GIOVANNELLI LAB

University of Naples "Federico II"

Marine Microbial Diversity

Nutritional Groups 1

donato.giovannelli@unina.it • @d_giovannelli • http://dgiovannelli.github.io

What is Life?

Erwin Schrodinger: What is Life? The Physical Aspects of the Living Cell
Cambridge University Press, London, 1944

- Life is the ability to self replicate
- Life is autocatalysis
- Life is chemical disequilibrium
- Life is electric
- All of the above

Life is Electric

Life uses energy in two forms, chemical and light. ***All*** life uses free energy released from one chemical reaction to drive another. More specifically, life catalyzes redox chemistry (*i.e.* electron transfer), capturing energy from the resulting flow of electrons to drive further reactions.

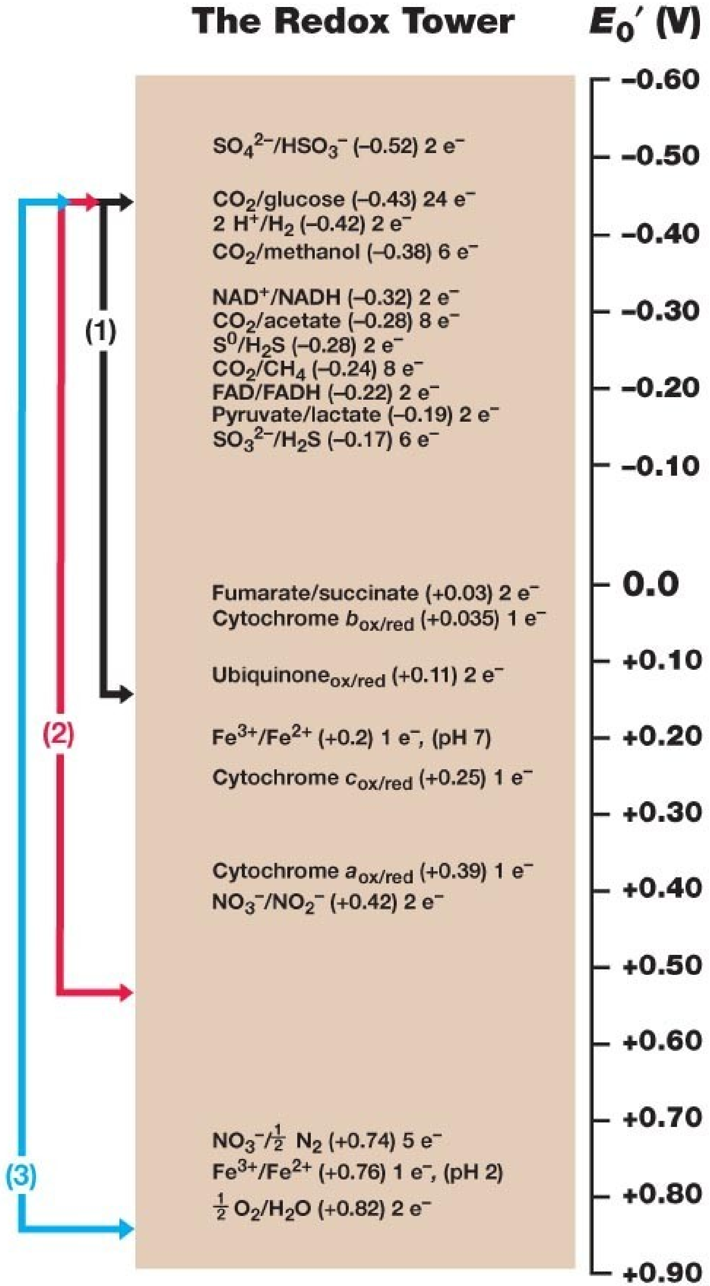
Life is Electric

Electron transfer (redox) reactions are most familiar as pairs of half-cell reactions, each half-reaction depicting an oxidized (electron poor) form of substrate having donated an electron, reversibly, to a reduced (electron rich) substrate (**Eq. 1)** or the reverse, **(Eq. 2**). Each half reaction has a given tendency to gain or lose electrons relative to a second half reaction.

$A \rightleftharpoons A^+ + e^-$	$E^o = A_E$	**Eq. 2**. Oxidation half reaction: A is oxidized to A^+
$B^+ + e^- \rightleftharpoons B$	$E^o = B_E$	**Eq. 3.** Reduction half reaction: B^+ is reduced to B

The standard reduction potential (E^o), the potential (in Volts) to gain an electron under standard conditions, has been tabulated for a large number of half reactions, using the standard hydrogen electrode (SHE) as reference, with $E^o_{SHE} = 0$ V.

If the reduction potential is negative for a half reaction, it will donate electrons when coupled to the SHE, or to any other half reaction with a less negative reduction potential.



(1) H_2 + fumarate $\longrightarrow$ succinate $\Delta G^{0\prime} = -86$ kJ

(2) $H_2 + NO_3^- \longrightarrow NO_2^- + H_2O$ $\Delta G^{0\prime} = -163$ kJ

(3) $H_2 + \frac{1}{2}\ O_2 \longrightarrow H_2O$ $\Delta G^{0\prime} = -237$ kJ

Free Energy of Formation and Calculating ΔG^0

Table A1.1 *Free energies of formation (G_f^0) for some substances (kJ/mol)*[a]

Carbon compound	*Carbon compound*	*Metal*	*Nonmetal*	*Nitrogen compound*
CO, −137.34	Glutamine, −529.7	Cu^+, +50.28	H_2, 0	N_2, 0
CO_2, −394.4	Glyceraldehyde, −437.65	Cu^{2+}, +64.94	H^+, 0 at pH 0; −39.83 at pH 7 (−5.69 per pH unit)	NO, +86.57
CH_4, −50.75	Glycerate, −658.1	CuS, −49.02	O_2, 0	NO_2, +51.95
H_2CO_3, −623.16	Glycerol, −488.52	Fe^{2+}, −78.87	OH^-, −157.3 at pH 14; −198.76 at pH 7; −237.57 at pH 0	NO_2^-, −37.2
HCO_3^-, −586.85	Glycine, −314.96	Fe^{3+}, −4.6	H_2O, −237.17	NO_3^-, −111.34
CO_3^{2-}, −527.90	Glycolate, −530.95	$FeCO_3$, −673.23	H_2O_2, −134.1	NH_3, −26.57
Acetaldehyde, −139.9	Glyoxalate, −468.6	FeS_2, −150.84	PO_4^{3-}, −1026.55	NH_4^+, −79.37
Acetate, −369.41	Guanine, +46.99	$FeSO_4$, −829.62	Se^0, 0	N_2O, +104.18
Acetone, −161.17	α-Ketoglutarate, −797.55	PbS, −92.59	H_2Se, −77.09	N_2H_4, +128
Alanine, −371.54	Lactate, −517.81	Mn^{2+}, −227.93	SeO_4^{2-}, −439.95	
Arginine, −240.2	Lactose, −1515.24	Mn^{3+}, −82.12	S^0, 0	
Aspartate, −700.4	Malate, −845.08	MnO_4^-, −506.57	SO_3^{2-}, −486.6	
Benzene, +124.5	Mannitol, −942.61	MnO_2, −456.71	SeO_4^{2-}, −744.6	
Benzoic acid, −245.6	Methanol, −175.39	$MnSO_4$, −955.32	$S_2O_3^{2-}$, −513.4	
n-Butanol, −171.84	Methionine, −502.92	HgS, −49.02	H_2S, −27.87	
Butyrate, −352.63	Methylamine, −40.0	MoS_2, −225.42	HS^-, +12.05	
Caproate, −335.96	Oxalate, −674.04	ZnS, −198.60	S^{2-}, +85.8	
Citrate, −1168.34	Palmitic acid, −305			
o-Cresol, −37.1	Phenol, −47.6			
Crotonate, −277.4	*n*-Propanol, −175.81			
Cysteine, −339.8	Propionate, −361.08			
Dimethylamine, −3.3	Pyruvate, −474.63			
Ethanol, −181.75	Ribose, −757.3			
Formaldehyde, −130.54	Succinate, −690.23			
Formate, −351.04	Sucrose, −370.90			
Fructose, −951.38	Toluene, +114.22			
Fumarate, −604.21	Trimethylamine, −37.2			
Gluconate, −1128.3	Tryptophan, −112.6			
Glucose, −917.22	Urea, −203.76			
Glutamate, −699.6	Valerate, −344.34			

[a]Values for free energy of formation of various compounds can be found in Dean, J. A. 1973. *Lange's Handbook of Chemistry*, 11th edition. McGraw-Hill, New York; Garrels, R. M., and C. L. Christ. 1965. *Solutions, Minerals, and Equilibria*. Harper & Row, New York; Burton, K. 1957. In Krebs, H. A., and H. L. Komberg. Energy transformation in living matter, *Ergebnisse der Physiologie* (appendix): Springer-Verlag, Berlin; and Thauer, R. K., K. Jungermann, and H. Decker. 1977. Energy conservation in anaerobic chemotrophic bacteria. *Bacteriol. Rev. 41*: 100–180.

Free Energy (*G*): defined as the energy released that is available to do useful work G^0_f indicates the free energy of formation for a given compound, defined as the energy yielded or required for the formation of a given molecule from its constituent elements

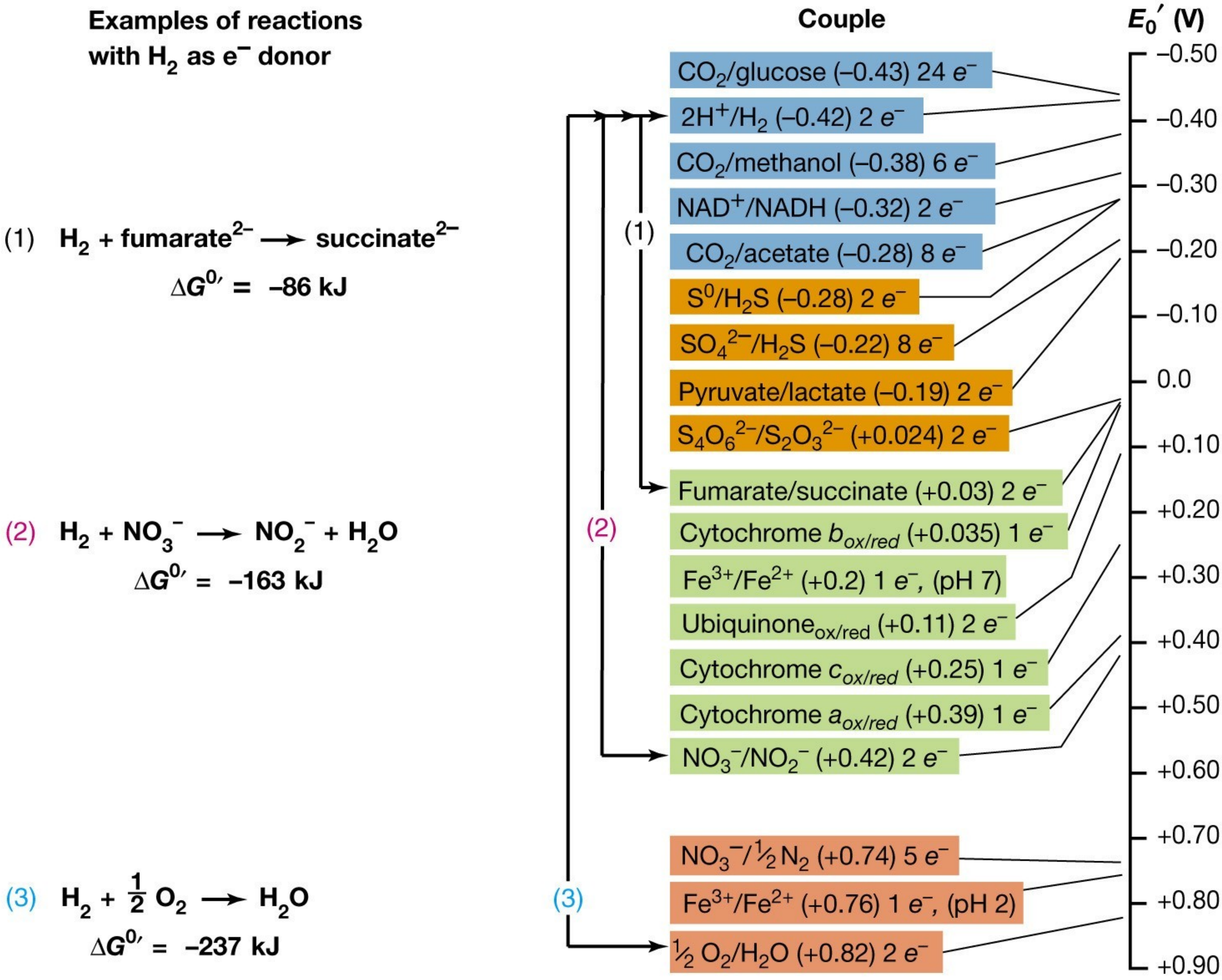
$\Delta G^{0'}$ is the change in free energy under standard conditions (pH 7, 25°C, 1 atm., [reactants] = 1 M)

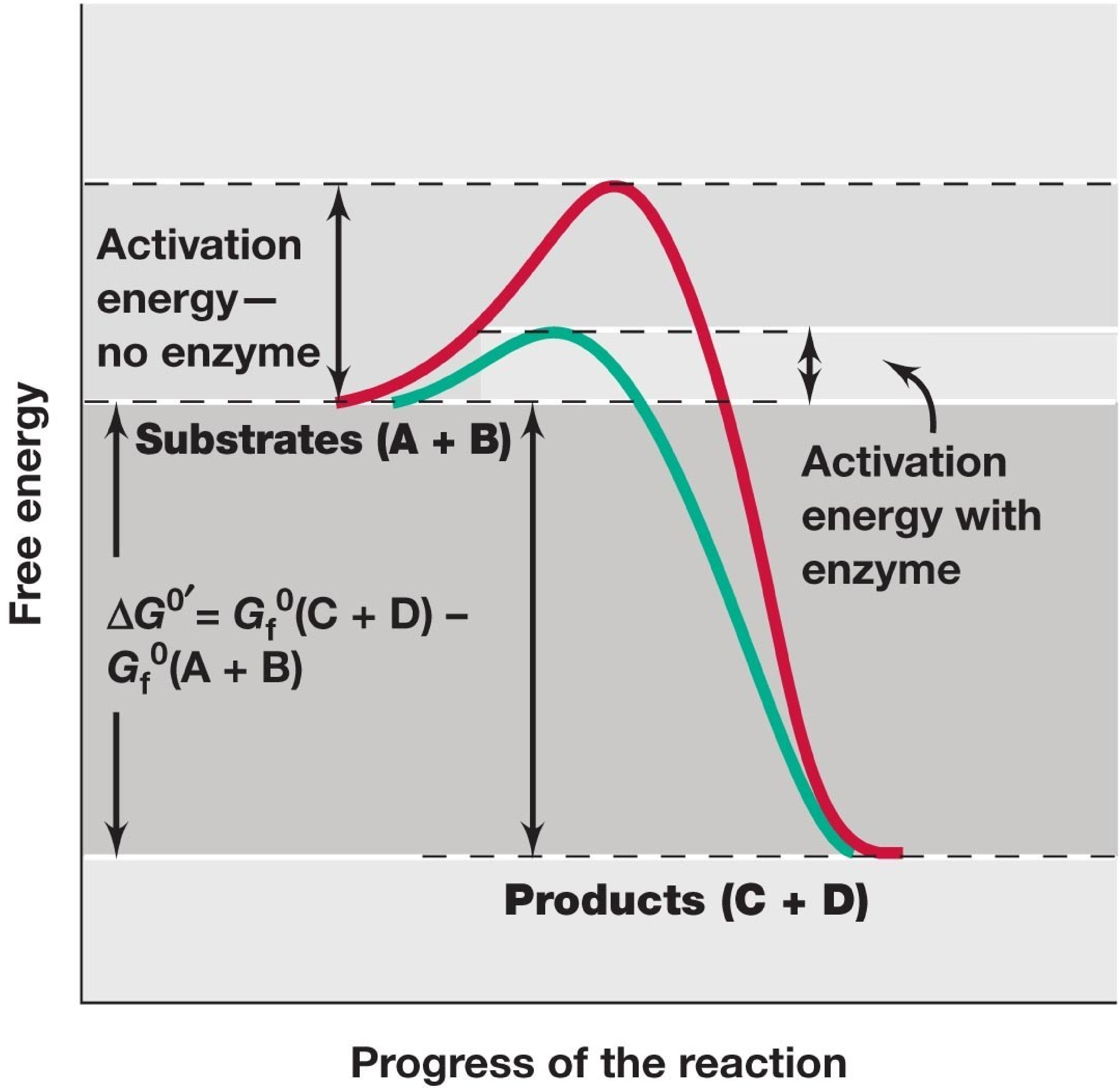
$\Delta G^0 = \Delta G_f^0$ (products) - ΔG_f^0 (reactants)

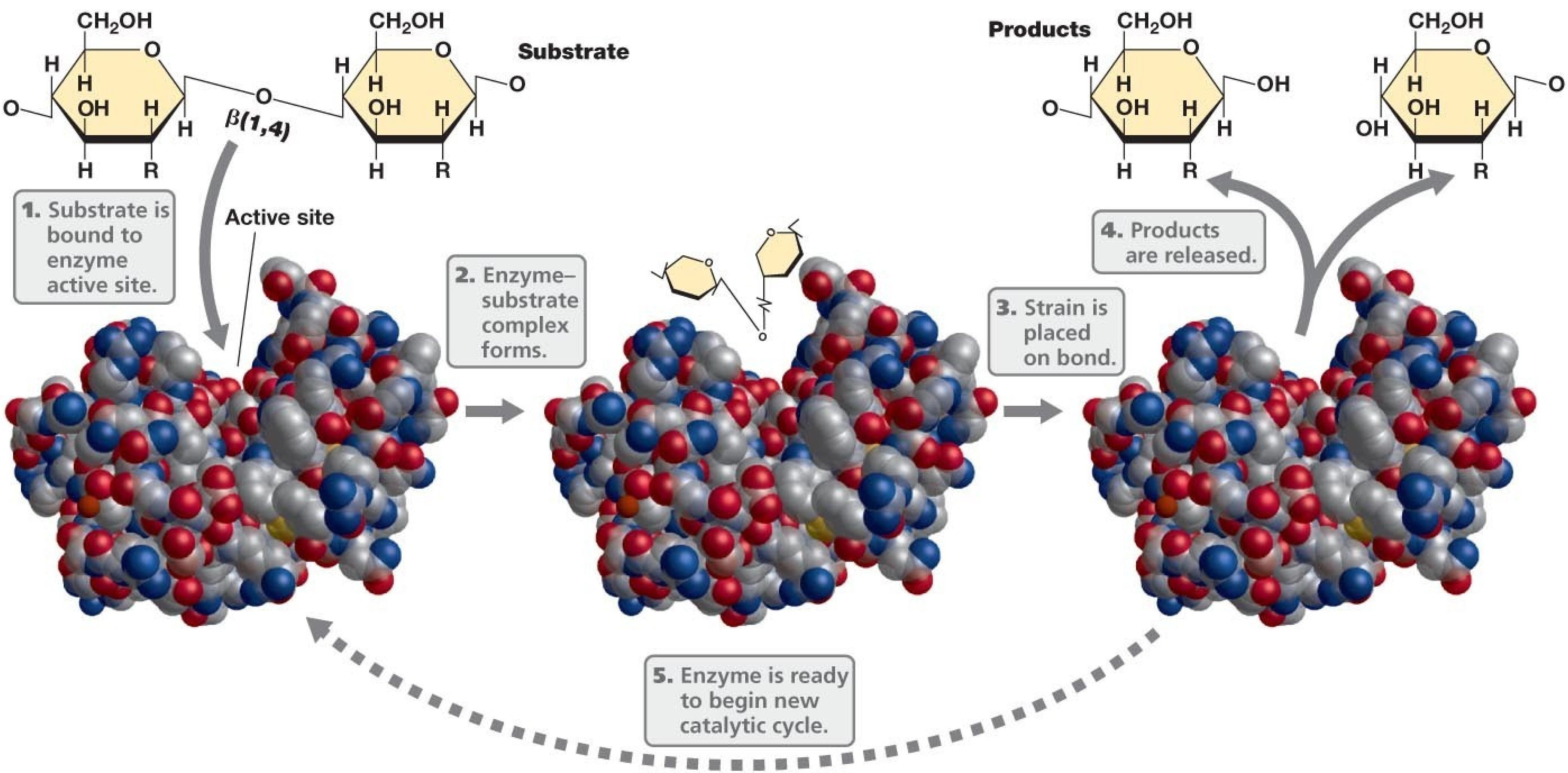
Neg. ΔG^0 / energy release / exergonic reaction
Pos. ΔG^0 / energy requirement / endergonic reaction

$$H_2S + 2O_2 \longrightarrow SO_4^{2-} + 2H^+$$

$$\Delta G^{0'} = (-744.6) + 2(-39.83) - (-27.87) + (0)$$
$$\Delta G^{0'} = -\ 796.39 \text{ kJ/rx}$$


Examples of reactions with H_2 as e^- donor
(1) H_2 + fumarate^{2-} → succinate^{2-}
$\Delta G^{0\prime}$ = –86 kJ
(2) $H_2 + NO_3^- \rightarrow NO_2^- + H_2O$
$\Delta G^{0\prime}$ = –163 kJ
(3) $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$
$\Delta G^{0\prime}$ = –237 kJ
Couple
$E_0{}'$ (V)
CO_2/glucose (–0.43) 24 e^-
$2H^+/H_2$ (–0.42) 2 e^-
CO_2/methanol (–0.38) 6 e^-
NAD^+/NADH (–0.32) 2 e^-
CO_2/acetate (–0.28) 8 e^-
S^0/H_2S (–0.28) 2 e^-
SO_4^{2-}/H_2S (–0.22) 8 e^-
Pyruvate/lactate (–0.19) 2 e^-
$S_4O_6^{2-}/S_2O_3^{2-}$ (+0.024) 2 e^-
Fumarate/succinate (+0.03) 2 e^-
Cytochrome $b_{ox/red}$ (+0.035) 1 e^-
Fe^{3+}/Fe^{2+} (+0.2) 1 e^-, (pH 7)
Ubiquinone$_{ox/red}$ (+0.11) 2 e^-
Cytochrome $c_{ox/red}$ (+0.25) 1 e^-
Cytochrome $a_{ox/red}$ (+0.39) 1 e^-
NO_3^-/NO_2^- (+0.42) 2 e^-
$NO_3^-/\frac{1}{2}N_2$ (+0.74) 5 e^-
Fe^{3+}/Fe^{2+} (+0.76) 1 e^-, (pH 2)
$\frac{1}{2} O_2/H_2O$ (+0.82) 2 e^-
–0.50
–0.40
–0.30
–0.20
–0.10
0.0
+0.10
+0.20
+0.30
+0.40
+0.50
+0.60
+0.70
+0.80
+0.90
(1)
(2)
(3)


Activation energy—no enzyme
Substrates (A + B)
Activation energy with enzyme
$\Delta G^{0\prime} = G_f^0(C + D) - G_f^0(A + B)$
Products (C + D)
Free energy
Progress of the reaction


Substrate
Products
β(1,4)
Active site
1. Substrate is bound to enzyme active site.
2. Enzyme–substrate complex forms.
3. Strain is placed on bond.
4. Products are released.
5. Enzyme is ready to begin new catalytic cycle.

Types of work carried out by organisms

chemical work

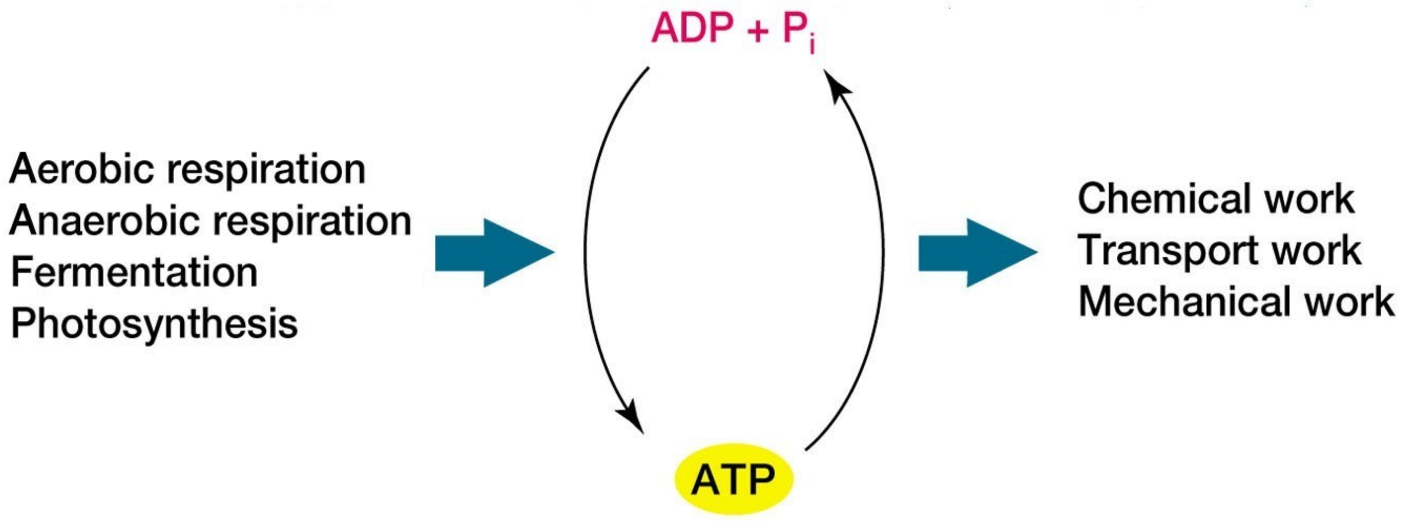
synthesis of complex molecules

transport work

take up of nutrients, elimination of wastes, and maintenance of ion balances

mechanical work

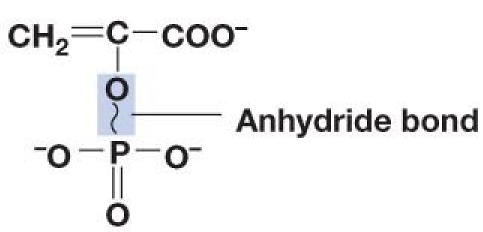
movement of organisms or cells and movement of internal structures



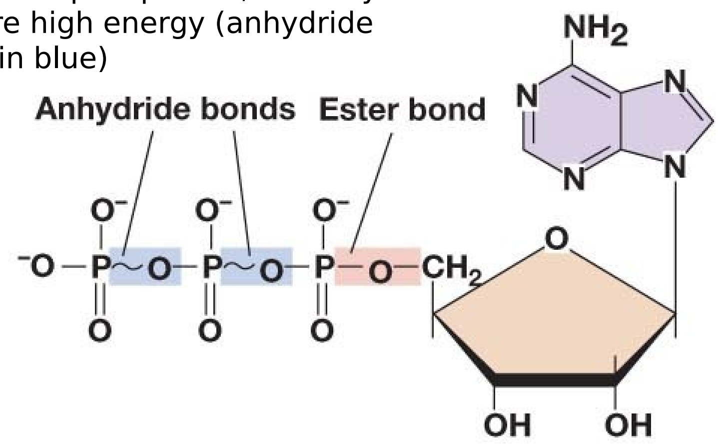
High-energy bonds

The energy released in redox reactions is conserved in the formation of certain compounds that contain energy-rich phosphate or sulfur bonds

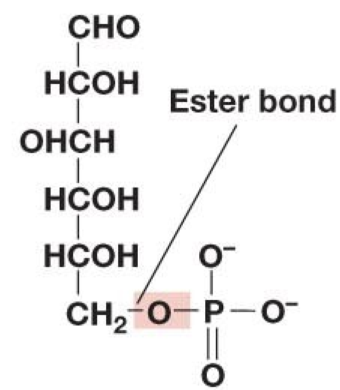
ATP contains three phosphates, but only two of them are high energy (anhydride bonds, shown in blue)



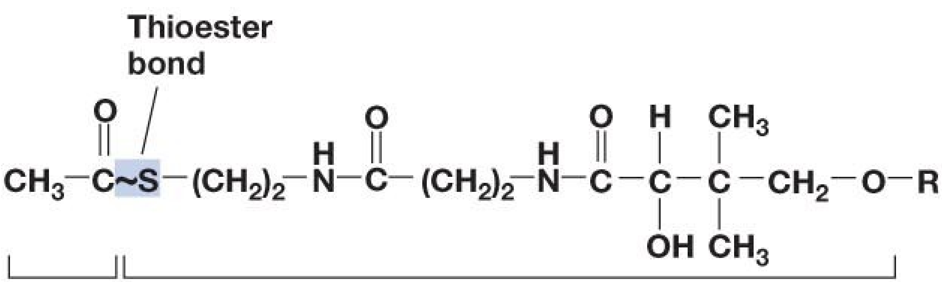
Phosphoenolpyruvate



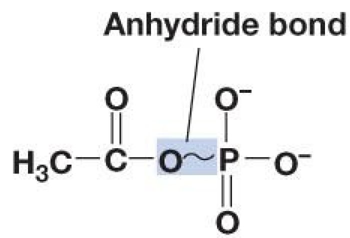
Adenosine triphosphate (ATP)



Glucose 6-phosphate

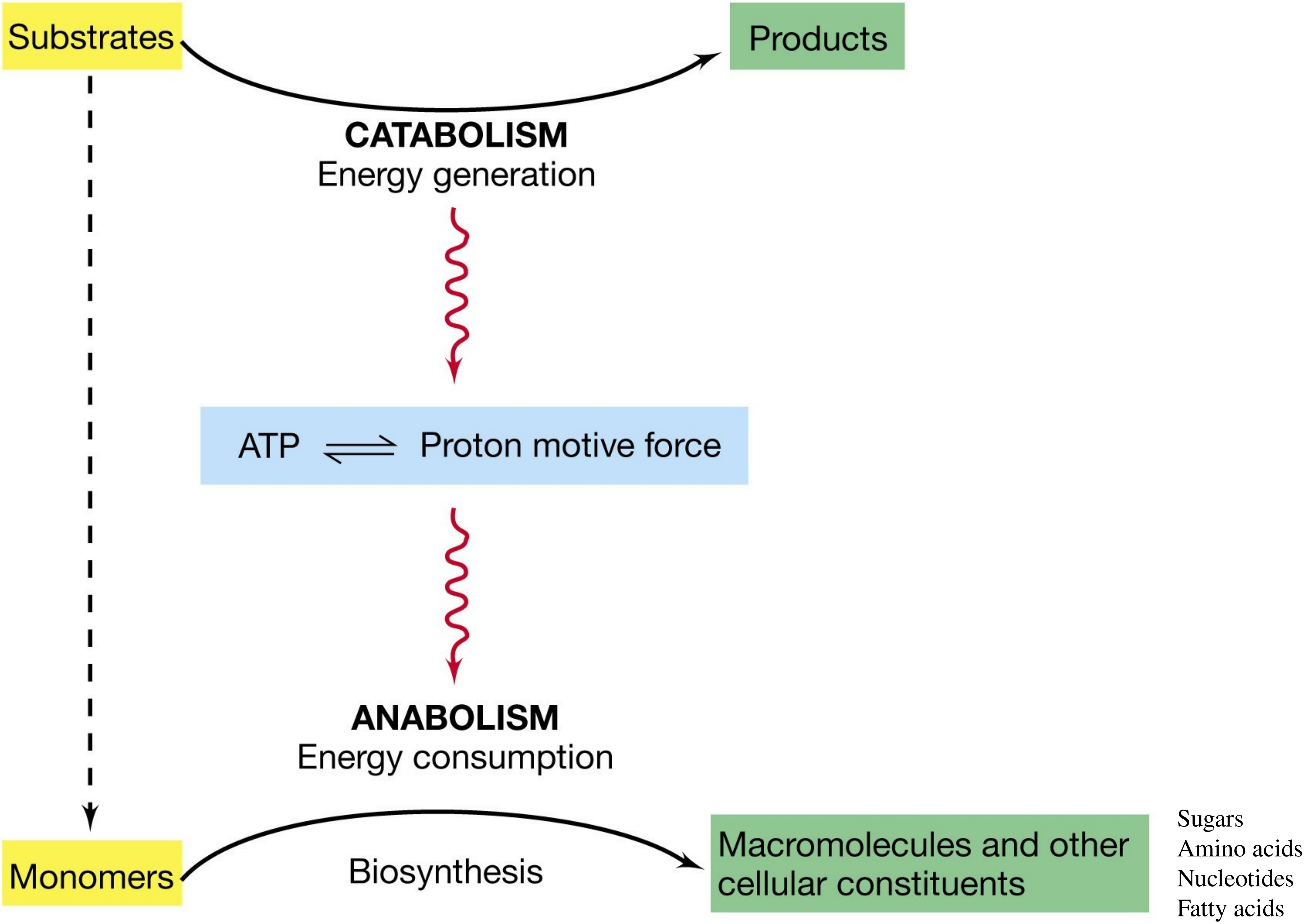


Acetyl-CoA



Acetyl phosphate

Compound	$G^{0\prime}$ kJ/mol
$\Delta G^{0\prime}$ > 30kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0\prime}$ < 30kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

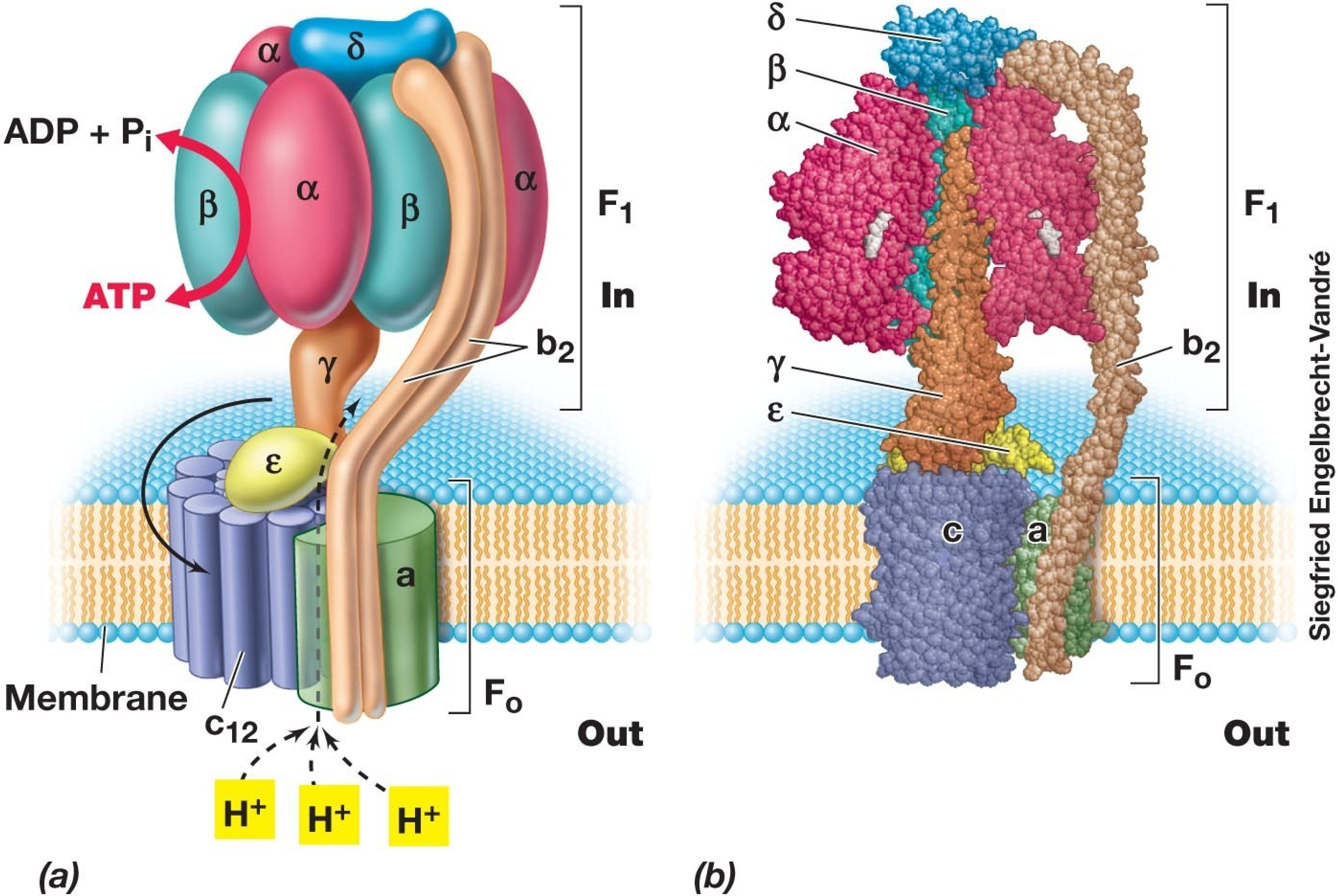


Scheme of anabolism and catabolism showing the key role of ATP and the proton motive force in integrating the processes. Monomers can come preformed as nutrients from the environment or from catabolic pathways like glycolysis and the citric acid cycle.

Proton Motive Force

With the exception of fermentation, in which substrate-level phosphorylation occurs, all other mechanisms for energy conservation employ the **proton motive force**. Whether electrons come from the oxidation of organic or inorganic chemicals or from light-driven processes, in all forms of respiration and photosynthesis, energy conservation is linked to the establishment of a PMF and its dissipation by ATPase to form ATP.

Respiration and anaerobic respiration can thus be viewed as variations on a theme of different electron acceptors. Likewise, **chemoorganotrophy**, **chemolithotrophy**, and **photosynthesis** are variations on a theme of different electron donors. Electron transport and the pmf link all of these processes, bringing these seemingly quite different forms of energy metabolism into a common focus.



ADP + Pi
ATP
α
δ
β
γ
ε
b2
F1
In
a
c12
Membrane
Fo
Out
H+
H+
H+
(a)
δ
β
α
γ
ε
c
a
b2
F1
In
Fo
Out
(b)
Siegfried Engelbrecht-Vandré

Basic Metabolism

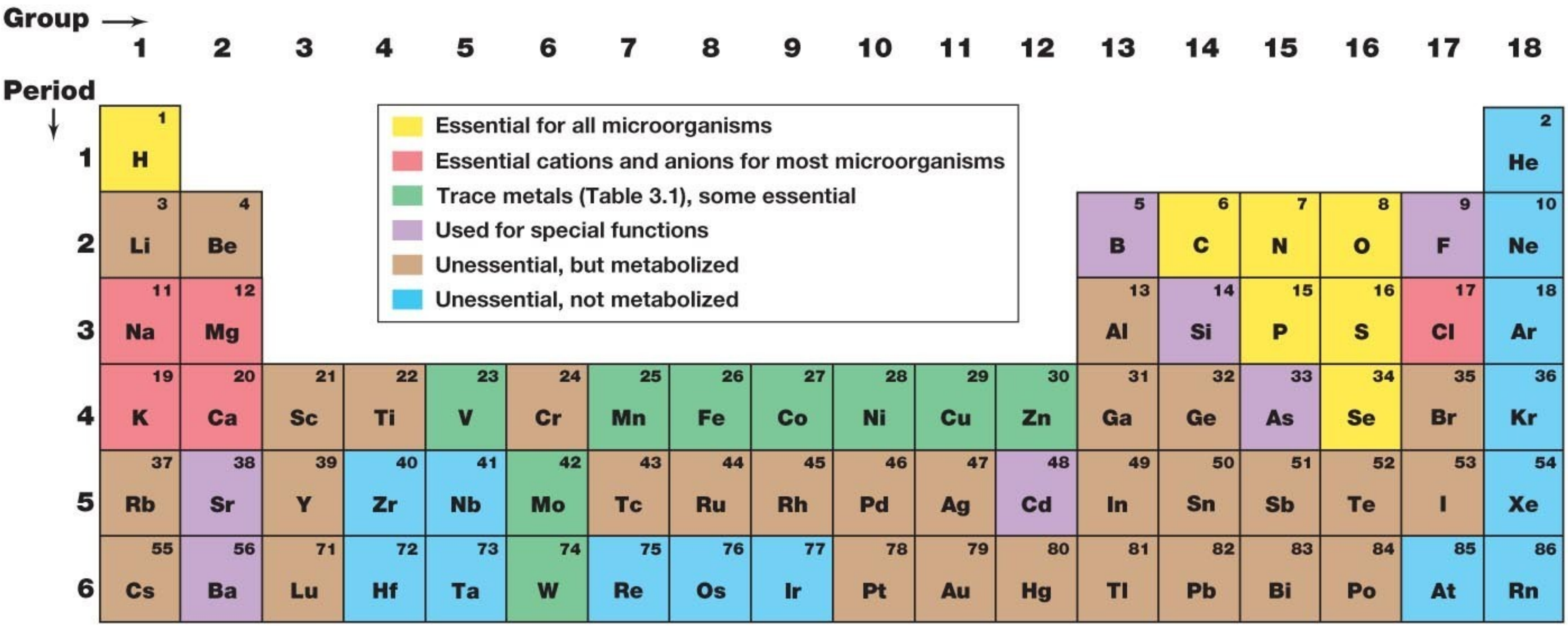
An **electron donor** (also known as energy source)

A **carbon source** (for biosynthesis)

An **electron acceptor**

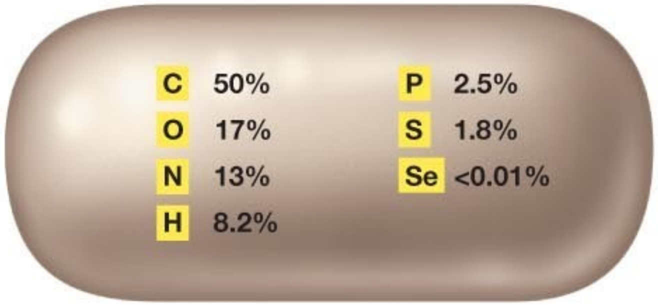
All type of metabolism, requires these three basic elements. An electron donor (the source of reducing power used to carry out redox reactions), a carbon source used as a donor of carbon for biosynthetic purposes, and an electron acceptor, used to dispose of excess reducing equivalents.

Microbial periodic table of elements



(a)

Essential elements as a percent of cell dry weight

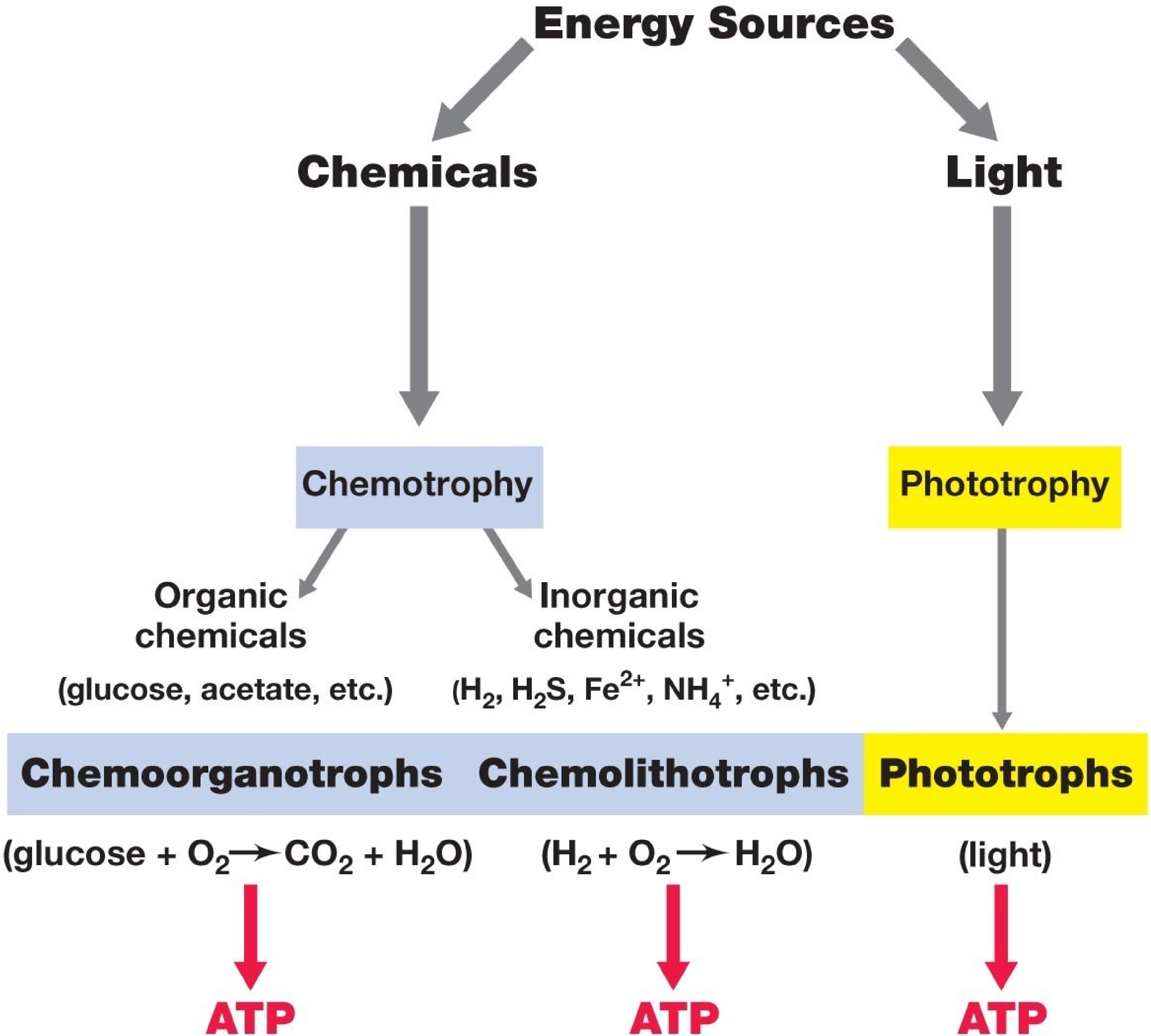


(b)

Macromolecular composition of a cell

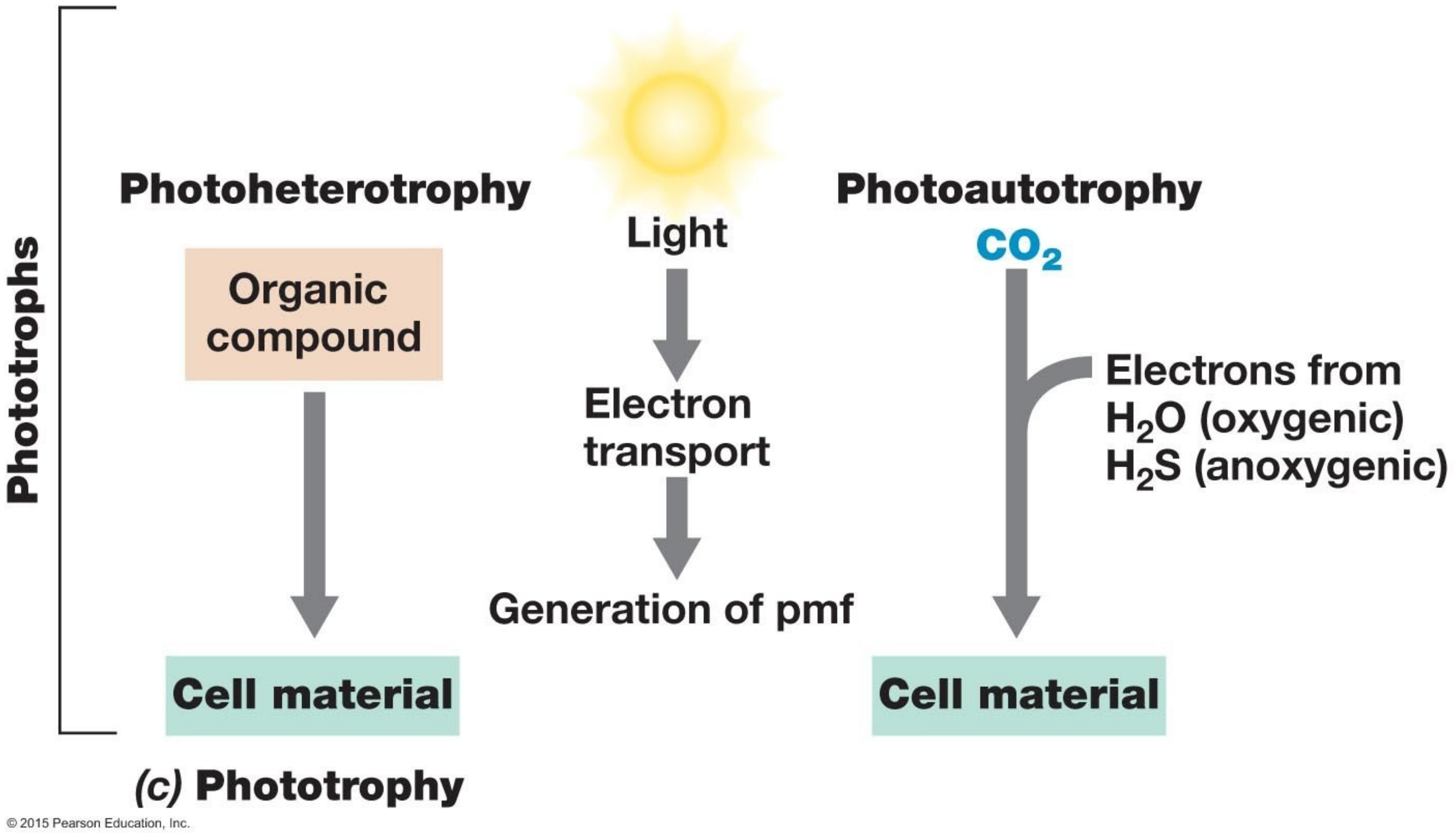
Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

(c)


Energy Sources
Chemicals
Light
Chemotrophy
Phototrophy
Organic chemicals
(glucose, acetate, etc.)
Inorganic chemicals
(H_2, H_2S, Fe^{2+}, NH_4^+, etc.)
Chemoorganotrophs
Chemolithotrophs
Phototrophs
(glucose + $O_2 \rightarrow CO_2 + H_2O$)
($H_2 + O_2 \rightarrow H_2O$)
(light)
ATP
ATP
ATP

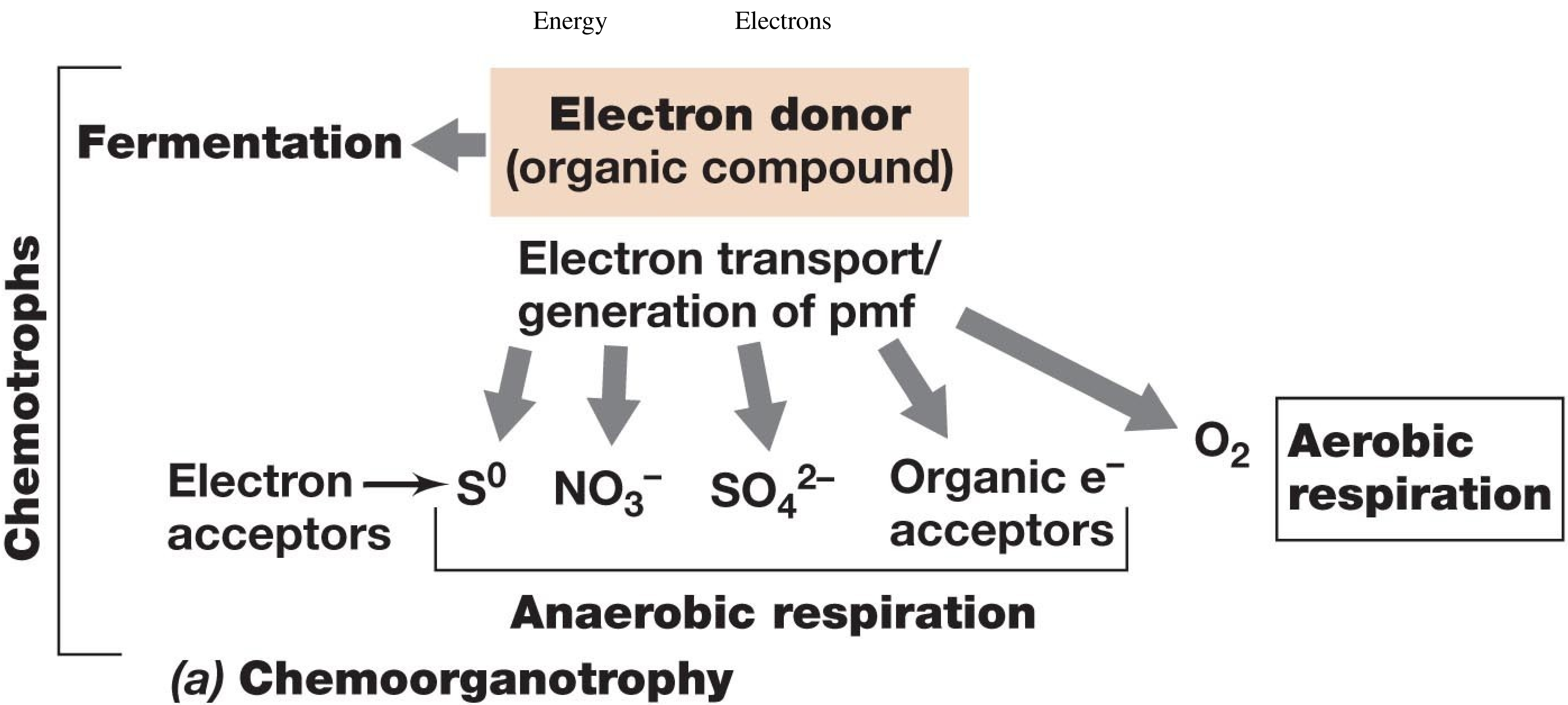
Phototrophy

Energy



Photoheterotrophs, Photoautotrophs: **Anoxygenic photoautotrophs** and **Oxygenic photoauthotrophs**

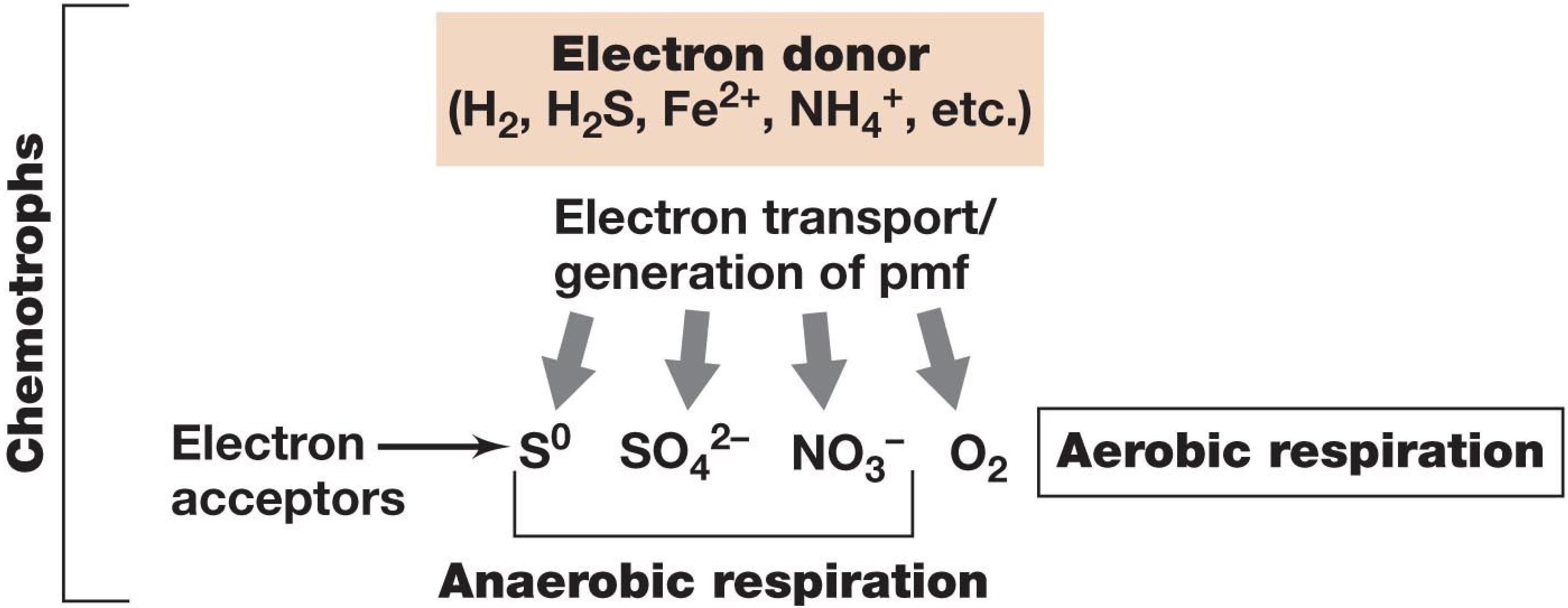
Chemoorganotrophy



Chemoorganotrophs can perform **Aerobic Respiration**, **Anaerobic Respiration** and **Fermentation**

Chemo<u>litho</u>trophy

Energy Electrons



(b) **Chemolithotrophy**

Chemolithotrophs can perform **Aerobic Respiration** and **Anaerobic Respiration**

Source of:

Energy	**Carbon**	**Electrons**		
Photo-	auto-	litho-	trophy	Bacteria
Chemo-	hetero-	organo-		

Photo-litho-auto-trophic
Chemo-litho-auto-trophic
Chemo-litho-hetero-trophic

Special cases:
Methylotrophic
Methanogenic
Syntrophic

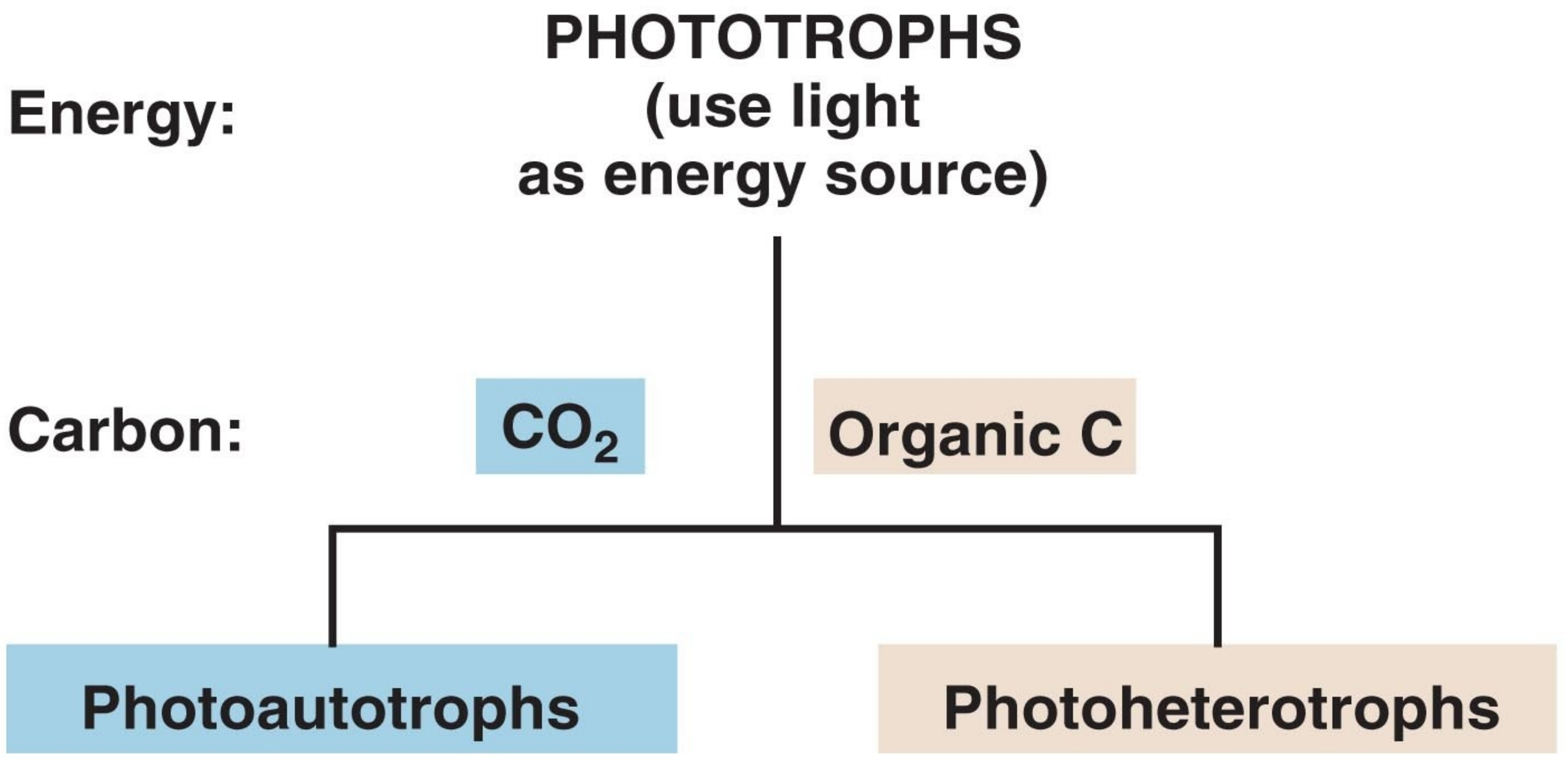
The Phototrophic Way of Life

- Anoxygenic Photosynthesis
- Oxygenic Photosynthesis
- Photoheterotrophy

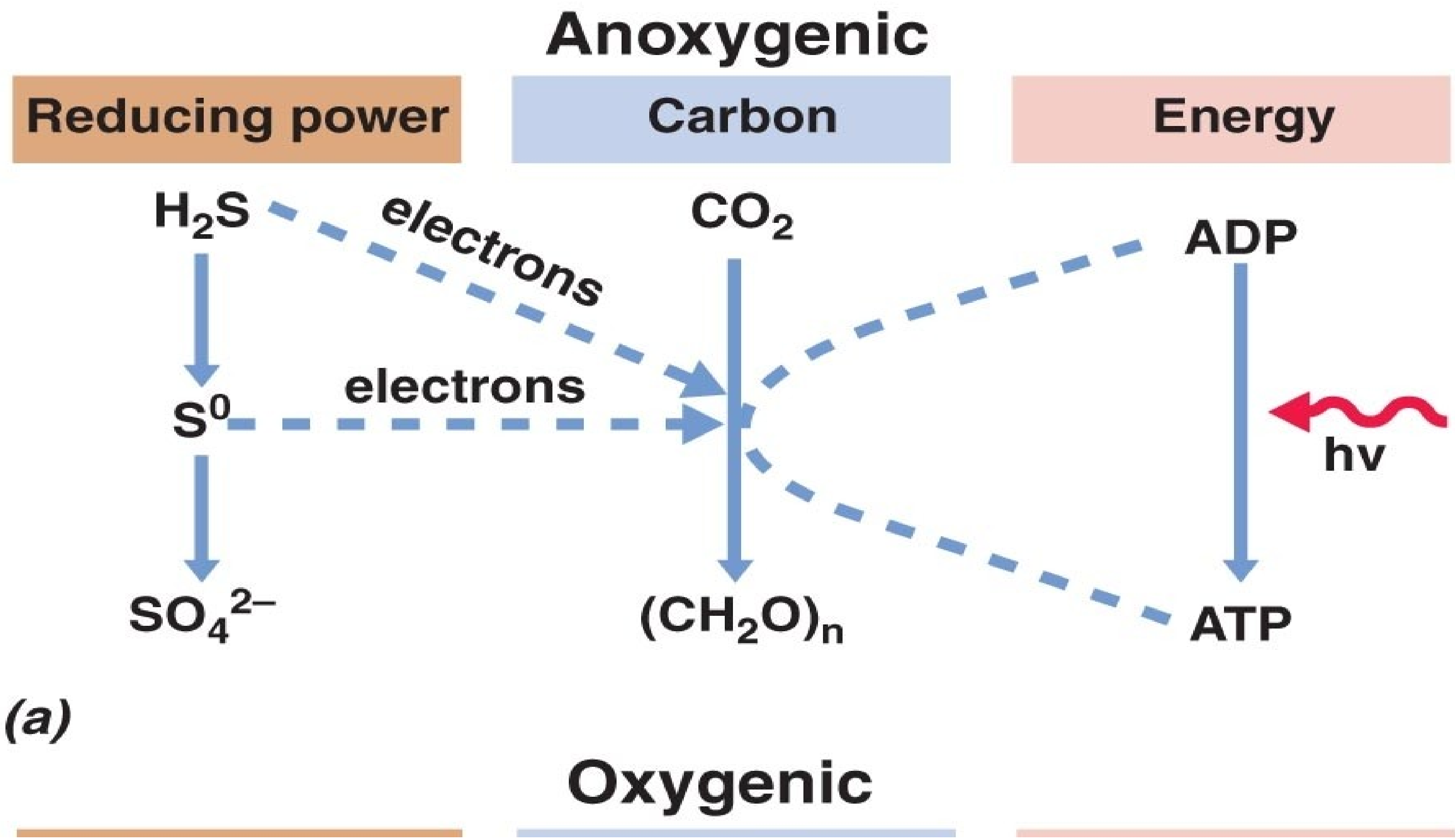
Photosynthesis

- Phototrophs are organisms that carry out photosynthesis
- Most phototrophs are also autotrophs
- Photosynthesis requires light-sensitive pigments called chlorophyll
- Photoautotrophy requires ATP production and CO_2 reduction
- Oxidation of H_2O produces O_2 (oxygenic photosynthesis)
- Oxygen not produced (anoxygenic photosynsthesis)
- Oxygenic Photosynthesis is the most important biological process on Earth

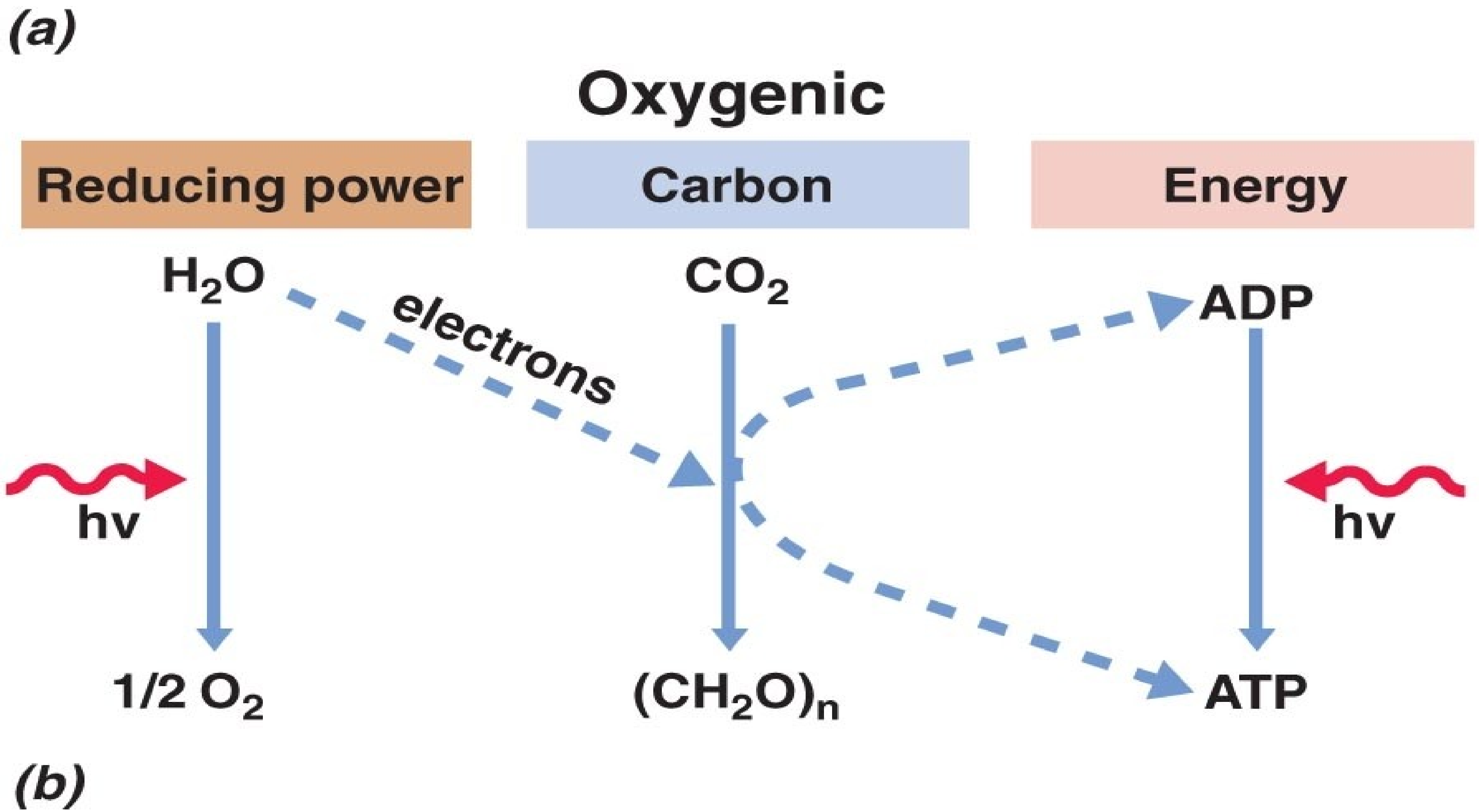
Classification of Phototrophic Organisms



Patterns of Photosynthesis



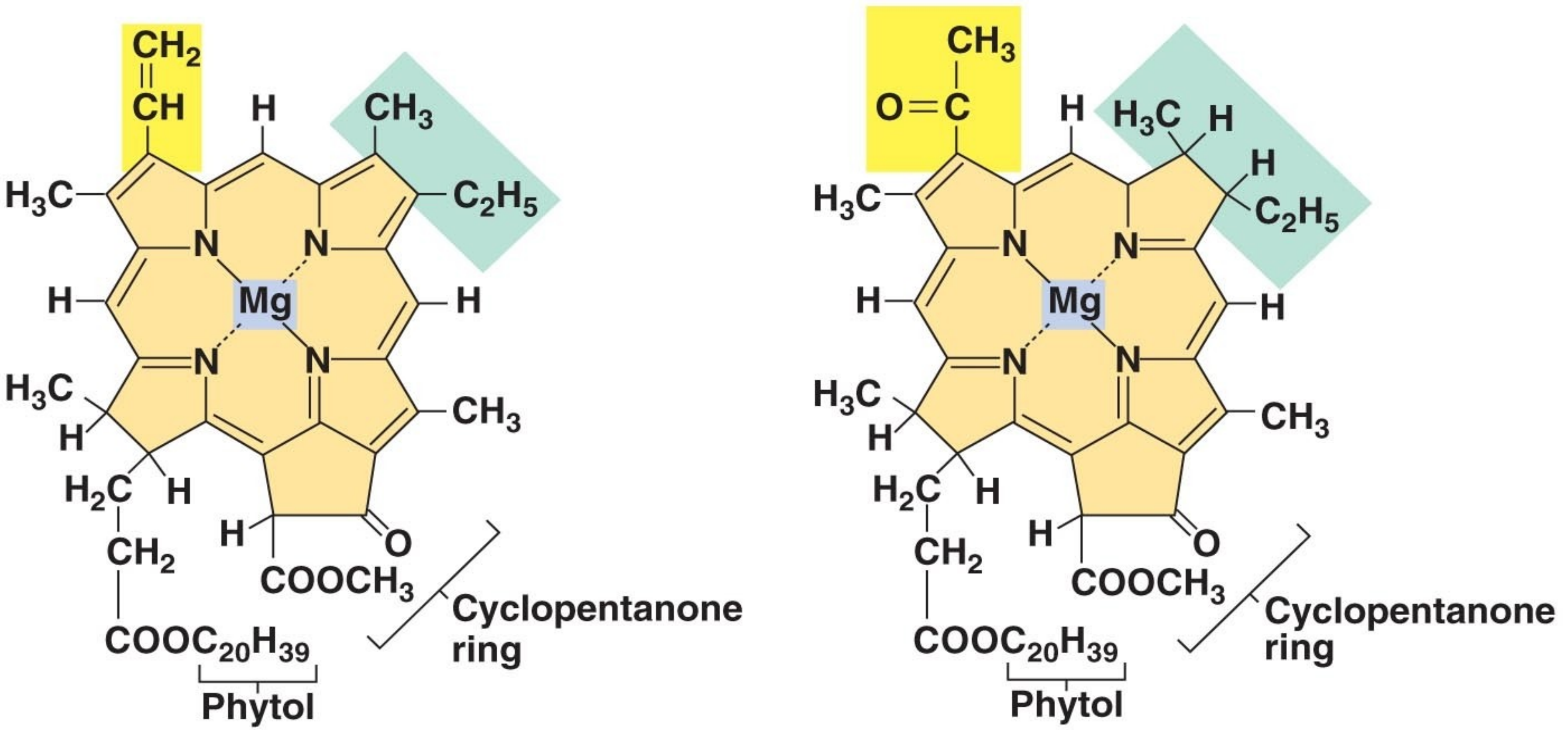
Patterns of Photosynthesis



Chlorophylls and Bacteriochlorophylls

- Phototrophy requires light-sensitive pigments
- Organisms must produce some form of chlorophyll (or bacteriochlorophyll) to be photosynthetic
- Chlorophyll is a porphyrin
- Number of different types of chlorophyll exist
  - Different chlorophylls have different absorption spectra
- Chlorophyll pigments are located within special membranes
  - In eukaryotes, called thylakoids
  - In prokaryotes, pigments are integrated into cytoplasmic membrane

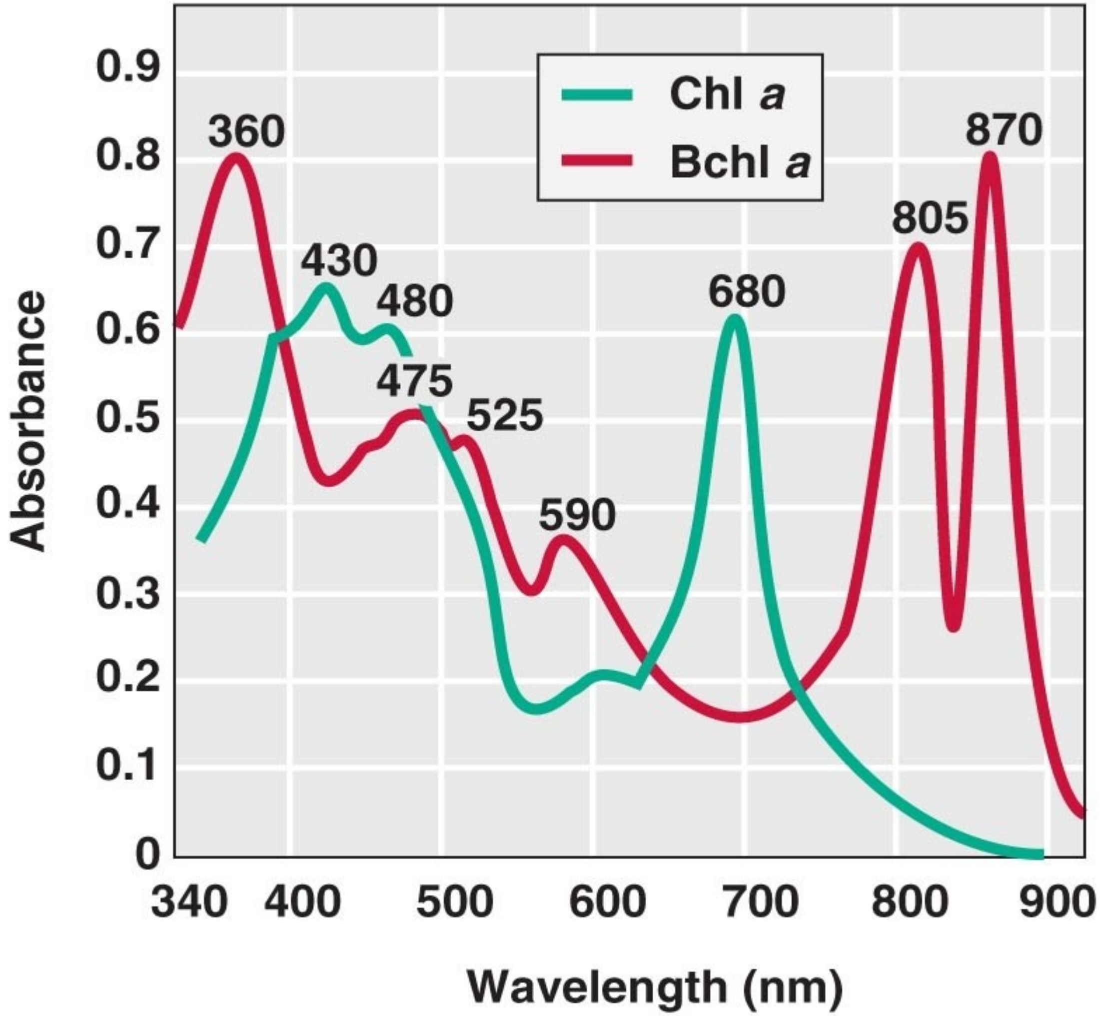
Chloro- and Bacteriochlorophyll



Chlorophyll *a*　　　Bacteriochlorophyll *a*

(a)

Spectra of Chloro- and Bacteriochlorophyll



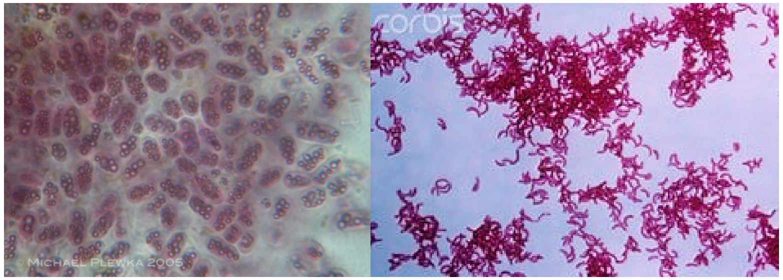
(b)

Oxygenic Photosynthesis:

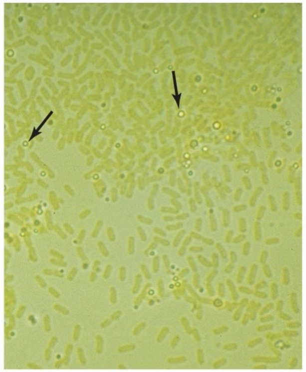
Cyanobacteria (formerly known also as *Cyanophyta* or Blue-Green Bacteria)

Anoxigenic Photosynthesis:

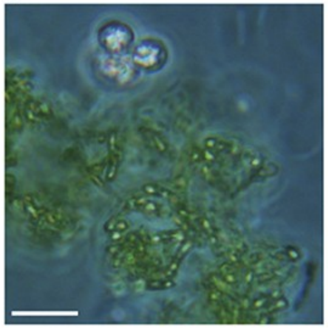
Purple Bacteria (*Chromatiaceae and Rhodospirillaceae*)



Green Sulfur Bacteria (*Chlorobi*)



Phototrophic Acidobacteria (*Chloracidobacterium*)



Green non-sulfur bacteria (*Chloroflexi*)

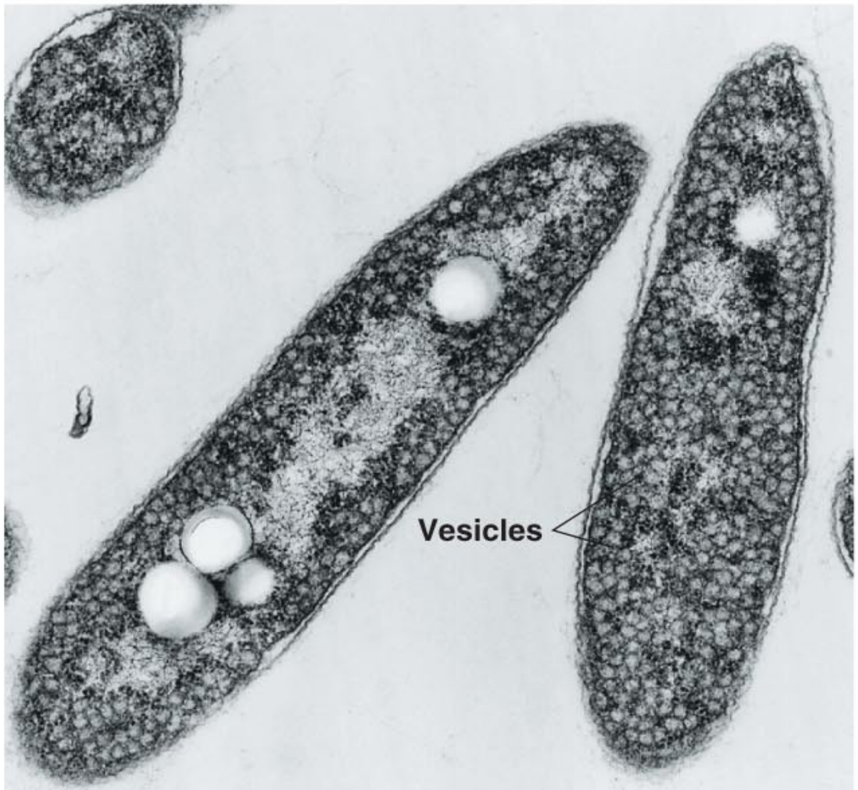
Photoheterotrophs:

Phototrophic Heliobacteria (*Heliobacteriaceae*)

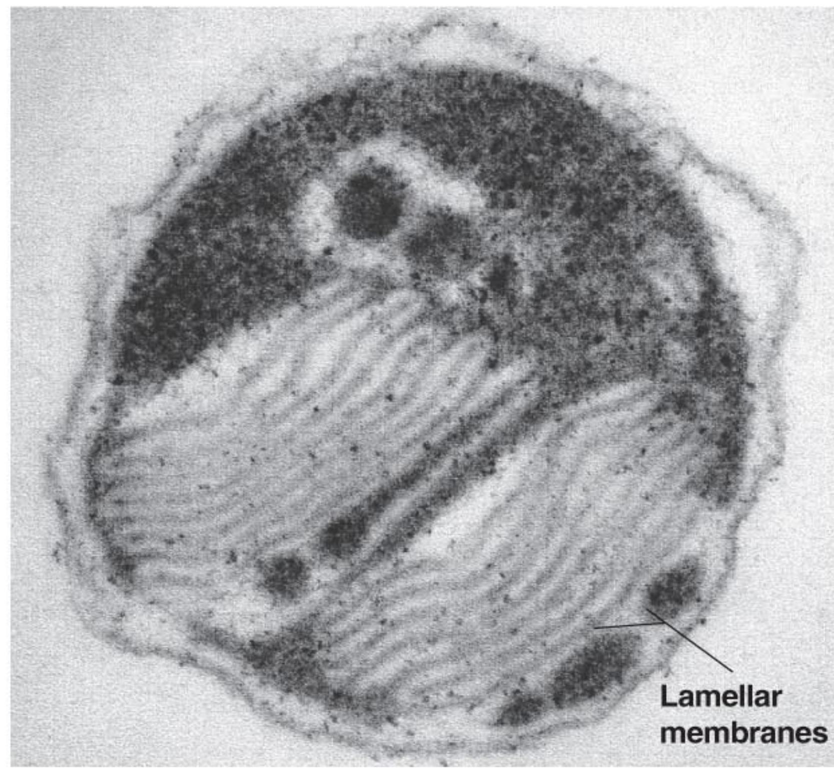
Anoxygenic Photosynthesis

- Anoxygenic photosynthesis is found in at least four phyla of *Bacteria*
- Electron transport reactions occur in the reaction center of anoxygenic phototrophs
- Reducing power for CO_2 fixation comes from reductants present in the environment (i.e., H_2S, Fe^{2+}, or NO^{2-})
- Requires reverse electron transport for NADH production in purple phototrophs

Membranes in Anoxygenic Phototrophs

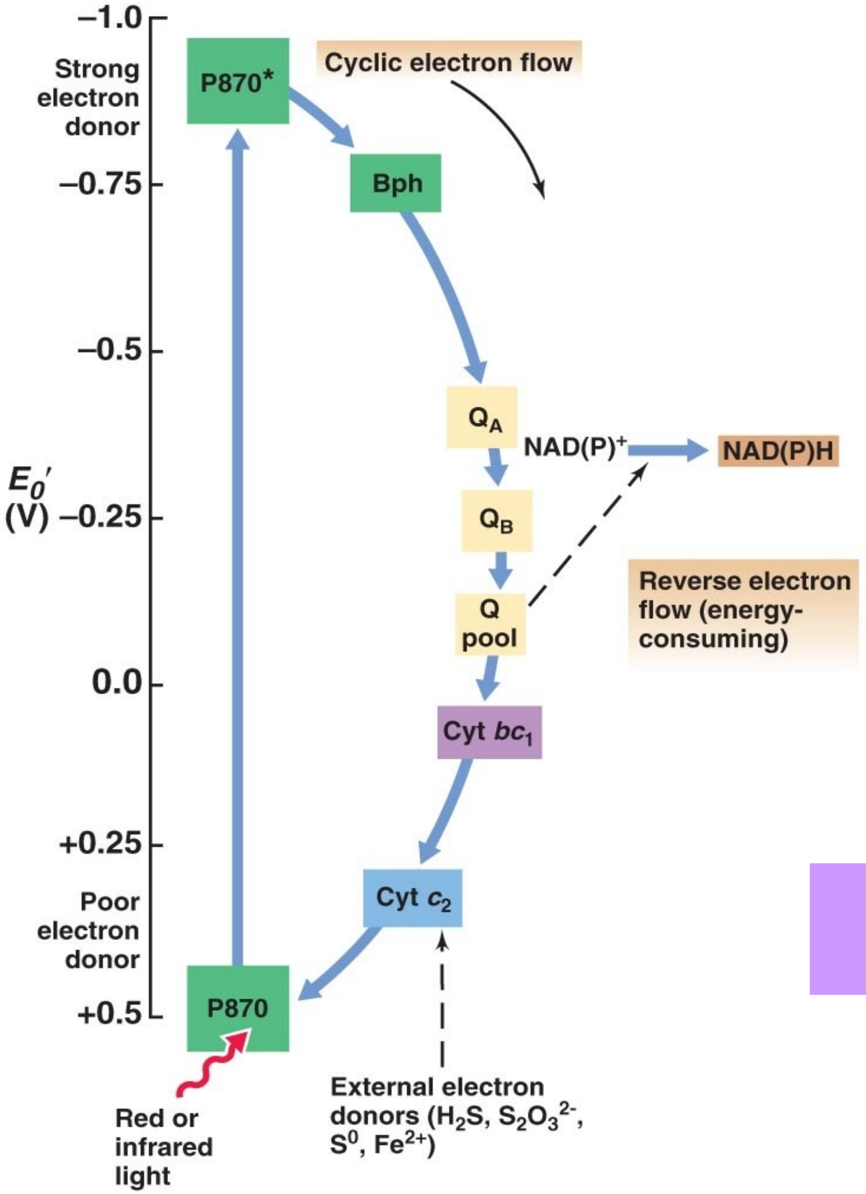


(a) Chromatophores

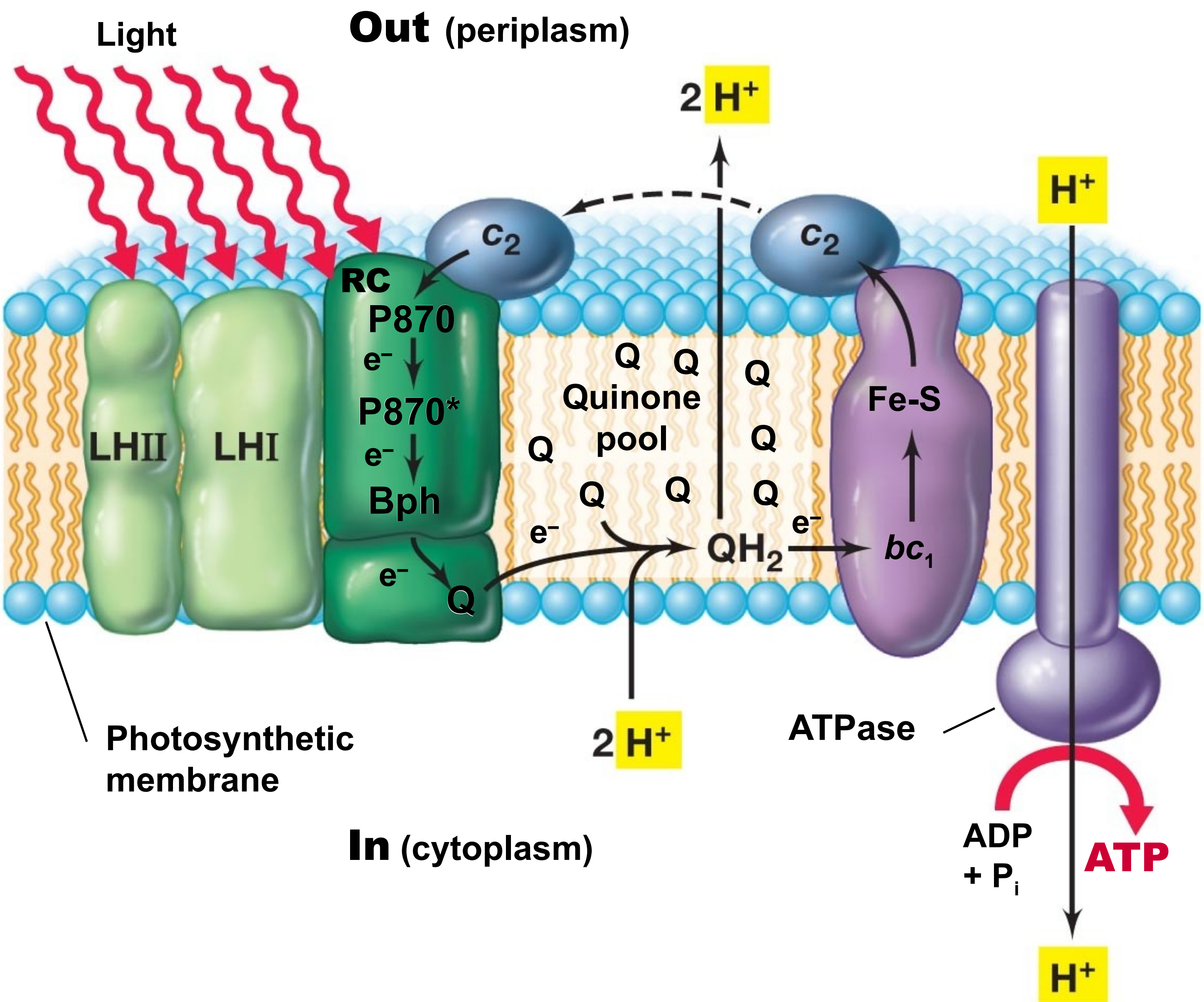


(b) Lamellar Membranes in the Purple Bacterium *Ectothiorhodospira*

Example of Electron Flow in Anoxygenic Photosynthesis

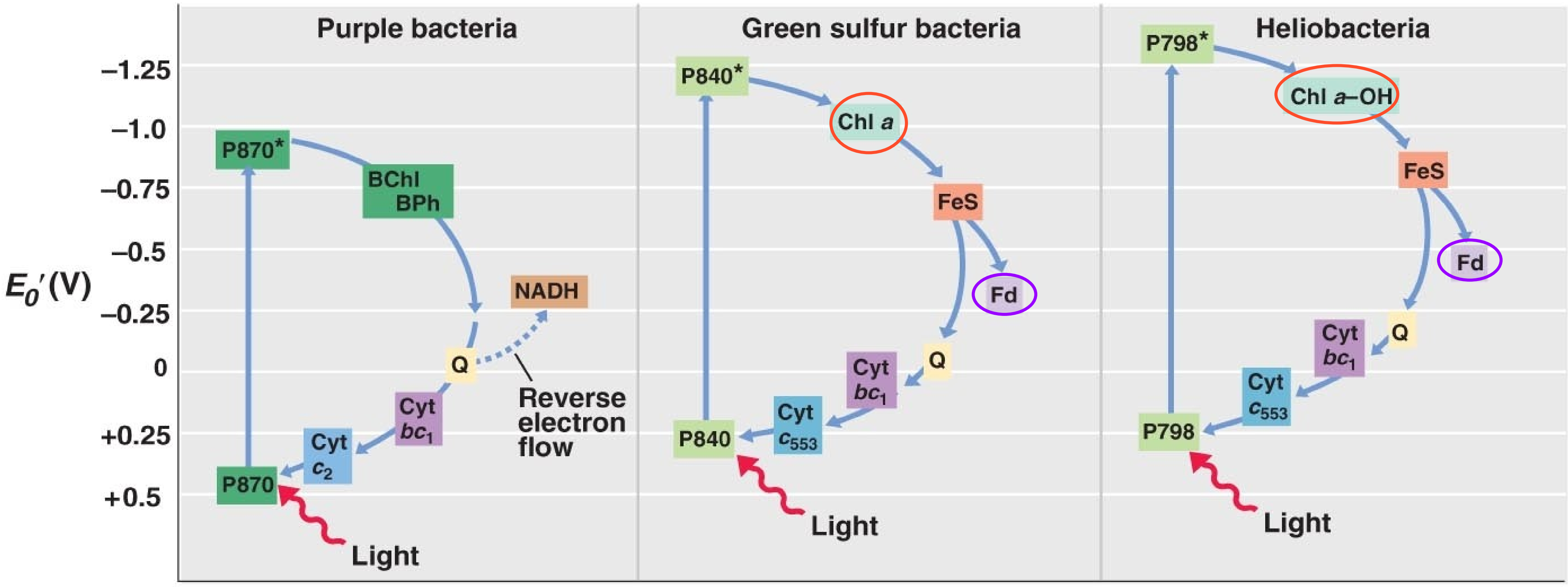


Purple bacterium



(b) **Arrangement of protein complexes in the purple bacterium reaction center**

Electron Flow in Purple, Green Sulfur and Heliobacteria



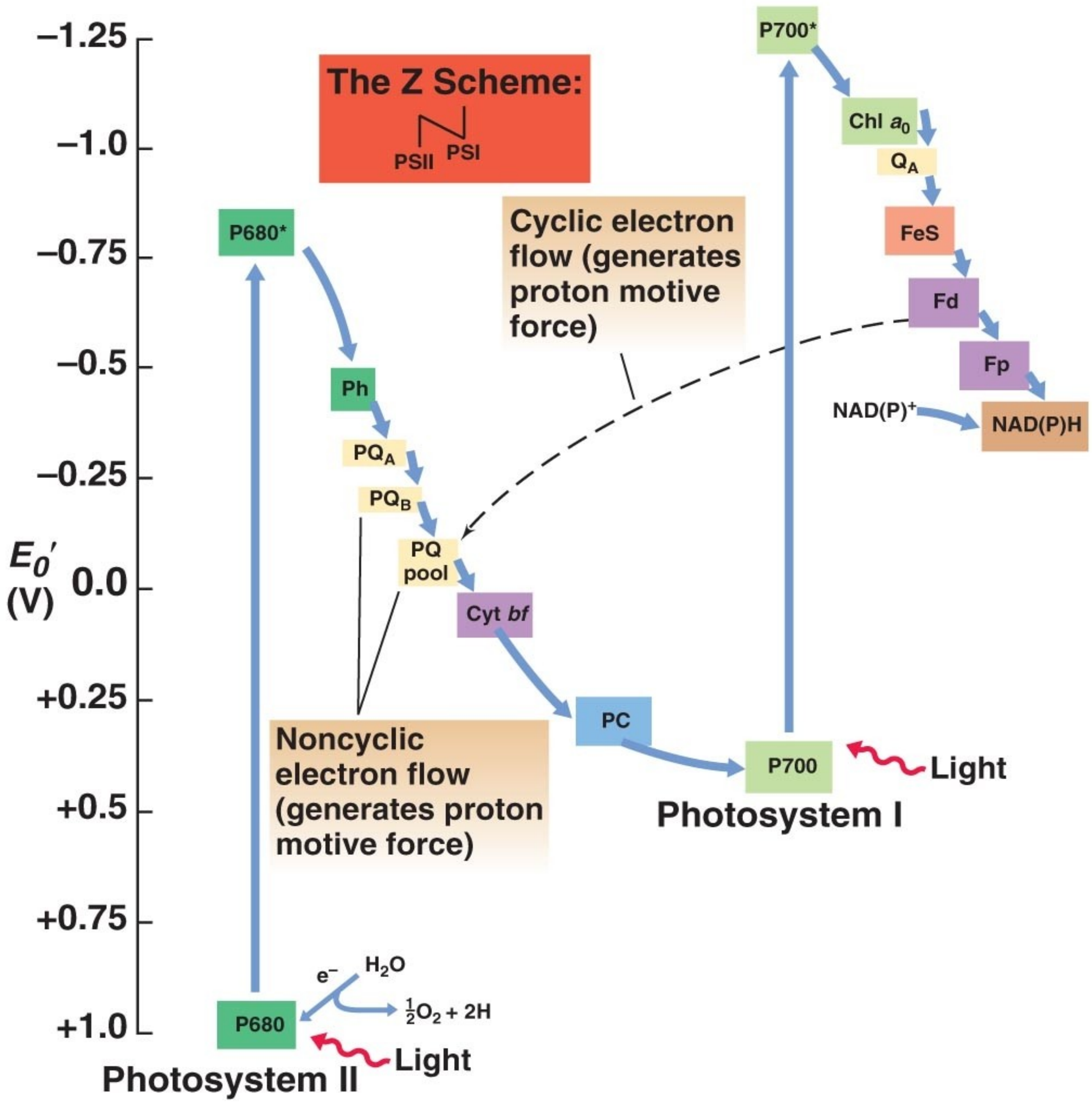
Bacteriochlorophyll *a*

Bacteriochlorophyll *g*

Oxygenic Photosynthesis

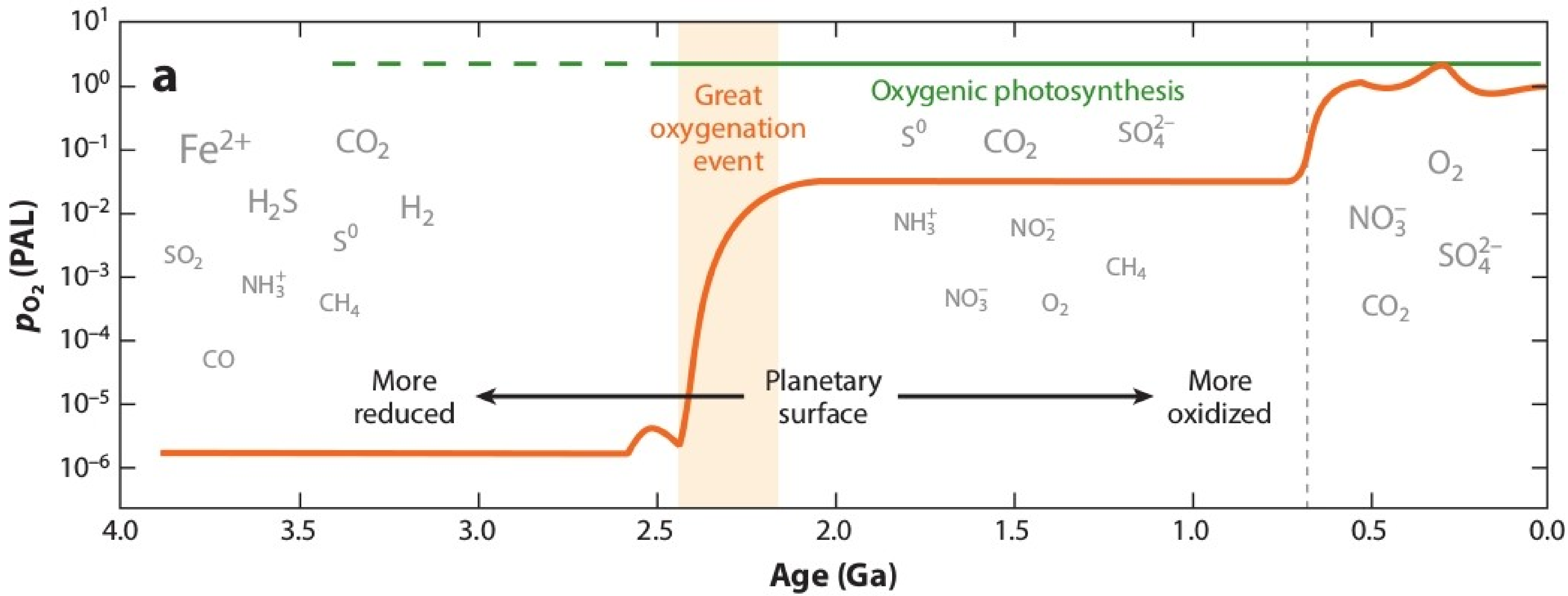
- Oxygen is the waste product of water oxidation
- Oxygenic phototrophs use light to generate ATP and NADPH
- The two light reactions are called photosystem I and photosystem II
- "Z scheme" of photosynthesis (photosystem II transfers energy to photosystem I)
- ATP can also be produced by cyclic photophosphorylation

The “Z scheme”



−1.25
−1.0
−0.75
−0.5
−0.25
E_0' (V) 0.0
+0.25
+0.5
+0.75
+1.0
The Z Scheme:
PSII PSI
P680*
Ph
PQ$_A$
PQ$_B$
PQ pool
Cyt *bf*
PC
P700
P700*
Chl a_0
Q$_A$
FeS
Fd
Fp
NAD(P)$^+$
NAD(P)H
Cyclic electron flow (generates proton motive force)
Noncyclic electron flow (generates proton motive force)
P680
e$^-$
H$_2$O
$\frac{1}{2}$O$_2$ + 2H
Light
Light
Photosystem I
Photosystem II

The Great Oxidation Event (GOE)



The Great Oxygenation Event (GOE) was the biologically induced appearance of dioxygen (O2) in Earth's atmosphere. Geological, isotopic, and chemical evidence suggest that this major environmental change happened around 2.3 billion years ago (2.3 Ga), however the actual causes and the exact date of the event are not clear.