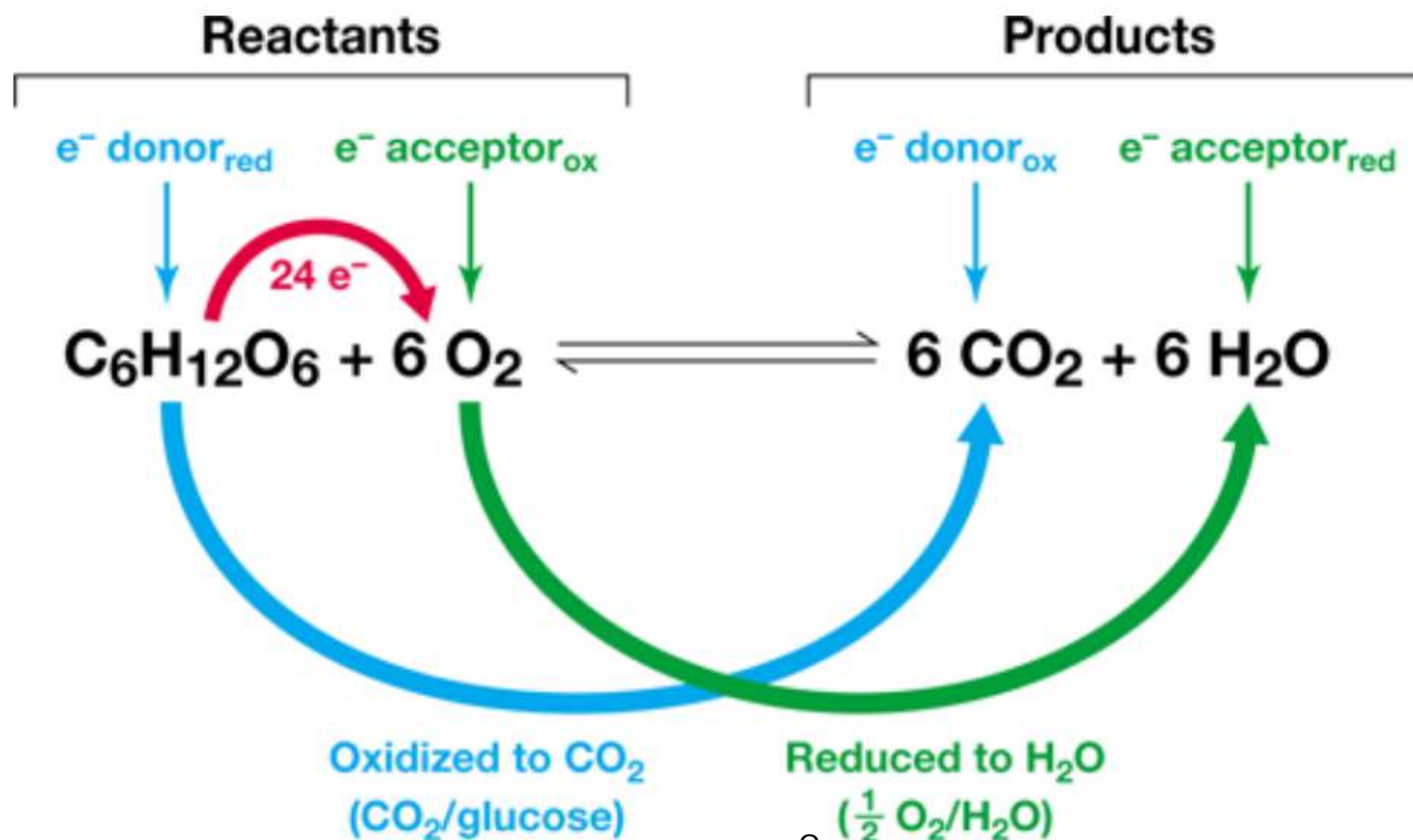


**212 SM L03b**

# Fundamentals in Metabolisms

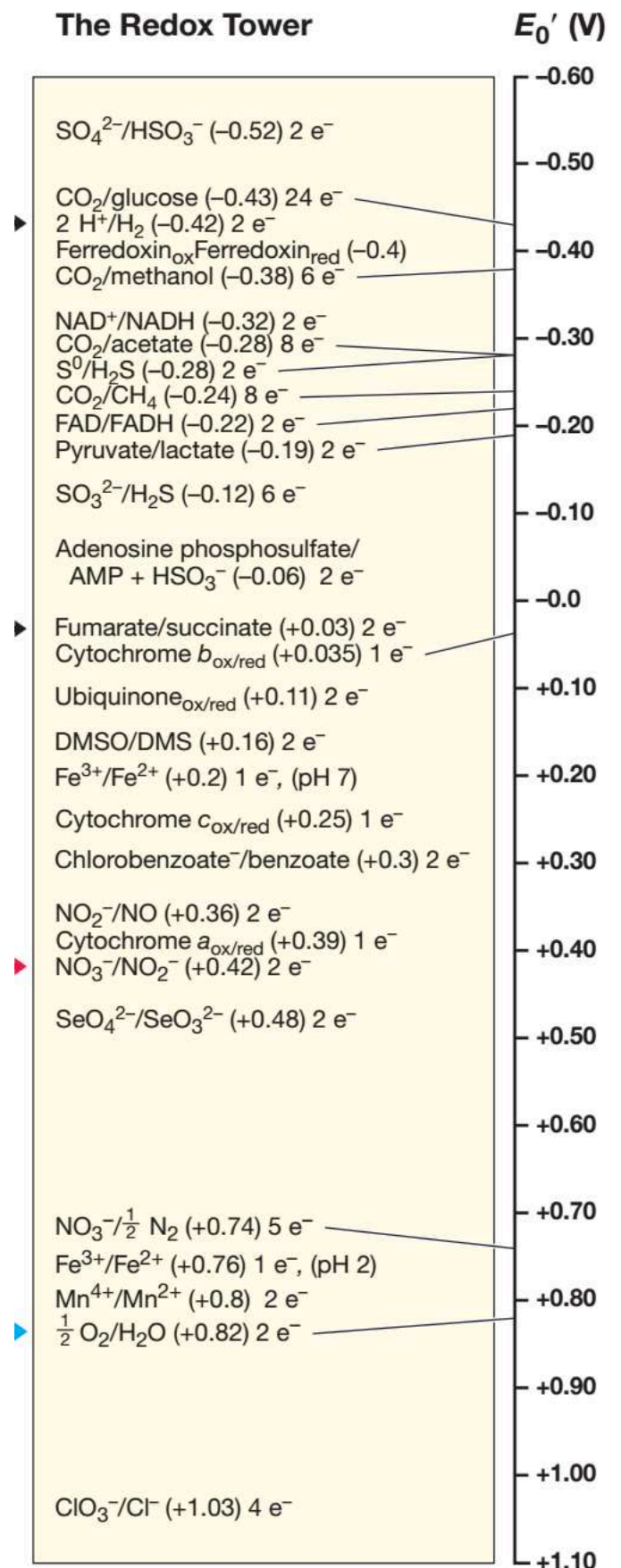
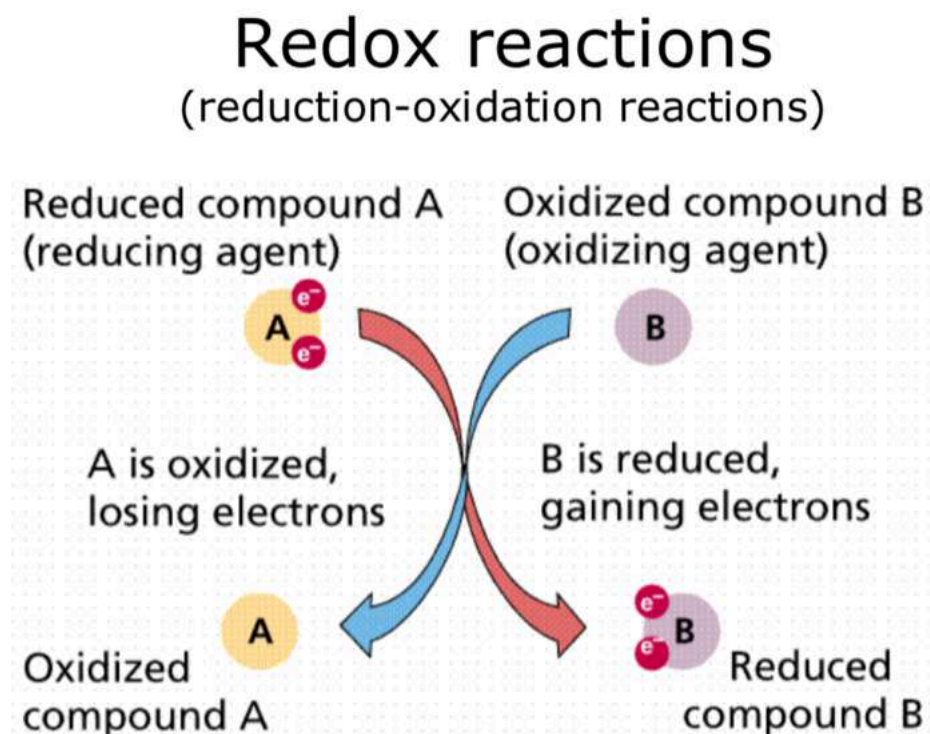
- Transfer  $e^-$  , production reducing power and conserve energy
- Reactions are not performed in single-step —> consecutive reactions in different part of the cells
- Need of soluble  $e^-$  and  $H^+$  carriers:  $NAD^+/NADH$ ,  $FAD^+/FADH_2$

Madigan et al. 2020



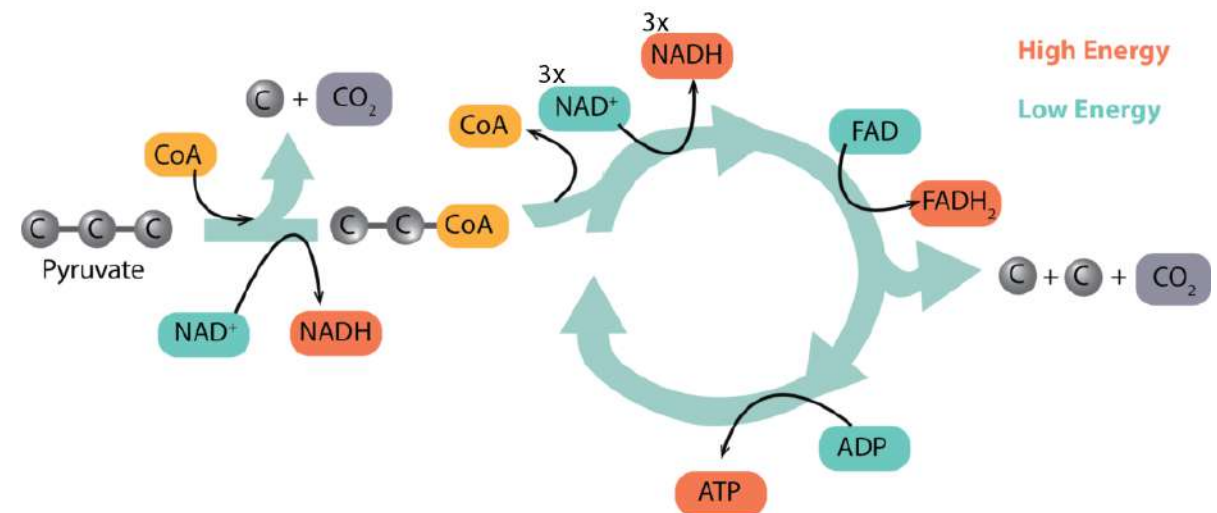
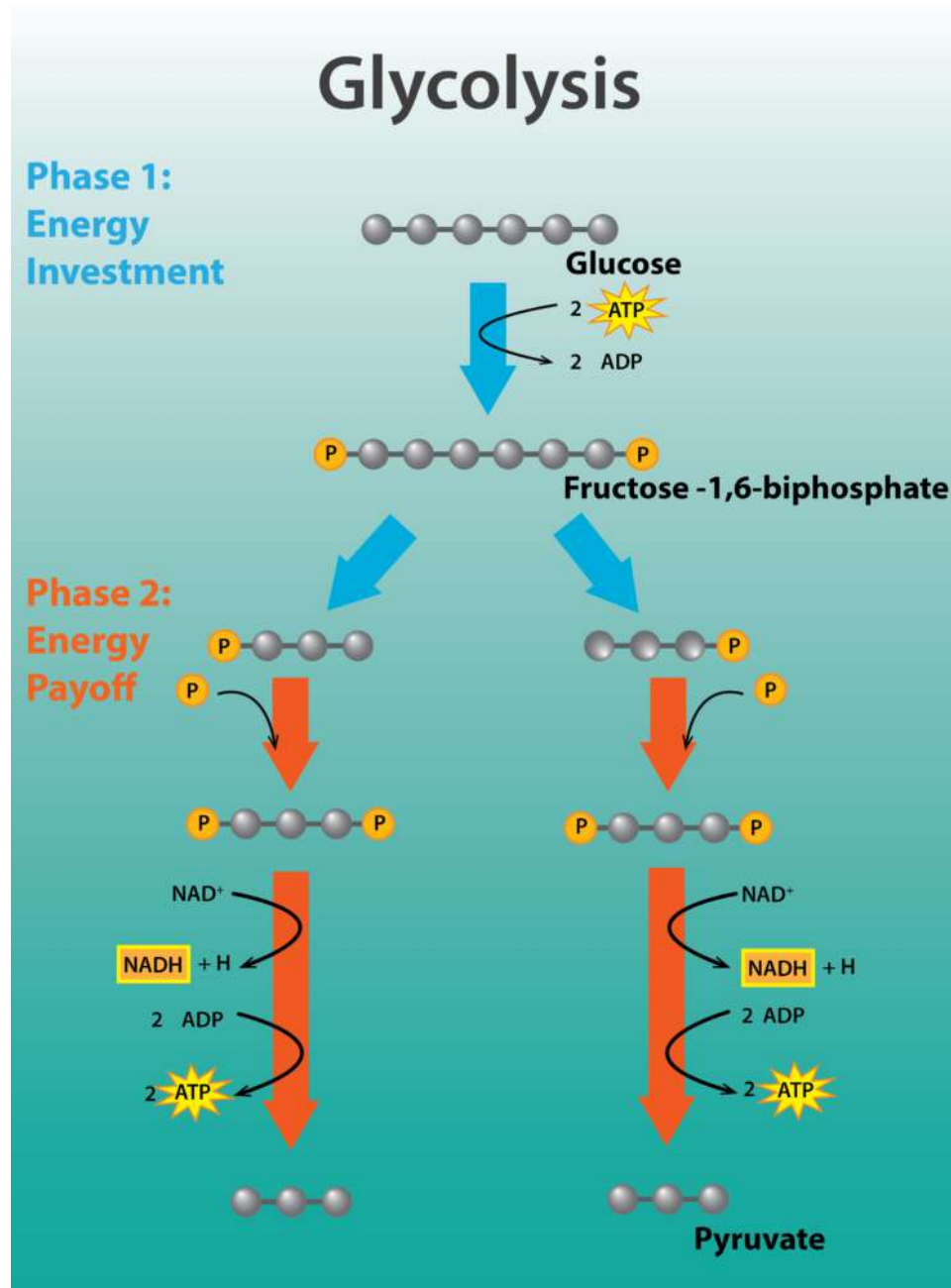
# The Redox Tower

- Redox couples are arranged from the **strongest e<sup>-</sup> donors at the top ( $E_0' < 0$ )** to the strongest e<sup>-</sup> 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 ( $\Delta G_0'$  can be computed via Nernst equation from reduction potential)



# **Aerobic respiration and ATP formation**

# Central metabolism



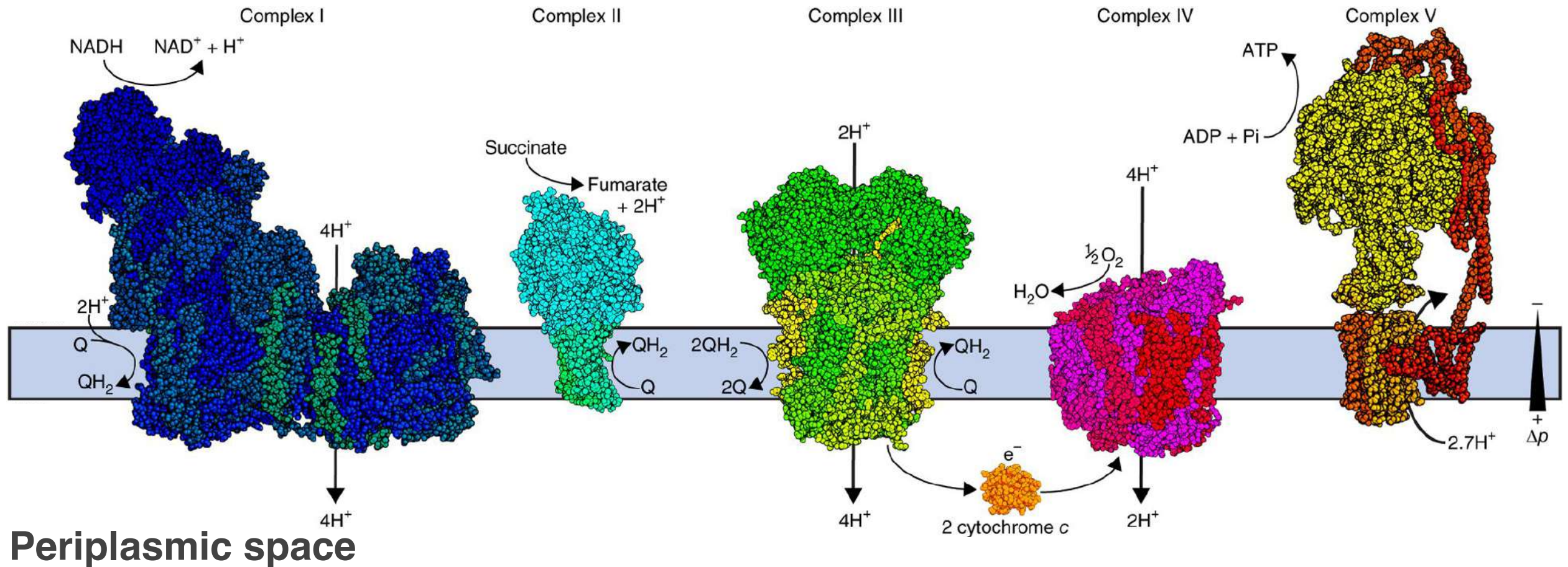
Tricarboxylic Acid Cycle (TSA)  
Citric acid cycle (CAC)  
Krebs cycle

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



# Electron transport chain (ETC), I

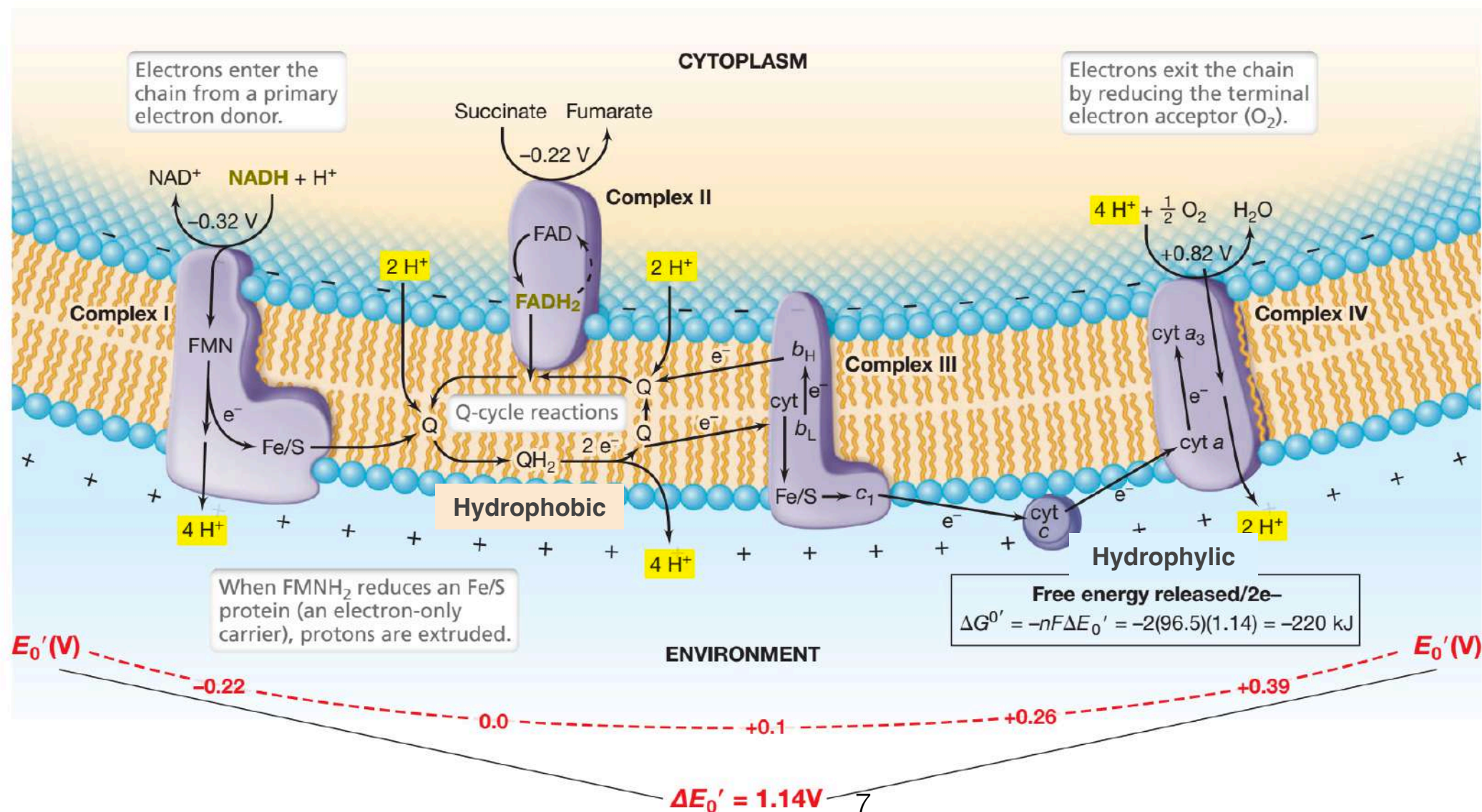
# Cytoplasm



- 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**

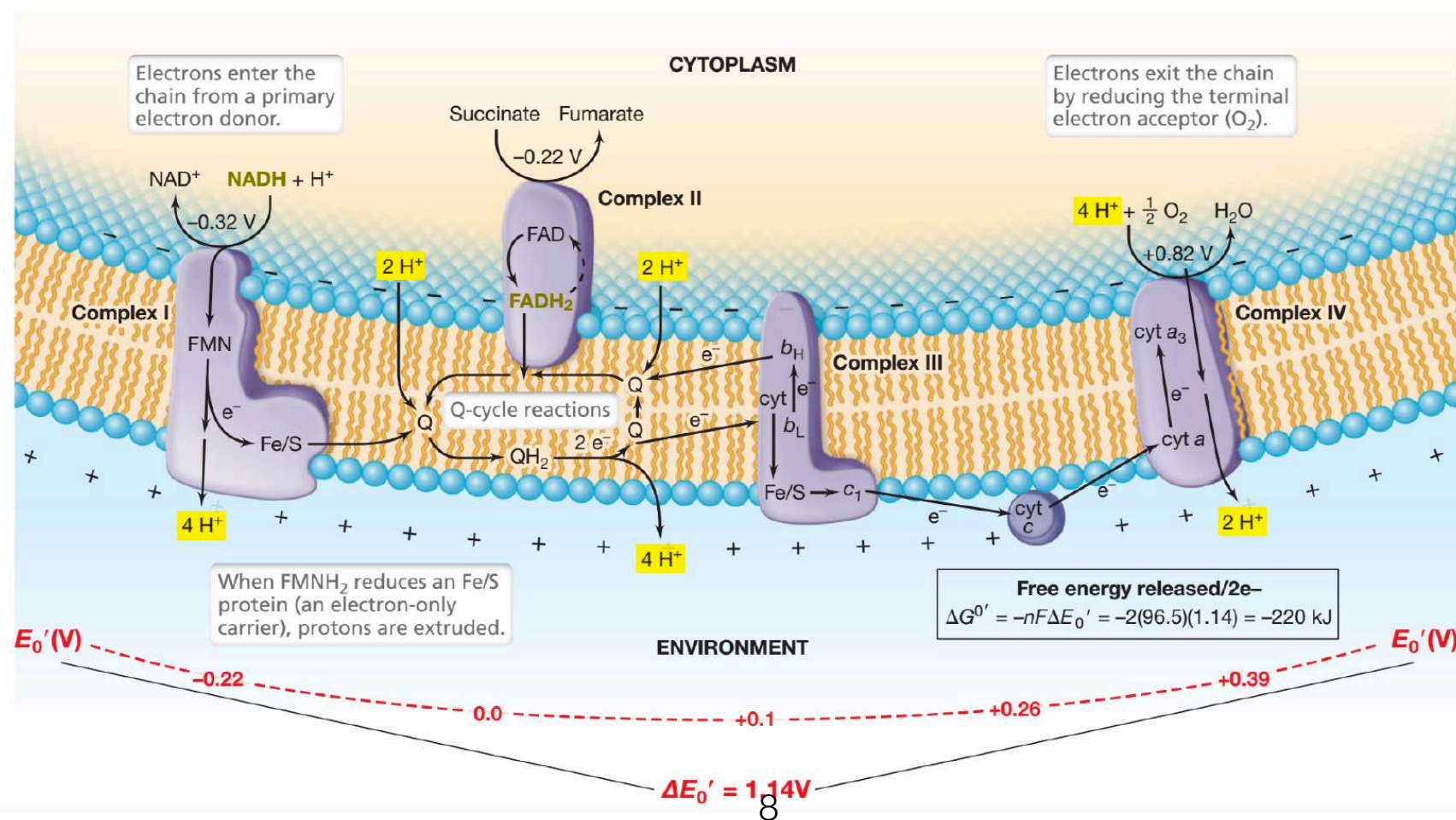




# Electron transport chain, III

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)

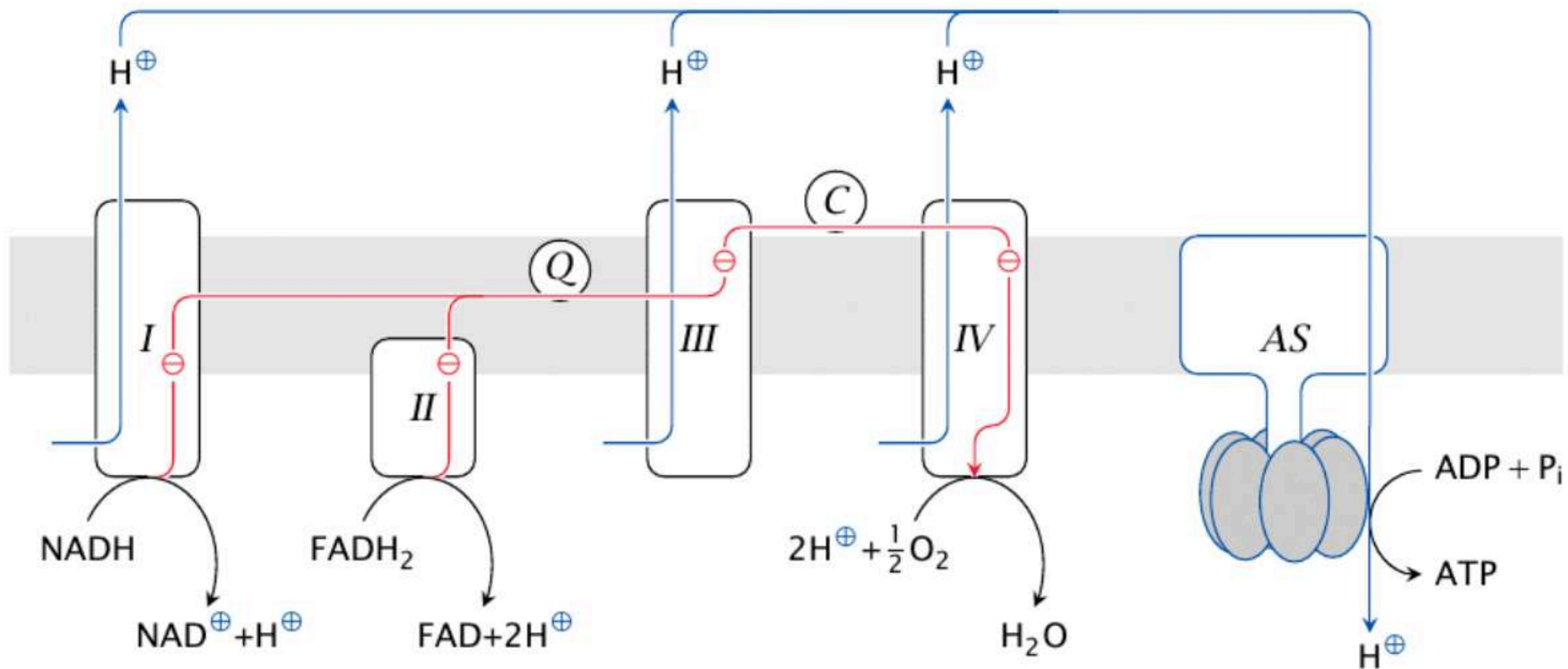




# Electron transport chain, IV

Environment

H<sup>+</sup> flow

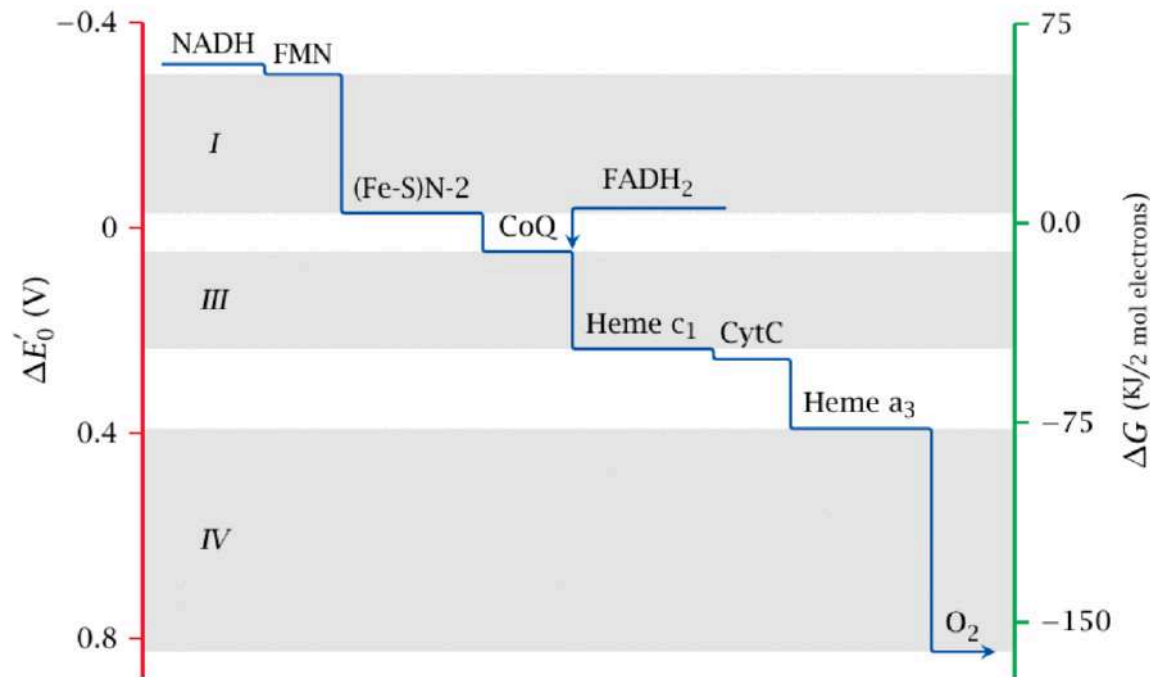


Cytoplasm

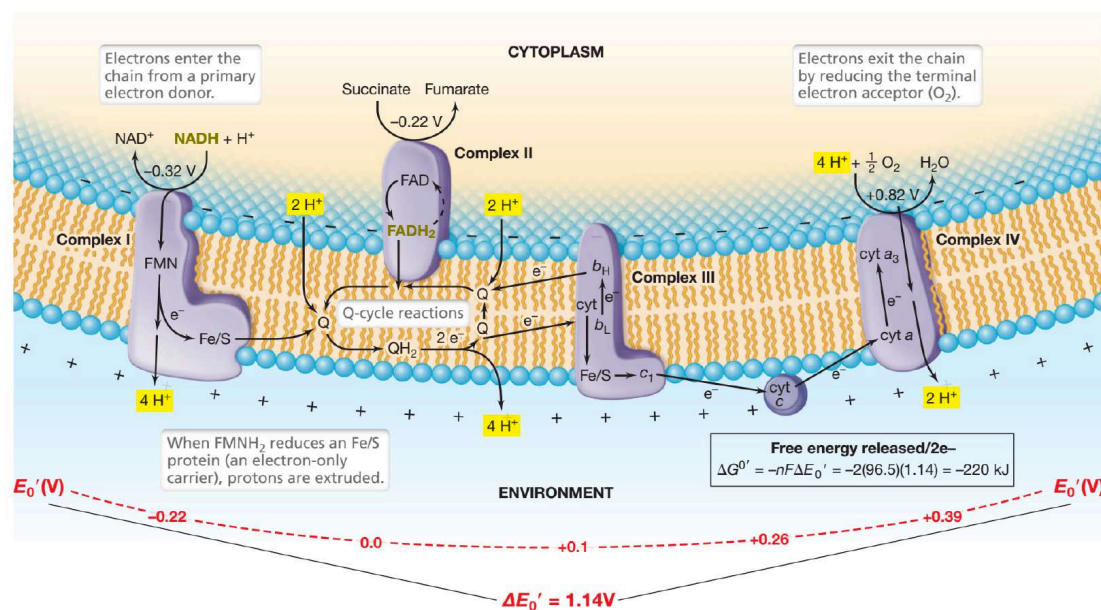
e<sup>-</sup> flow

# Structural orientation for ATP production

## Redox potentials and free energies in the respiratory chain

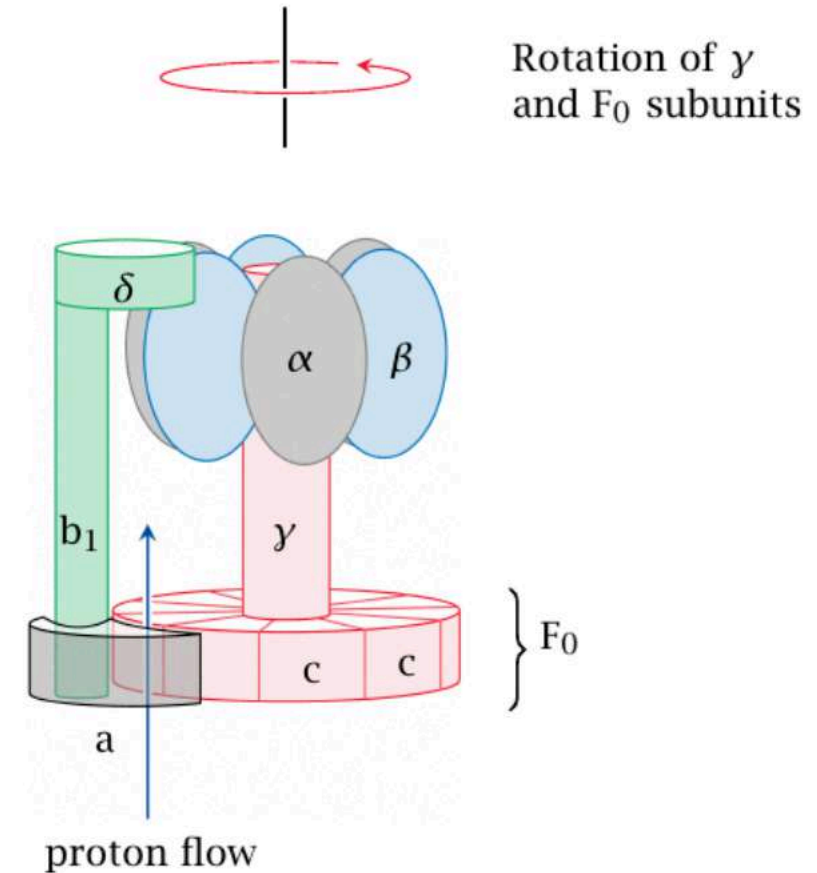
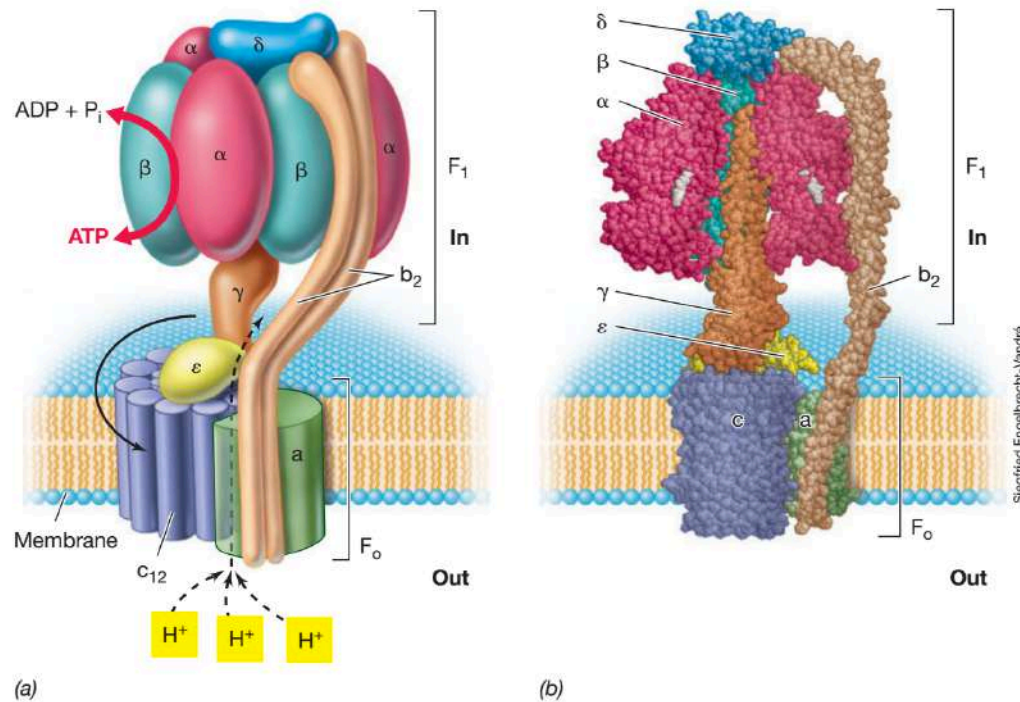


- Spontaneous flow of electrons ( $E_0'$ )
- $H^+$  are separated from  $e^-$  across membrane (spatial localization ETC)
- Inner and outer surfaces of the membrane differ in charge, pH, and electrochemical potential
- Electrochemical potential is proton motive force (PMF) and energizes the membrane, 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



# ATP production, I

Madigan et al. 2018

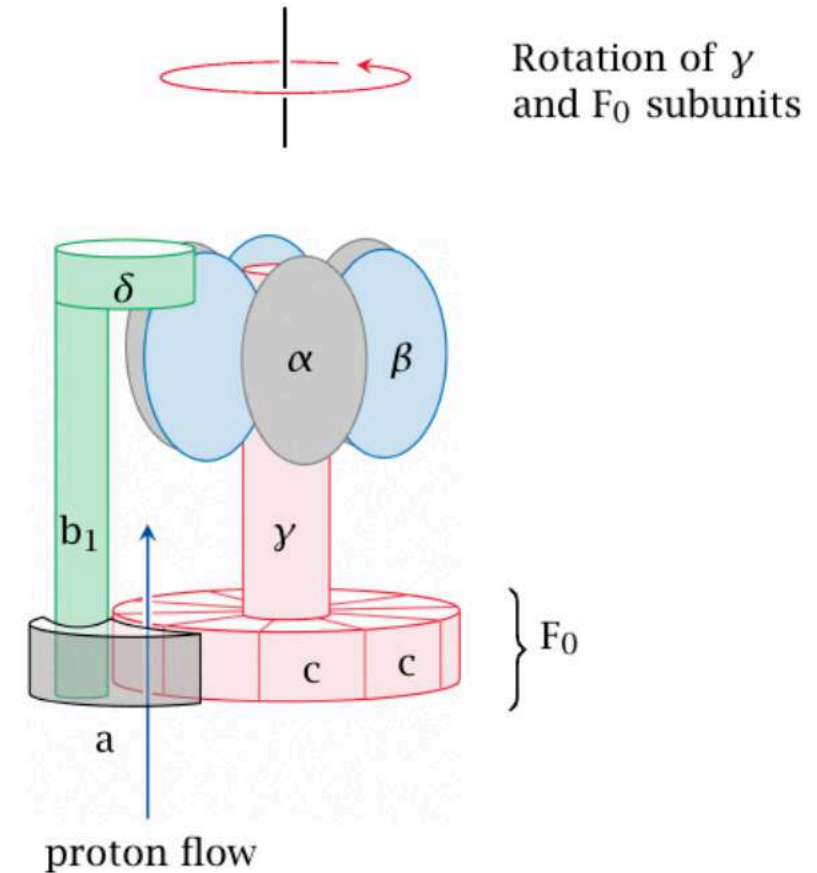
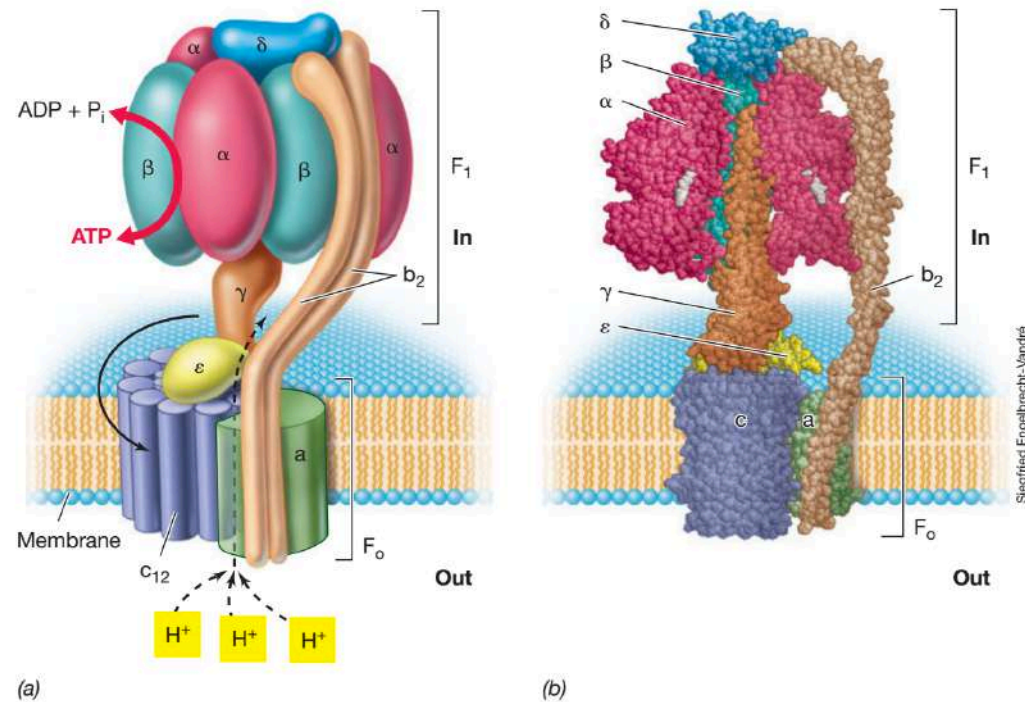


- $H^+$  gradient that drives phosphorylation of ADP to ATP as well as several other important transport systems (nutrient transport, flagellar rotation, and other energy-requiring reactions)
- $3 H^+ \rightarrow ATP$  (Noguchi et al., 2004):  $F_1$  is the catalytic complex responsible for the interconversion of  $ADP + P_i$  and ATP.  $F_0$ , the rotor, is integrated in the membrane



# ATP production, II

Madigan et al. 2018



<http://wcut.uwaterloo.ca/webnotes/Metabolism/RespiratoryChain.html>

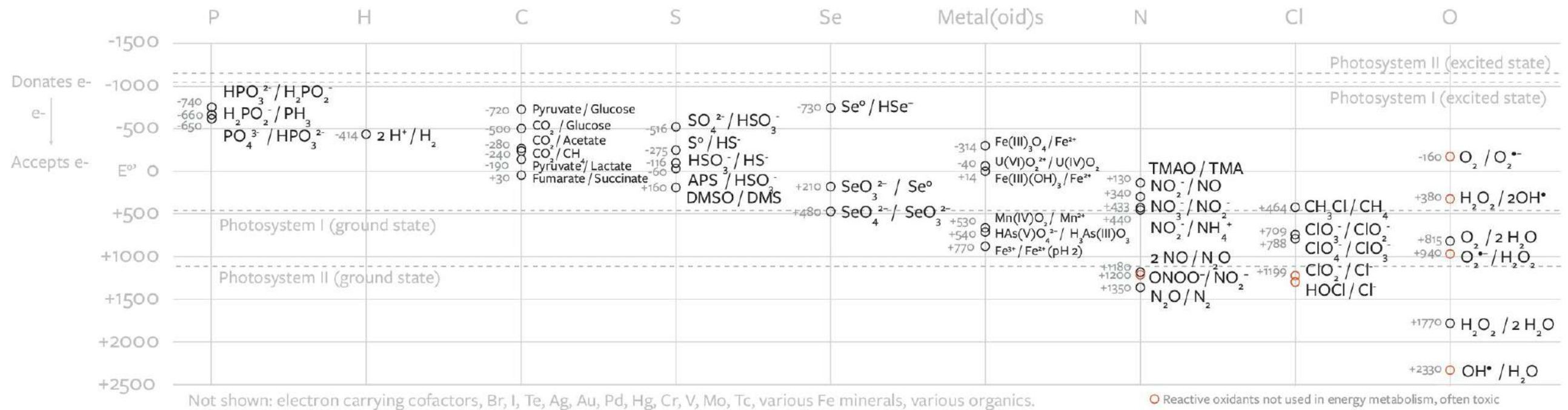
- In analogy to how dissipation of the pmf applies torque that rotates the bacterial flagellum, the pmf also creates torque in the large membrane protein complex that synthesizes ATP
- This complex is called ATP synthase (ATPase)
- The activity of ATPase is driven by the pmf, and the formation of ATP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

# 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<sup>-</sup>).

**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.

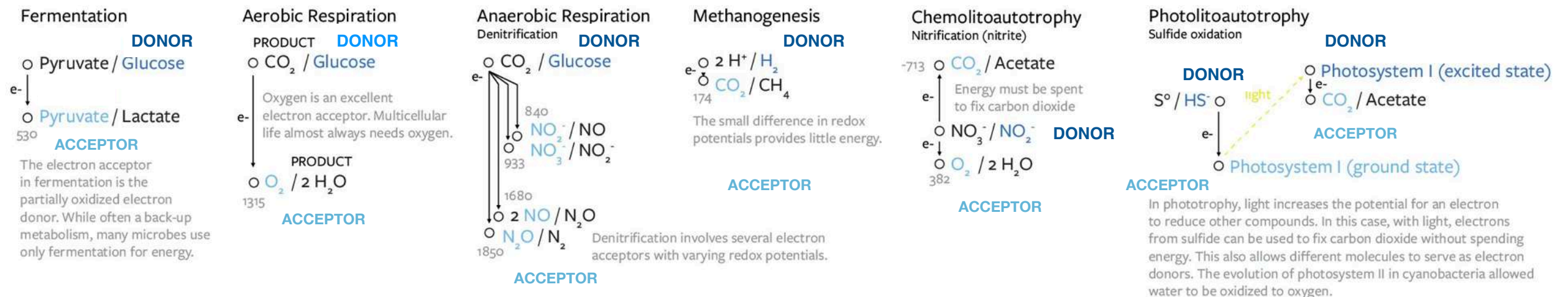


- Across periodic table
- P, H, C, S, Se, Fe, U, Mn, As, N, Cl, O

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

# Microbial Redox couples structure the metabolism

## Examples of energetically favorable redox metabolisms



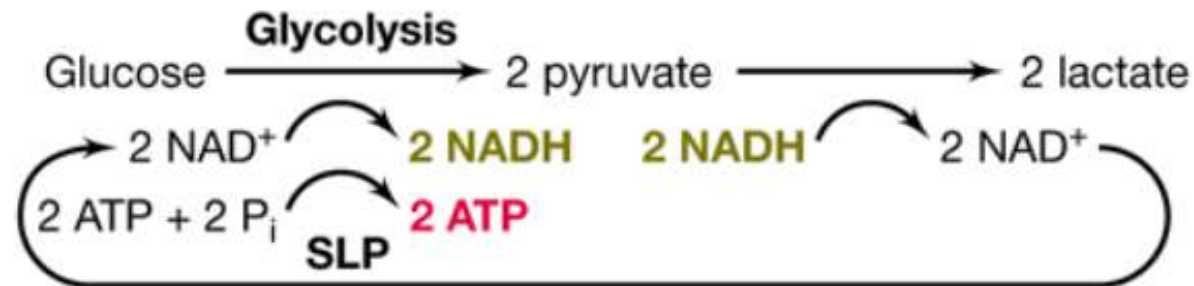
\* For teaching purposes only. Consult the scientific literature for exact values.

Image produced by Tyler Barnum @tylerbarnumphd

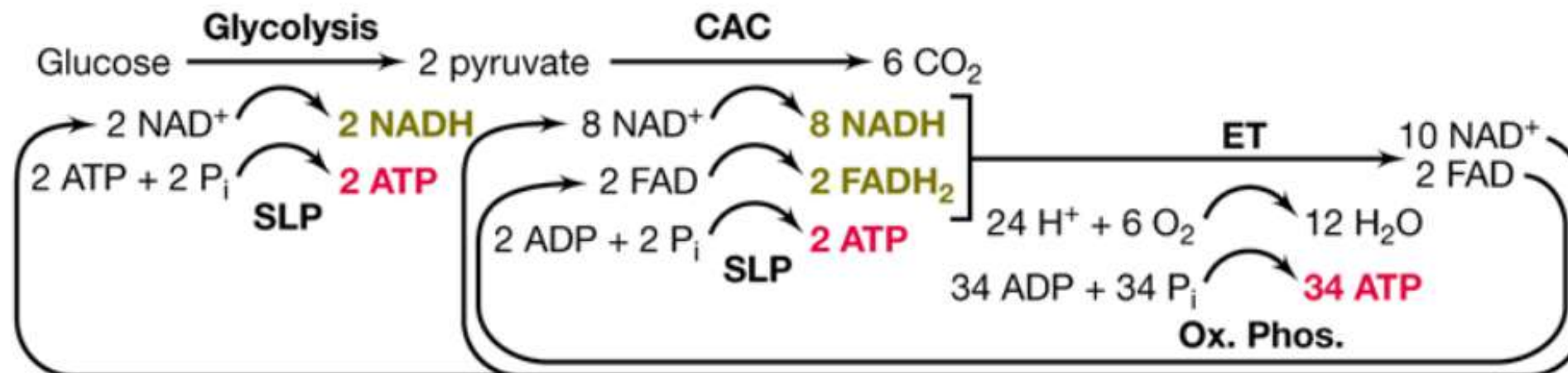


# Figure 3.21 Energetics in fermentation and aerobic respiration.

## Lactic acid fermentation

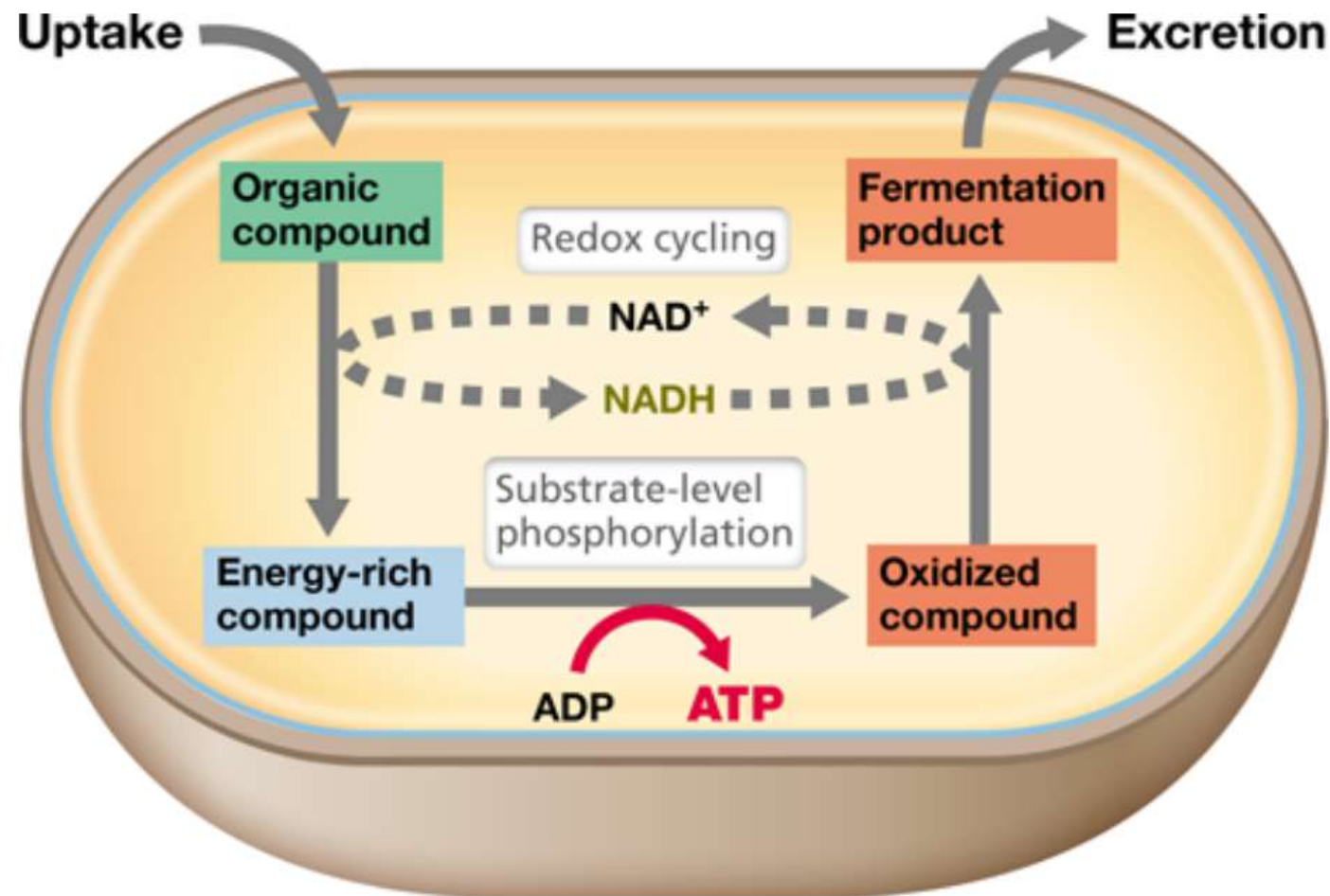


## Aerobic respiration



# Fermentation

**Figure 3.14 The essentials of fermentation.**



- 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 because an external e-acceptor is lacking
- Product is excreted from the cell and ATP is produced by substrate-level phosphorylation

# 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*



# Fermentation, II

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

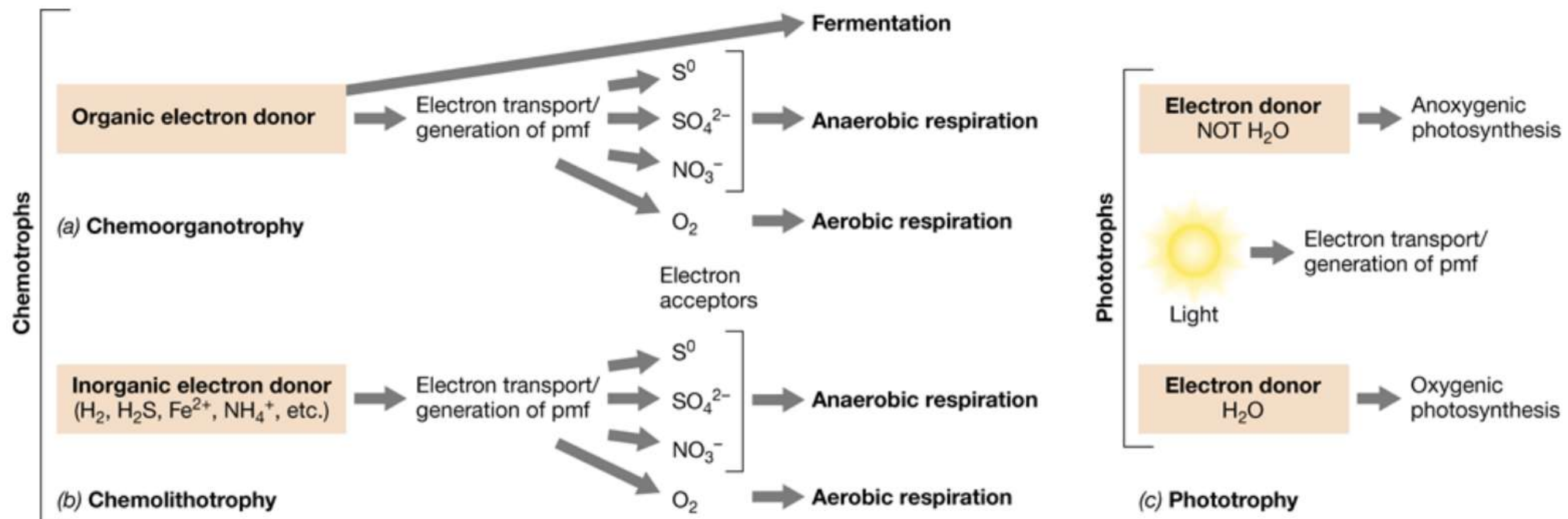
Type	Reaction (substrate → products)	Organisms
Alcoholic	Hexose <sup>a</sup> → 2 ethanol + 2 CO <sub>2</sub>	Yeast, <i>Zymomonas</i>
Homolactic	Hexose → 2 lactate <sup>-</sup> + 2 H <sup>+</sup>	<i>Streptococcus</i> , some <i>Lactobacillus</i>
Heterolactic	Hexose → lactate <sup>-</sup> + ethanol + CO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

- 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)

# 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,  $P_i$ , as is done in oxidative phosphorylation (ET)

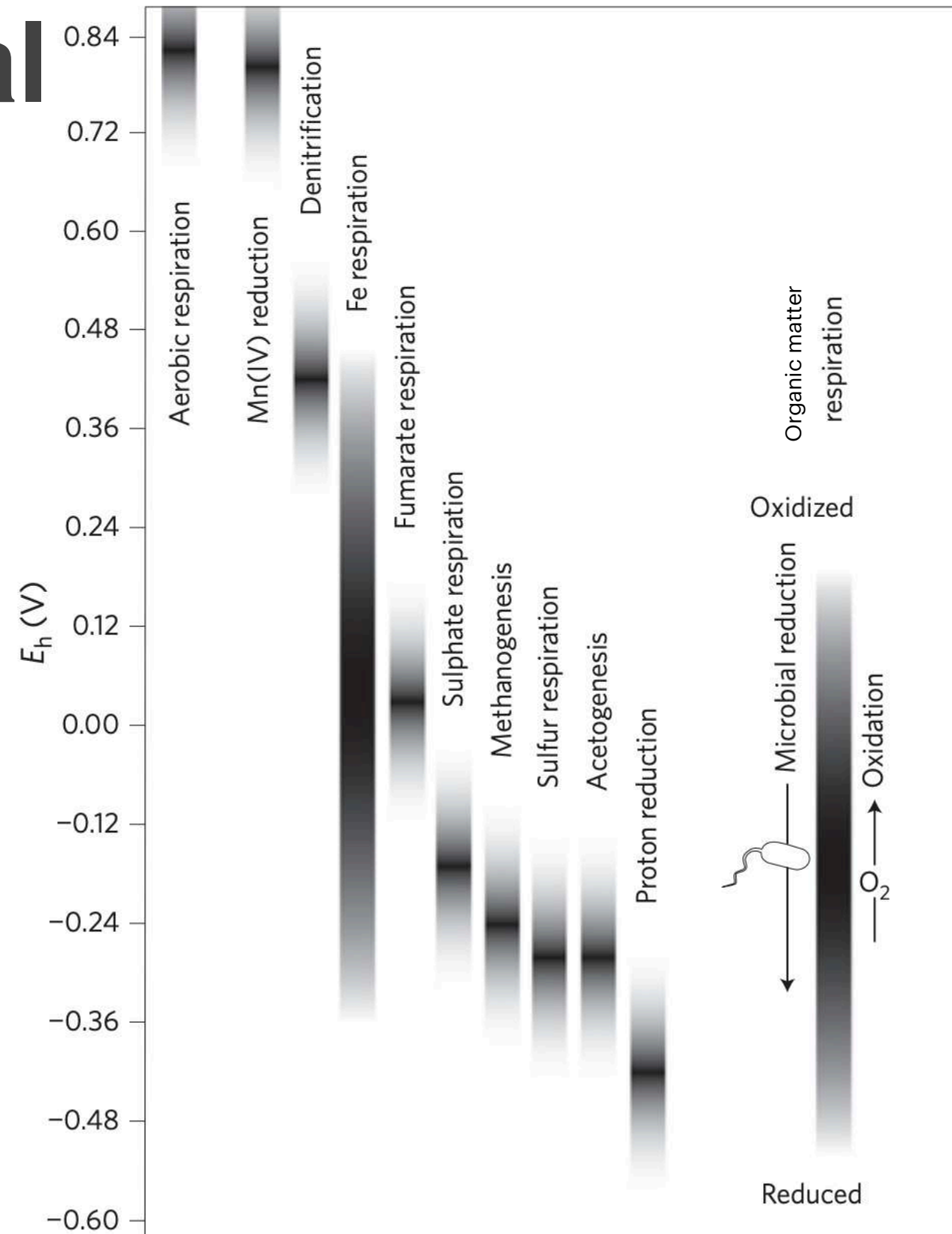
# Figure 3.22 Metabolic diversity and its relationship to oxygen.



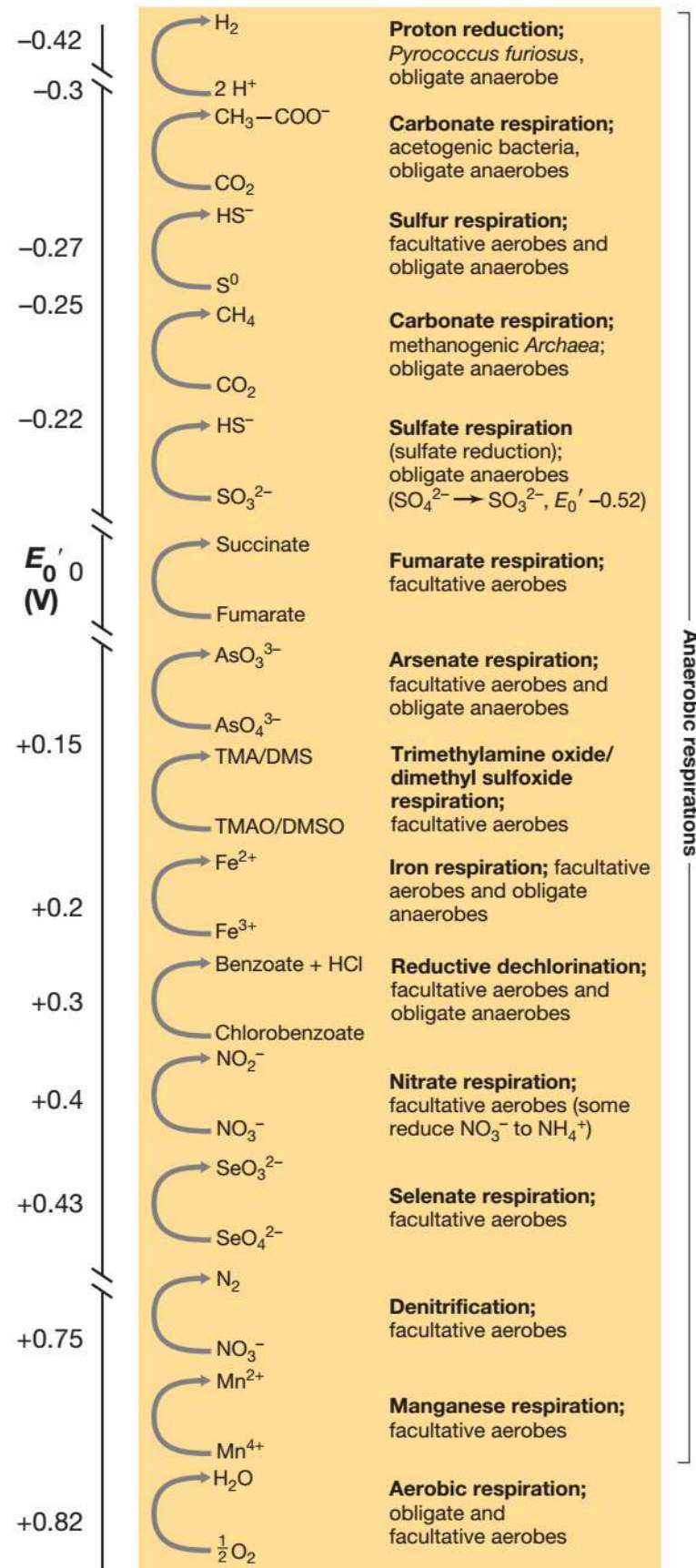


# Reduction potential ranges of microbial respiration

- 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*  $O_2 > NO_3^- > \text{fumarate}$ )



# Anaerobic respiration



## Microbially mediated reactions

### Microaerophiles

$4\text{Fe}^{2+} + 10\text{H}_2\text{O} + \text{O}_2 \rightarrow 4\text{Fe}(\text{OH})_3 + 8\text{H}^+$   
*Gallionella* spp., *Leptothrix* spp.,  
*Mariprofundus* spp., *Sideroxydans* spp.

### Photoferrotrophs

$\text{HCO}_3^- + \text{Fe}^{2+} + 10\text{H}_2\text{O} \xrightarrow{h\nu} (\text{CH}_2\text{O}) + 4\text{Fe}(\text{OH})_3 + 7\text{H}^+$   
*Rhodospseudomonas palustris* TIE-1  
*Rhodobacter* sp. SW2  
*Chlorobium ferrooxidans* (KoFox)  
*Thiodictyon* sp. F4

### NO<sub>3</sub><sup>-</sup>-reducing Fe(II)-oxidizers

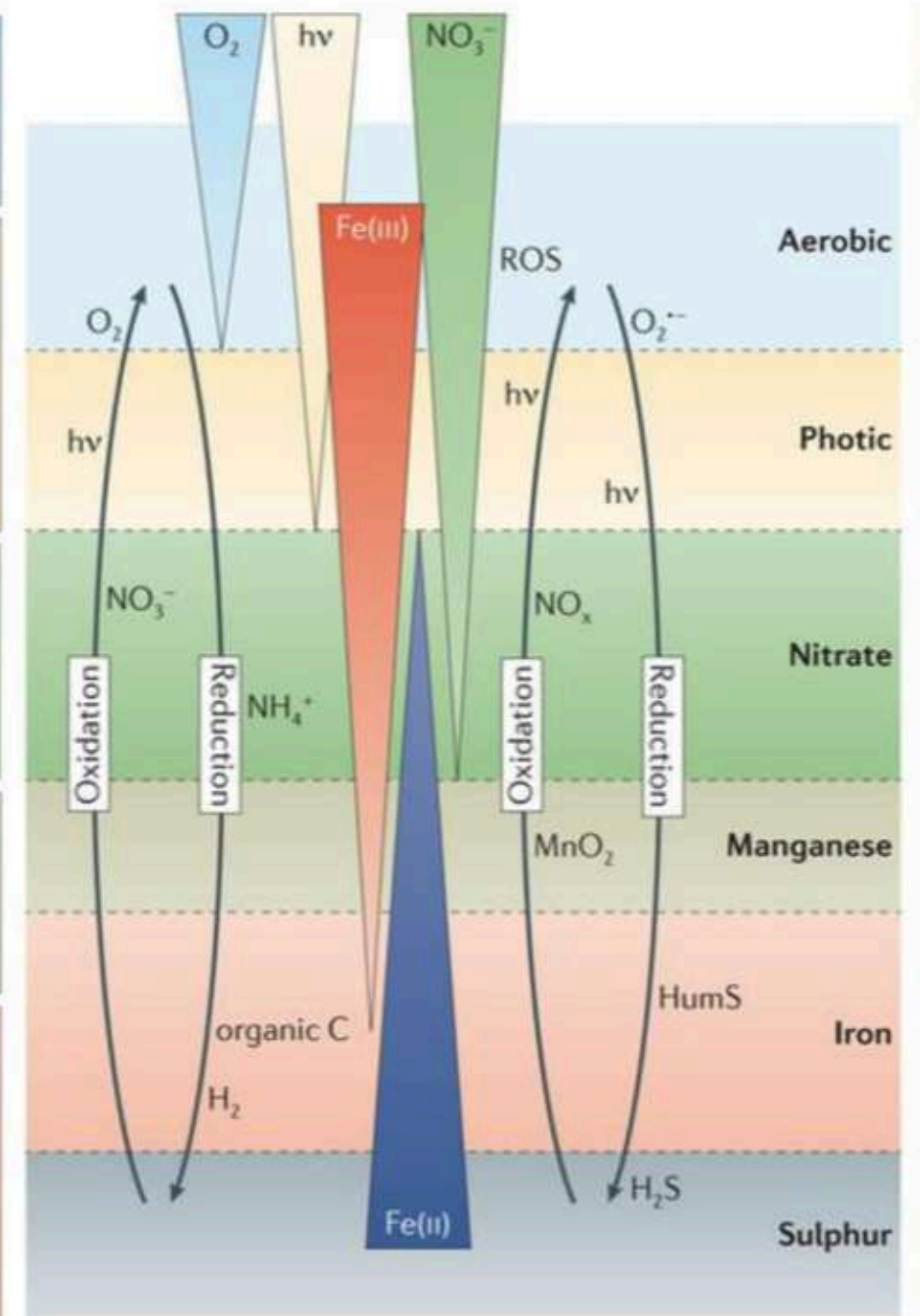
$10\text{Fe}^{2+} + 2\text{NO}_3^- + 24\text{H}_2\text{O} \rightarrow 10\text{Fe}(\text{OH})_3 + \text{N}_2 + 18\text{H}^+$   
*Acidovorax* spp., KS, 2002  
*Thiobacillus denitrificans*

### Fe-ammox

$\text{NH}_4^+ + 6\text{FeOOH} + 10\text{H}^+ \rightarrow \text{NO}_2^- + 6\text{Fe}^{2+} + 10\text{H}_2\text{O}$   
 Unknown

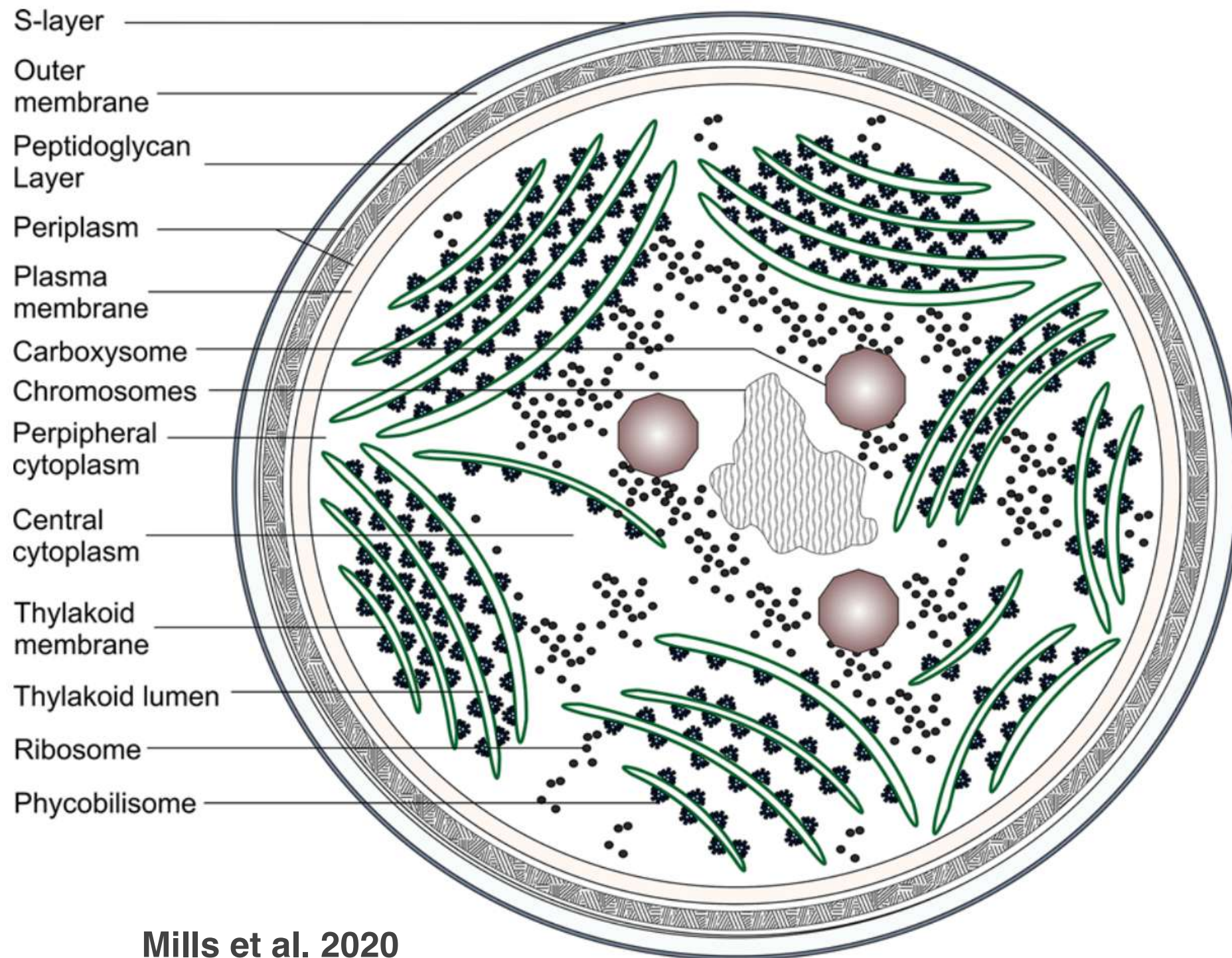
### Fe(III)-reducing organic C and/or H<sub>2</sub>-oxidizers

$4\text{FeOOH} + \text{CH}_3\text{CHOHCOO}^- + 7\text{H}^+ \rightarrow 4\text{Fe}^{2+} + \text{CH}_3\text{COO}^- + \text{HCO}_3^- + 6\text{H}_2\text{O}$   
 $2\text{Fe}(\text{OH}) + \text{H}_2 \rightarrow 2\text{Fe}^{2+} + 2\text{H}_2\text{O}$   
*Geobacter* spp., *Shewanella* spp.,  
*Albidoferax ferrireducens*, *Geothrix* spp.





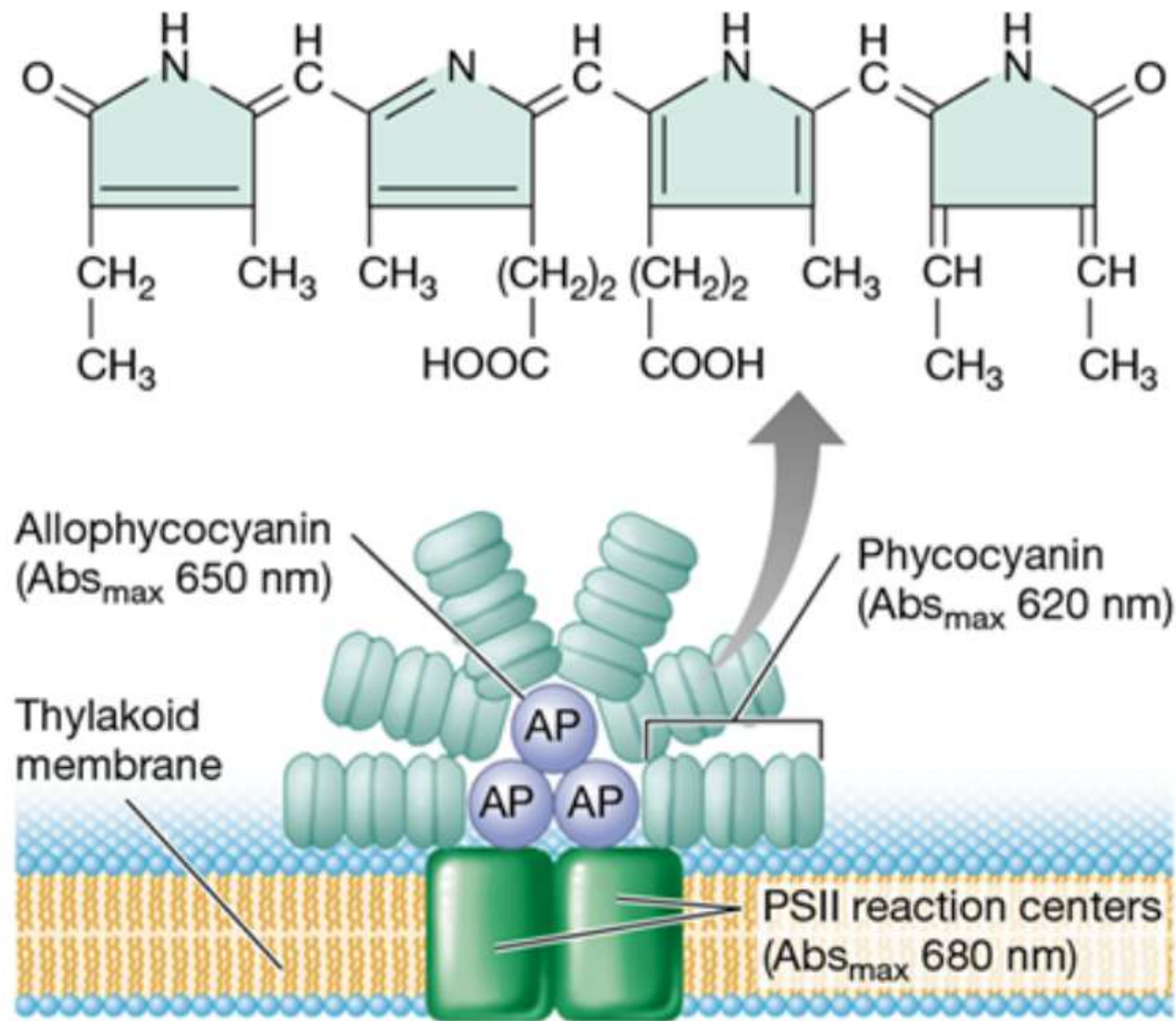
# Photosynthesis: Calvin–Benson–Bassham



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



# Oxygenic photosynthesis

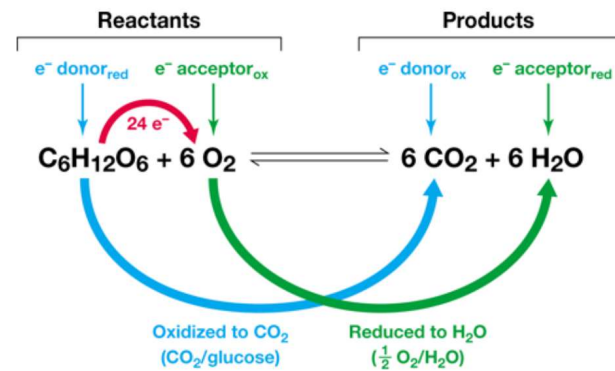


Madigan et al. 2020

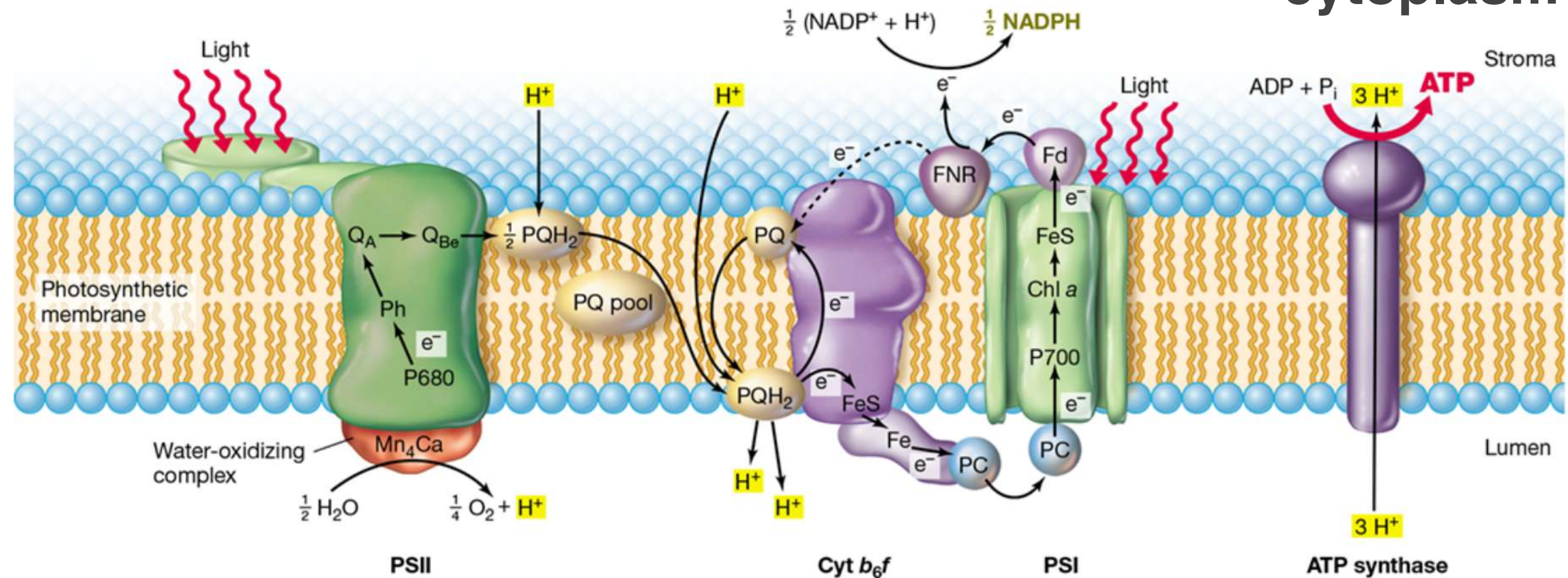
- Physical location within the cell (Cyanobacteria)
- Bilayer w. proteins and complex that capture light, phycobilisome



# Photo



# cytoplasm

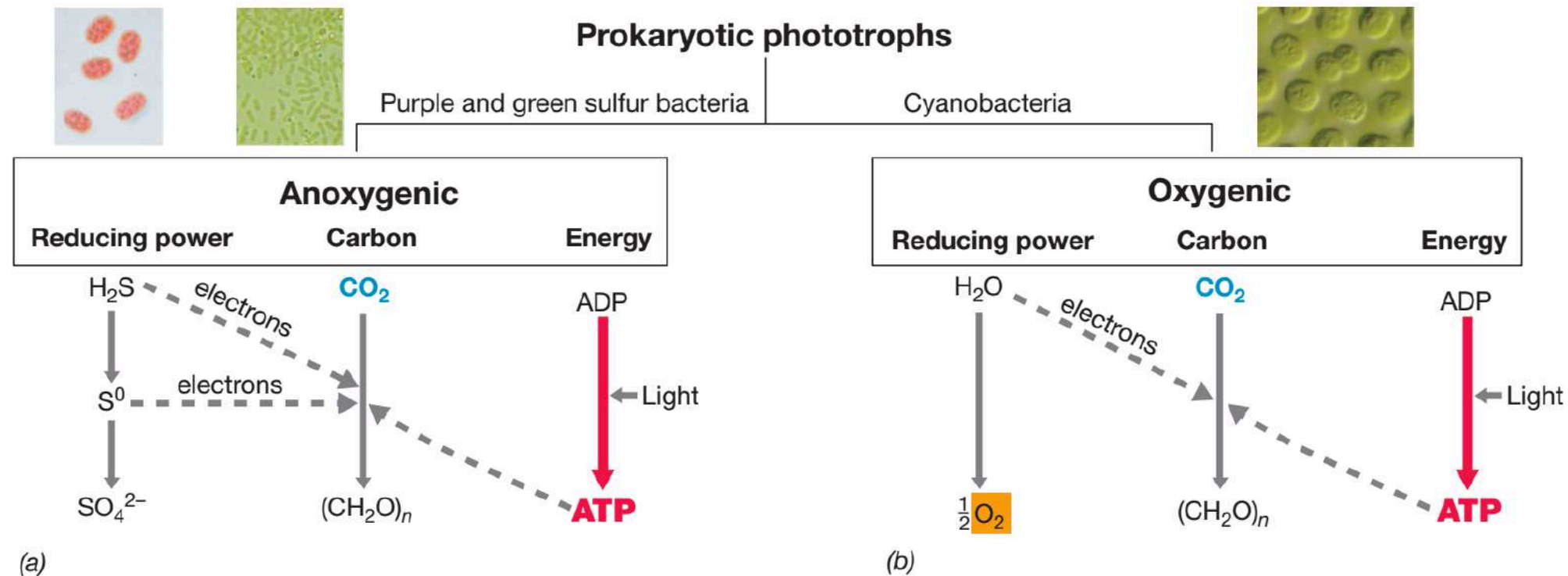


- Splitting of  $\text{H}_2\text{O}$
- Generation  $\text{H}^+$  motive force
- Generation of NADPH  $\rightarrow$  **C fixation (from  $\text{CO}_2$ )** via Calvin–Benson–Bassham cycle
- ATP production

Madigan et al. 2020



# Light driven processes



Madigan et al. 2018

## Winogradsky columns

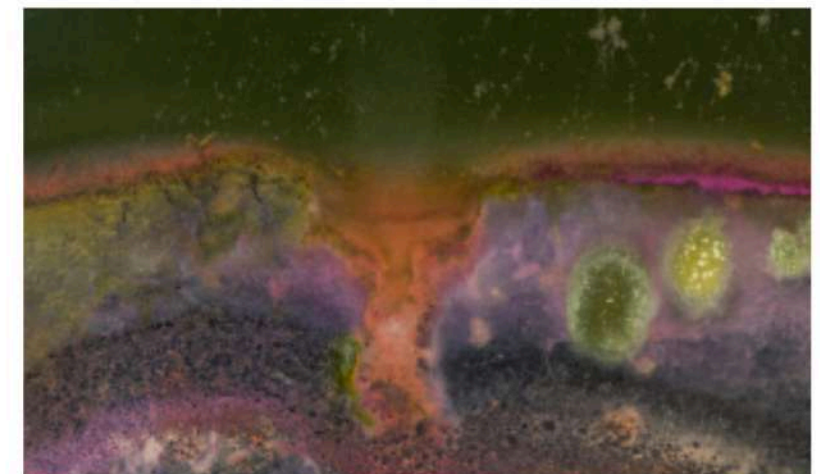
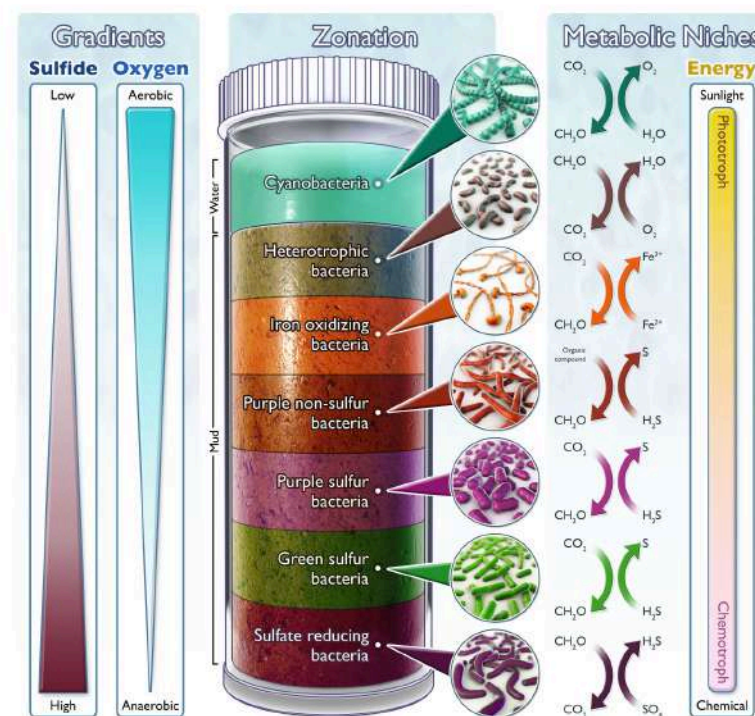


Figure 3. The upper sediment interface on day 15. Aerobic cyanobacteria and algae (upper aqueous phase), yellow-orange microaerophilic iron-oxidizing bacteria, and anaerobic green and purple photosynthetic bacteria develop into layered communities.

# Rewiring of exhibiting membrane-associated micromachines

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

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

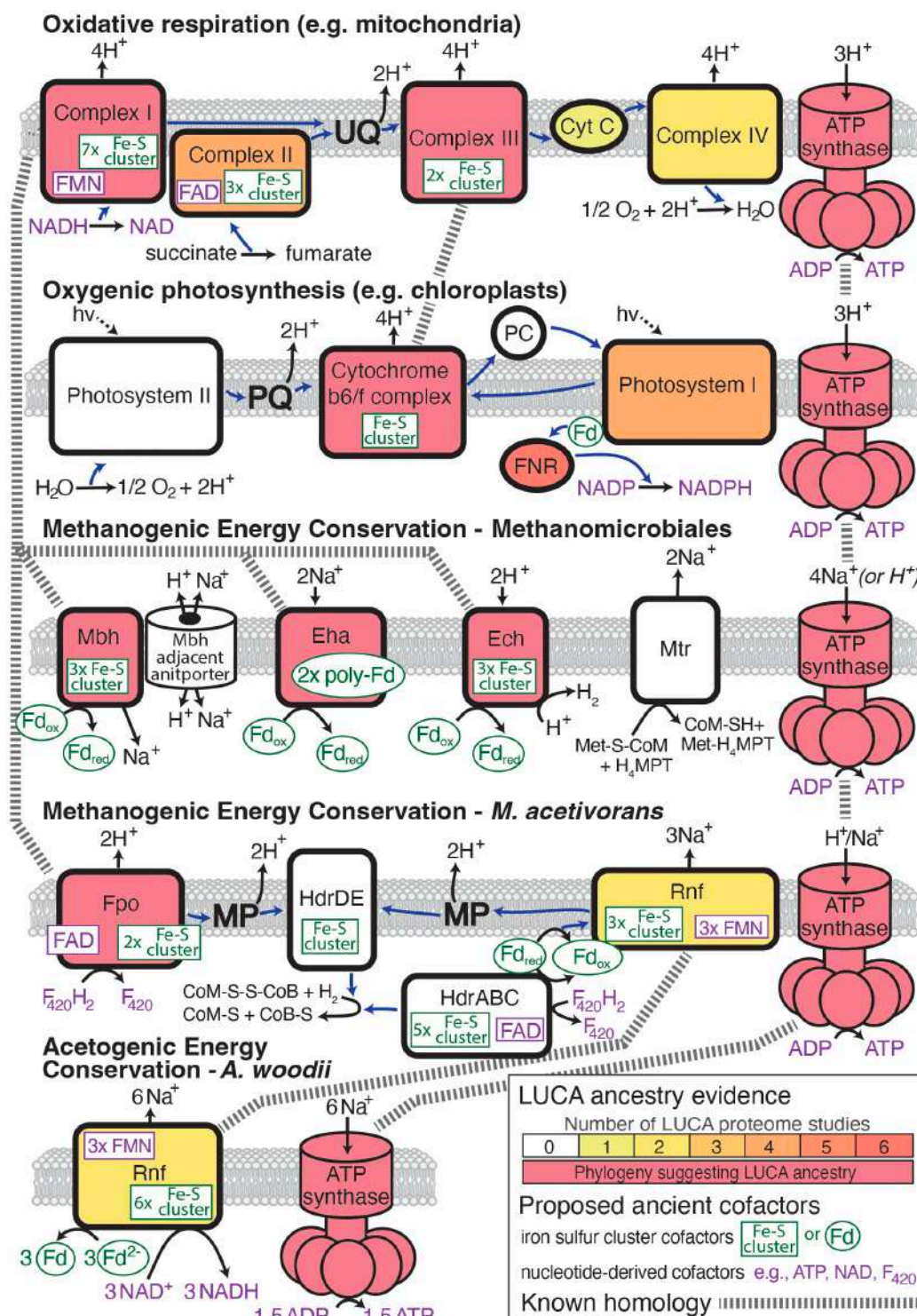
*Electron flow is shown as blue arrows.*

*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.*

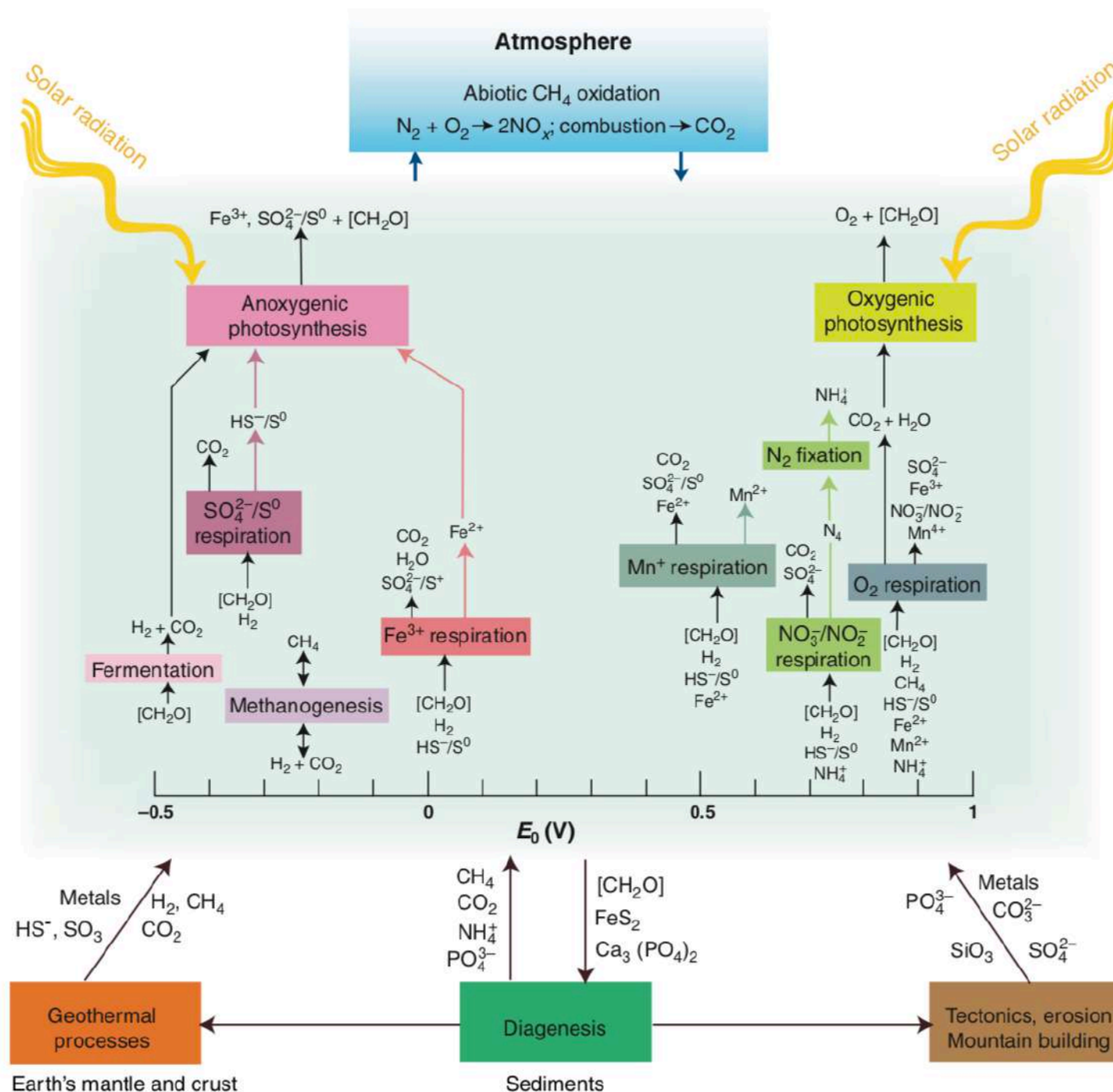


# Recap



# Biosphere model of energy fluxes and elemental cycles

Falkowski et al. 2008



Microbial microscale actions structure planet-scale functioning

# 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

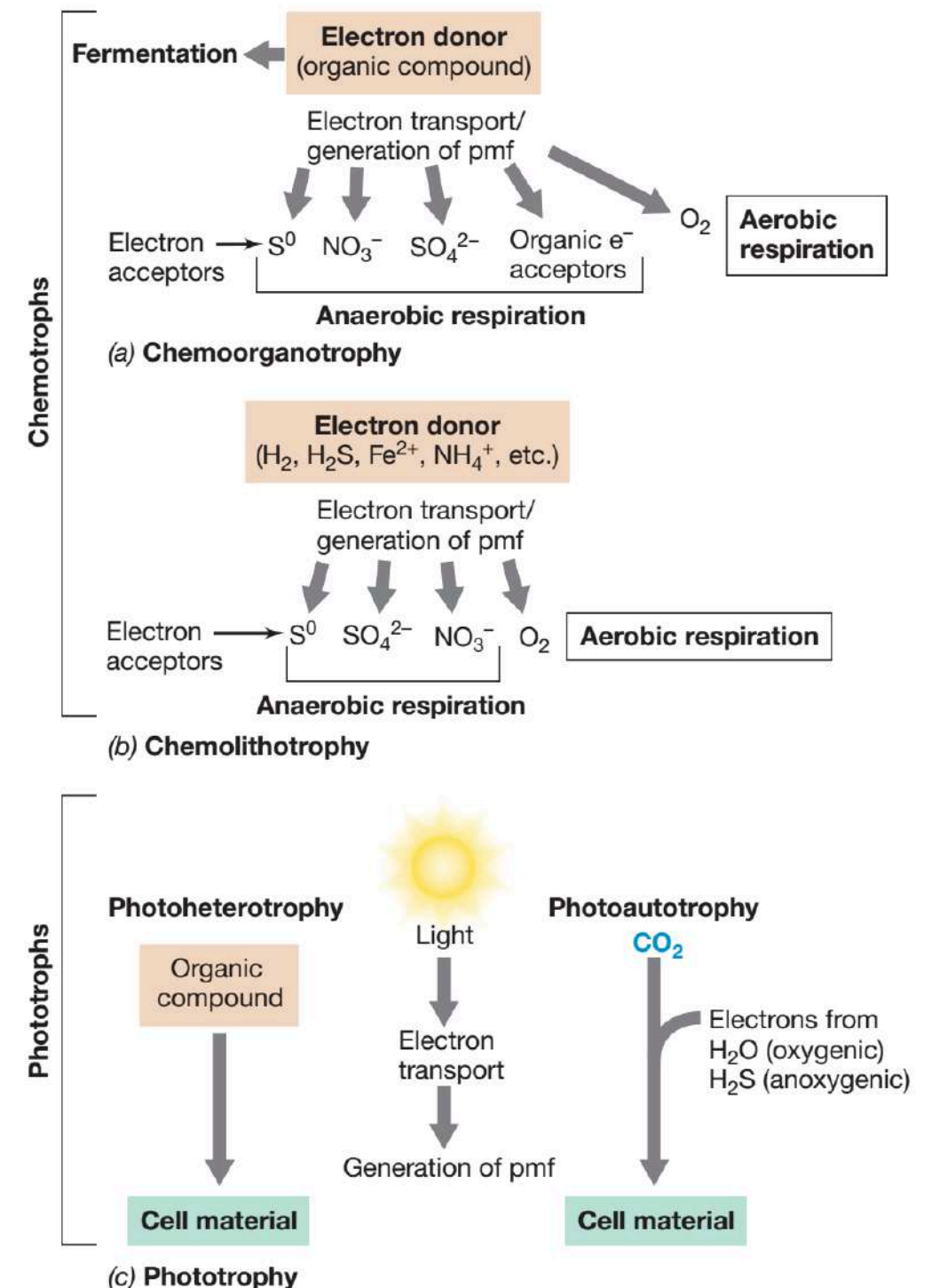
## •Anaerobic Respiration

Lower ATP yield than aerobic respiration; NAD easily reduced

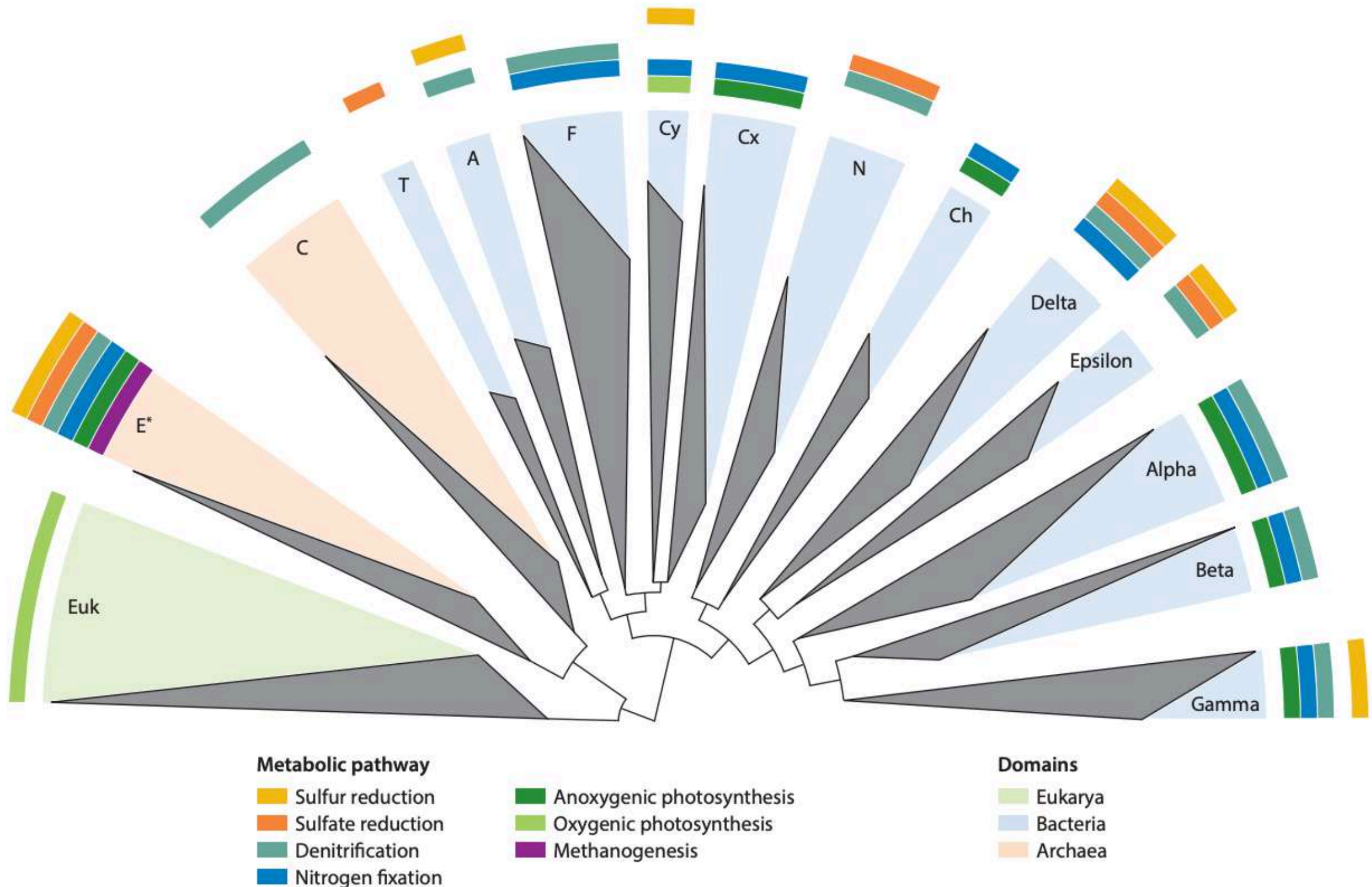
Requires electron acceptor other than oxygen

## Fermentation

Little ATP, no net NAD reduction, MOST SIMPLE SYSTEM

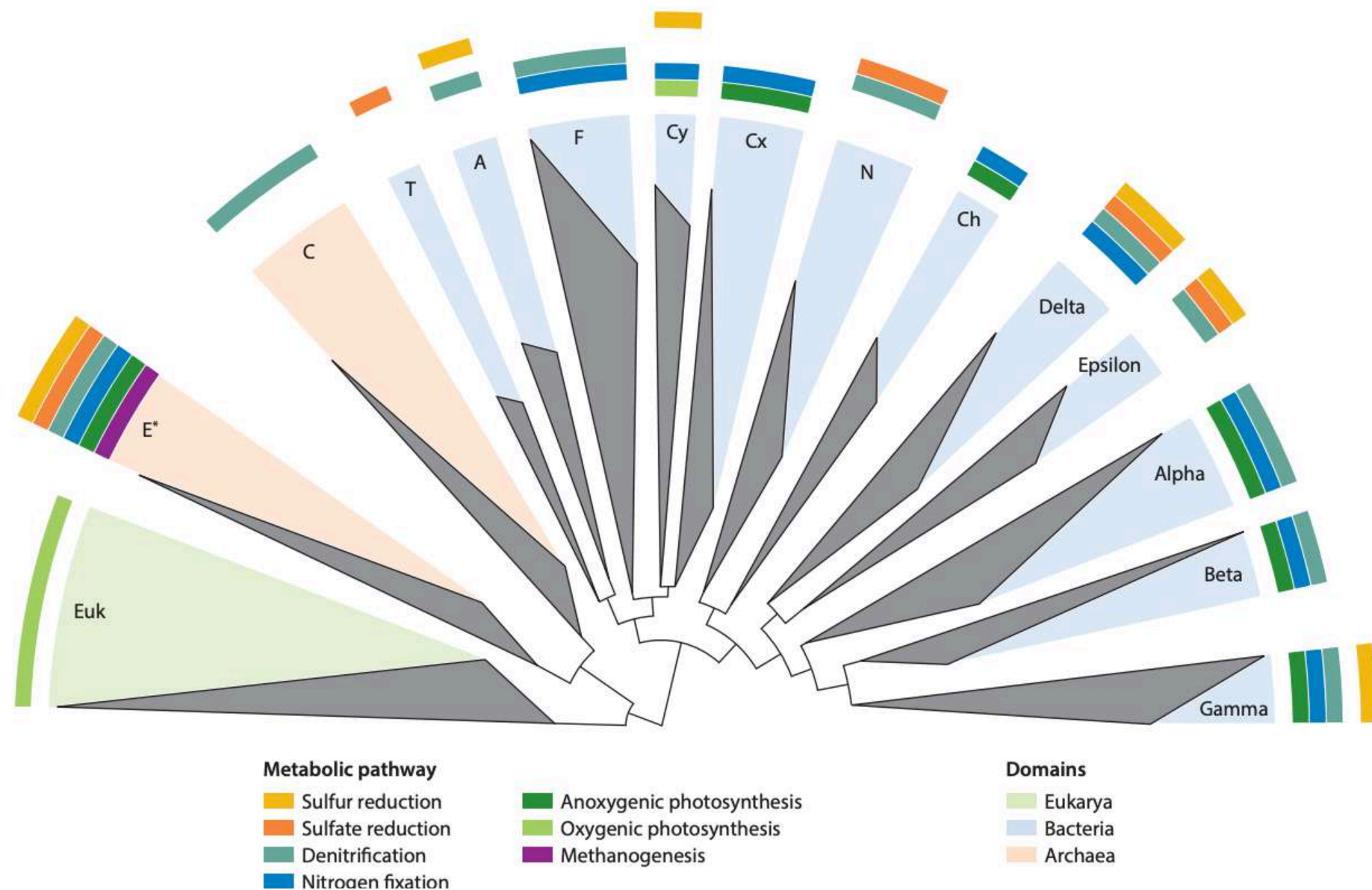


# Microbial diversity and metabolic pathways to survive in the environment





# Microbial diversity and metabolic pathways to survive in the environment



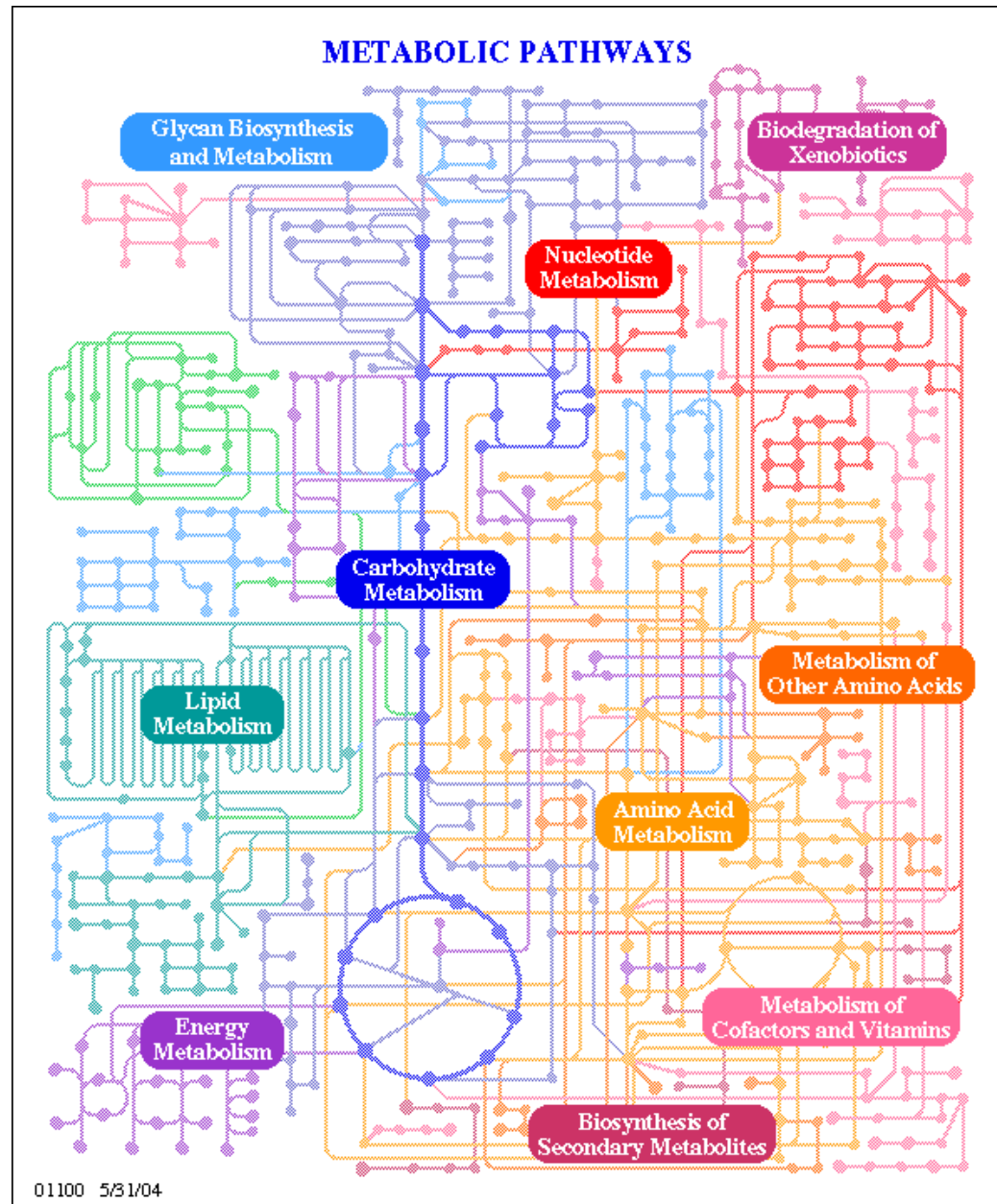
**Figure 1**

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. \**Euryarchaeota* are capable of bacteriorhodopsin-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*.

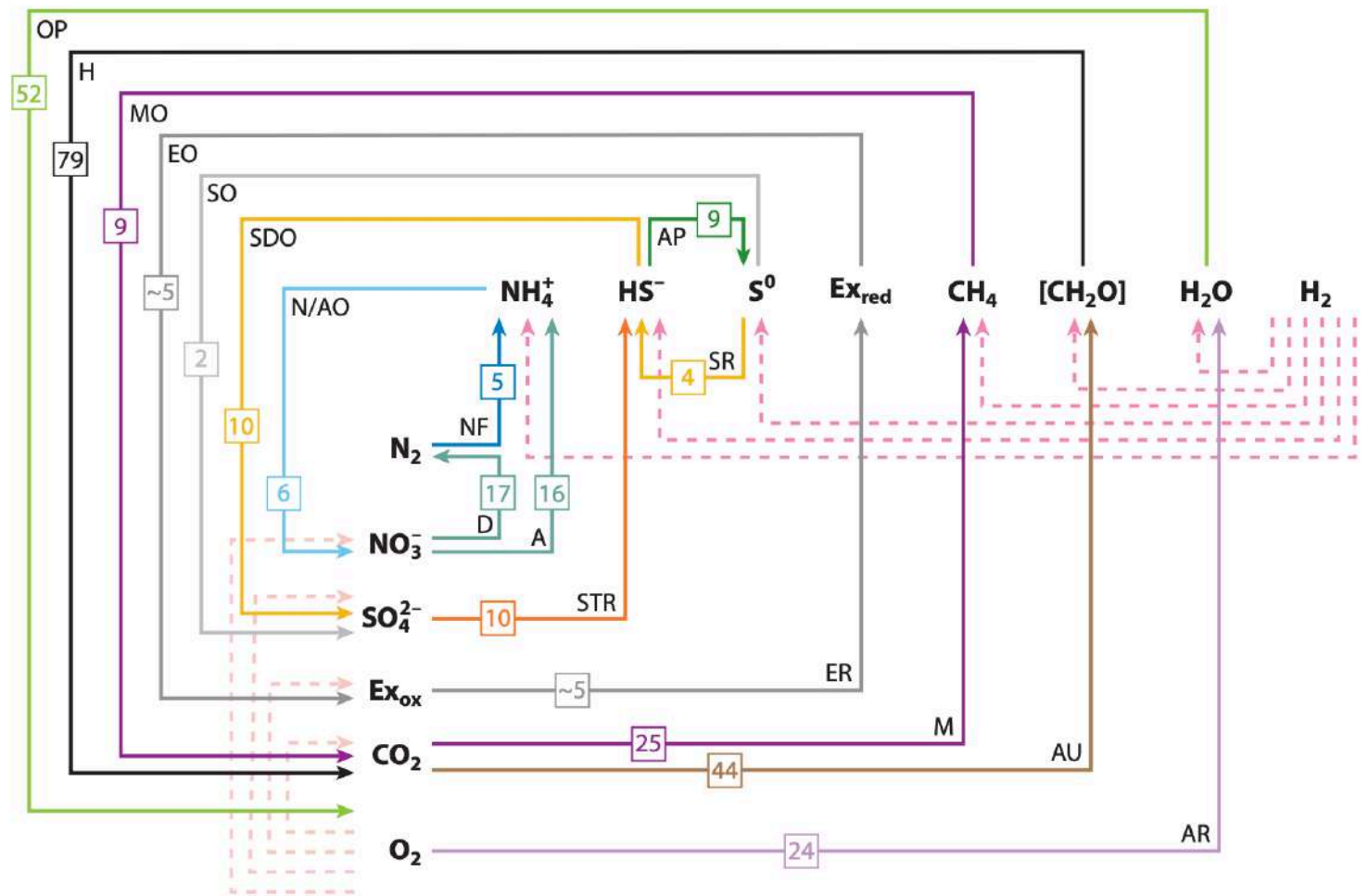


# Integrative approach

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



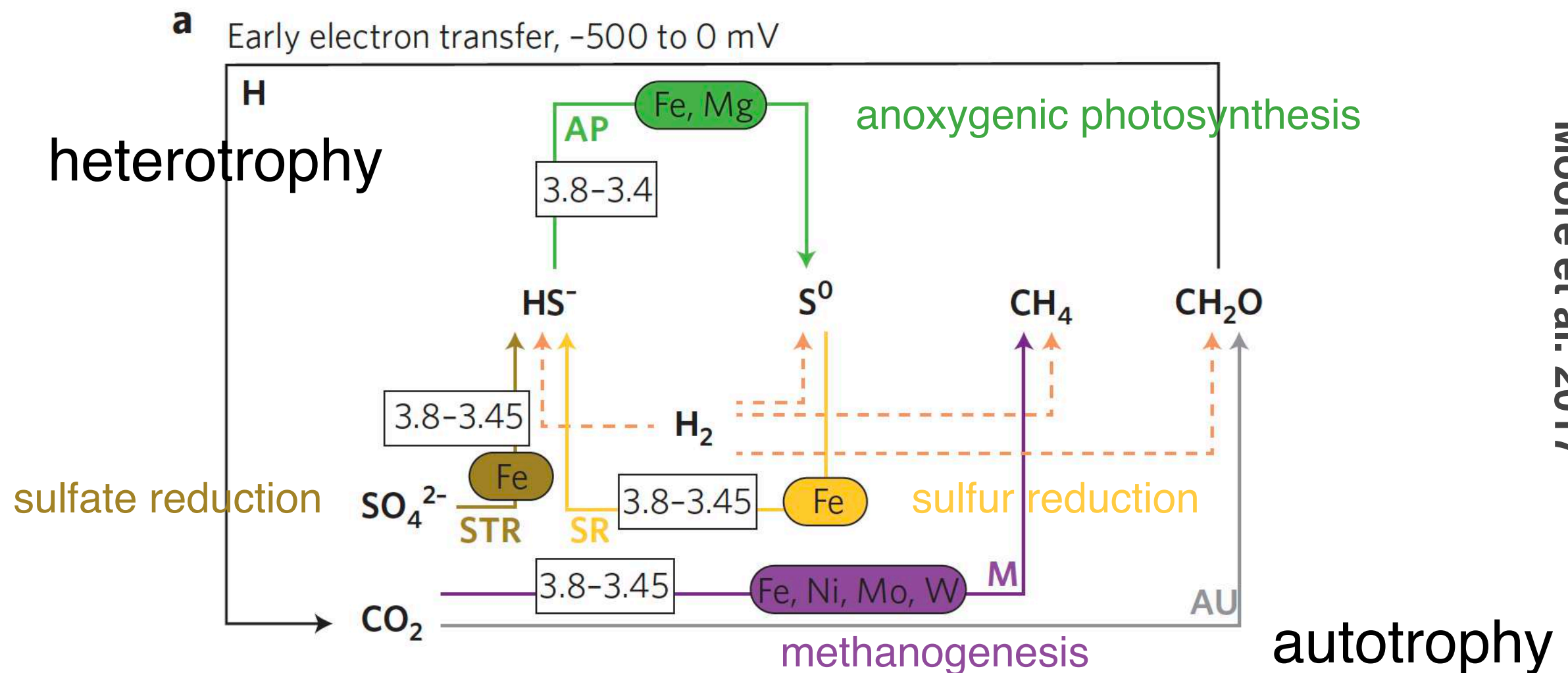
## Oxidative reaction



## Reductive reaction

A, ammonification; AP, anoxygenic photosynthesis; AR, aerobic respiration; AU, autotrophy; D, denitrification; Ex<sub>ox</sub>, other elements oxidation; Ex<sub>red</sub>, 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

# Microbial metabolic pathways during Earth history: *anoxxygenic world*



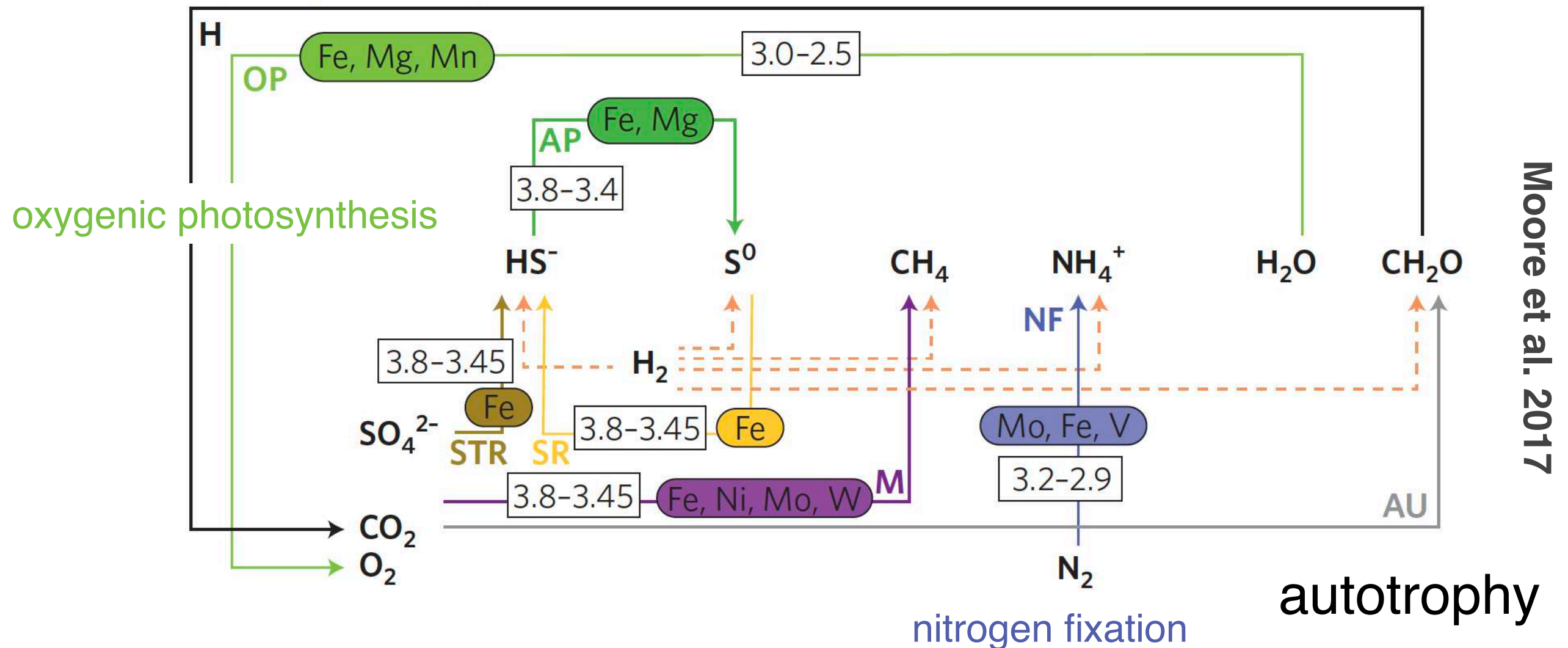
Moore et al. 2017

Molecules listed horizontally ( $\text{HS}^-$ ,  $\text{S}^0$ ,  $\text{CH}_4$ ,  $\text{NH}_4^+$ ,  $\text{H}_2\text{O}$ ,  $\text{CH}_2\text{O}$ , Exred) are reduced substrates, molecules listed vertically ( $\text{SO}_4^{2-}$ ,  $\text{CO}_2$ ,  $\text{NO}_3^-$ ,  $\text{O}_2$ , Exox) are oxidized substrates, and  $\text{N}_2$  is an intermediate and atmospheric reservoir of unreactive nitrogen

# Microbial metabolic pathways during Earth history: *oxygenic world*

## heterotrophy

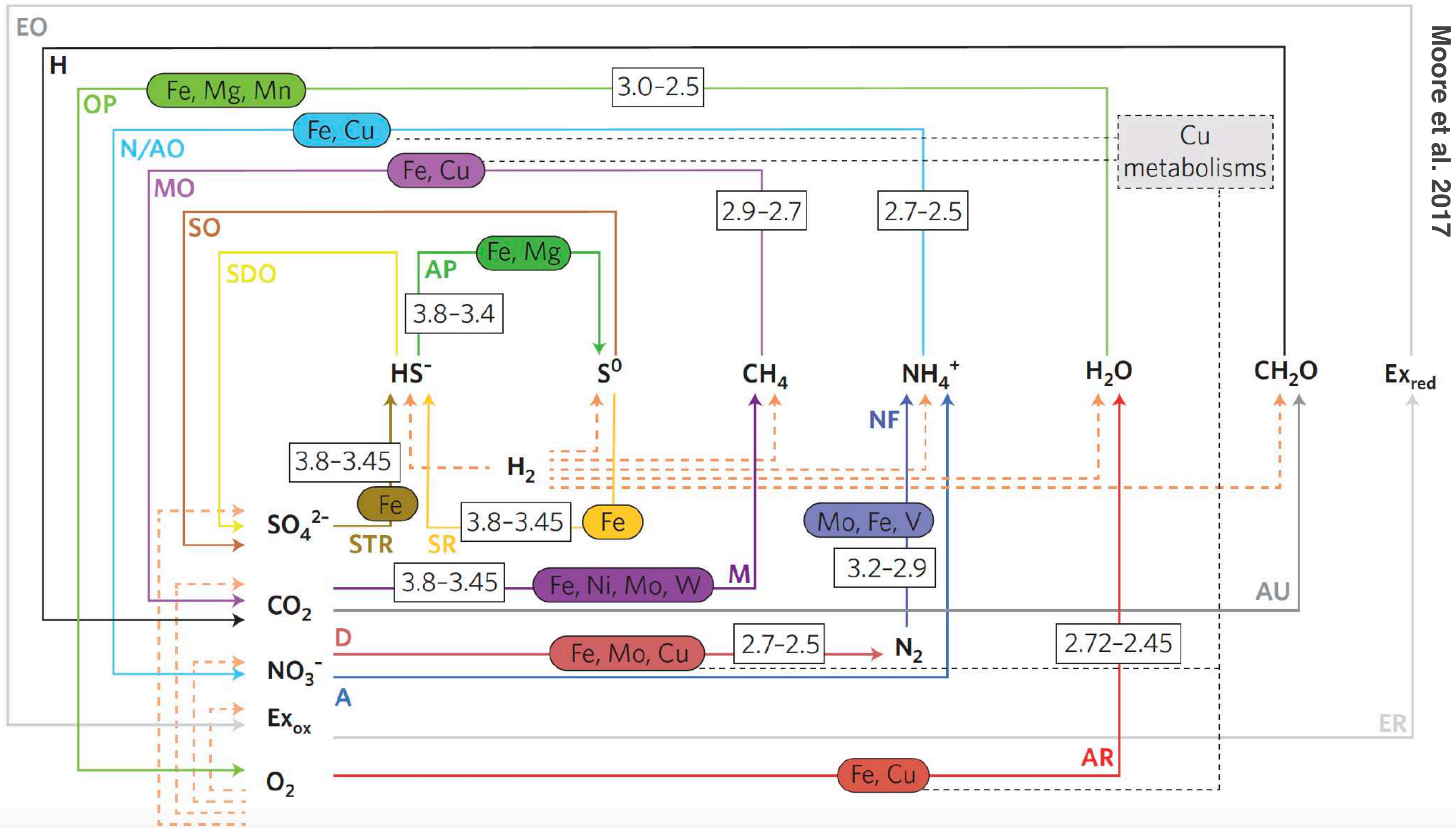
**b** Exploiting the atmosphere and ocean, -500 to 800 mV





# Present microbial metabolic pathways

**d** Closing the carbon cycle, -500 to 1,200 mV





A, ammonification; AP, anoxygenic photosynthesis; AR, aerobic respiration; AU, autotrophy; D, denitrification; EO, other elements oxidation; ER, other elements reduction (EO and ER include Fe and Mn oxidation and 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

# Energy conservation

- The achievable energy gain (Gibbs free energy,  $\Delta G$ ) of ETC depends on the redox potential difference ( $\Delta E$ ) of all reactions 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*  $O_2 > NO_3^- \rightarrow$  fumarate)
- **Cellular metabolism coordinate the production, management and re-distribution of carbon building blocks and energy (ATP and NADPH) between various electron and carbon sinks**
- ATP and NAD(P)H are **essential energy carriers** for numerous biochemical reactions occurring
- With the exception of fermentation, in which substrate-level phosphorylation occurs all other mechanisms of microbial energy conservation are linked to the proton motive force (or gradient of sodium ions,  $Na^+$ , instead of protons)
- Whether electrons come from the oxidation of organic or inorganic chemicals or are mediated by light-driven processes, in both respiration and photosynthesis, **energy conservation is the result of electron transport reactions and the formation of a PMF  $\rightarrow$  ATP**
- **The oxidation of NADH and FADH, to  $NAD^+$  and FAD, respectively, is linked to energy conservation via ETC**