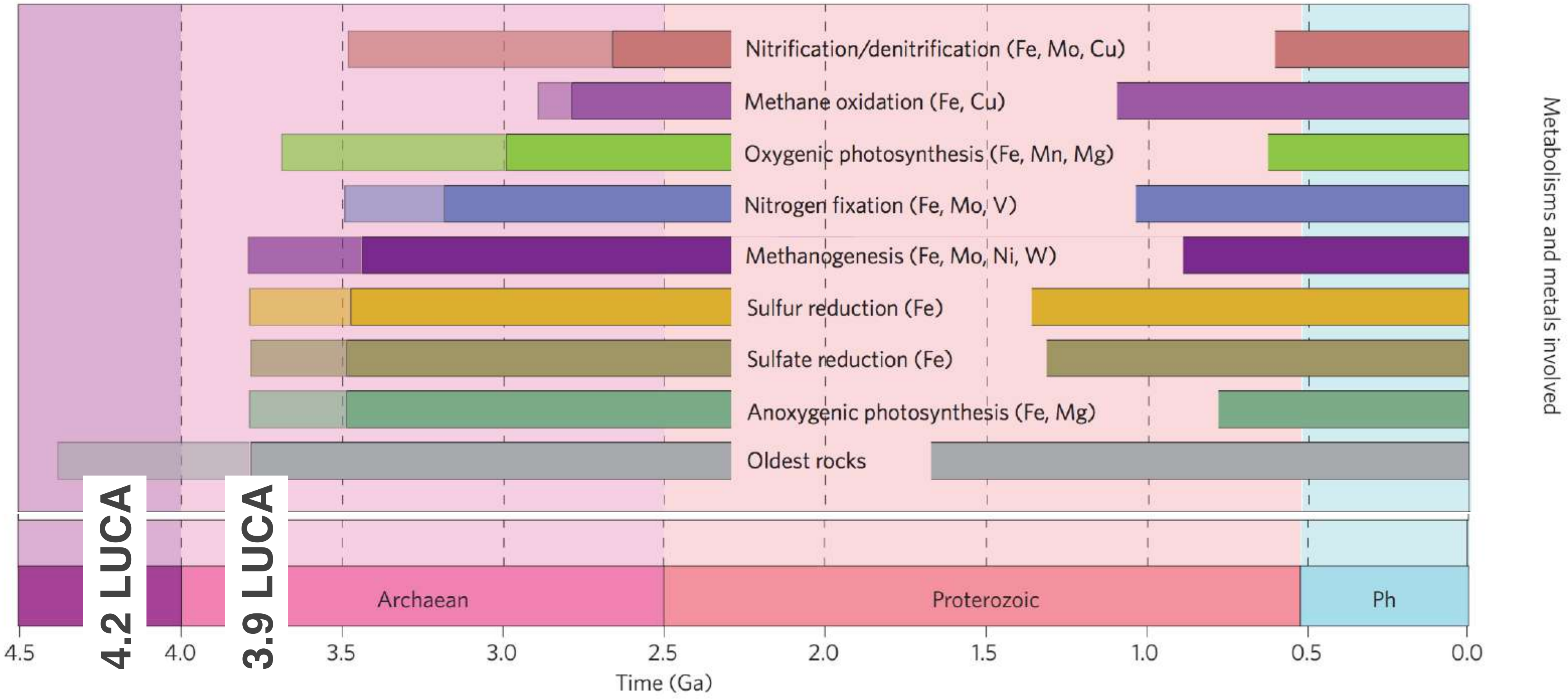
3.9 LUCA

Jelen et al., 2016

Standard reduction potential at pH 7 (E 0) of biologically relevant redox pairs. Redox halfreactions represent the reductive side (i.e., terminal electron \acceptor) of given pathways

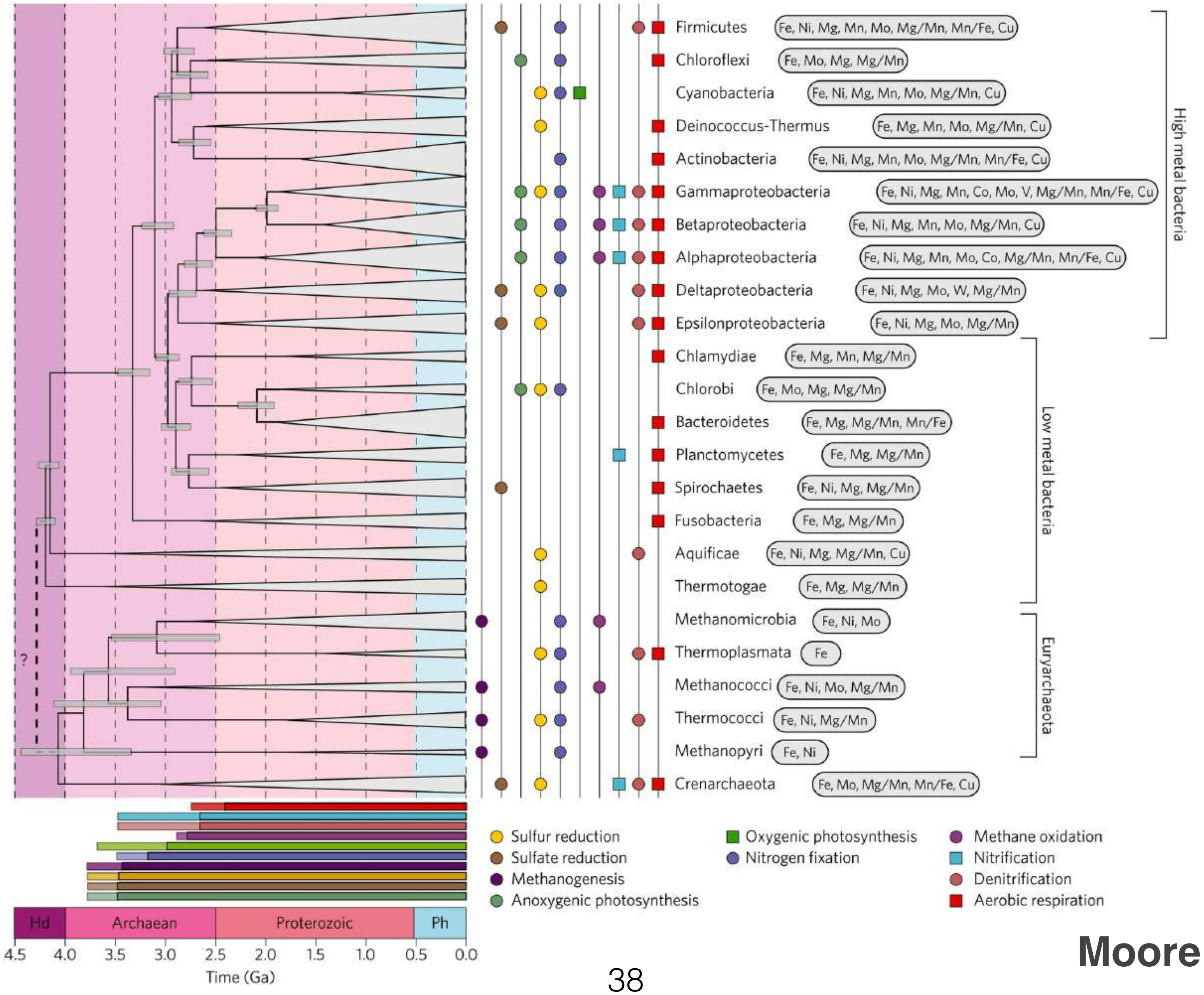
Emerging microbial metabolisms

Moore et al., 2017



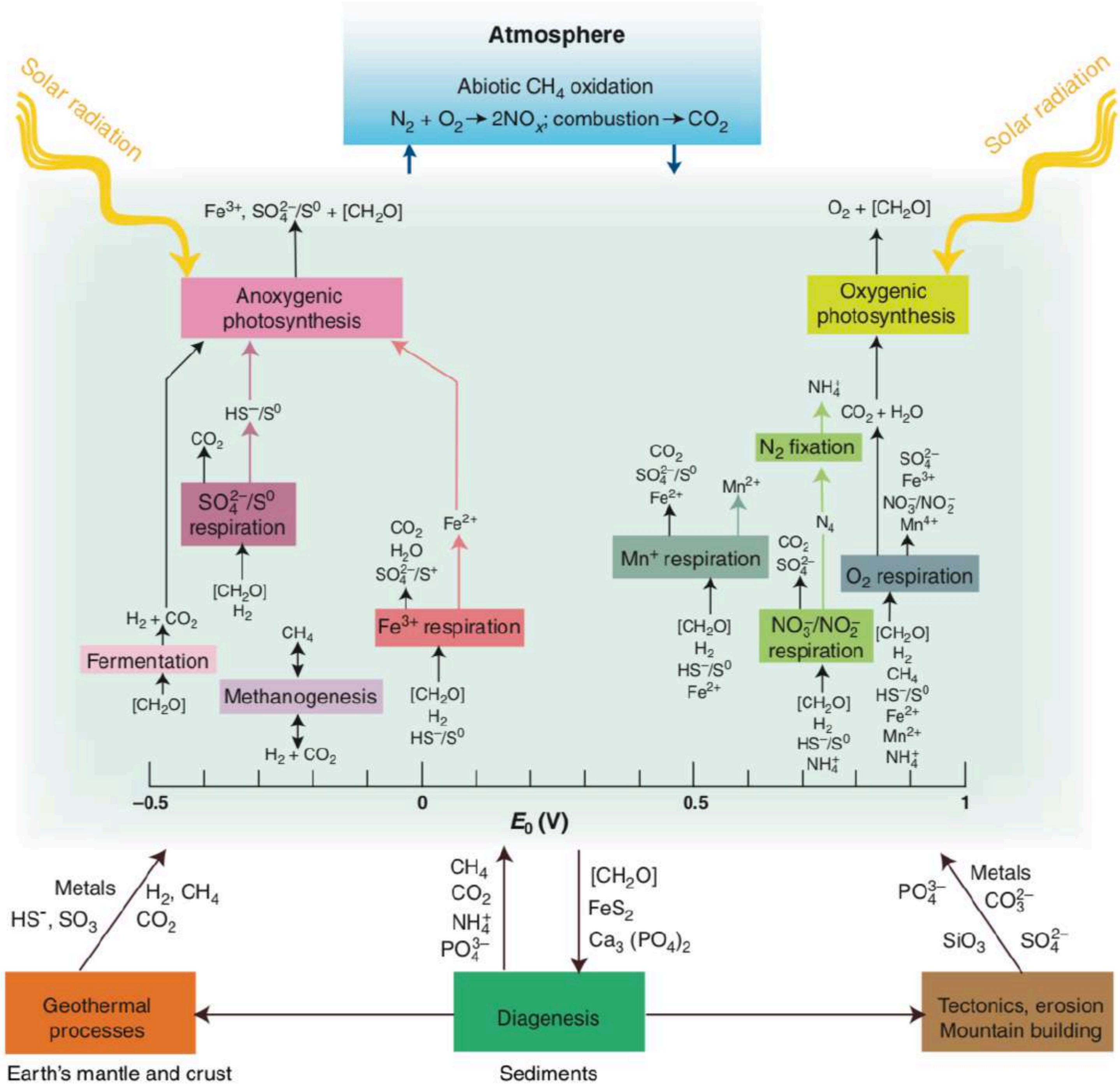
The oxidoreductases responsible for these metabolisms incorporated metals that were readily available in Archaean oceans: iron and iron–sulfur clusters

Phylogenetic tree of the main lineages of Bacteria and Archaea and their putative divergence times



Moore et al., 2017

Biosphere model of energy fluxes and elemental cycles



Falkowski, Fenchel and Delong, 2008

Microbial microscale actions structure planet-scale functioning

Microbial diversity and metabolic pathways to survive in the environment

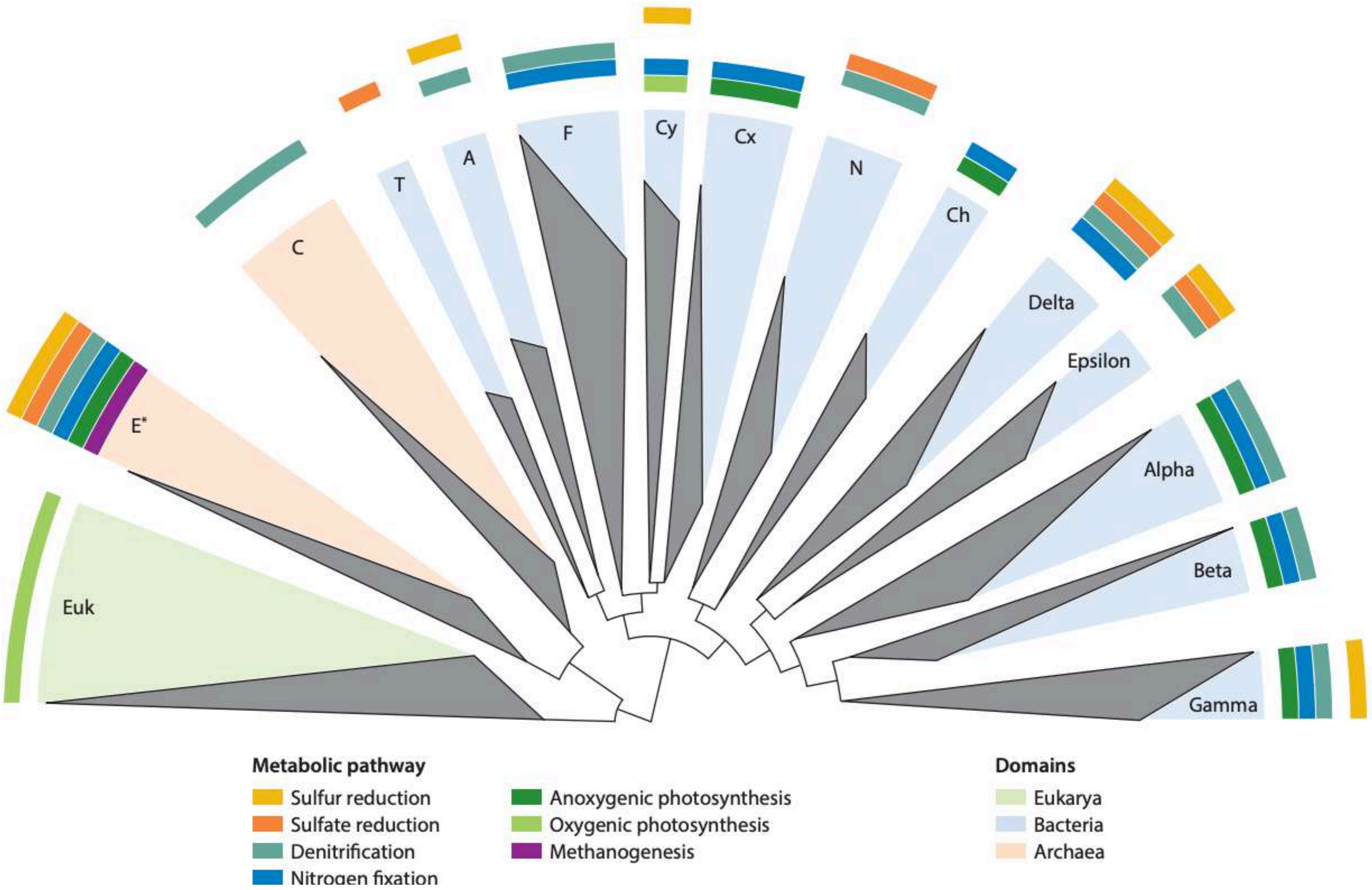
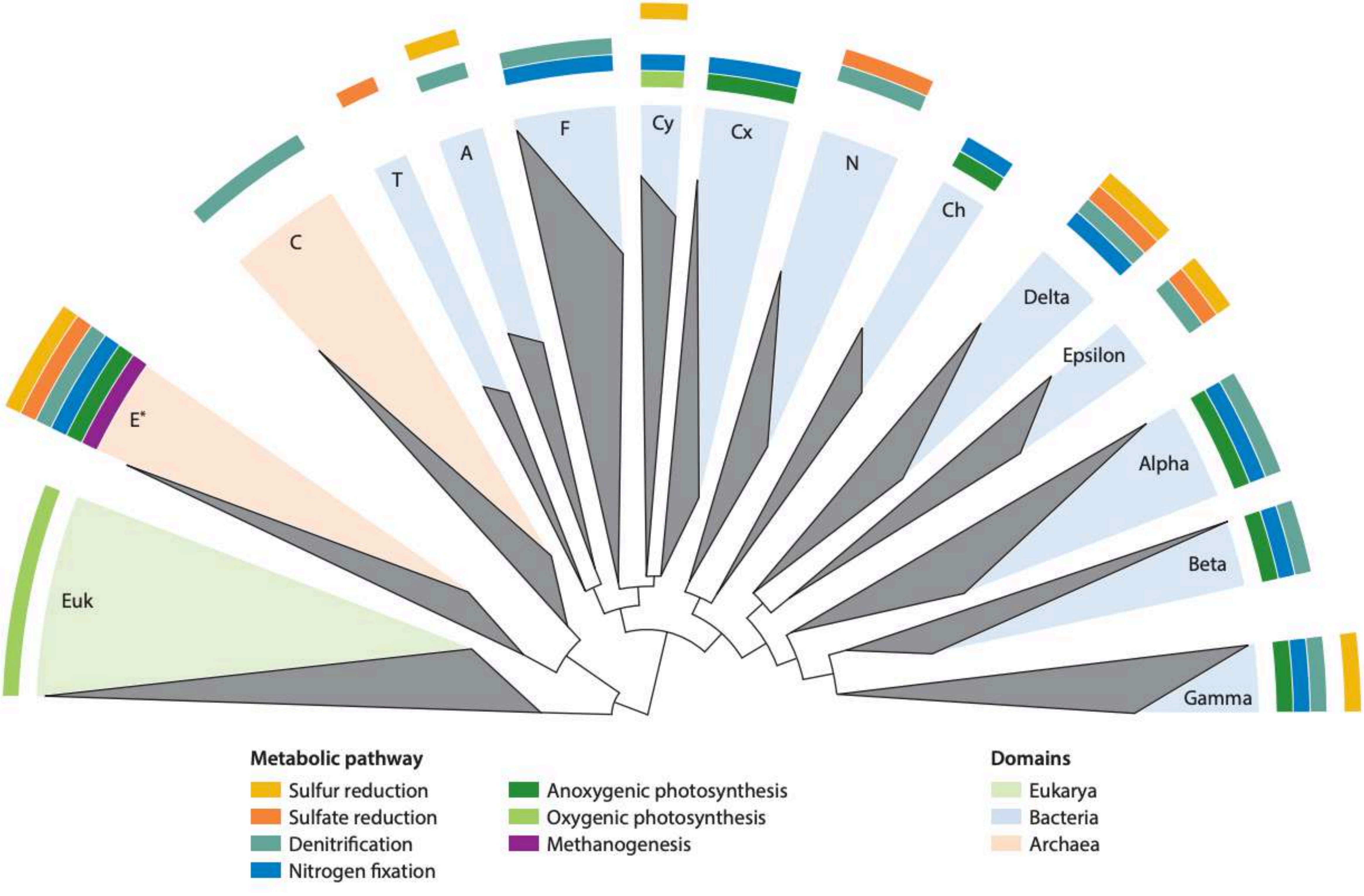


Figure 1

Distribution of selected metabolic pathways on the 16S rRNA tree of life. The tree (constructed with ARB; 104) was edited for clarity and shows selected bacterial and archaeal taxa. The area of each branch is proportional to the total number of 16S rRNA sequences present in the database. Metabolic pathways were assigned based on physiological data (**Supplemental Table 2**). Sulfate reduction includes sulfite and thiosulfate reduction pathways. **Euryarcheata* are capable of bacteriorhodpsin-based photosynthesis only. Abbreviations: A, *Aquificae*; Alpha, *Alphaproteobacteria*; Beta, *Betaproteobacteria*; C, *Crenarchaeota*; Ch, *Chlorobi*; Cx, *Chloroflexi*; Cy, *Cyanobacteria*; Delta, *Deltaproteobacteria*; E, *Euryarchaeota*; Epsilon, *Epsilonproteobacteria*; Euk, *Eukarya*; F, *Firmicutes*; Gamma, *Gammaproteobacteria*; N, *Nitrospirae*; T, *Thermodesulfobacteria*.

Jelen et al. 2016

Microbial diversity and metabolic pathways to survive in the environment



Jelen et al. 2016