MICROBIOLOGY OF EXTREME ENVIRONMENTS

CHEMOLITHOAUTOTROPHY 2: ENERGY METABOLISM

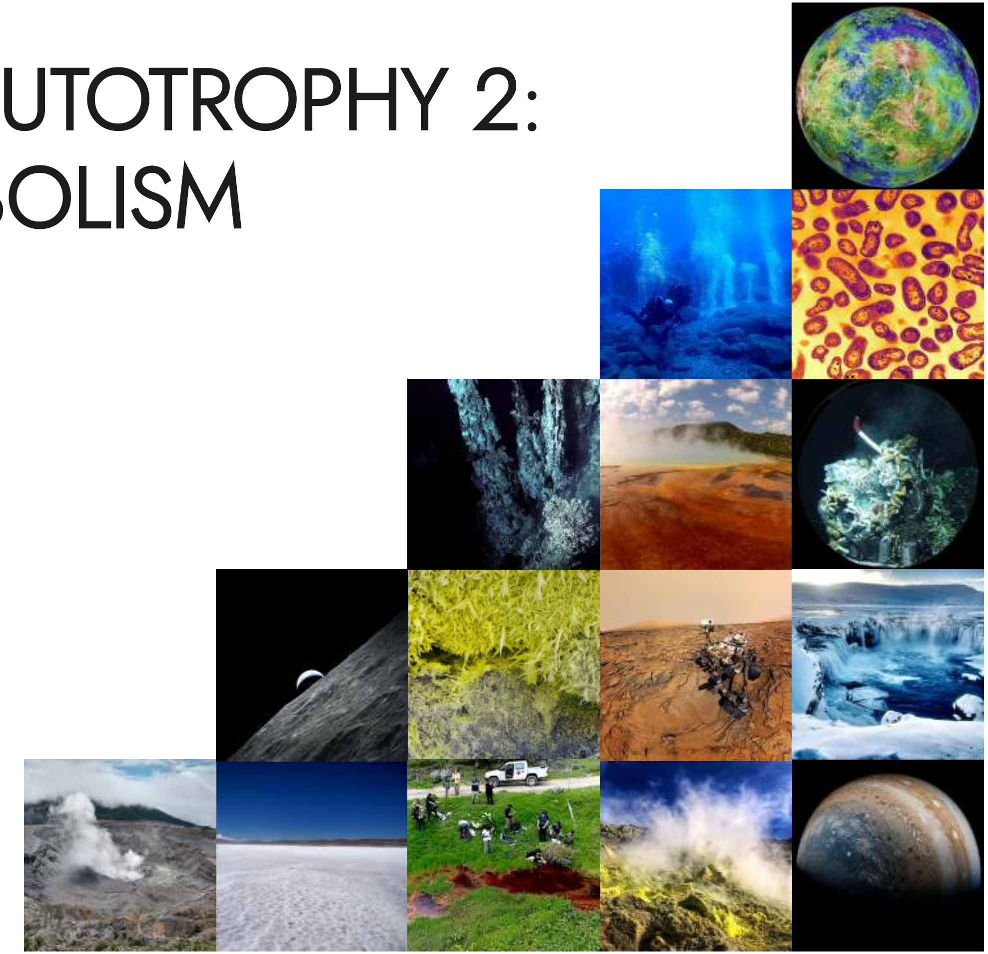
Donato Giovannelli

donato.giovannelli@unina.it

www.donatogiovannelli.com

@d_giovannelli

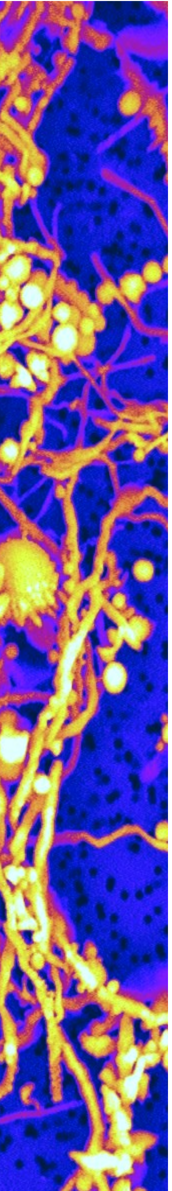
@donatogiovannelli



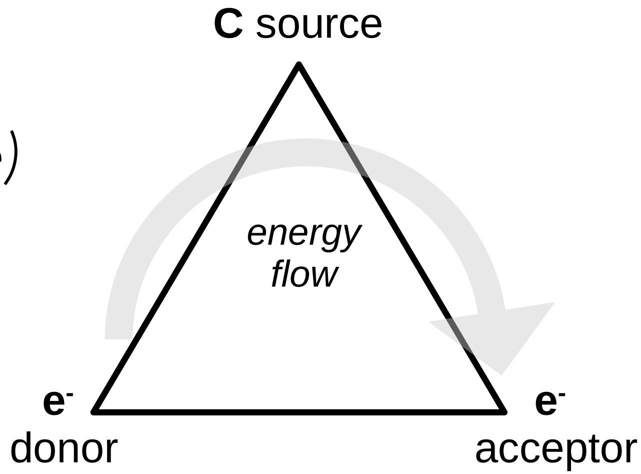
GIOVANNELLI LAB

BIOLOGY OF EXTREME ENVIRONMENTS

DIPARTIMENTO DI BIOLOGIA



Metabolism 101



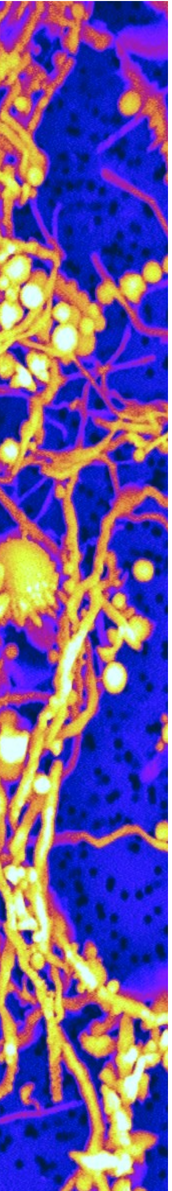
An ***electron donor*** *(also known as energy source)*

A ***carbon source*** *(for biosynthesis)*

An ***electron acceptor***

<u>All type of metabolism, requires these three basic elements.</u>

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.

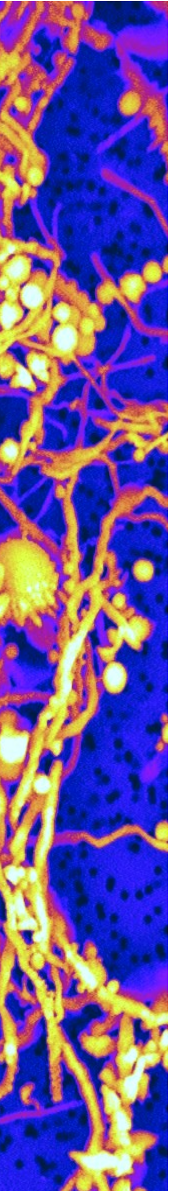


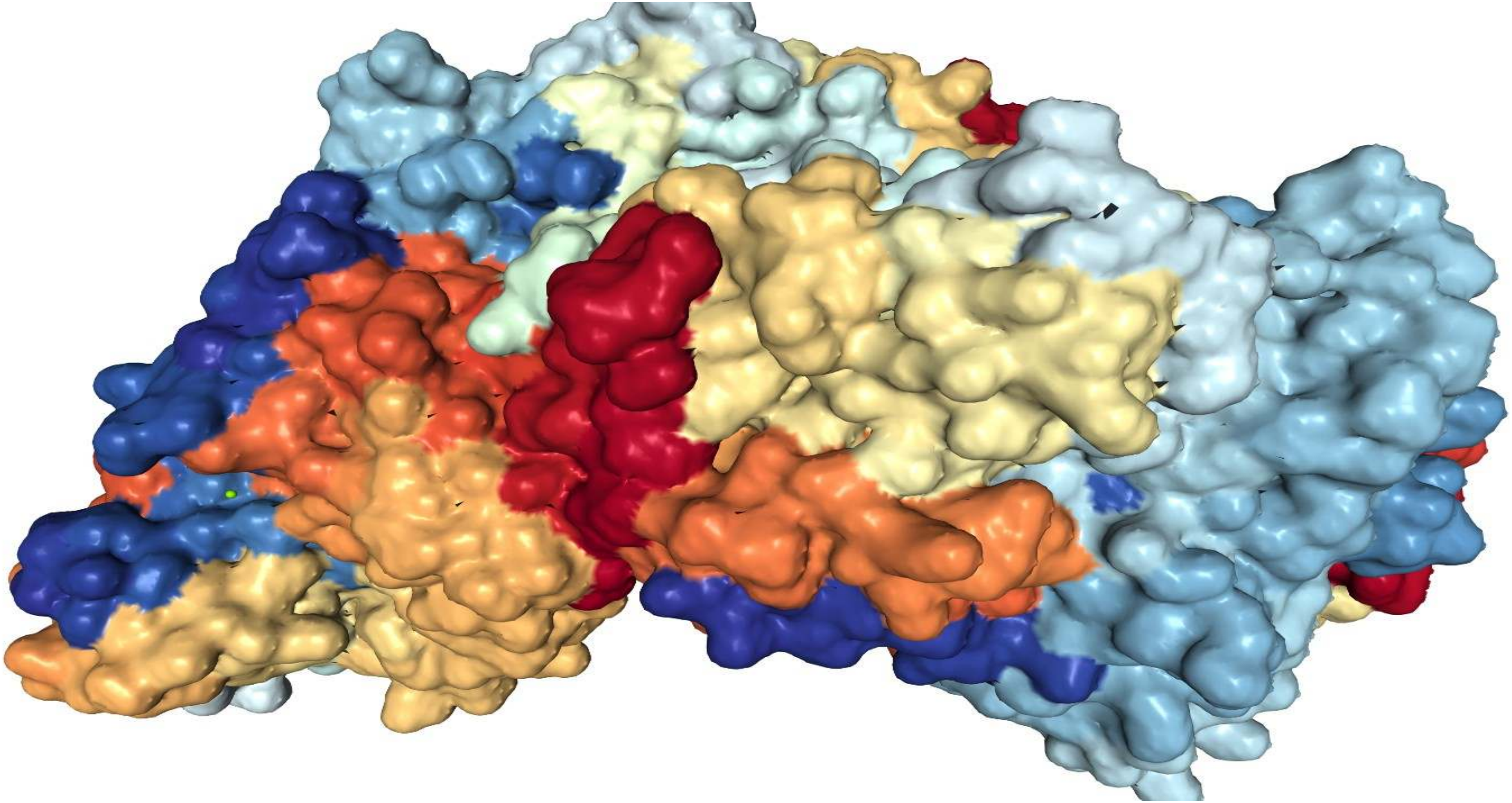
Life is based on RedOx reactions

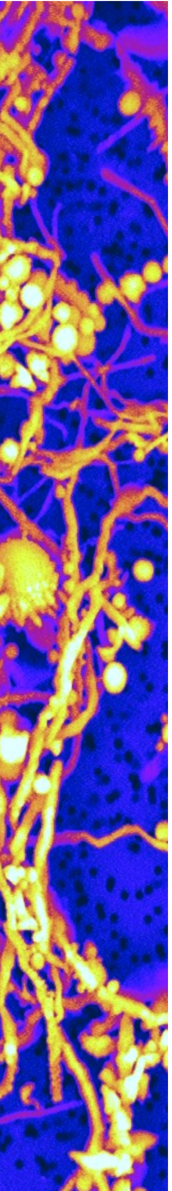
Refresher:

An ***oxidation-reduction*** *(redox)* ***reaction*** *is a type of chemical reaction that involves a* ***transfer of electrons*** *between two species. An oxidation-reduction reaction is any chemical reaction in which the oxidation number of a molecule, atom, or ion changes by gaining or losing an electron.*

Life is literally electric







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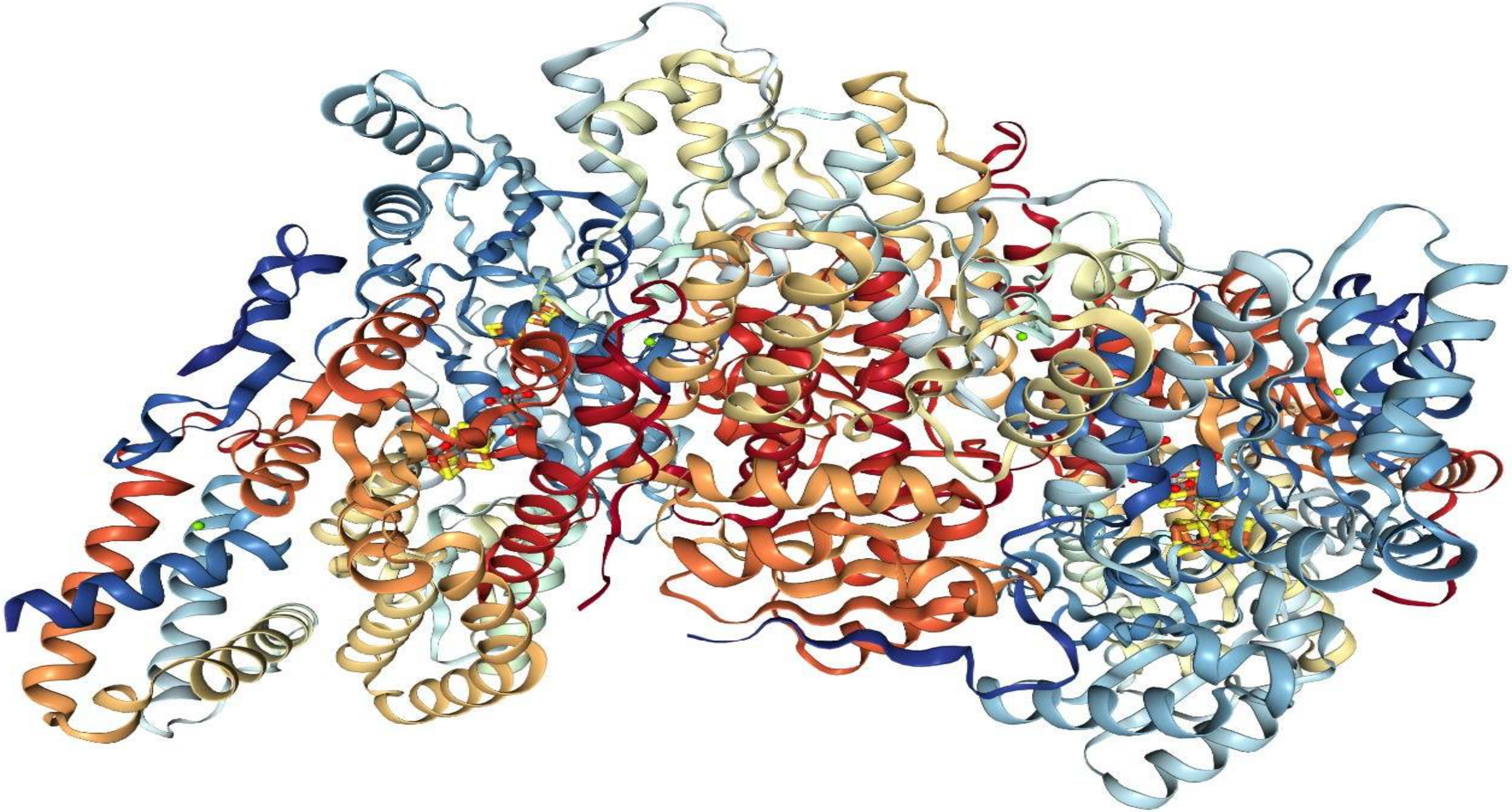

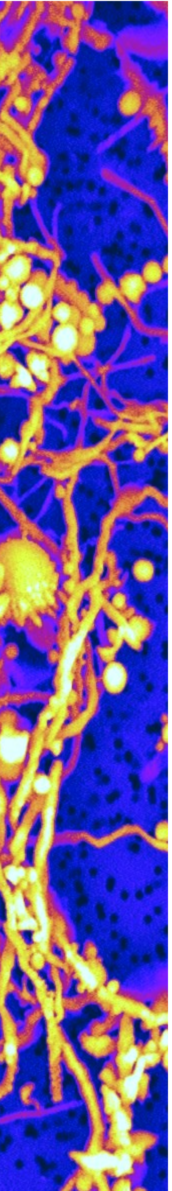

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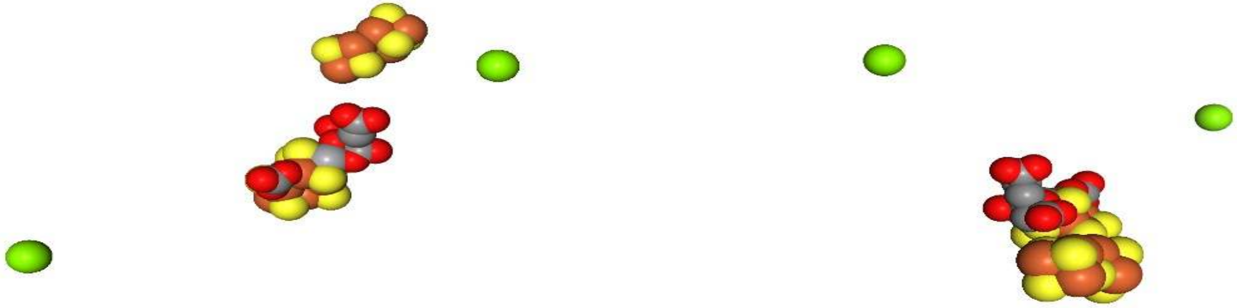

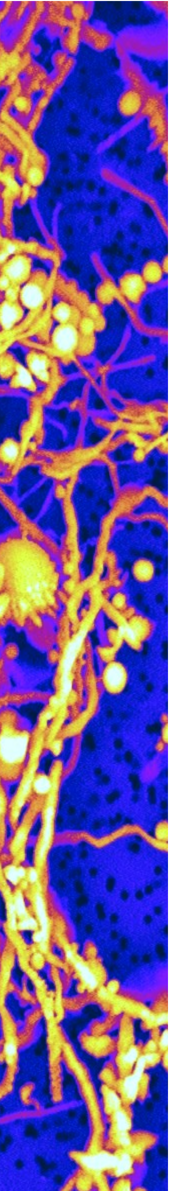

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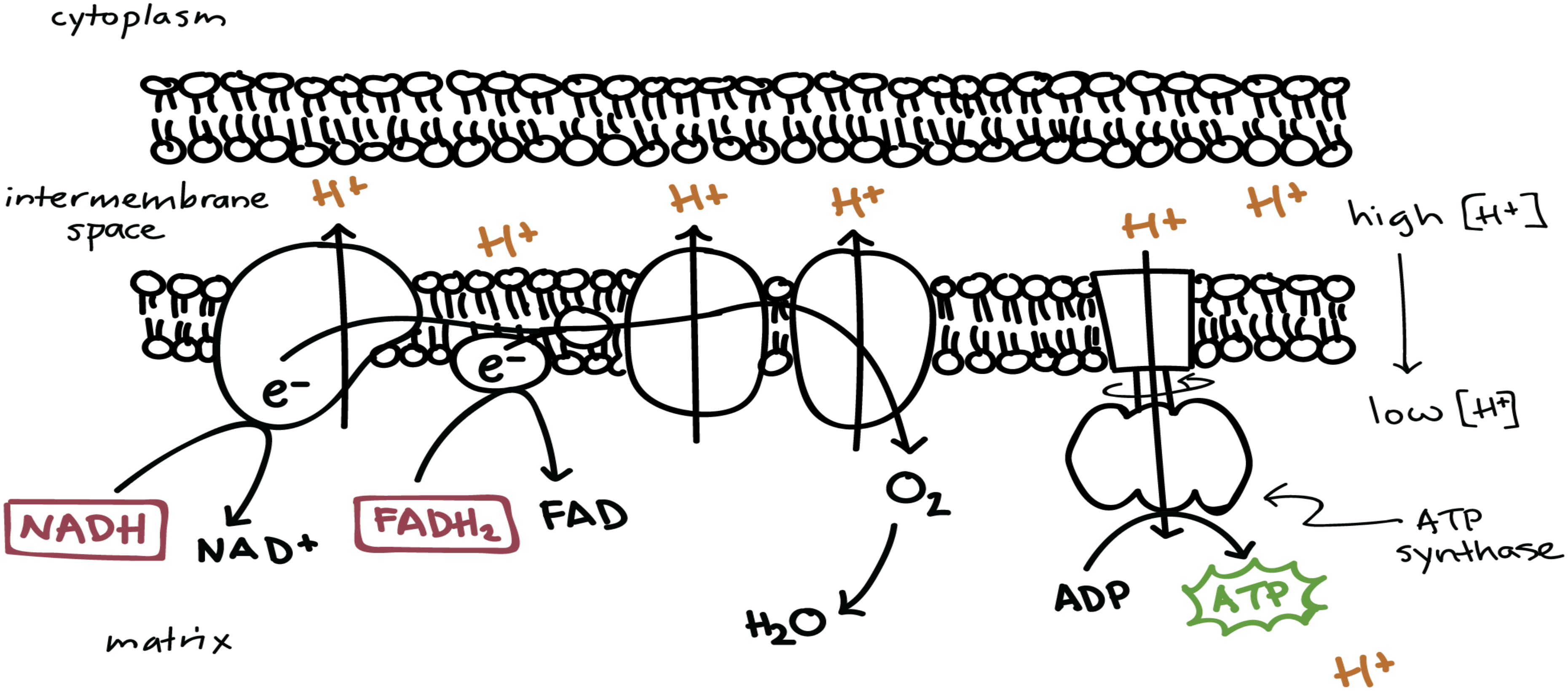

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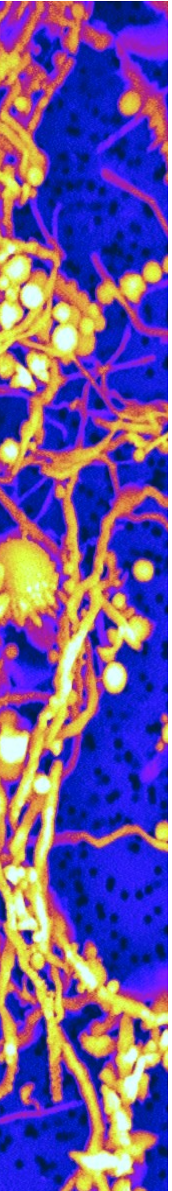
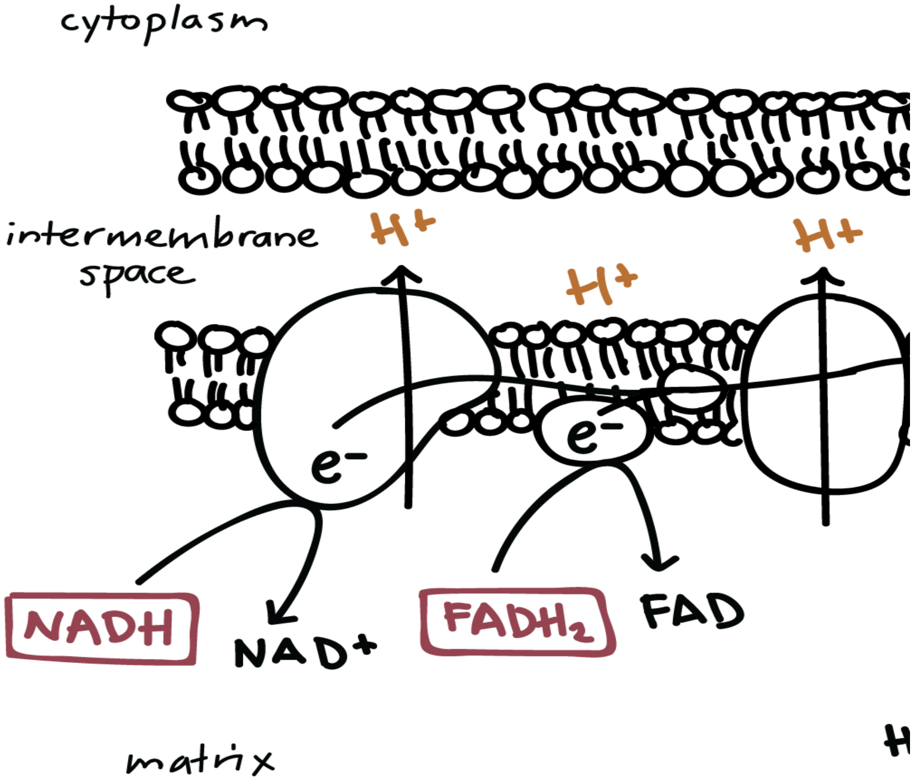
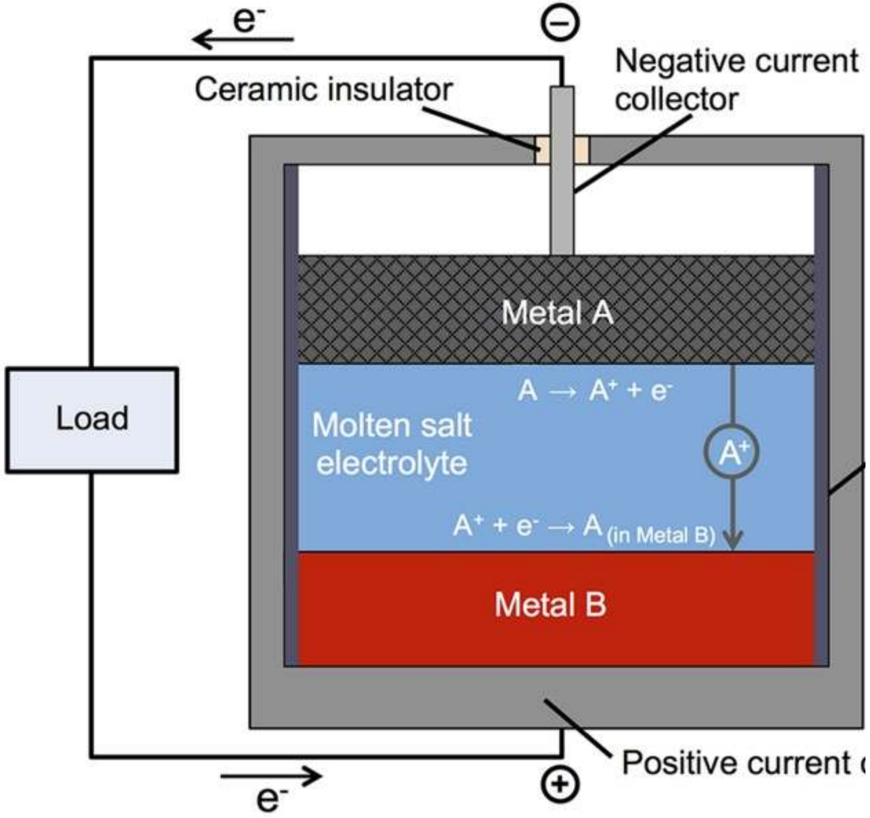
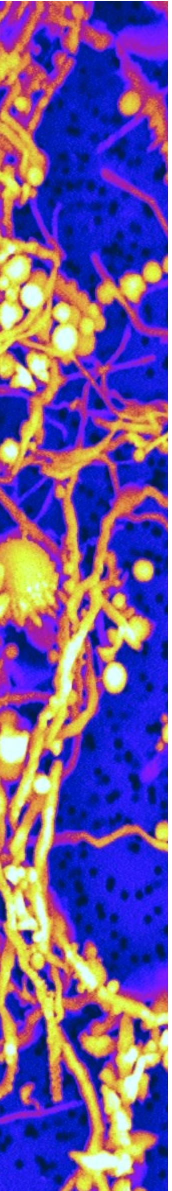
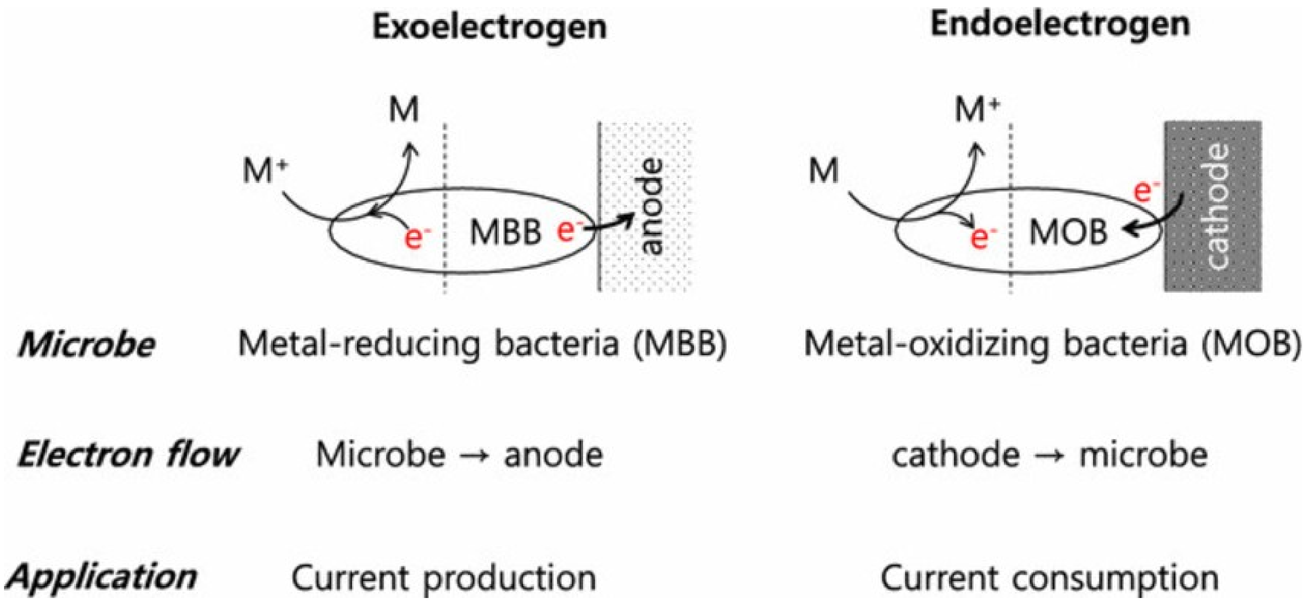


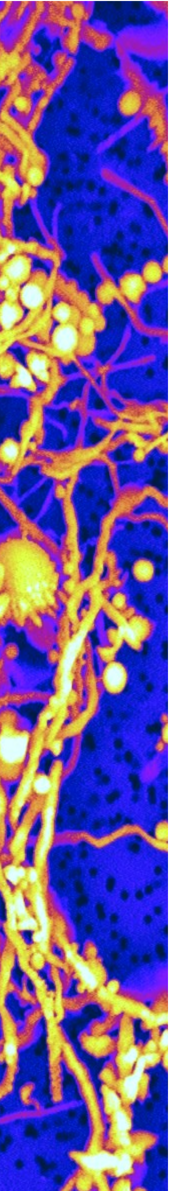




Life as a battery







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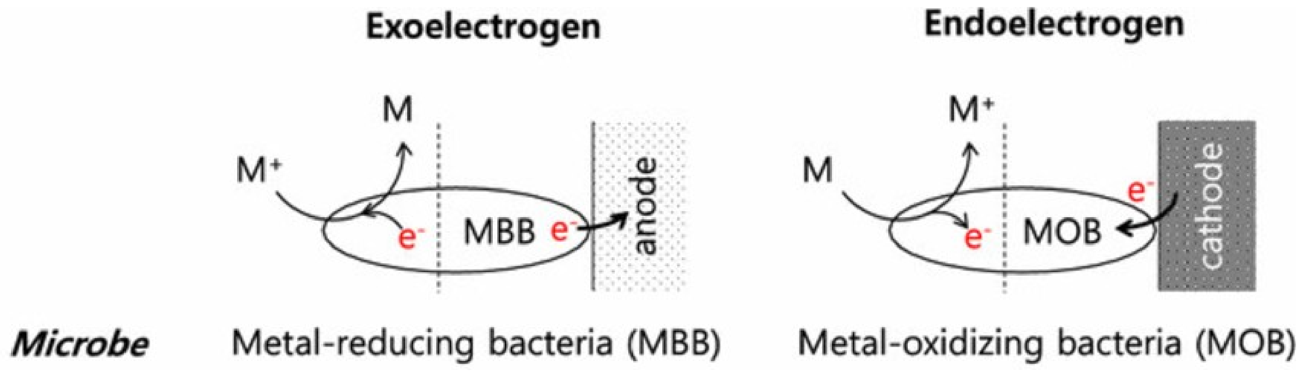
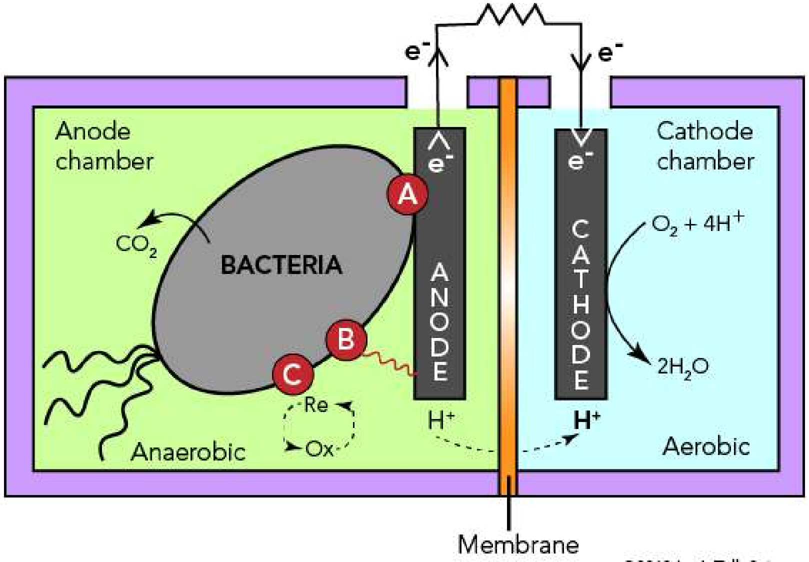
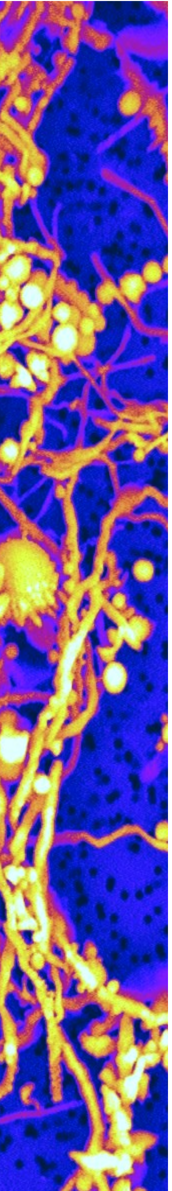






Life is Electric

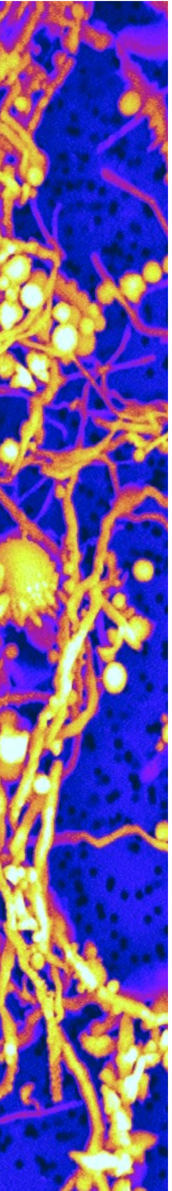
*Electron transfer (redox) reactions are most familiar as pairs of half-cell reactions, each half-reaction depicting an oxidized (electron poor) form of substrate having donated an electron, reversibly, to a reduced (electron rich) substrate (**Eq. 1)** or the reverse, **(Eq. 2**). Each half reaction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

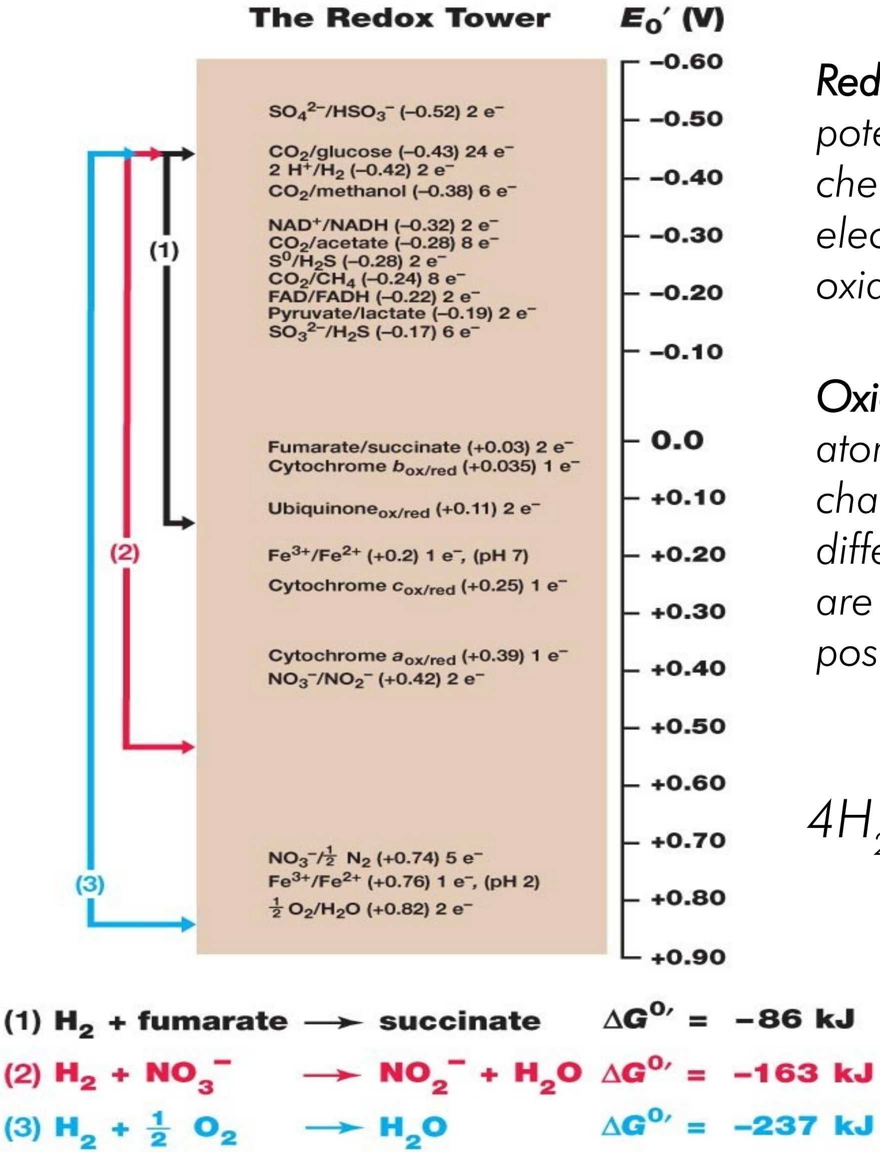
$A \rightleftharpoons A^{+} + e^{-}$ $\quad E^{\circ} = A_E$ $\quad$ ***Eq. 2***. *Oxidation half reaction: A is oxidized to* A^{+}

$B^{+} + e^{-} \rightleftharpoons B$ $\quad E^{\circ} = B_E$ $\quad$ ***Eq. 3.*** *Reduction half reaction:* B^{+} *is reduced to B*

The standard reduction potential (E°), 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 $E^{\circ}_{SHE} = 0$ 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.

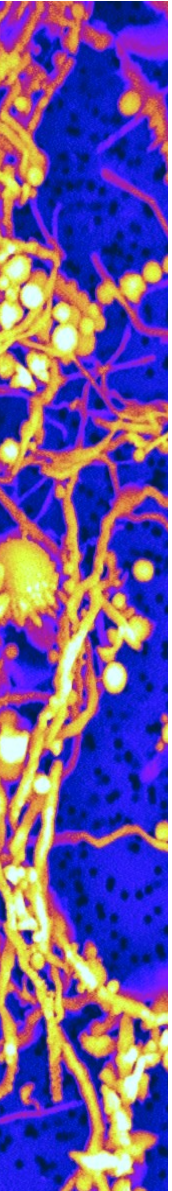




Redox potential *(also known as oxidation / reduction potential, 'ORP', E0',}) is a measure of the tendency of a chemical species to acquire electrons from or lose electrons to an electrode and thereby be reduced or oxidised respectively*

Oxidation state *indicates the degree of oxidation for an atom in a chemical compound. it is the hypothetical charge that an atom would have if all bonds to atoms of different elements were completely ionic. Oxidation states are typically represented by integers, which can be positive, negative, or zero.*

$$4H_2 + SO_4^{2-} + H = HS^- + 4H_2S \; \Delta G^{0\prime} = -151.9$$



ORIGINAL RESEARCH ARTICLE

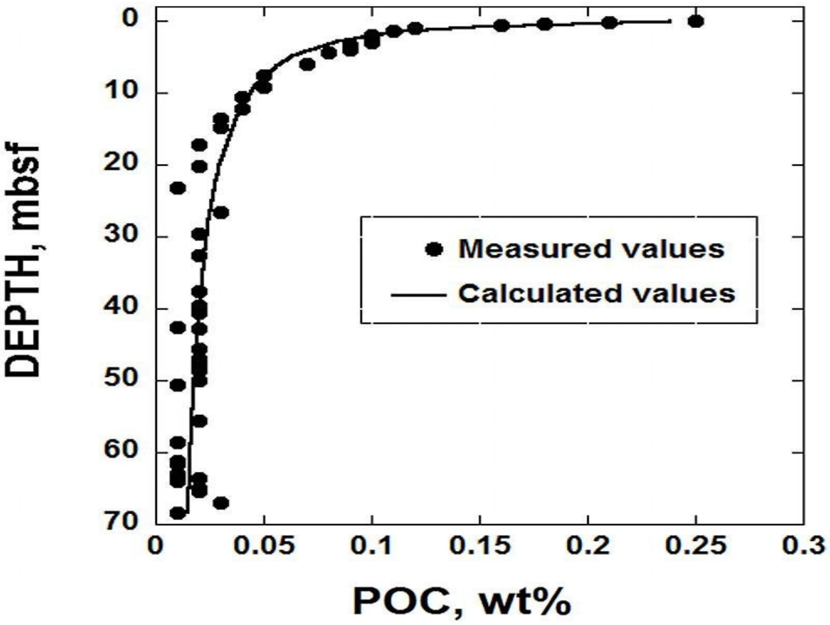
Front. Microbiol., 15 July 2015 | https://doi.org/10.3389/fmicb.2015.00718

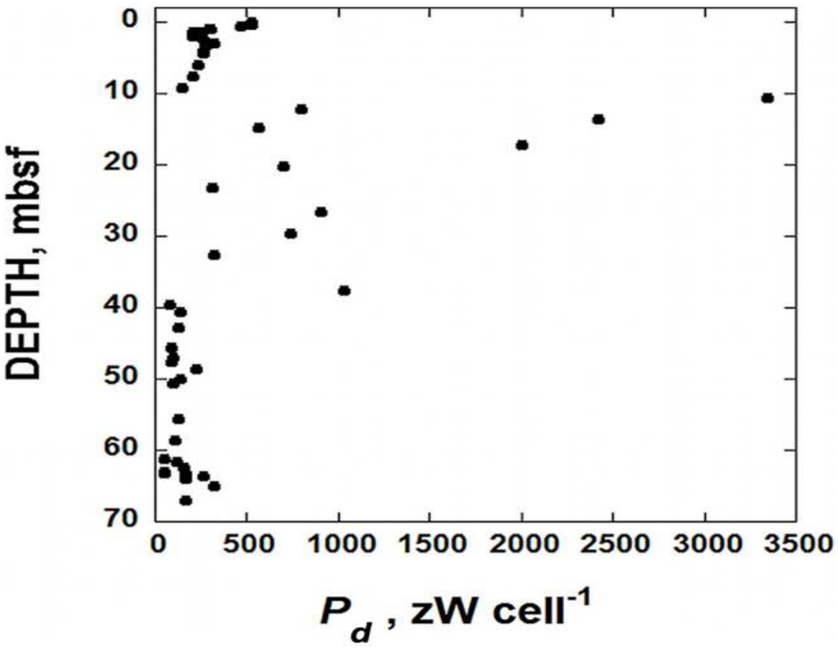
Power limits for microbial life

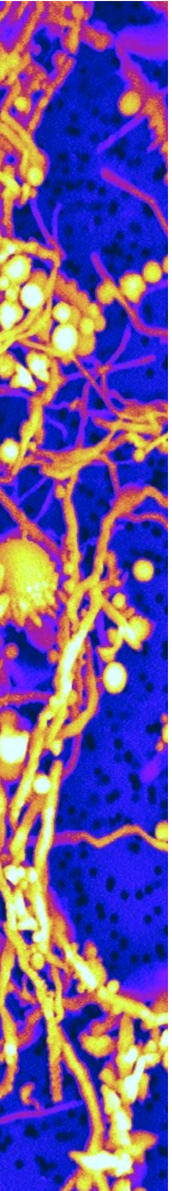
Douglas E. LaRowe[1*] and Jan P. Amend[1,2]

[1]Department of Earth Sciences, University of Southern California, Los Angeles, CA, USA

[2]Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA







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.

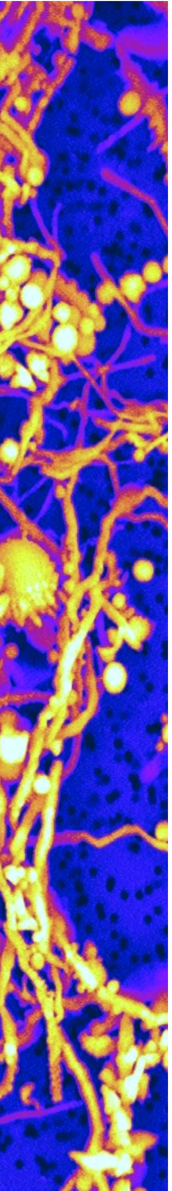
Free Energy (G): defined as the energy released that is available to do useful work G^0_f indicates the free energy of formation for a given compound, defined as the energy yielded or required for the formation of a given molecule from its constituent elements

$\Delta G^{0'}$ 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

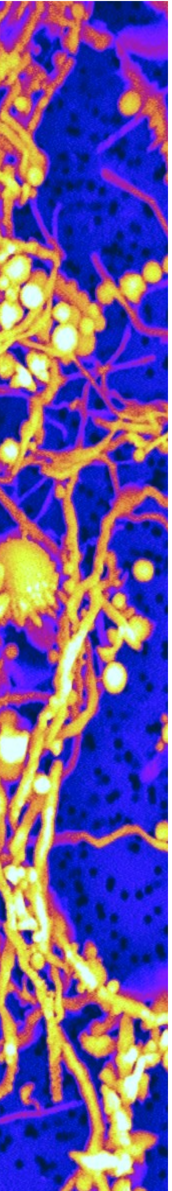


Electron donor or acceptor?

Compound	Formula	Oxidation state of sulfur	R/NR
sulfate	SO_4^{2-}	+6	R
sulfite	SO_3^{2-}	+4	R
tetrathionate	$S_4O_6^{2-}$	+2.5	R
thiosulfate	$S_2O_3^{2-}$	+2	R
elemental sulfur	S_8 (S^0)	0	R?
polysulfides	HS_x^-, S_x^{2-}	~−0.5	R
organic sulfur (disulfide)	R-S-S-R	−1	NR
pyrite	FeS_2	−1	NR
iron monosulfide	FeS	−2	R?
organic sulfur (thiol)	R-SH	−2	NR
bisulfide	HS^-	−2	R
hydrogen sulfide	H_2S	−2	R?

Electron acceptors

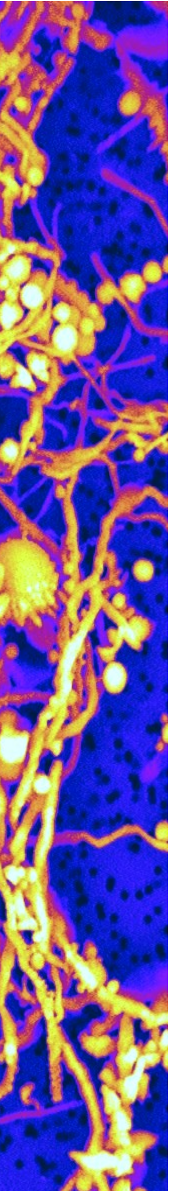
Electron donors

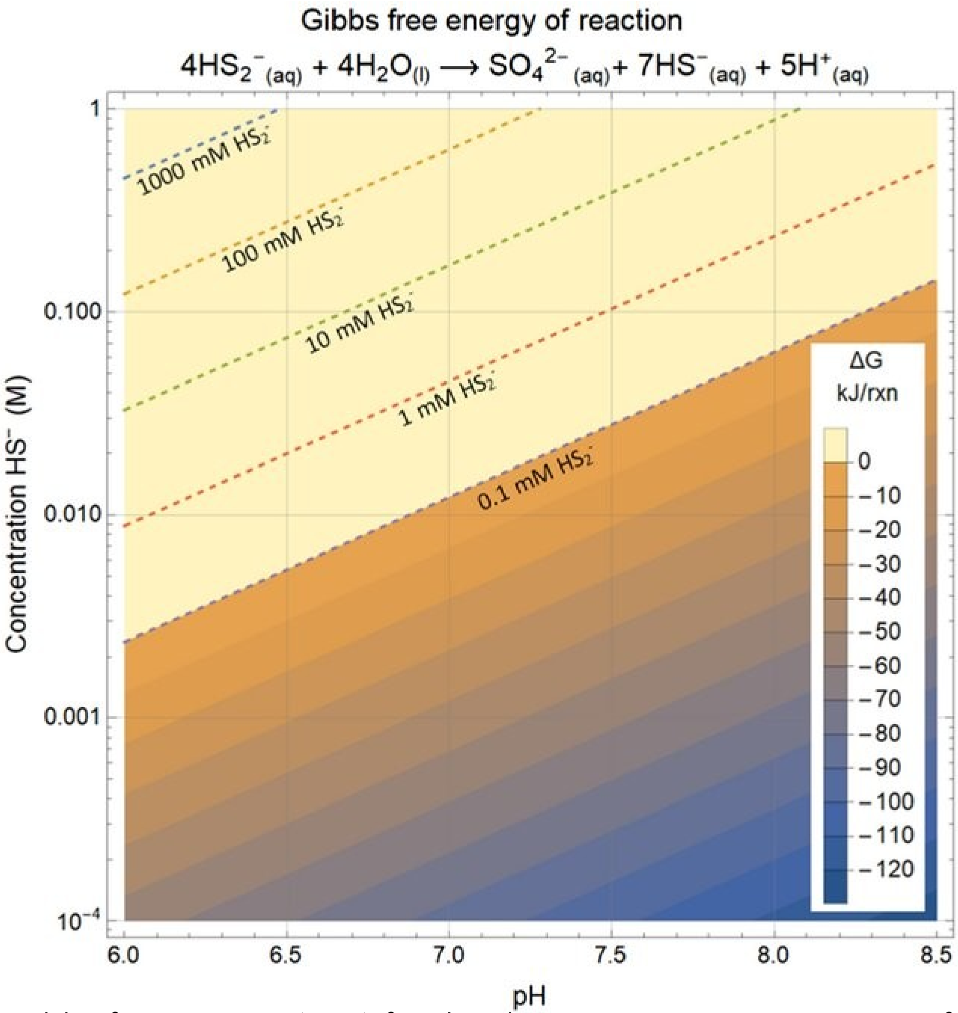


Electron donor or acceptor?

Oxidation States

	-6	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6	+7
Hydrogen							H_2	H^+						
Carbon			CO_2		CO		C		CH_2H_4		CH_4			
Oxygen					H_2O		O_2							
Nitrogen				NH_4^+		NH_2OH	N_2	N_2O	NO	NO_2^-	NO_2	NO_3^-		
Sulfur					H_2S	HS^-	S		$S_2O_3^{2-}$		SO_3^{2-}		SO_4^{2-}	
Iron							Fe		Fe^{2+}	Fe^{3+}				
Manganese									MnO	Mn_2O_3	MnO_2		MnO_3	Mn_2O_7
Arsenic										AsO_3^{3-}	AsO_4^{3-}			
Selenium											SeO_3^{2-}		SeO_4^{3-}	

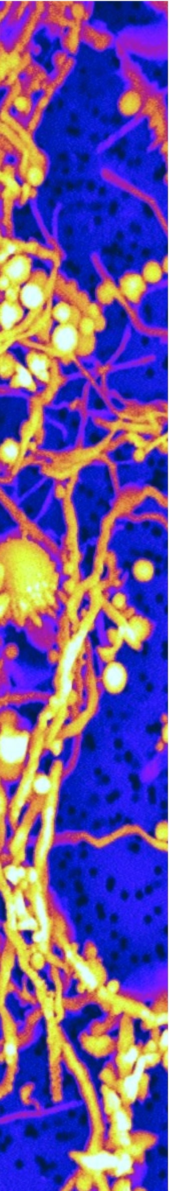


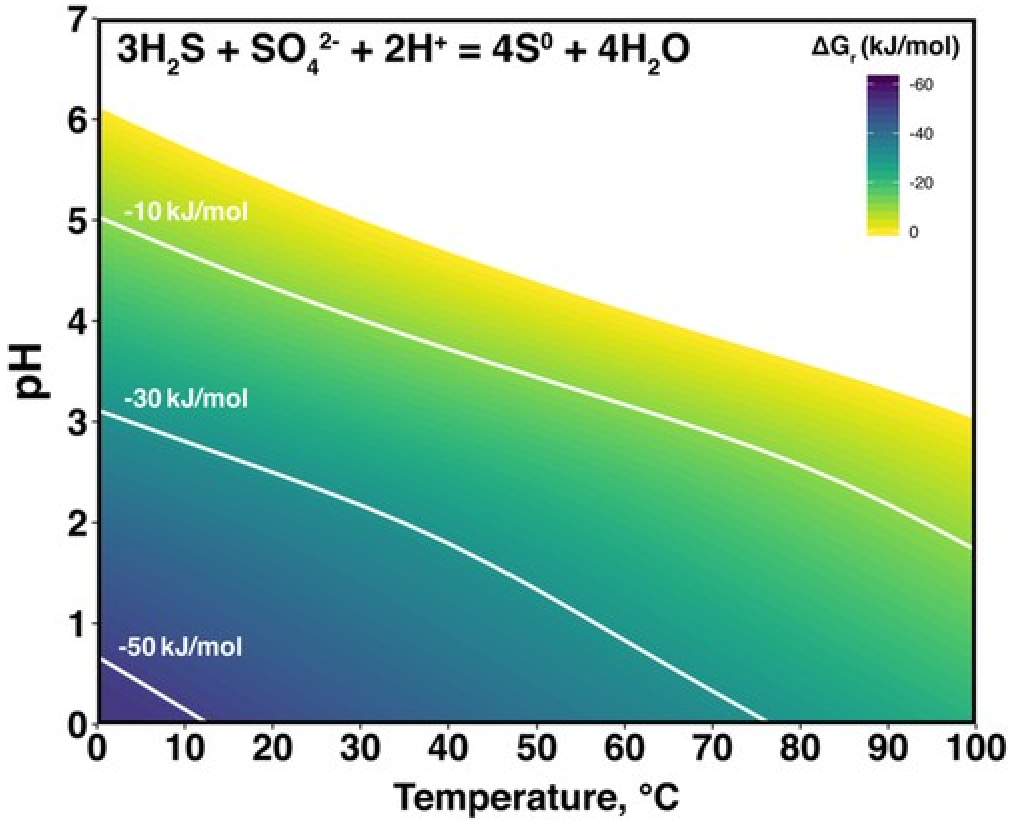


Gibbs free energy (ΔG) for the disproportionation reaction of HS2− to SO4²− and HS− at 25 °C and 25 mM SO4²− (typical concentration of sulfate in seawater). Dashed lines represent zero Gibbs free energy values at different concentrations of HS2−

Hatzikioseyian et al. 2021 Bioproc and Biosyst Eng

The Gibbs free energy of reaction depends on the concentrations of reactants, products and the environmental conditions (pH, temperature, pressure)

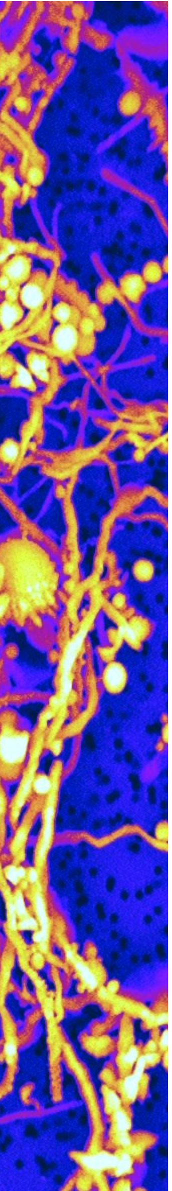


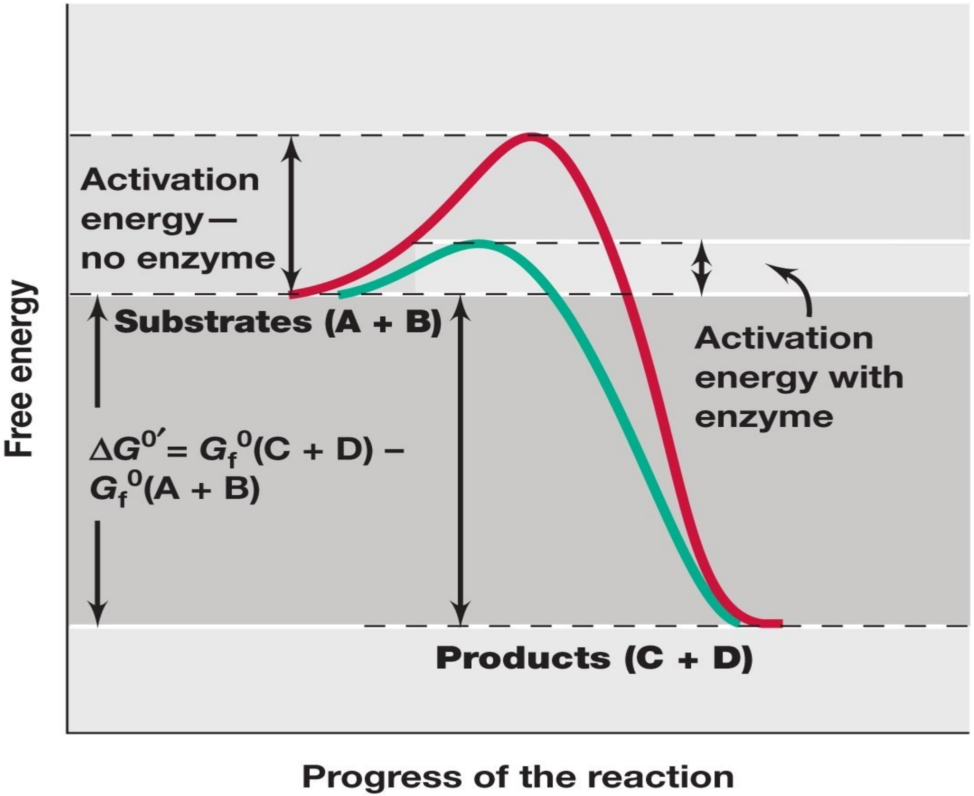


Values of ΔG r for Reaction 1 calculated with Equation 3 as a function of temperature and pH for sulfur disproportionation, a confirmed metabolism found in several natural environments

Amend et al. 2020 Env Microbiol

Thermodynamic drive vs activation vs rates





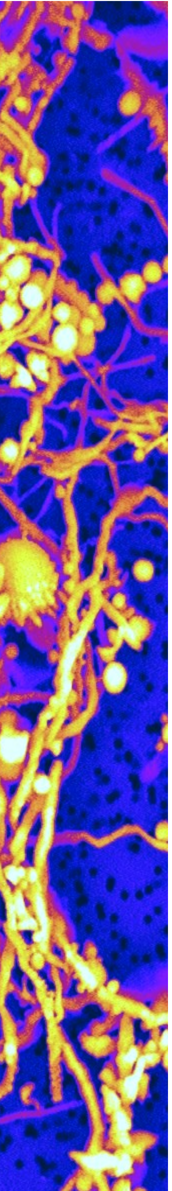
Lets look at a favorable redox reaction

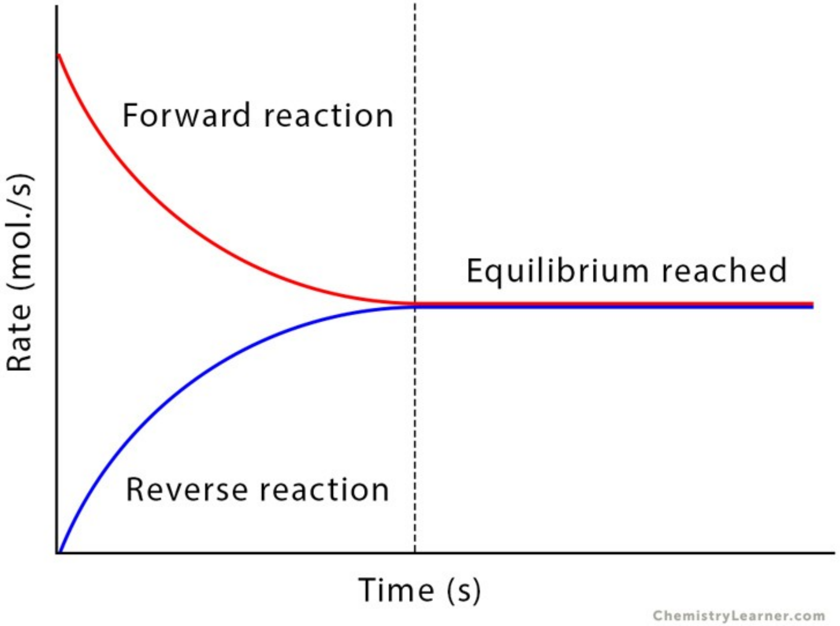
$$4Fe^{2+} + 10H_2O + O_2 = 4Fe(OH)_3 + 8H^+$$

The free Gibbs energy tells us the the reaction is thermodynamically favorable, but nothing about the energy of activation or the rate of the reaction

And while the energy of activation is a property of the reaction considered (and can be lowered using a catalyst/enzyme) the rate is a complex function of many things, including the concentrations of the reactants and the products

Biology likes it slow!





$$-\frac{d[Fe^{2+}]}{dt} = k[Fe^{2+}][OH^-]^2[O_2]$$

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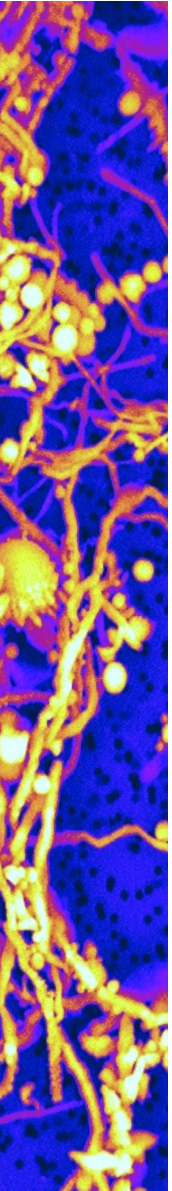

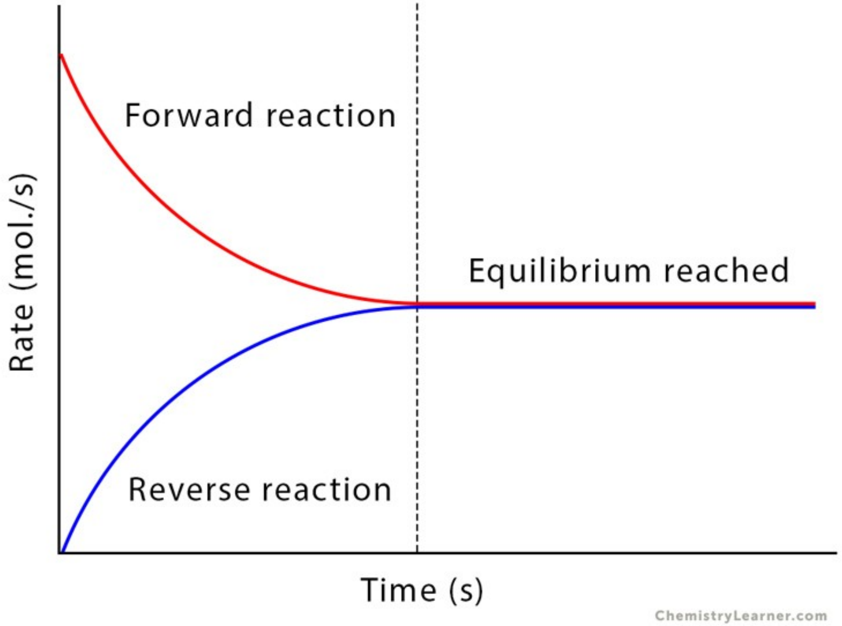

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For biology to be able to use a reaction ***the abiotic rate must be slower than the biotic rate****, otherwise the reaction runs so fast that energy release cannot be controlled*

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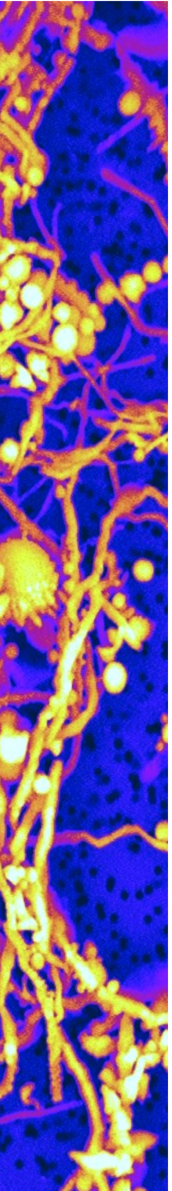

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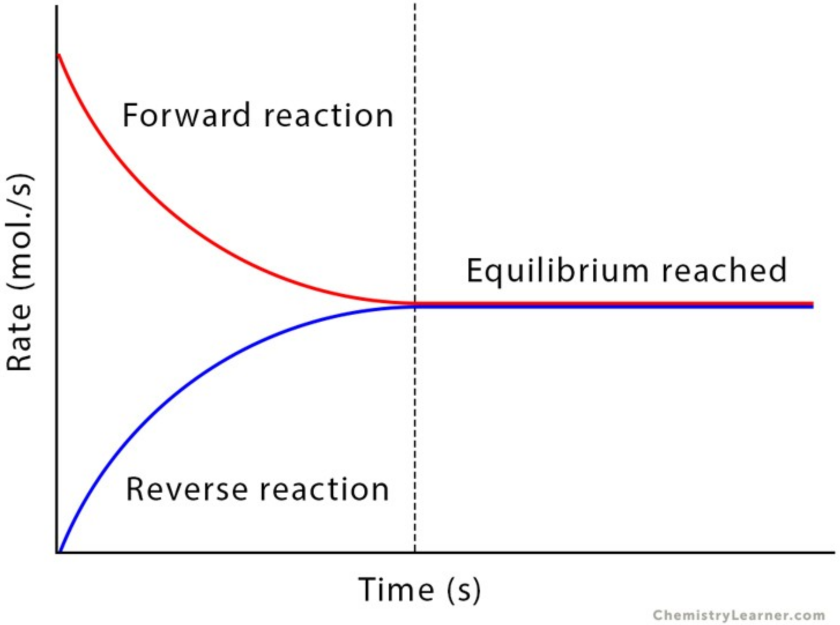

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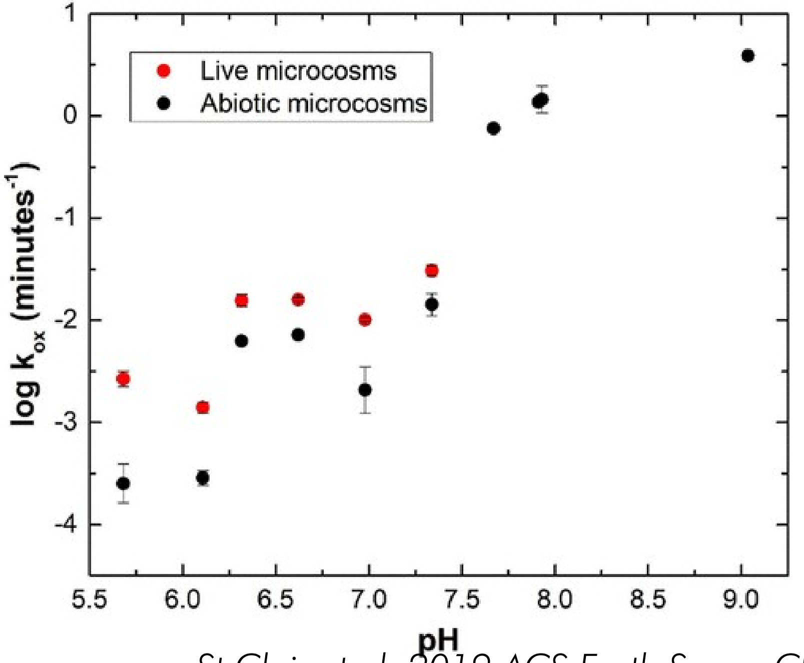

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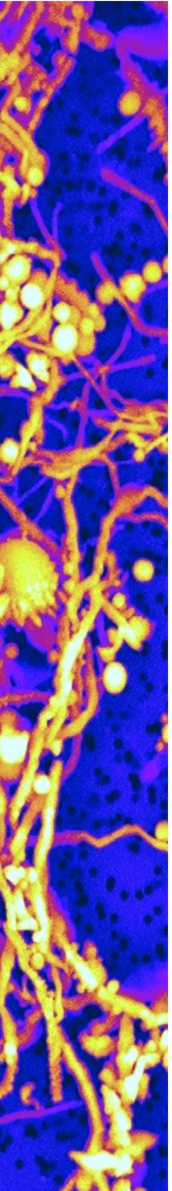

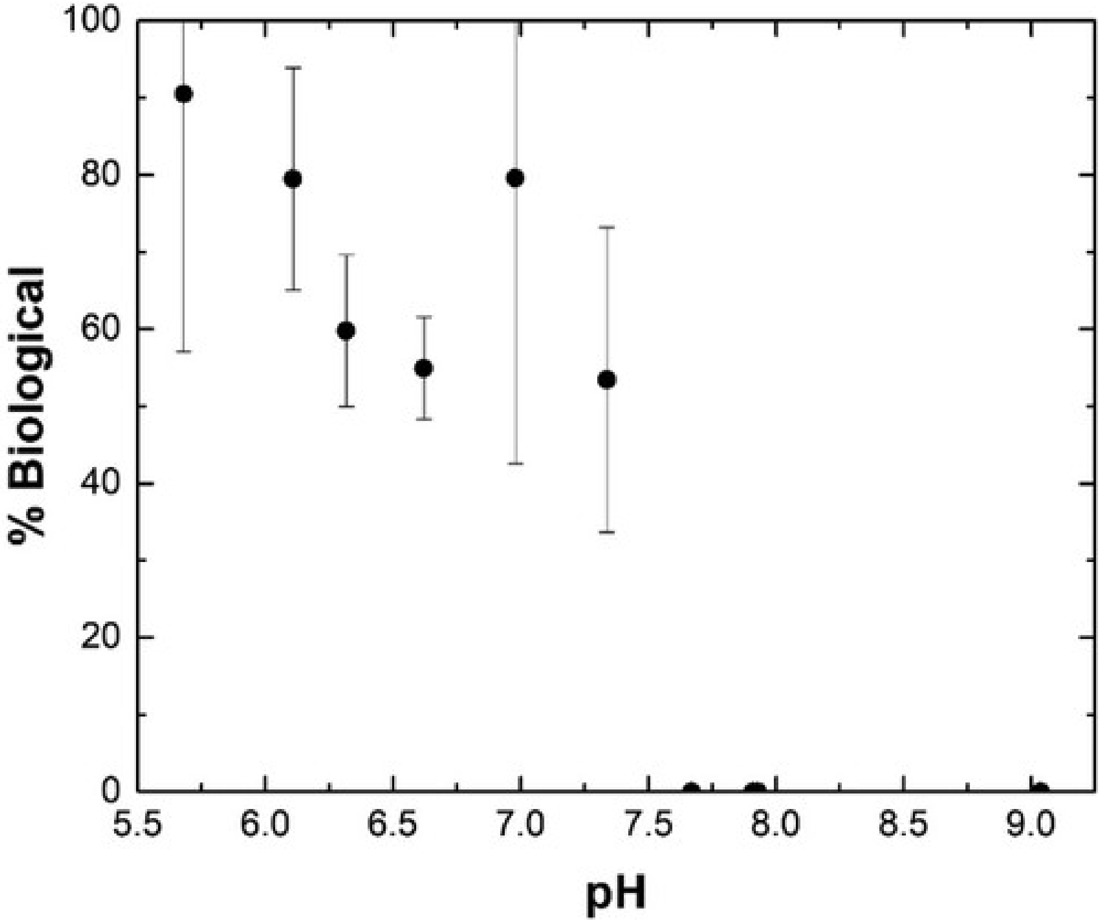

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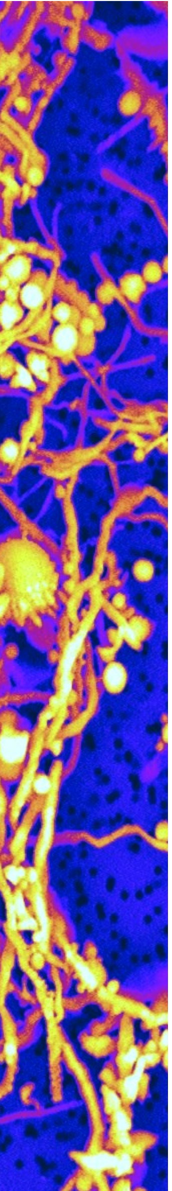
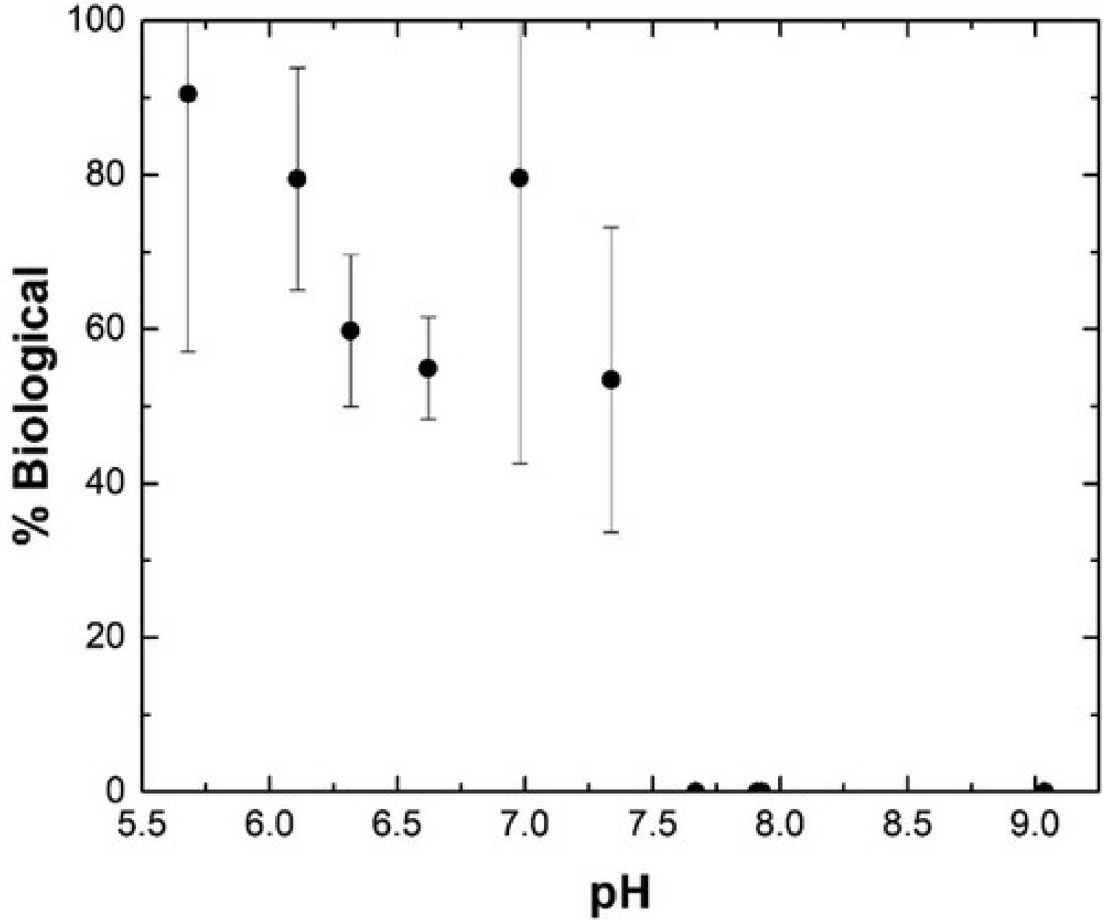
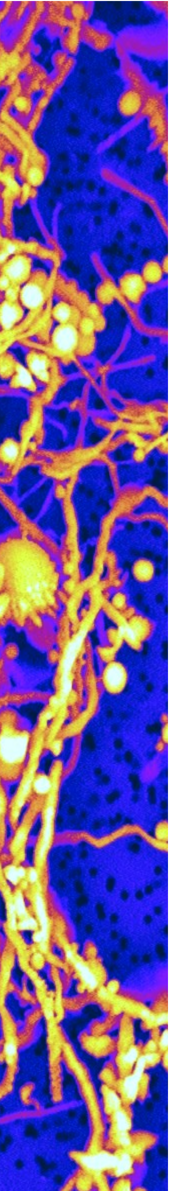


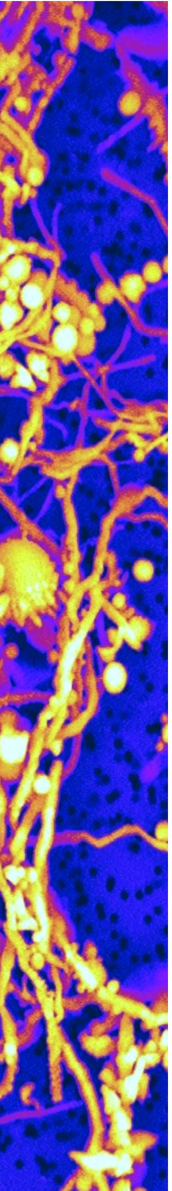


Things that burst into flames are not good to eat!

Everett Shock



Nitrogen compound	Oxidation state
NO_3^-	+5
NO_2^-	+3
NO	+2
N_2O	+1
N_2	0
NH_2O_2	-1
N_2H_4	-2
NH_4^+	-3



Nitrate **Reduction** to Ammonia

$$5H_2 + NO_3^- = NH_4^+ + 3H_2O$$

Nitrogen compound	Oxidation state
NO_3^-	+5
NO_2^-	+3
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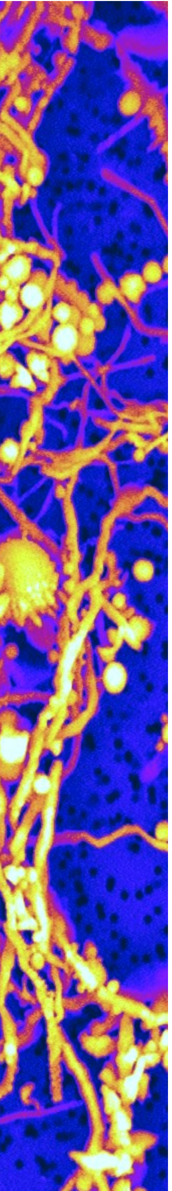
NH_4^+	-3



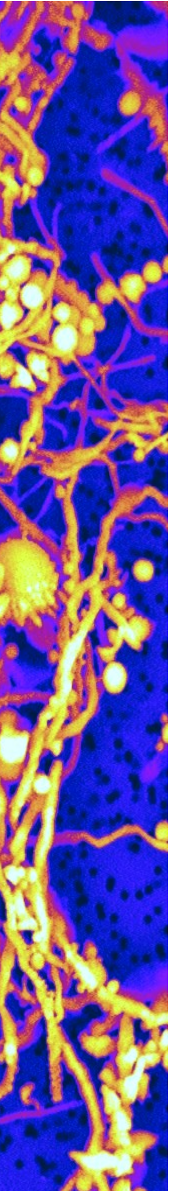
Nitrate **Reduction** to Ammonia

$$5H_2 + NO_3^- = NH_4^+ + 3H_2O$$

Ammonia **Oxidation** to Nitrite

$$2NH_4^+ + 3O_2 = 2NO_2^- + 4H^+ + 2H_2O$$

Nitrogen compound	Oxidation state
NO_3^-	+5
NO_2^-	+3
NO	+2
N_2O	+1
N_2	0
NH_2O_2	-1
N_2H_4	-2
NH_4^+	-3



Nitrate **Reduction** to Ammonia

$$5H_2 + NO_3^- = NH_4^+ + 3H_2O$$

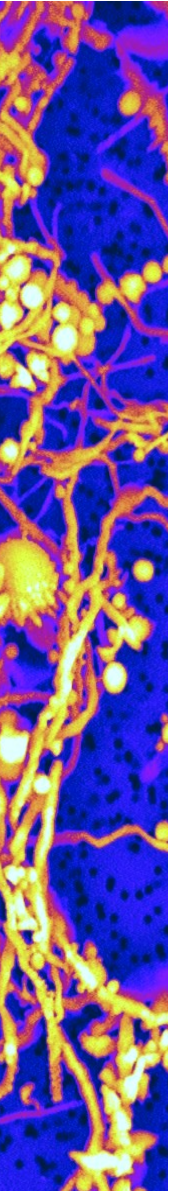
Ammonia **Oxidation** to Nitrite

$$2NH_4^+ + 3O_2 = 2NO_2^- + 4H^+ + 2H_2O$$

Nitrogen **comproportionation**

$$NO_2^- + NH_4^+ = N_2 + 2H_2O$$

Nitrogen compound	Oxidation state
NO_3^-	+5
NO_2^-	+3
NO	+2
N_2O	+1
N_2	0
NH_2O_2	-1
N_2H_4	-2
NH_4^+	-3



Nitrate Reduction to Ammonia

$$5H_2 + NO_3^- = NH_4^+ + 3H_2O$$

Ammonia Oxidation to Nitrite

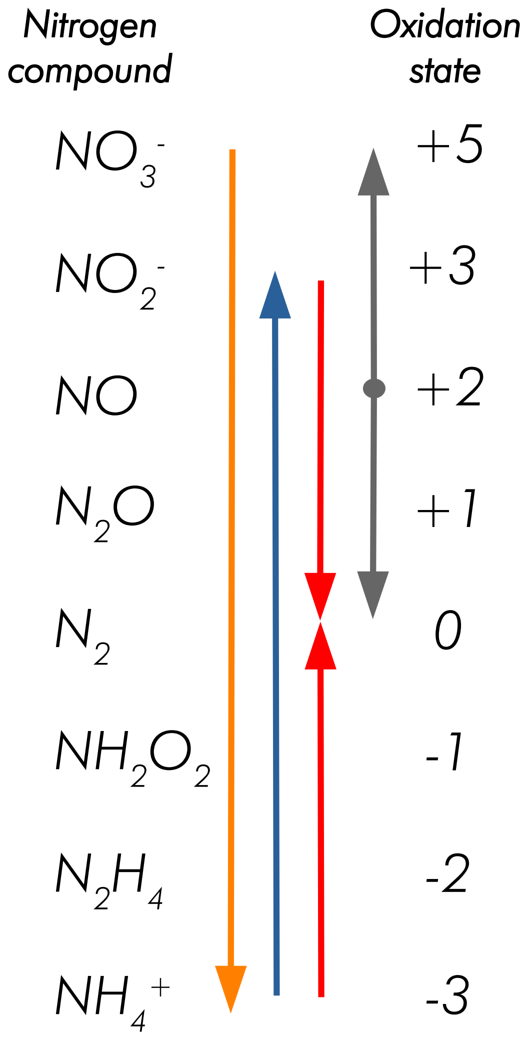
$$2NH_4^+ + 3O_2 = 2NO_2^- + 4H^+ + 2H_2O$$

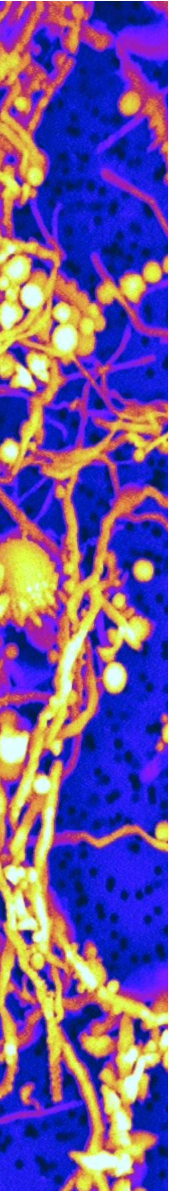
Nitrogen comproportionation

$$NO_2^- + NH_4^+ = N_2 + 2H_2O$$

Nitrogen disproportionation

$$2NO_2^- + H_2O = NO_3^- + N_2$$





DNRA

Nitrate Reduction to Ammonia

$$5H_2 + NO_3^- = NH_4^+ + 3H_2O$$

AO

Ammonia Oxidation to Nitrite

$$2NH_4^+ + 3O_2 = 2NO_2^- + 4H^+ + 2H_2O$$

ANAMMOX

Nitrogen comproportionation

$$NO_2^- + NH_4^+ = N_2 + 2H_2O$$

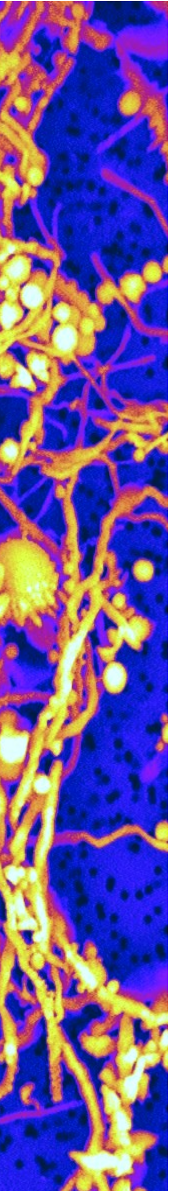
Unknown

Nitrogen disproportionation

$$2NO_2^- + H_2O = NO_3^- + N_2$$

Nitrogen compound	Oxidation state
NO_3^-	+5
NO_2^-	+3
NO	+2
N_2O	+1
N_2	0
NH_2O_2	-1
N_2H_4	-2
NH_4^+	-3

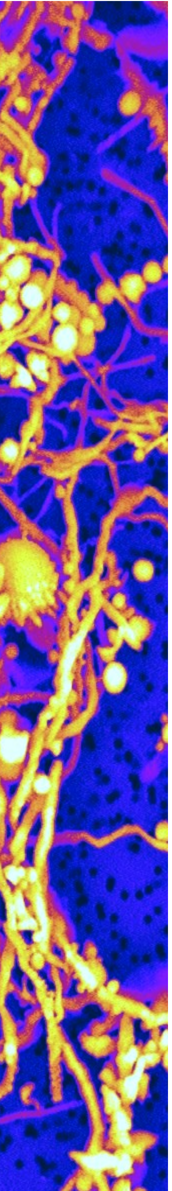
Life interacts with a lot of elements!



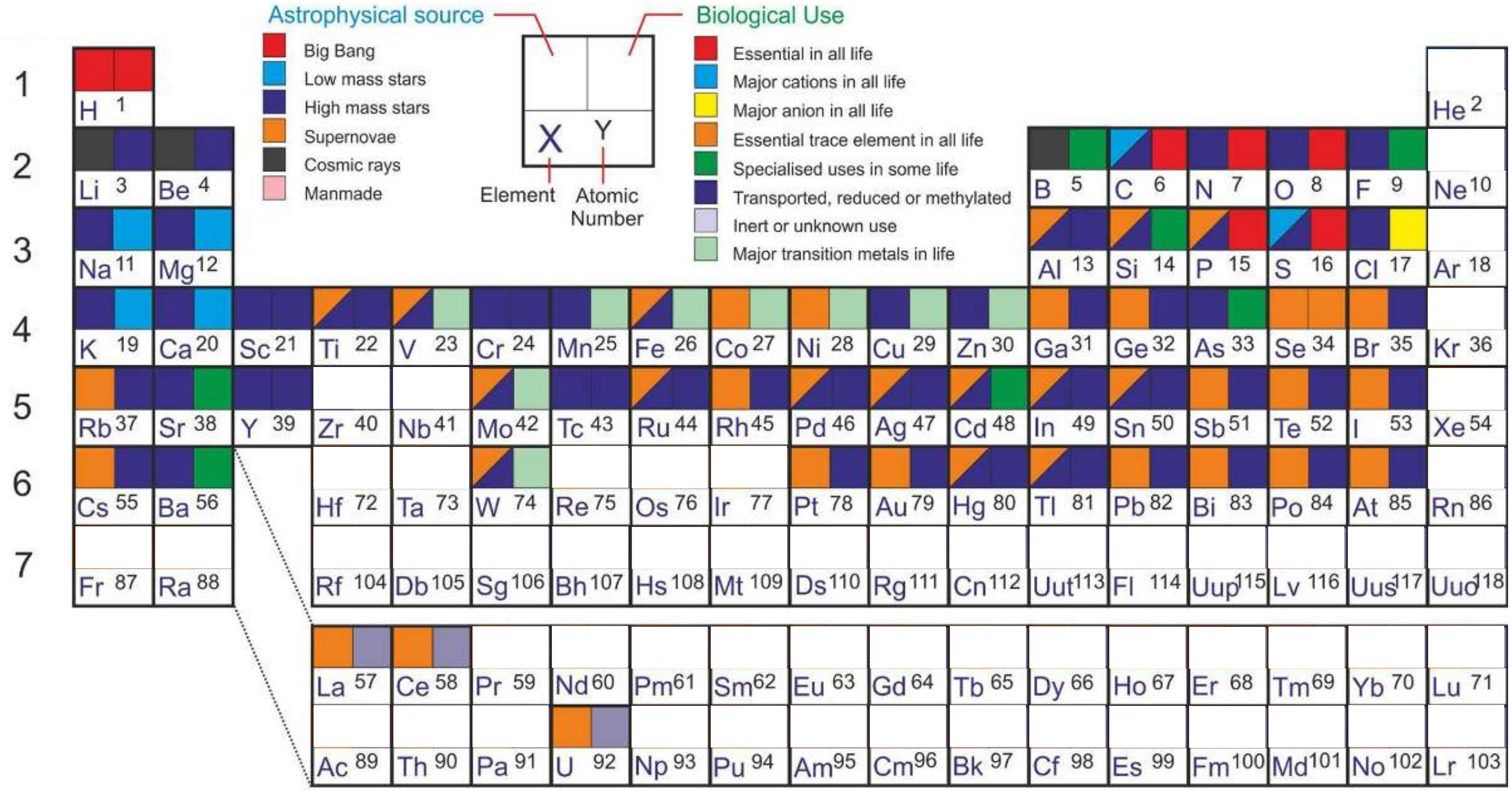
1	H 1																	He 2
2	Li 3	Be 4											B 5	C 6	N 7	O 8	F 9	Ne 10
3	Na 11	Mg 12											Al 13	Si 14	P 15	S 16	Cl 17	Ar 18
4	K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26	Co 27	Ni 28	Cu 29	Zn 30	Ga 31	Ge 32	As 33	Se 34	Br 35	Kr 36
5	Rb 37	Sr 38	Y 39	Zr 40	Nb 41	Mo 42	Tc 43	Ru 44	Rh 45	Pd 46	Ag 47	Cd 48	In 49	Sn 50	Sb 51	Te 52	I 53	Xe 54
6	Cs 55	Ba 56		Hf 72	Ta 73	W 74	Re 75	Os 76	Ir 77	Pt 78	Au 79	Hg 80	Tl 81	Pb 82	Bi 83	Po 84	At 85	Rn 86
7	Fr 87	Ra 88		Rf 104	Db 105	Sg 106	Bh 107	Hs 108	Mt 109	Ds 110	Rg 111	Cn 112	Uut 113	Fl 114	Uup 115	Lv 116	Uus 117	Uuo 118
				La 57	Ce 58	Pr 59	Nd 60	Pm 61	Sm 62	Eu 63	Gd 64	Tb 65	Dy 66	Ho 67	Er 68	Tm 69	Yb 70	Lu 71
				Ac 89	Th 90	Pa 91	U 92	Np 93	Pu 94	Am 95	Cm 96	Bk 97	Cf 98	Es 99	Fm 100	Md 101	No 102	Lr 103

Biological data from Wackett, L.P., Dodge, A.G., Ellis, L.B.M. (2004) *Applied and Environmental Microbiology* **70**, 647-655.

Cockell 2015



Life interacts with a lot of elements!



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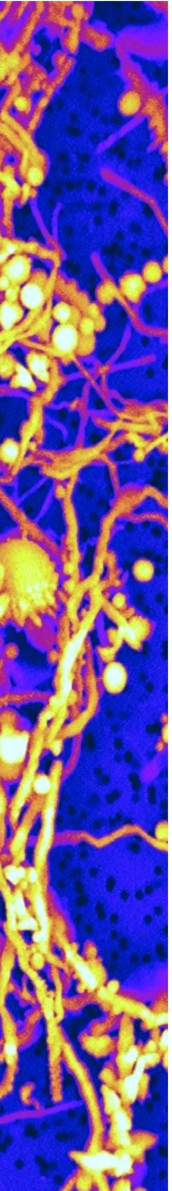
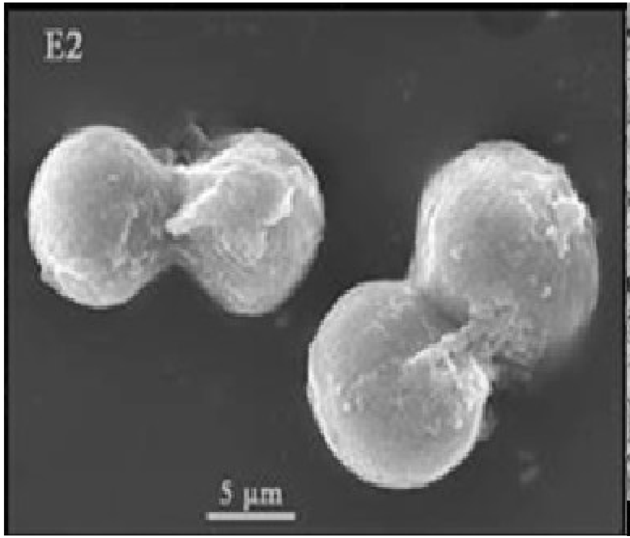
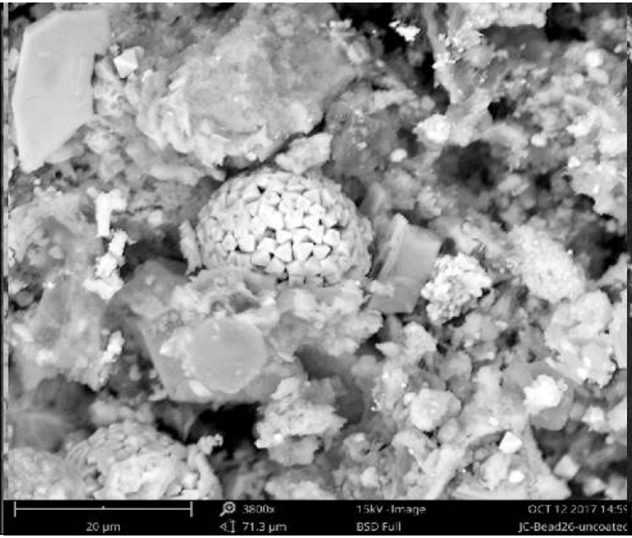


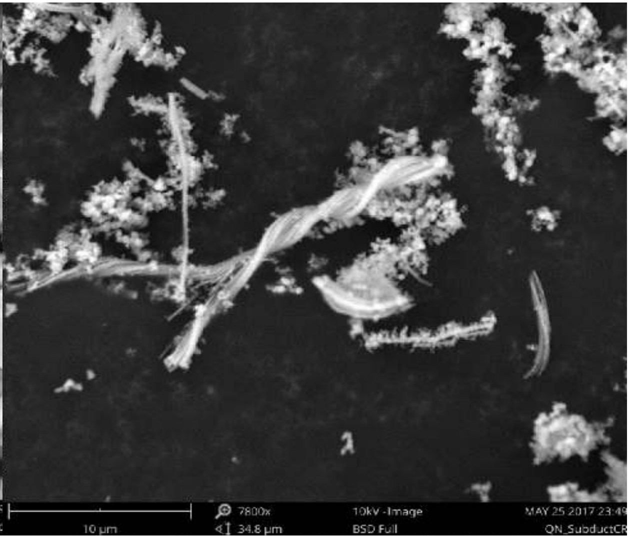
Minerals: biodissolved and bioprecipitated



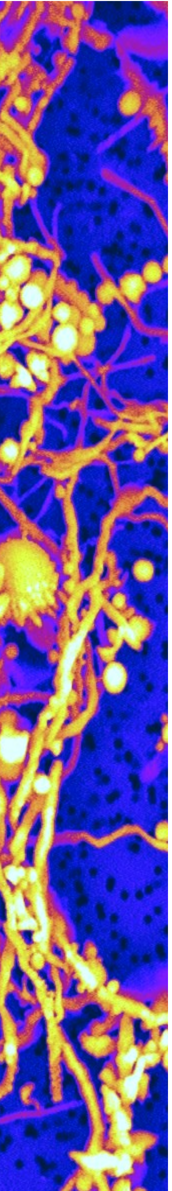
Calcite



Pyrite



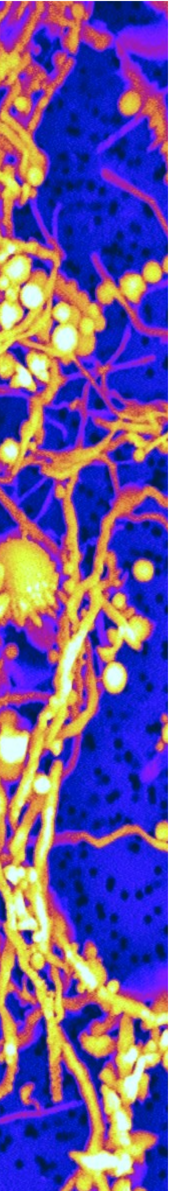
Iron hydroxide



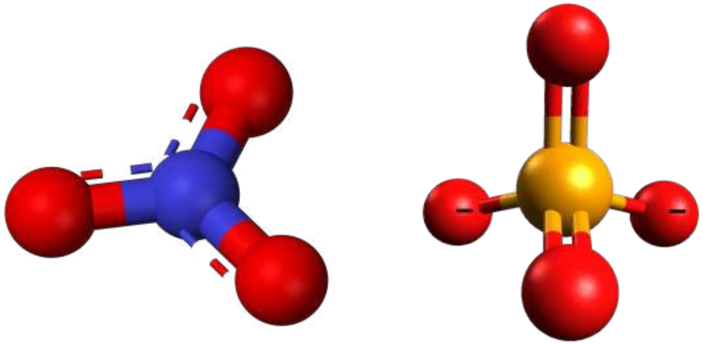
Chemical formula	Mineral name	Involved microorganism
$Fe(OH)_3$	Ferric/iron oxyhydroxide	Fe-oxidizing bacteria
$2Fe(OH)_3 \cdot Fe(OH)_2$	Green rust	*Shewanella putrefaciens*
α-$FeO(OH)$	Goethite	*Fe-oxidizing bacteria*
γ-$FeO(OH)$	Lepidocrocite	Marine bacteriophage, Bacillus subtilis
$5Fe_2O_3 \cdot 9H_2O$	Ferrihydrite	*Fe-oxidizing bacteria, Firmicutes*
Fe_2O_3	Hematite	*Fe-oxidizing bacteria*
Fe_3O_4	Magnetite	*Fe(III)-reducing bacteria, magnetotactic bacteria, thermophilic Fe(III)-reducing bacteria*
γ-Fe_2O_3	Maghemite	*Thermophilic Fe(III)-reducing bacteria*
$FeCO_3$	Siderite	*Thermophilic Fe(III)-reducing bacteria*
$FePO_4 \cdot nH_2O$	Hydrous ferric phosphate	*Acidovorax sp.*
$Fe_3(PO_4)_2 \cdot 2H_2O$	Vivianite	Sulfate Reducing Bacteria
FeS	Cubic FeS (Sphalerite-type)	Magnetotactic bacteria
FeS	Mackinawite (tetragonal FeS)	Magnetotactic bacteria, Sulfate Reducing Bacteria
Fe_3S_4	Greigite	Magnetotactic bacteria, Sulfate Reducing Bacteria
$Fe_{1-x}S$	Pyrrhotite	Magnetotactic bacteria
FeS_2	Pyrite	Magnetotactic bacteria, Sulfate Reducing Bacteria
$KFe_3(SO_4)_2(OH)_6$	Jarosite	*Fe-oxidising bacteria*
$Fe_8O_8SO_4(OH)_6$	Schwertmanite	*Acidithiobacillus ferrooxidans*
$MnCO_3$	Rhodochrosite	*Leptothrix discophora*
MnO_2	Manganese oxides	*Pseudomonas putida, Leptothrix discophora, Bacillus sp.*
$Na_4Mn_{14}O_{27} \cdot 9H_2O$	Birnessite	*Pseudomonas putida*
S^0	Elemental sulfur	*Chromatiaceae, Beggiatoa spp., Thiothrix, Thiovulum, Thioploca*
Au^0	Elemental gold	*Bacillus sp., Rhodopseudomonas capsulate, Shewanella algae, SRB*
	Calcite	Sulfate Reducing Bacteria and archaea, cyanobacteria, soil bacteria, Algae
$CaCO_3$	Aragonite	Cyanobacteria, Halomonas eurihalina, Halophiles
	Vaterite	*Kocuria, Myxococcus Xanthus, Bacillus sphaericus*
$CaMg(CO_3)_2$	Dolomite	*Nesterenkonia halobia*
$SiO_2 \cdot nH_2O$	Amorphous silica	*Calothrix, Fischerella sp., Shewanella oneidensis*
SiO_2	Silica	Diatoms, radiolarians, Thiobacillus, Bacillus subtilis
$Ca_5(PO_4)_3(OH)$	Hydroxyapatite/calcium phosphate	*Actinobacteria, Gammaproteobacteria*
$MgNH_4PO_4 \cdot 6H_2O$	Struvite	*Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes*

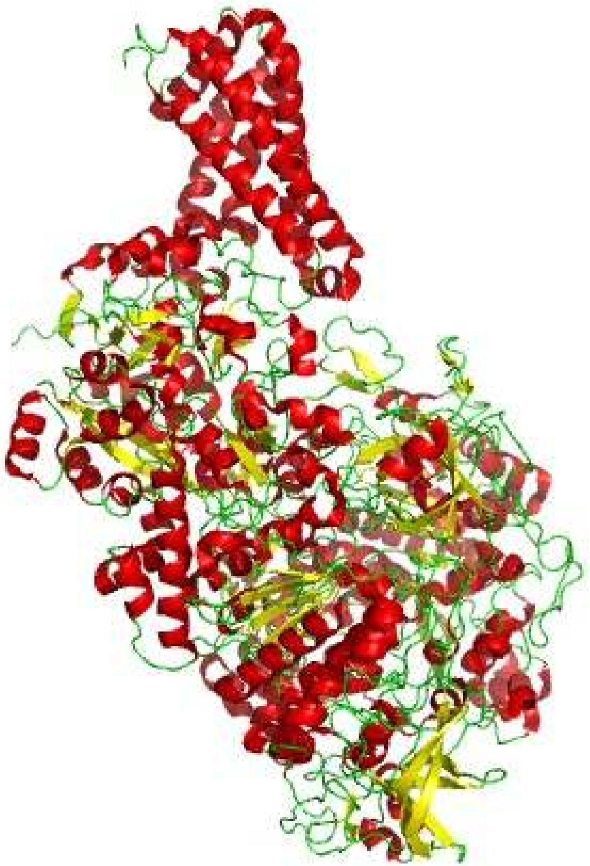
Heim et al 2011 Encyclop Geomicrobiol

And remember promiscuity!



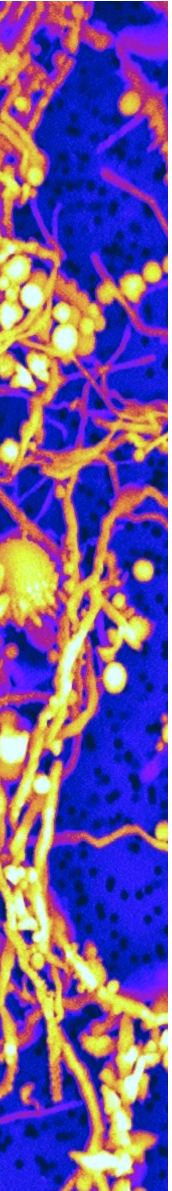
*While working primarily on **Nitrate**, this reductase enzyme can also act on **Selenate**, thus being promiscuous with regard to the substrate*





nitrite reductase enzyme $NO_3^- \rightarrow NO_2^-$

Substrate promiscuity is widespread in nature



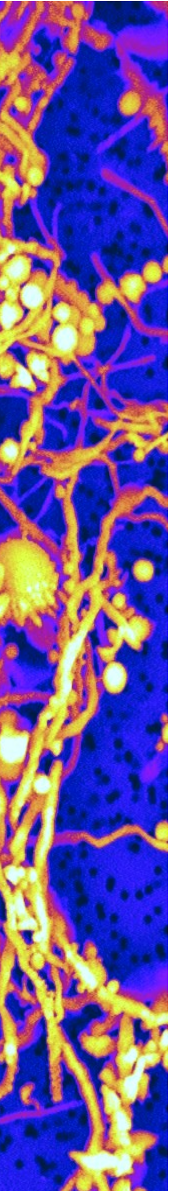
Types of Chemolithotrophs

e^- donors

H_2, NH_4^+, H_2S, $S^{-}_{(n)}$, S^0, $S_2O_3^{2-}$, S^{2-}, CH_4, CO, Fe^{2+}, As^{3+}, etc...

e^- acceptors

O_2, NO_3^-, NO_2^-, N_2O, NO, SO_4^{2-}, SO_3^{2-}, S^0, CO_2, Fe^{3+}, Mn^{2+}, SeO_4^{2-}, AsO_4^{3-}, UO_3^{2-}, TeO_4^{2-}, etc...



$$2NH_4^+ + 1.5O_2 \rightarrow N_2 + 2H^+ + 3H_2O$$

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

$$H_2S + 0.5O_2 \rightarrow S^\circ + H_2O$$

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

$$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$$

$$S^\circ + H_2 \rightarrow H_2S$$

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$$

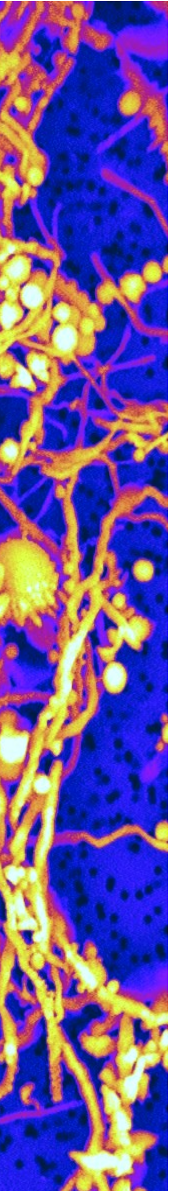
$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$$

$$2NO_2^- + 3H_2 + 2H^+ \rightarrow N_2 + 4H_2O$$

$$3Fe^{2+} + 0.5O_2 + 3H_2O \rightarrow Fe_3O_4 + 6H^+$$

$$Fe_3O_4 + H_2 + 6H^+ \rightarrow 3Fe^{2+} + 4H_2O$$

Amend et al. 2003 Geobiology



Thousands of thermodynamically permissive reactions

Reaction
$2NH_4^+ + 1.5O_2 \rightarrow N_2 + 2H^+ + 3H_2O$
$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$
$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
$N_2 + 1.5O_2 + H_2O \rightarrow 2NO_2^- + 2H^+$
$N_2 + 2.5O_2 + H_2O \rightarrow 2NO_3^- + 2H^+$
$NO_2^- + 0.5O_2 \rightarrow NO_3^-$
$NO_3^- + H_2 \rightarrow NO_2^- + H_2O$
$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$
$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$
$2NO_2^- + 3H_2 + 2H^+ \rightarrow N_2 + 4H_2O$
$NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$
$N_2 + 3H_2 + 2H^+ \rightarrow 2NH_4^+$
$4N_2 + 2H^+ + 9H_2O \rightarrow 3NO_3^- + 5NH_4^+$
$N_2 + 2H_2O \rightarrow NO_2^- + NH_4^+$
$4NO_2^- + 2H^+ + H_2O \rightarrow 3NO_3^- + NH_4^+$
$5NO_2^- + 2H^+ \rightarrow 3NO_3^- + N_2 + H_2O$

$CH_4 + 1.5O_2 \rightarrow CO + 2H_2O$
$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$
$CO + 0.5O_2 \rightarrow CO_2$
$CO_2 + H_2 \rightarrow CO + H_2O$
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$
$CO + 3H_2 \rightarrow CH_4 + H_2O$
$4CO + 2H_2O \rightarrow 3CO_2 + CH_4$

$H_2S + 0.5O_2 \rightarrow S^\circ + H_2O$
$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$
$S^\circ + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$
$SO_4^{2-} + 3H_2 + 2H^+ \rightarrow S^\circ + 4H_2O$
$SO_4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$
$S^\circ + H_2 \rightarrow H_2S$
$4S^\circ + 4H_2O \rightarrow SO_4^{2-} + 3H_2S + 2H^+$

$3Fe^{2+} + 0.5O_2 + 3H_2O \rightarrow Fe_3O_4 + 6H^+$
$Fe_3O_4 + H_2 + 6H^+ \rightarrow 3Fe^{2+} + 4H_2O$

$2NH_4^+ + SO_4^{2-} \rightarrow N_2 + S^\circ + 4H_2O$
$8NH_4^+ + 3SO_4^{2-} \rightarrow 4N_2 + 3H_2S + 2H^+ + 12H_2O$
$NH_4^+ + SO_4^{2-} \rightarrow NO_2^- + S^\circ + 2H_2O$
$4NH_4^+ + 3SO_4^{2-} \rightarrow 4NO_2^- + 3H_2S + 2H^+ + 4H_2O$
$3NH_4^+ + 4SO_4^{2-} + 2H^+ \rightarrow 3NO_3^- + 4S^\circ + 7H_2O$
$NH_4^+ + SO_4^{2-} \rightarrow NO_3^- + H_2S + H_2O$
$2NH_4^+ + 3S^\circ \rightarrow N_2 + 3H_2S + 2H^+$
$NH_4^+ + 3S^\circ + 2H_2O \rightarrow NO_2^- + 3H_2S + 2H^+$
$NH_4^+ + 4S^\circ + 3H_2O \rightarrow NO_3^- + 4H_2S + 2H^+$
$N_2 + SO_4^{2-} \rightarrow 2NO_2^- + S^\circ$
$4N_2 + 3SO_4^{2-} + 4H_2O \rightarrow 8NO_2^- + 3H_2S + 2H^+$
$3N_2 + 5SO_4^{2-} + 4H^+ \rightarrow 6NO_3^- + 5S^\circ + 2H_2O$
$4N_2 + 5SO_4^{2-} + 2H^+ + 4H_2O \rightarrow 8NO_3^- + 5H_2S$
$N_2 + 3S^\circ + 4H_2O \rightarrow 2NO_2^- + 3H_2S + 2H^+$
$N_2 + 5S^\circ + 6H_2O \rightarrow 2NO_3^- + 5H_2S + 2H^+$
$3NO_2^- + SO_4^{2-} + 2H^+ \rightarrow 3NO_3^- + S^\circ + H_2O$
$4NO_2^- + SO_4^{2-} + 2H^+ \rightarrow 4NO_3^- + H_2S$
$NO_2^- + S^\circ + H_2O \rightarrow NO_3^- + H_2S$

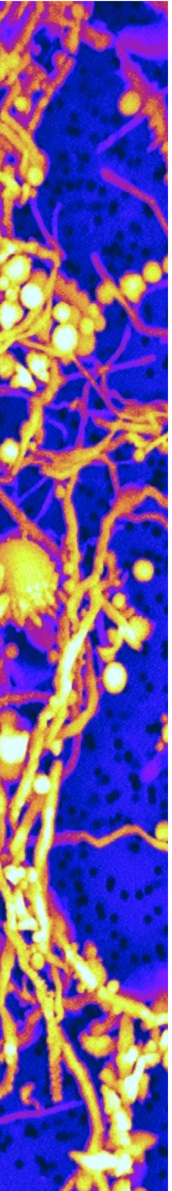
$2NH_4^+ + 3CO_2 \rightarrow N_2 + 3CO + 2H^+ + 3H_2O$
$8NH_4^+ + 3CO_2 \rightarrow 4N_2 + 3CH_4 + 8H^+ + 6H_2O$
$NH_4^+ + 3CO_2 \rightarrow NO_2^- + 3CO + 2H^+ + H_2O$
$4NH_4^+ + 3CO_2 + 2H_2O \rightarrow 4NO_2^- + 3CH_4 + 8H^+$
$NH_4^+ + 4CO_2 \rightarrow NO_3^- + 4CO + 2H^+ + H_2O$
$NH_4^+ + CO_2 + H_2O \rightarrow NO_3^- + CH_4 + 2H^+$
$2NH_4^+ + CO \rightarrow N_2 + CH_4 + 2H^+ + H_2O$
$NH_4^+ + CO + H_2O \rightarrow NO_2^- + CH_4 + 2H^+$
$3NH_4^+ + 4CO + 5H_2O \rightarrow 3NO_3^- + 4CH_4 + 6H^+$
$N_2 + 3CO_2 + H_2O \rightarrow 2NO_2^- + 3CO + 2H^+$
$4N_2 + 3CO_2 + 10H_2O \rightarrow 8NO_2^- + 3CH_4 + 8H^+$
$N_2 + 5CO_2 + H_2O \rightarrow 2NO_3^- + 5CO + 2H^+$
$4N_2 + 5CO_2 + 14H_2O \rightarrow 8NO_3^- + 5CH_4 + 8H^+$
$N_2 + CO + 3H_2O \rightarrow 2NO_2^- + CH_4 + 2H^+$
$3N_2 + 5CO + 13H_2O \rightarrow 6NO_3^- + 5CH_4 + 6H^+$
$NO_2^- + CO_2 \rightarrow NO_3^- + CO$
$4NO_2^- + CO_2 + 2H_2O \rightarrow 4NO_3^- + CH_4$
$3NO_2^- + CO + 2H_2O \rightarrow 3NO_3^- + CH_4$

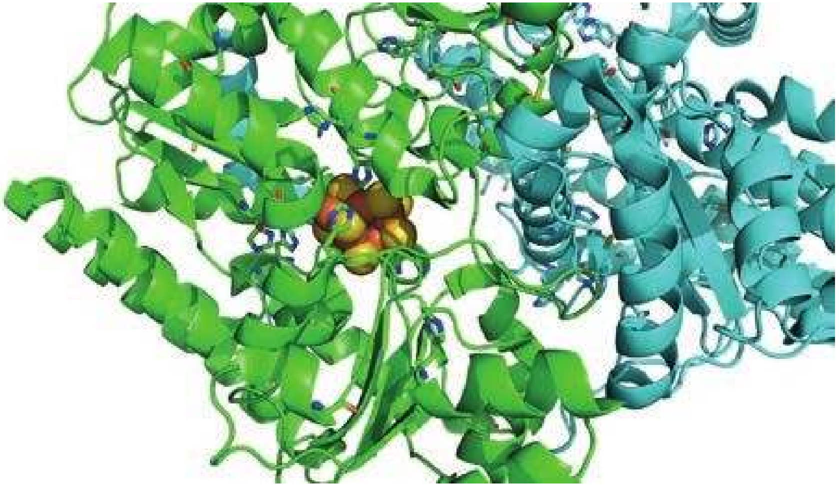
$2NH_4^+ + 3Fe_3O_4 + 16H^+ \rightarrow N_2 + 9Fe^{2+} + 12H_2O$
$NH_4^+ + 3Fe_3O_4 + 16H^+ \rightarrow NO_2^- + 9Fe^{2+} + 10H_2O$
$NH_4^+ + 4Fe_3O_4 + 22H^+ \rightarrow NO_3^- + 12Fe^{2+} + 13H_2O$
$N_2 + 3Fe_3O_4 + 16H^+ \rightarrow 2NO_2^- + 9Fe^{2+} + 8H_2O$
$N_2 + 5Fe_3O_4 + 28H^+ \rightarrow 2NO_3^- + 15Fe^{2+} + 14H_2O$
$NO_2^- + Fe_3O_4 + 6H^+ \rightarrow NO_3^- + 3Fe^{2+} + 3H_2O$

$H_2S + CO_2 \rightarrow S^\circ + CO + H_2O$
$4H_2S + CO_2 \rightarrow 4S^\circ + CH_4 + 2H_2O$
$H_2S + 4CO_2 \rightarrow SO_4^{2-} + 4CO + 2H^+$
$H_2S + CO_2 + 2H_2O \rightarrow SO_4^{2-} + CH_4 + 2H^+$
$3H_2S + CO \rightarrow 3S^\circ + CH_4 + H_2O$
$3H_2S + 4CO + 8H_2O \rightarrow 3SO_4^{2-} + 4CH_4 + 6H^+$
$S^\circ + 3CO_2 + H_2O \rightarrow SO_4^{2-} + 3CO + 2H^+$
$4S^\circ + 3CO_2 + 10H_2O \rightarrow 4SO_4^{2-} + 3CH_4 + 8H^+$
$S^\circ + CO + 3H_2O \rightarrow SO_4^{2-} + CH_4 + 2H^+$

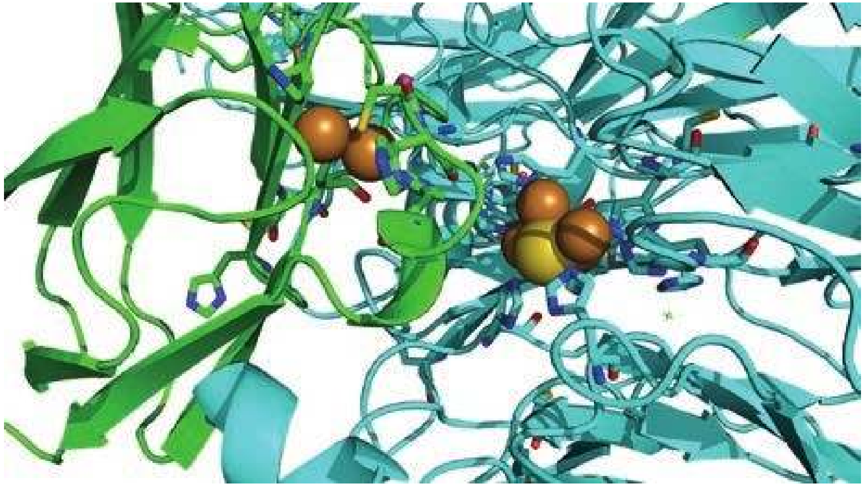
Amend et al. 2003 Geobiology

A multitude of metal centers



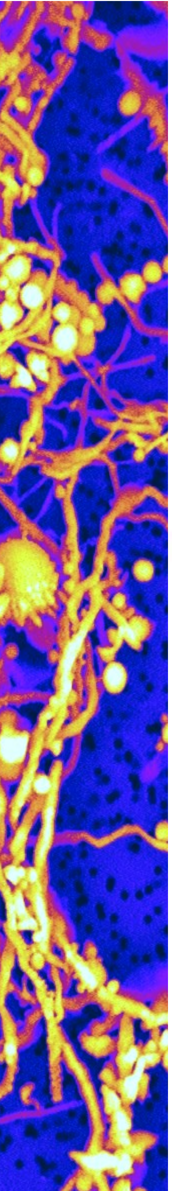


Nitrogenase
$MoFe_7S_8$

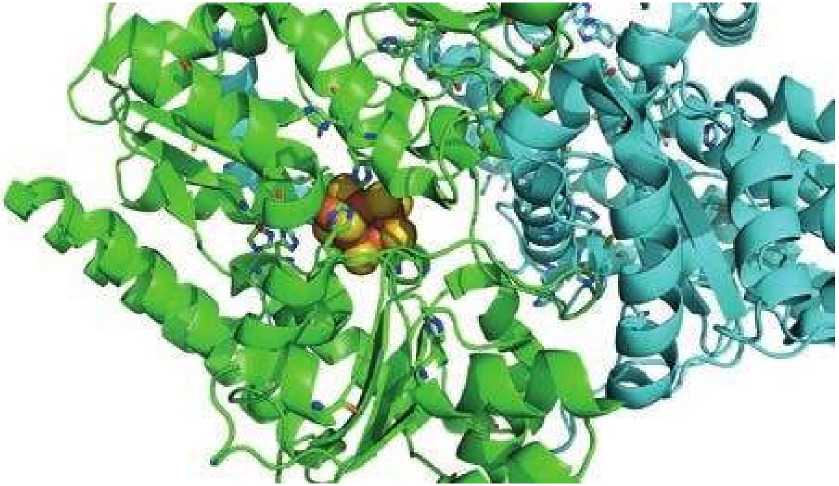


Nitrous Oxide Reductase
Cu_4S

Moore et al 2017 Nature Geoscience



A multitude of metal centers



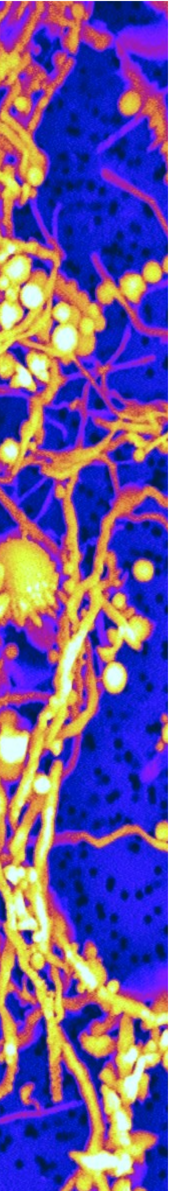
Nitrogenase

$MoFe_7S_8$

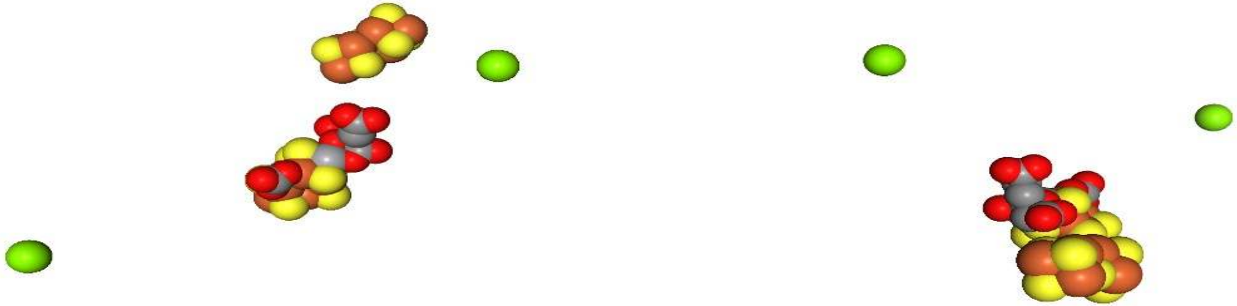
The exact mid point potential of an enzyme can be changed in biology with a number of mechanisms:

- by changing the metal cluster
- by changing the metal redox state
- by changing the coordination sphere
- by changing the depth in the pocket
- by changing water activity in the pocket
- by the overall protein charge
- etc...

Moore et al 2017 Nature Geoscience



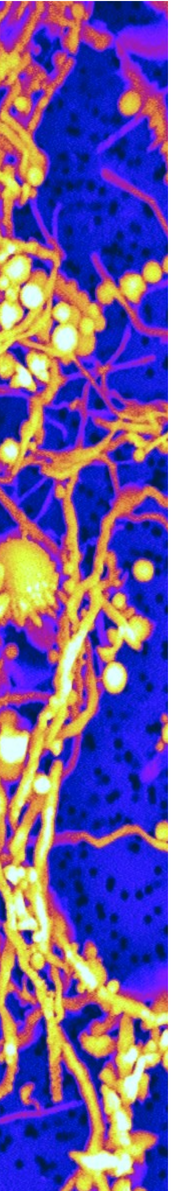
Electron tunneling in Biology

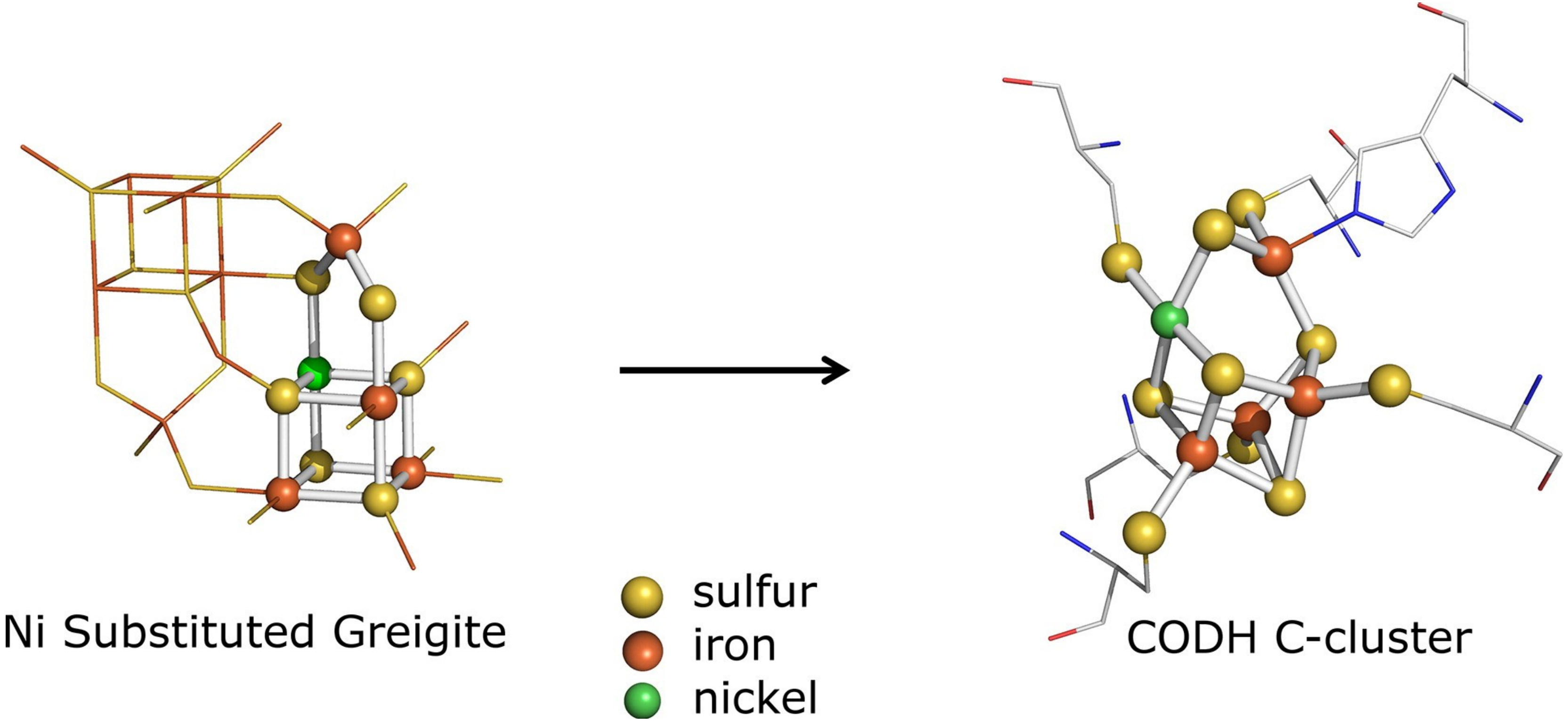


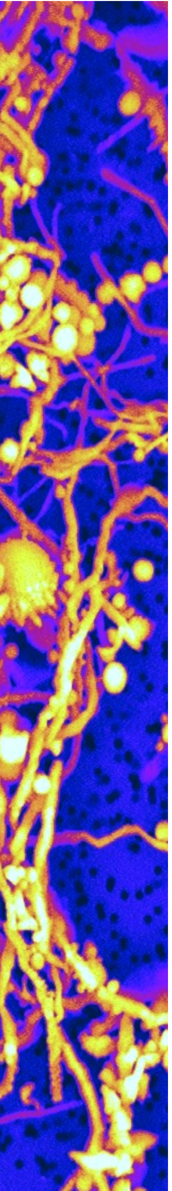
(Long-distance) electron tunneling is a fundamental process which is involved in energy generation in cells. Almost all productive electron transfer reactions in biology fall within the distance range of 4 to 14 Å, and can sometimes arrive to 20-25 Å

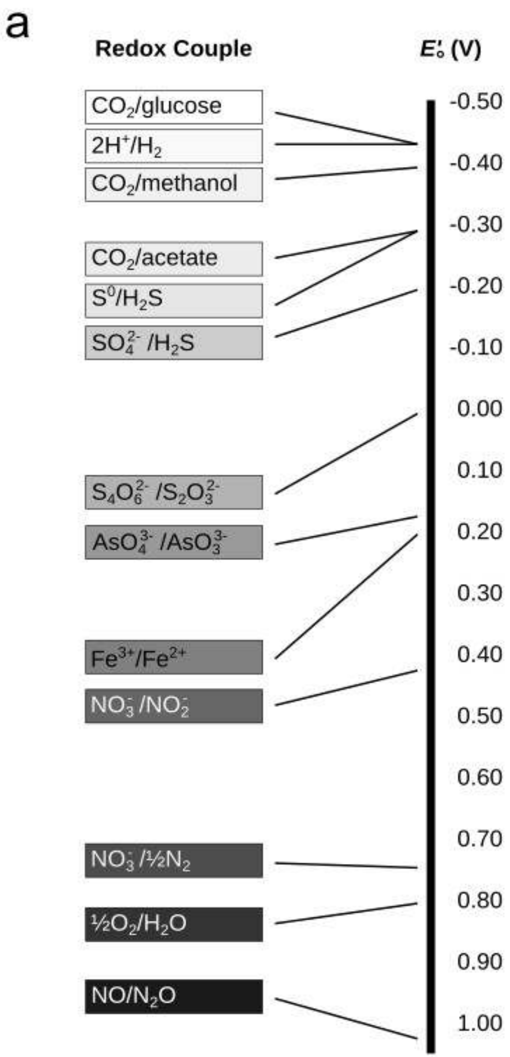
Metal center in oxidoreductase proteins (EC1) are placed optimizing the distance for electron tunneling following the redox potentials of the acceptors

Where do metal cofactors come from?

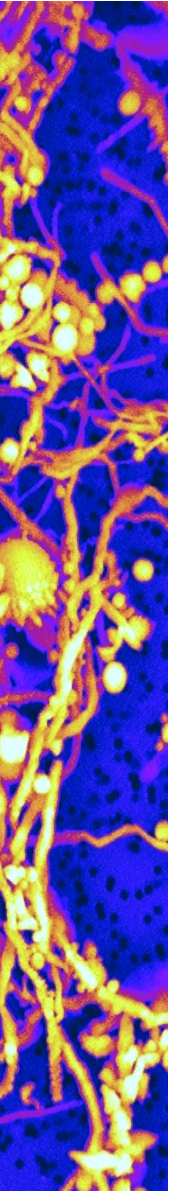


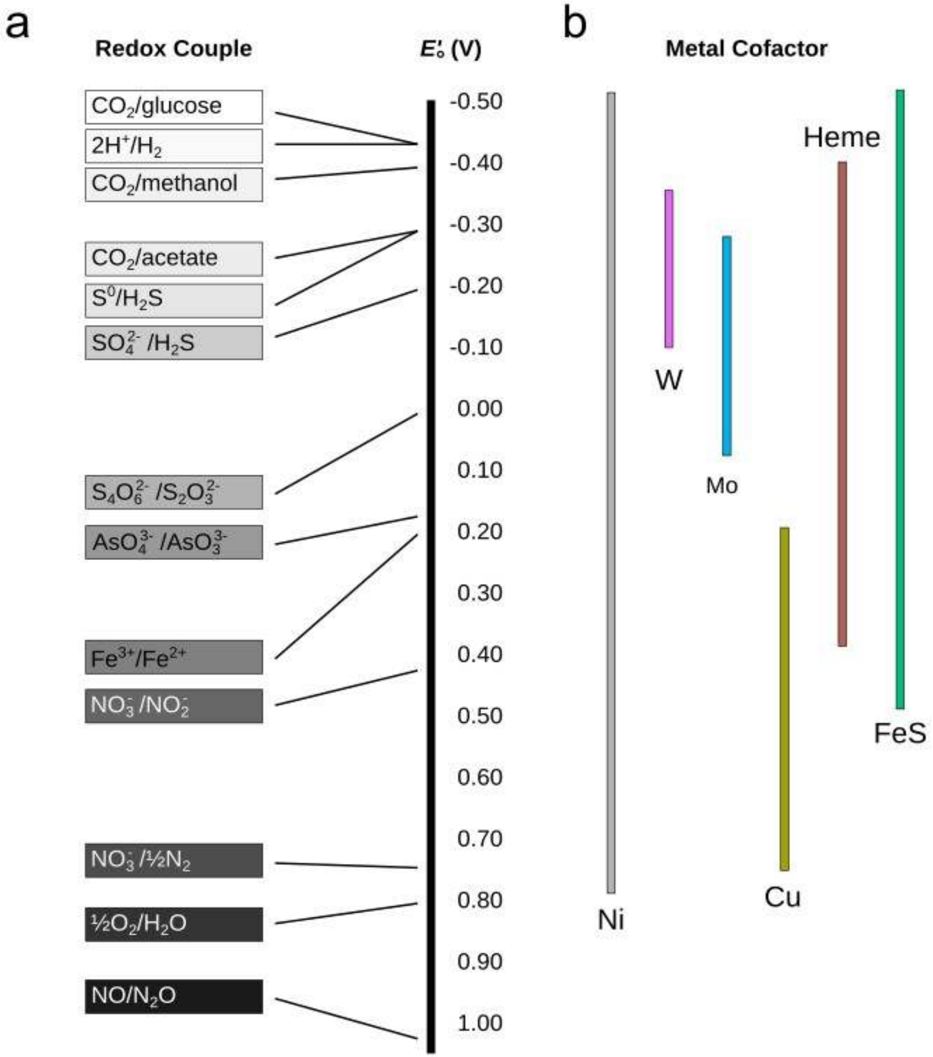




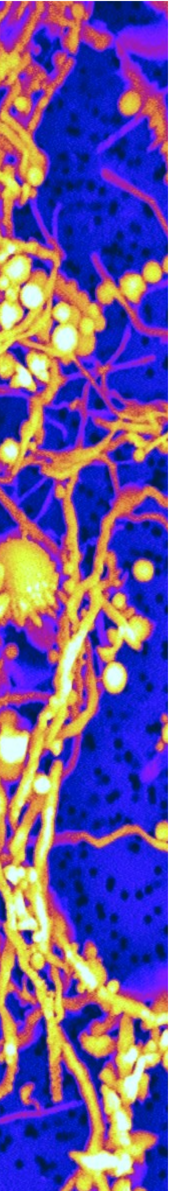


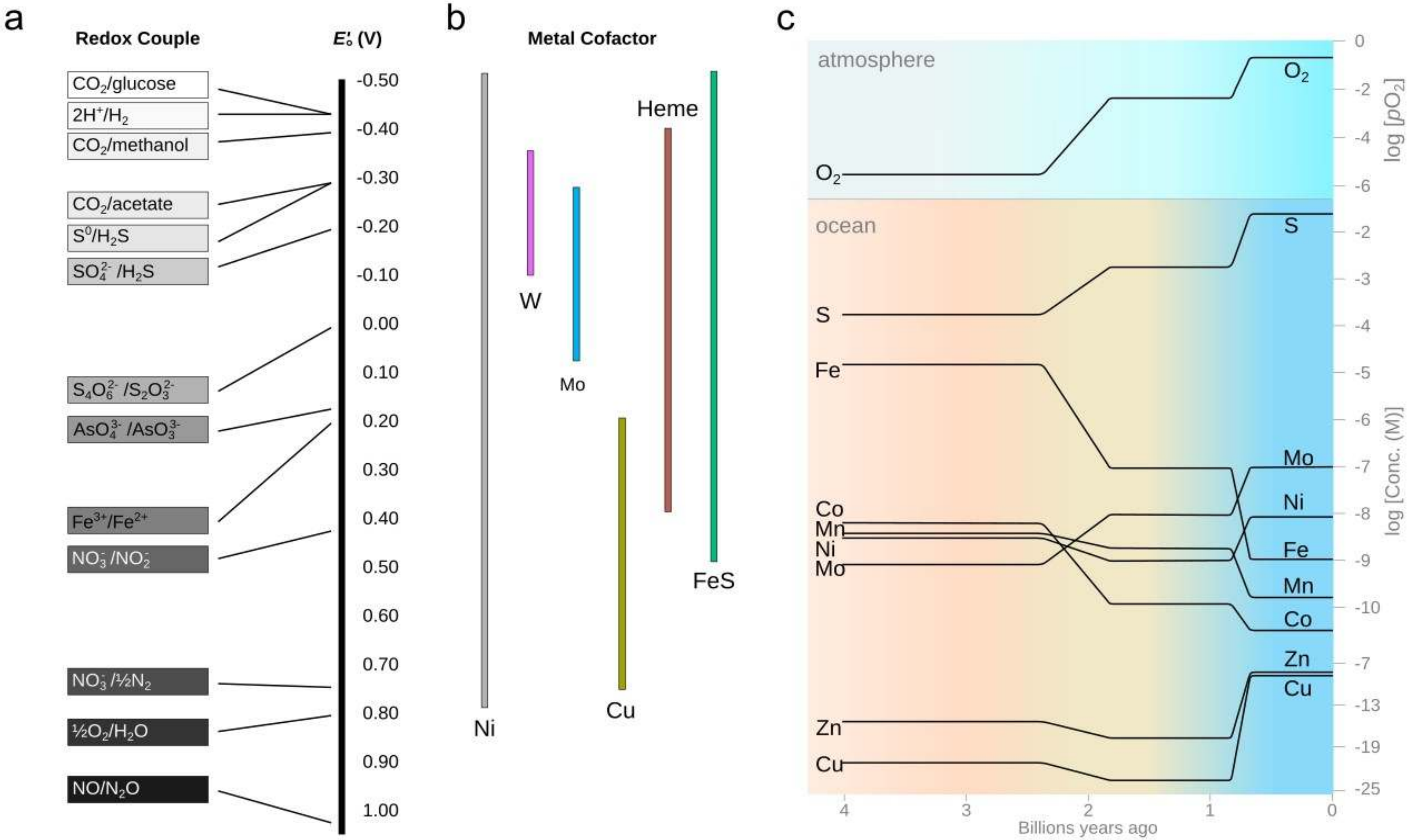
Giovannelli, in prep



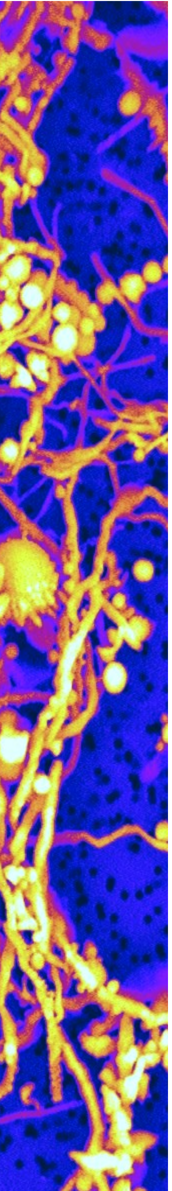


Giovannelli, in prep



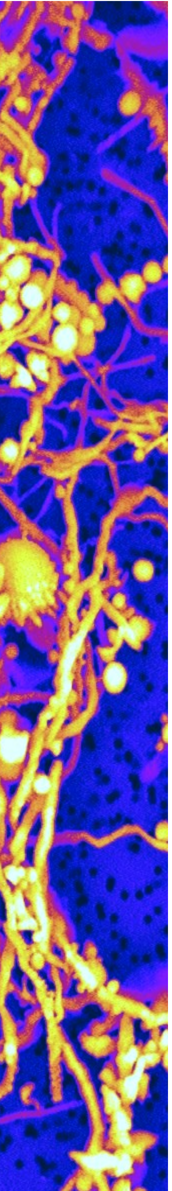


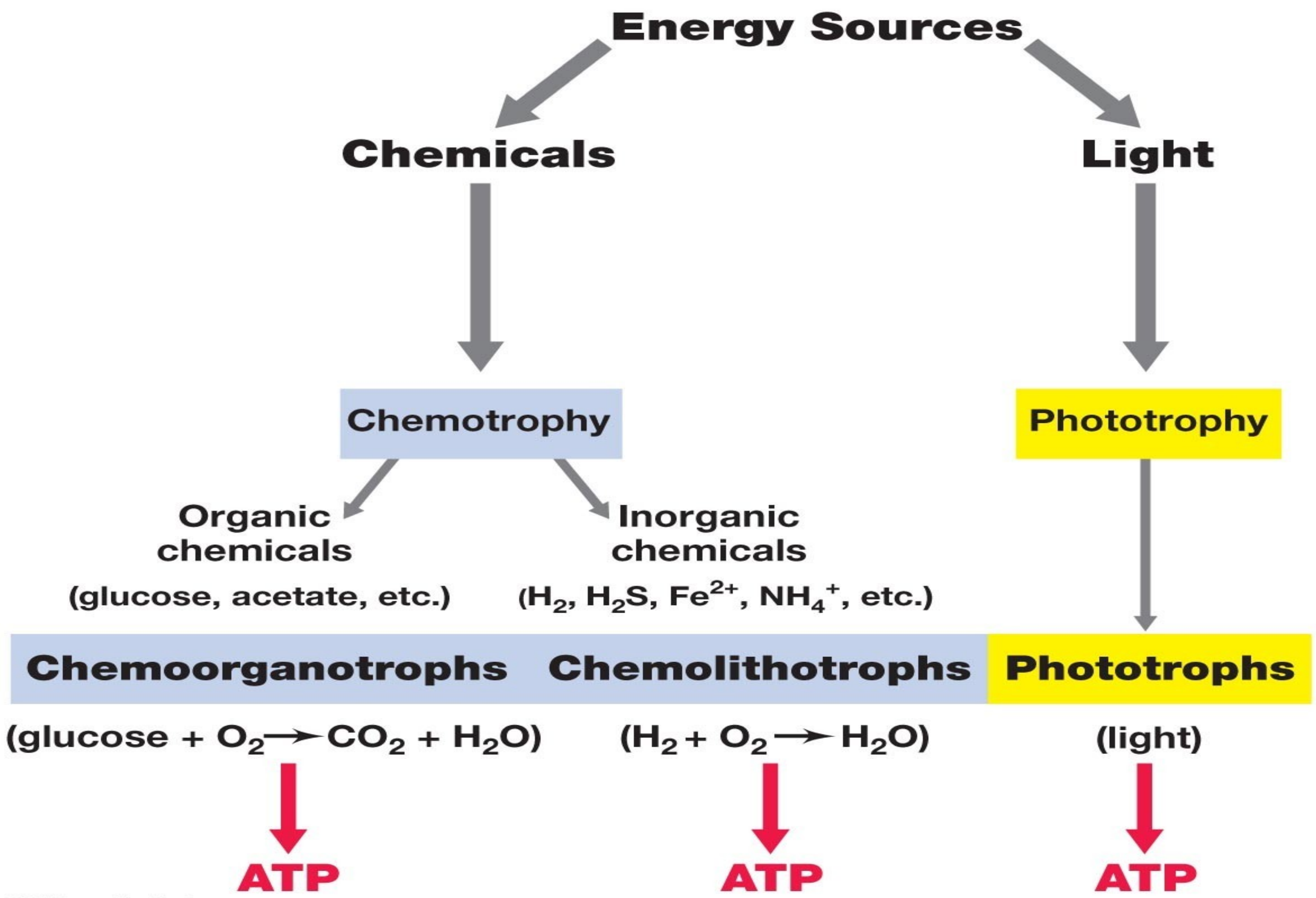
Giovannelli, in prep

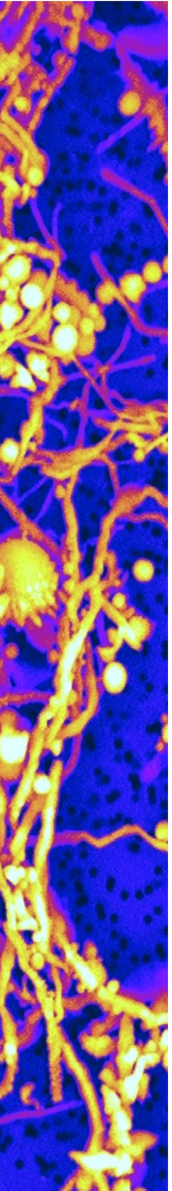


BACK TO ENERGY-CONSERVING METABOLIC DIVERSITY

Metabolic diversity 101

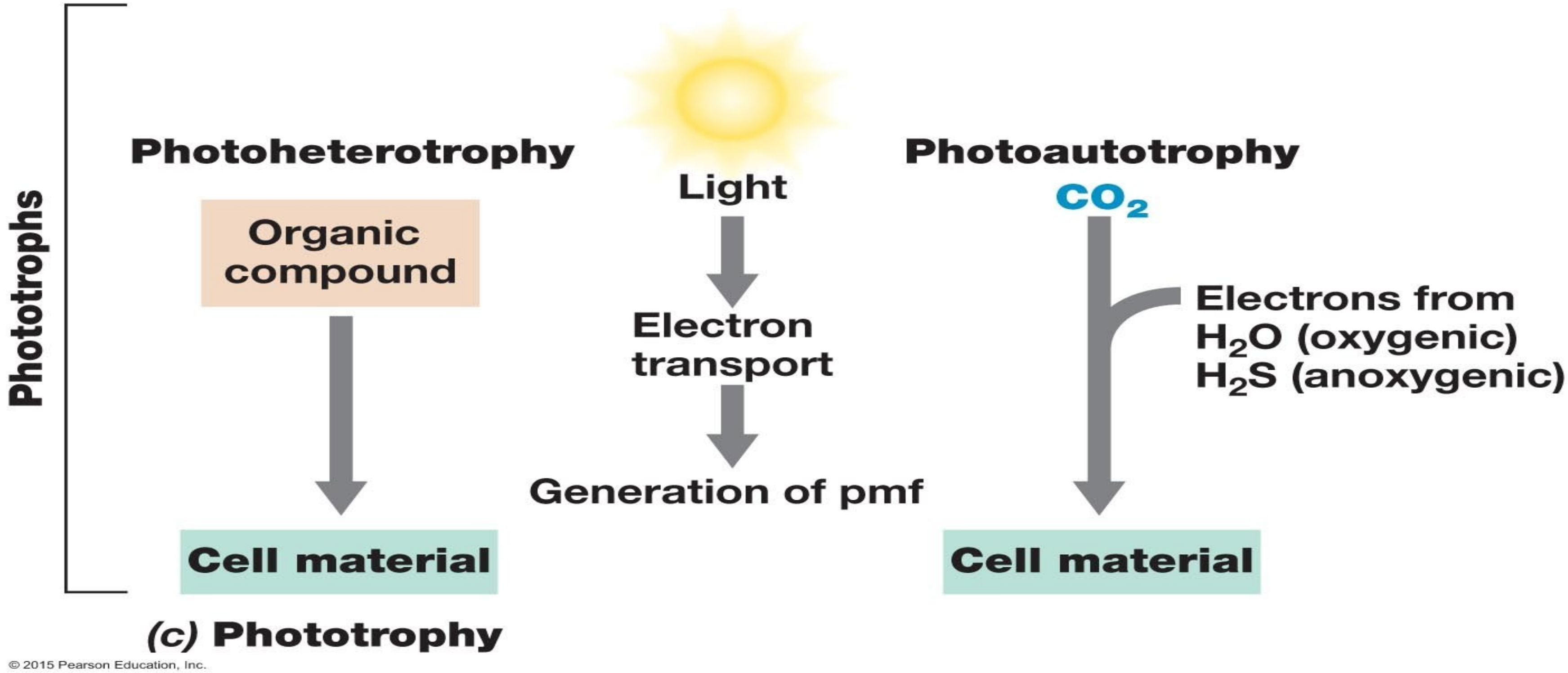






Phototrophy

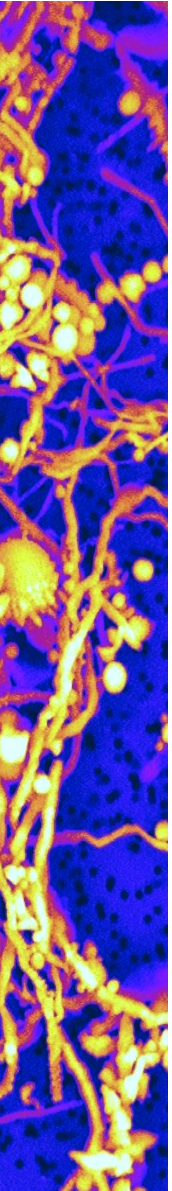
Energy

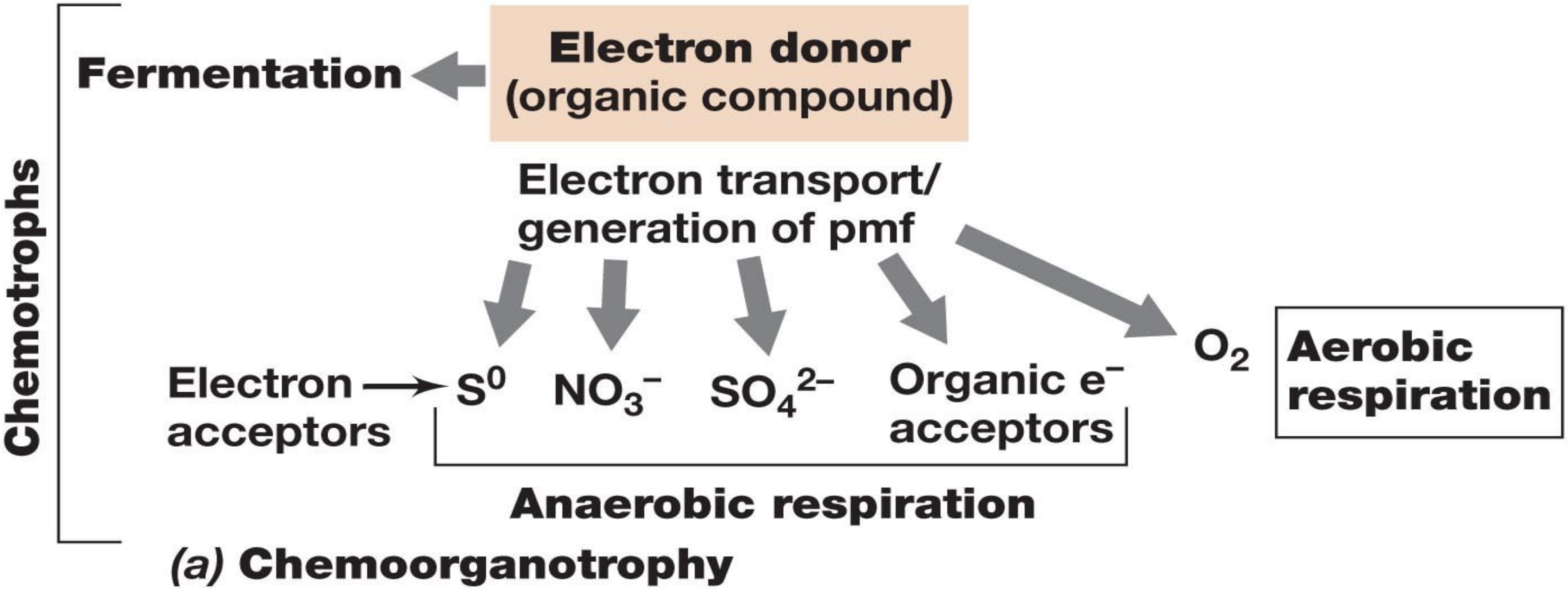


Photoheterotrophs, Photoautotrophs: *Anoxygenic photoautotrophs* and *Oxygenic photoauthotrophs*

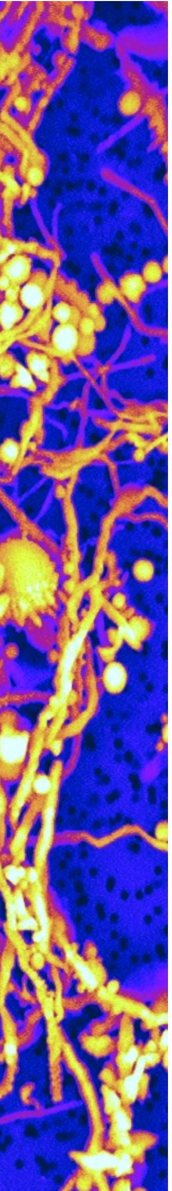
Chemoorganotrophy

Energy Electrons



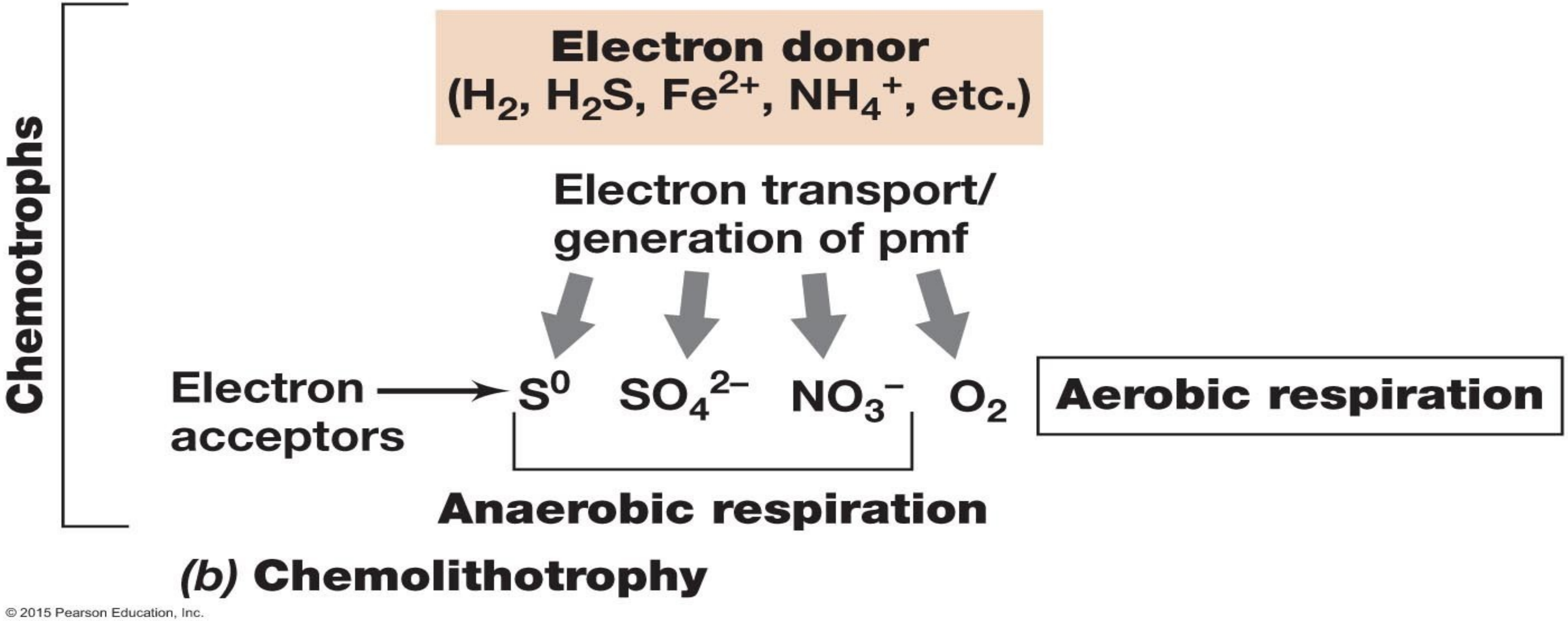


Chemoorganotrophs can perform *Aerobic Respiration*, *Anaerobic Respiration* and *Fermentation*

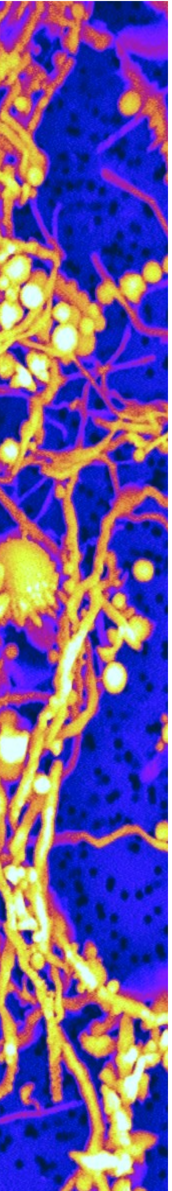


Chemolithotrophy

Energy Electrons

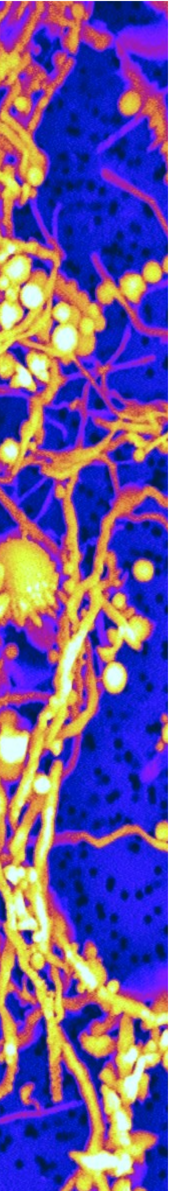


Chemolithotrophs can perform *Aerobic Respiration* and *Anaerobic Respiration*



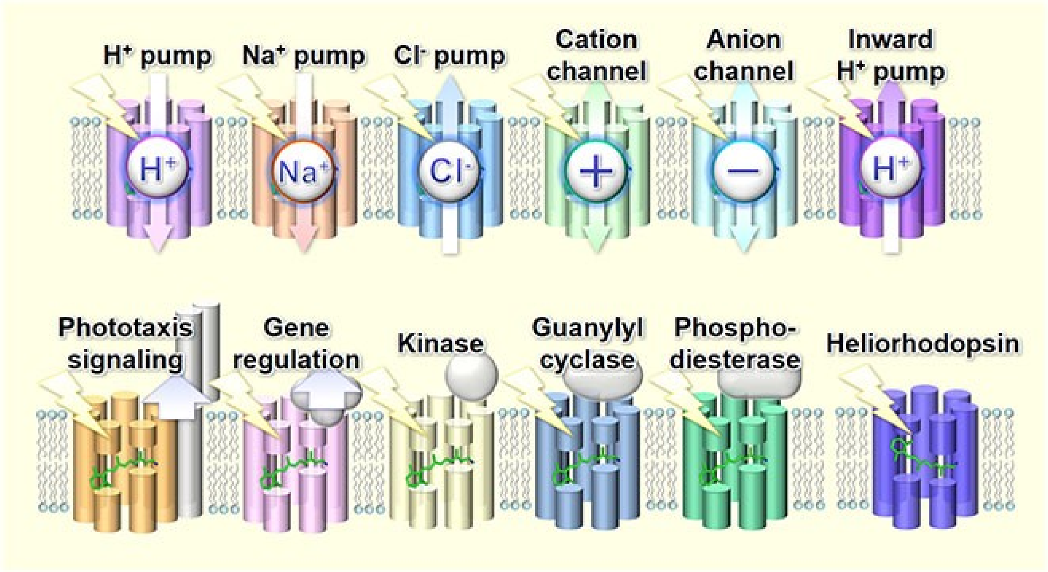
PHOTOTROPHY

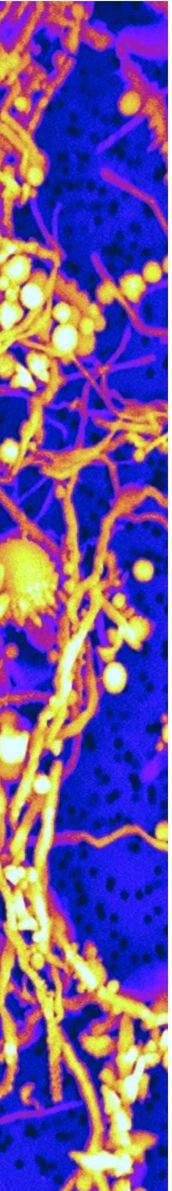
Biology interacting with light



Interacting with light is carried out in biology using a variety of different mechanisms that often include the use of light-sensitive proteins and molecules of different types

While phototrophy, and specifically photosynthesis, are the most well know light-dependent energy metabolisms, light interacts with biology for a number of other functions, for example through rhodopsins proteins





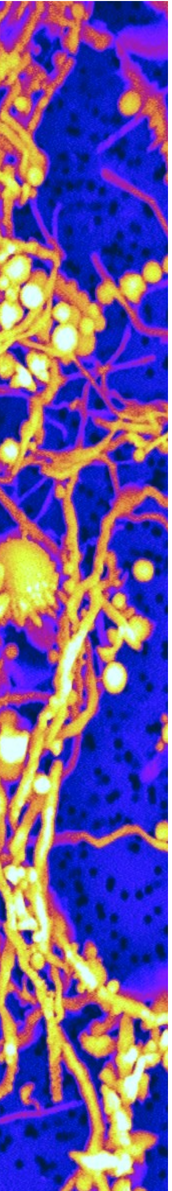
Phototrophy: The Sunny side of life

Energy can be obtained from photons using a number of different metabolisms. The key component are proteins or pigments capable of interacting with photons by absorbing their energy

- *Photoheterotrophy*
- *Anoxygenic Photosynthesis*
- *Oxygenic Photosynthesis*

In many cases, but not all, phototrophy also implies a close coupling to the energy requirement for reduction of CO2 for assimilation into organic matter (photosynthesis) according to the general scheme

$$2H_2A + CO_2 = 2[CH_2O] + 2A$$



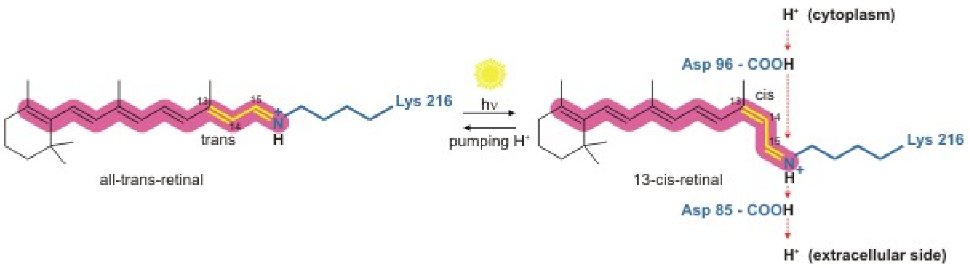
Phototrophy: Photoheterotrophy

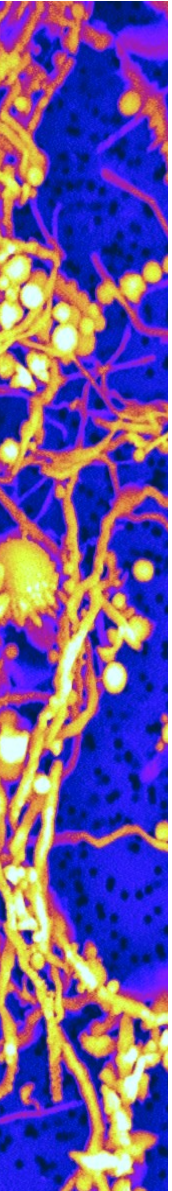
Photoheterotrophs are organisms that use light for energy, but cannot use carbon dioxide as their sole carbon source

Examples of photoheterotrophic organisms include purple non-sulfur bacteria (Chromatiales), green non-sulfur bacteria (Chloroflexi), heliobacteria (Firmicutes) and Halobacteria (Archaea)

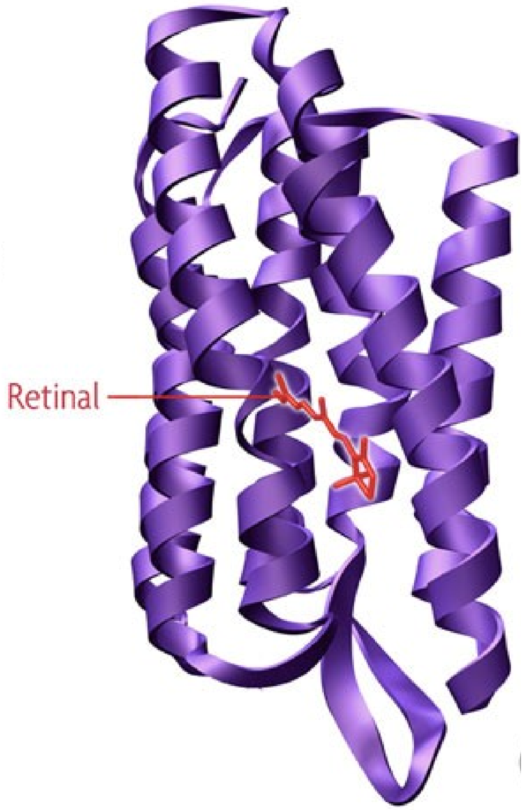
A variety of different proteins, collective called rhodopsins, are used to interact with light to obtain energy, usually in the form of a proton gradients, used for ATP synthesis

Rhodopsins work thanks to a light sensitive compound known as retinal that undergoes structural changes when absorbing photons

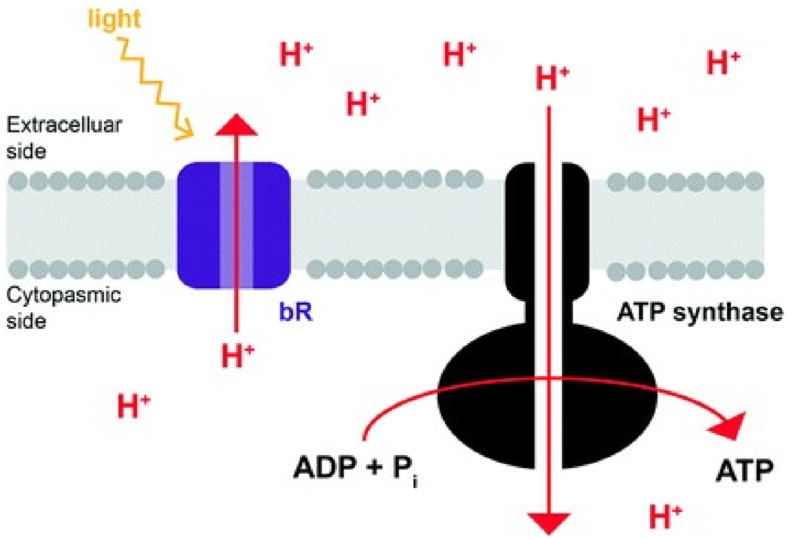


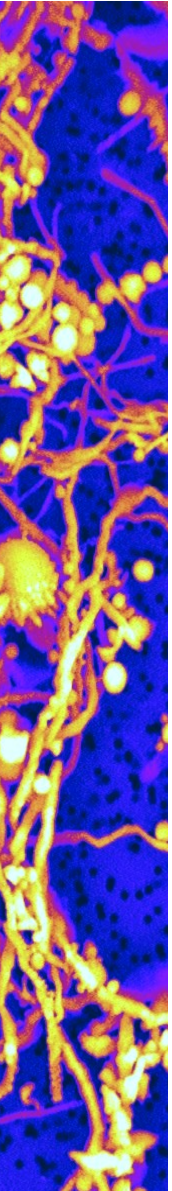


Bacteriorhodopsin and Proteorhodopsin



Bacteriorhodopsin (Archaea), Archaerhodopsin (Archaea), Halorhodopsin (Haloarchaea) and Proteorhodopsin (Bacteria, Archaea, Protist) are light-sensitive proton-pumping proteins found on the membrane of several phototrophic microorganisms





Phototrophy: Photosynthesis

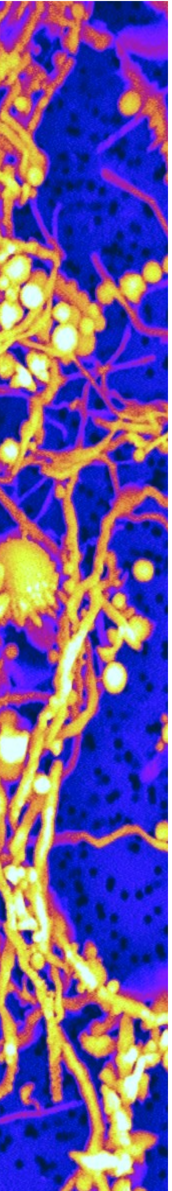
Photosynthetic organsims carry out phototrophy couple to carbon fixation

Photosynthesis requires light-sensitive pigments called chlorophyll. Two main variety are known Chlorophyll and Bacteriocholoriphyll. Beside this a large variety of different chlorophylls and pigments exists, with large differences in their absorption spectra

Photosynthetic organisms can be either Anoxygenic Phototrophs or Oxygenic Phototrophs depending on the electron donor used in the reaction

Oxidation of H_2O produces O_2 (Oxygenic Photosynthesis). Using alternative electron donors oxygen is not produced (Anoxygenic Photosynsthesis)

Oxygenic Photosynthesis is the most important biological process on the surface of Earth and it has been active for the past 3 to 2.5 billion of years



Chlorophyll and Bacteriochlorophyll

Photoautotrophy requires light-sensitive pigments, collectively called chlorophylls

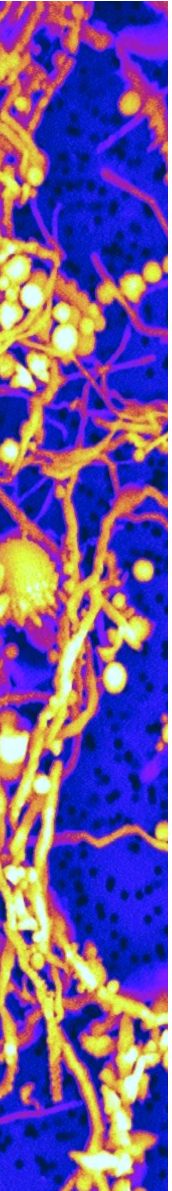
These pigments sometime are either weakly associated to protein complexes, like chlorophyll and carotenoids, or covalently bound to proteins, like in the case of phycobiliprotein and phycocyanin while other times

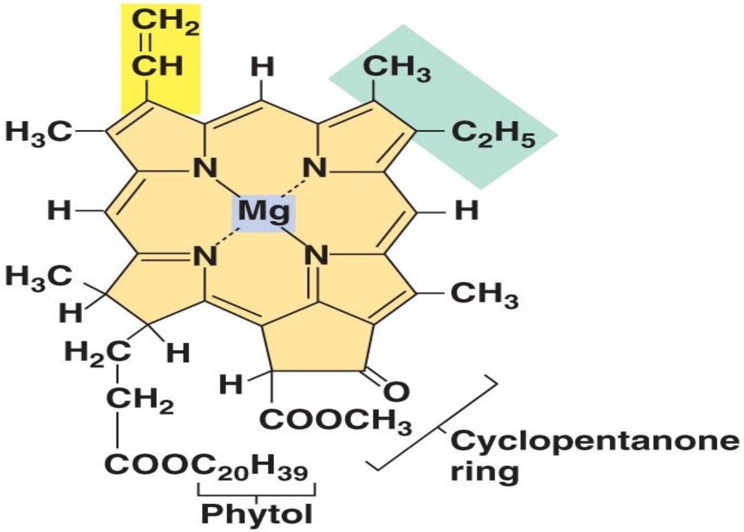
Organisms must produce some form of chlorophyll (or bacteriochlorophyll) to be photosynthetic

The main types of chlorophylls are: ***Chlorophyll a****, found in all higher plants, algae and cyanobacteria;* ***Chlorophyll b****, found in higher plants and green algae;* ***Chlorophyll c****, found in diatoms, dinoflagellates and brown algae; and* ***Chlorophyll d****, found only in red algae*

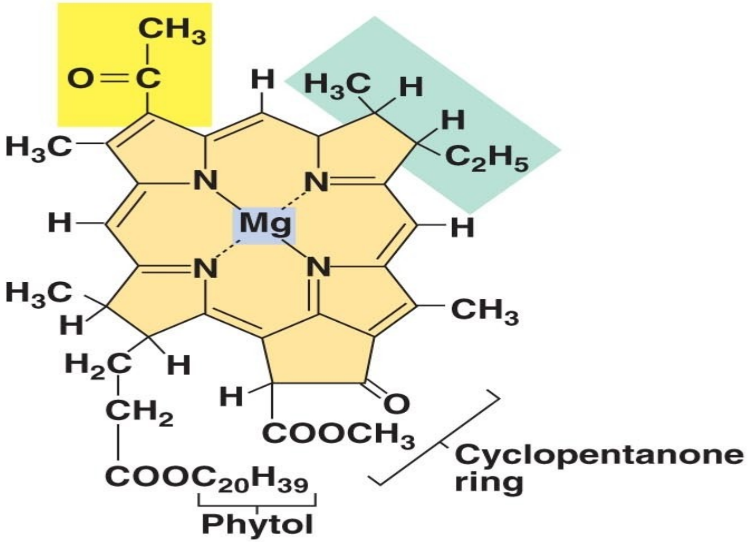
Beside these a large variety of Chlorophylls exists, having different absorption spectra. The majority absorb in the visible light or photosynthetically active radiation (PAR) ranging from 400 to 700 nm

Chlorophyll and Bacteriochlorophyll



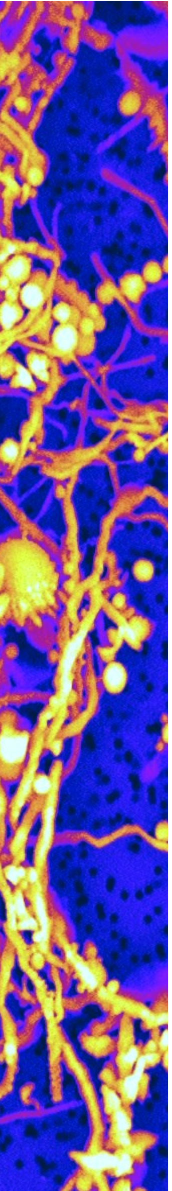


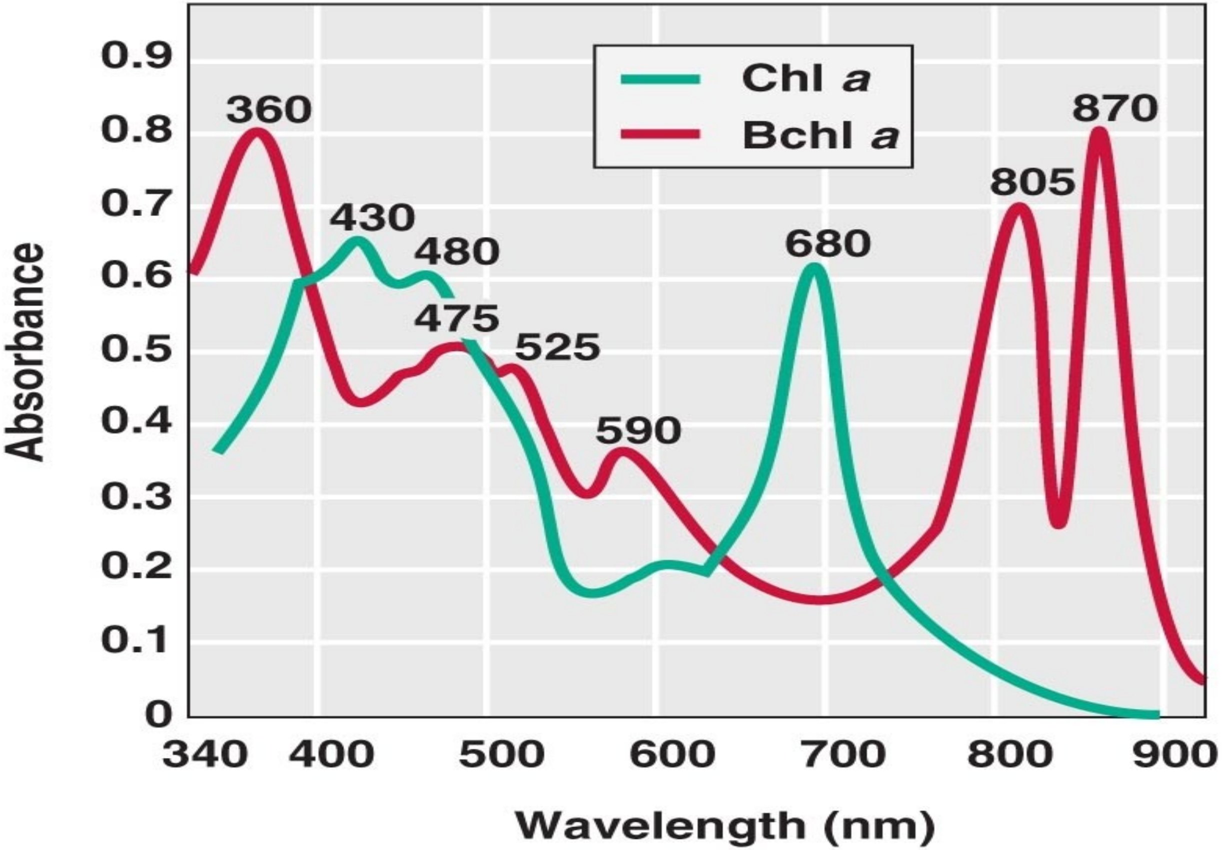
Chlorophyll *a*

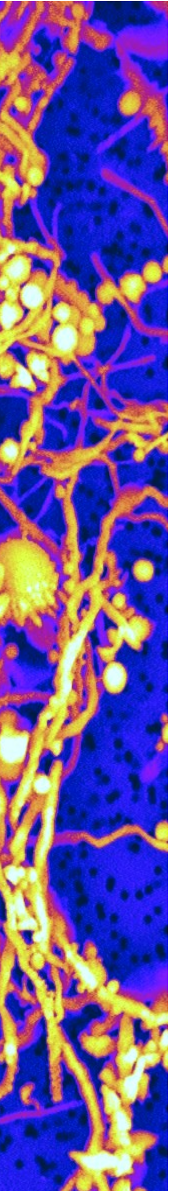


Bacteriochlorophyll *a*

Chlorophyll and Bacteriochlorophyll absorption spectra







Anoxygenic phototrophs

Anoxygenic photosynthesis gets the name from the absence of free oxygen generated as a byproduct

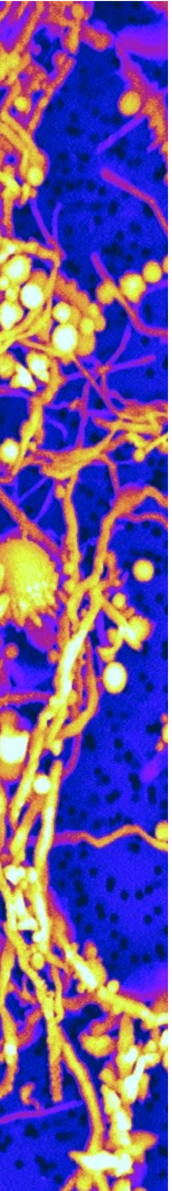
Anoxygenic photosynthesis is found in at least four phyla of Bacteria, which are generally strict anaerobes due to the type of electron donor they use in their reaction

Reducing power for CO2 fixation comes from reductants present in the environment. A variety of electron donor can be used, with the major being H_2, H_2S or Fe^{2+}

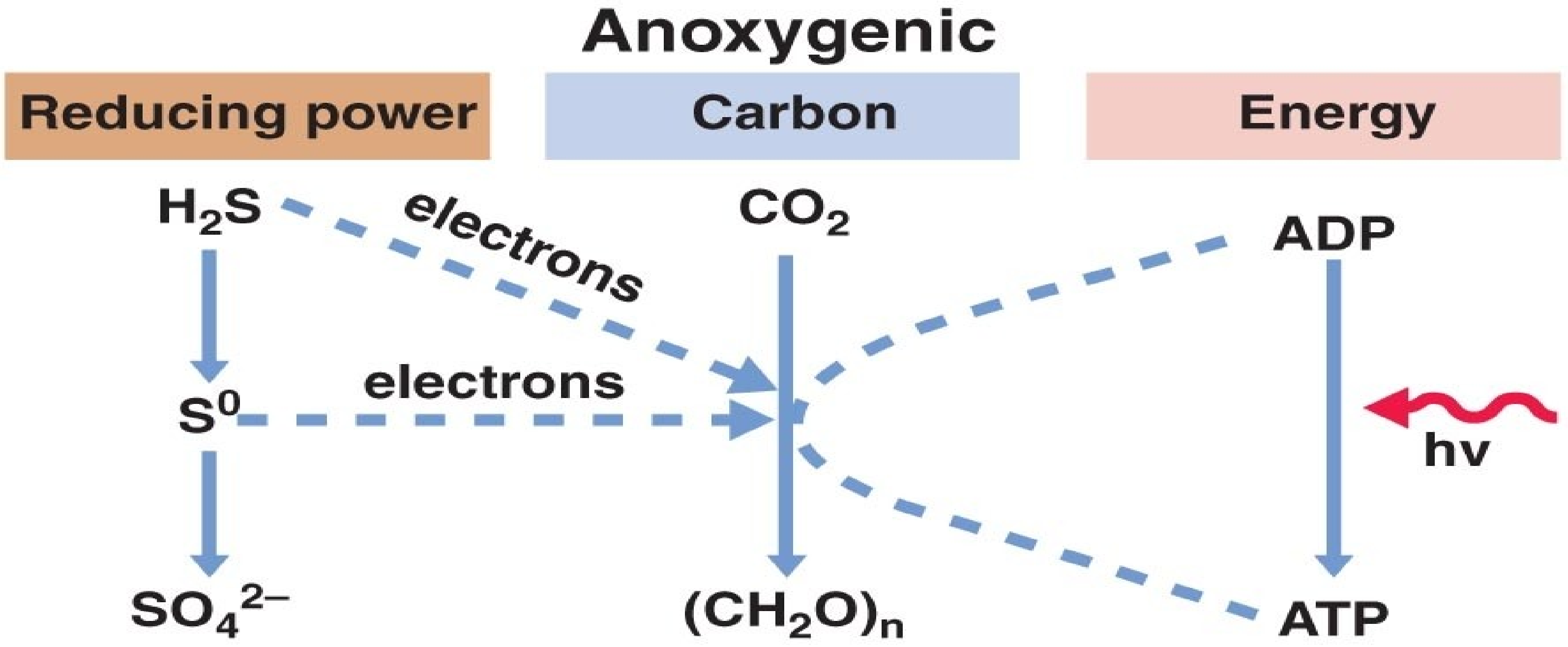
Anoxigenic phototrophs can be abundant in stratified lakes and waters, microbial mats, arid deserts, hot springs and soils

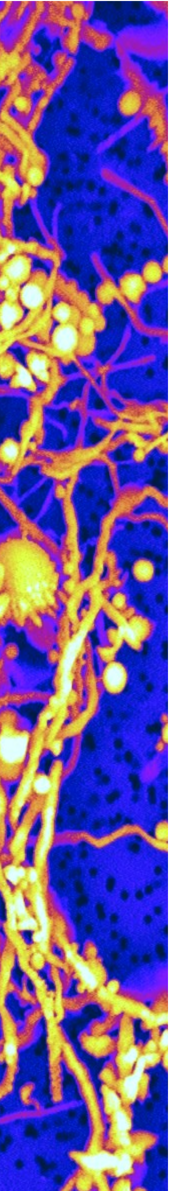
The formation of NADH in certain groups, like the purple phototrophs, requires reverse electron transport

Evolutionary anoxygenic photosynthesis predates oxygenic photosynthesis, with the latter derived from the former



Anoxygenic Photosynthesis



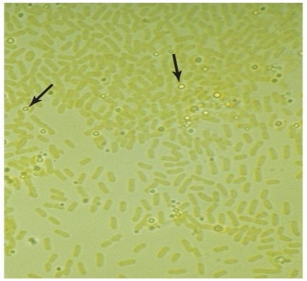


Diversity of anoxygenic phototrophs

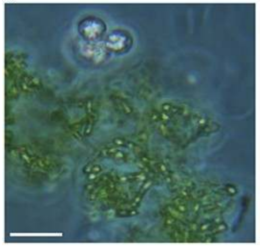
Anoxygenic Phototroph diversity

Chromatiaceae and Rhodospirillaceae in the Proteobacteria (Purple Bacteria)

Chlorobi (Green Sulfur Bacteria)

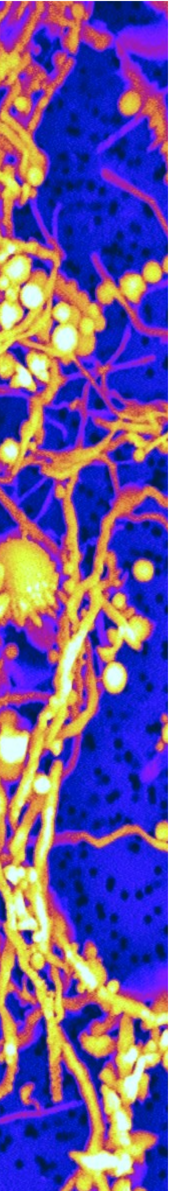


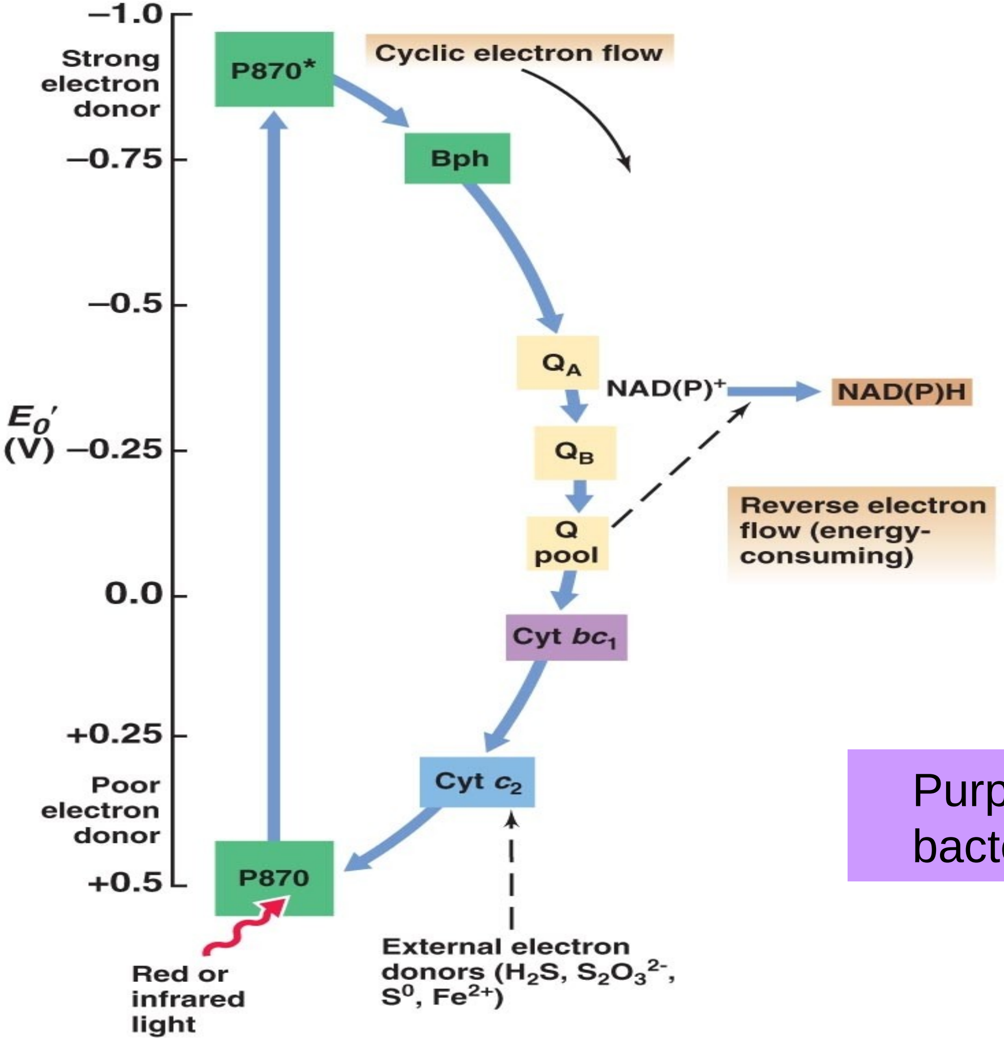
Chloracidobacterium in the Acidobacteria



Chloroflexi (Green non-sulfur bacteria)

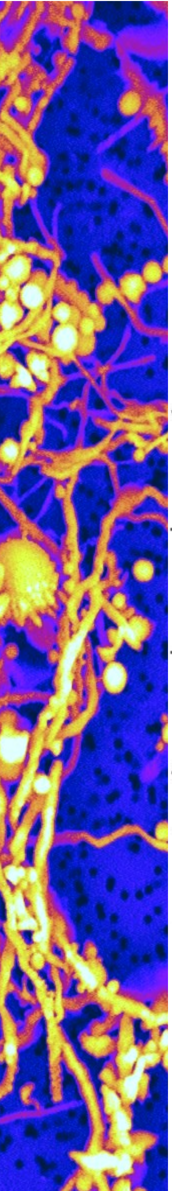
Example of electron flow in Anoxygenic Phototrophs

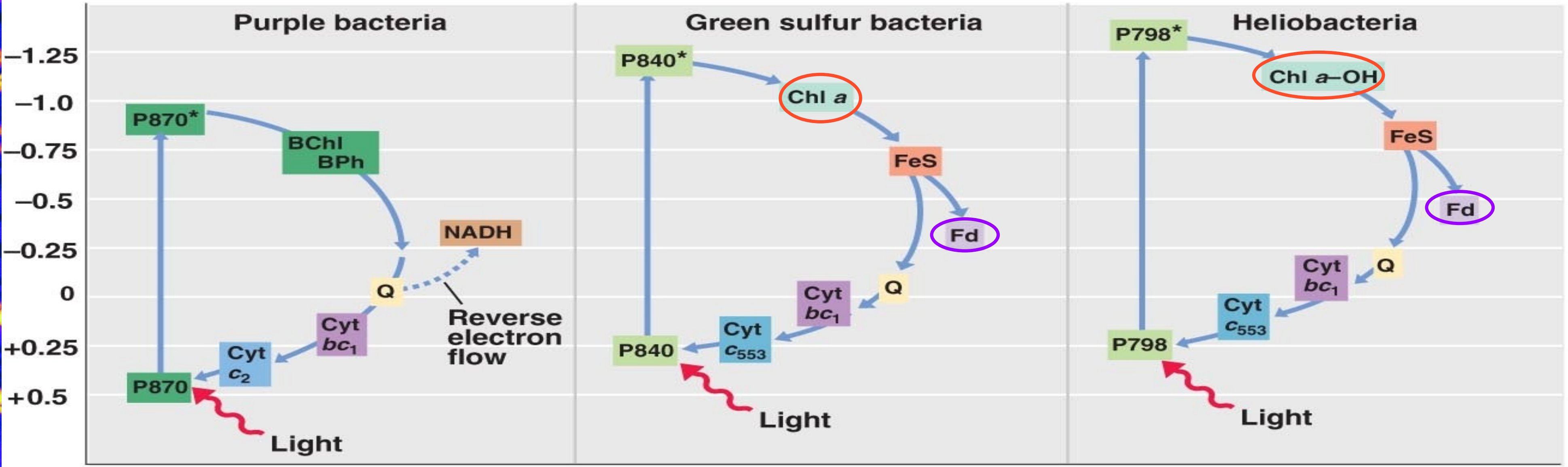




Purple bacterium

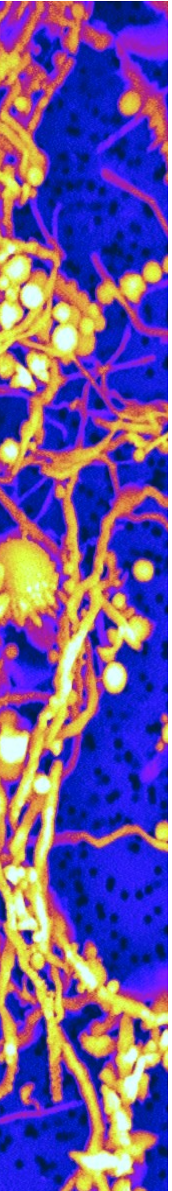
Electron flow in Purple, Green Sulfur and Heliobacteria



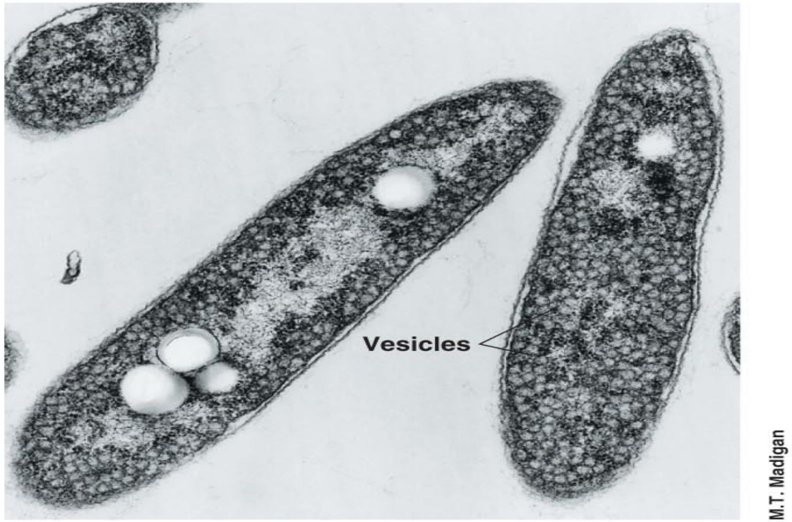


Bacteriochlorophyll *a*

Bacteriochlorophyll *g*

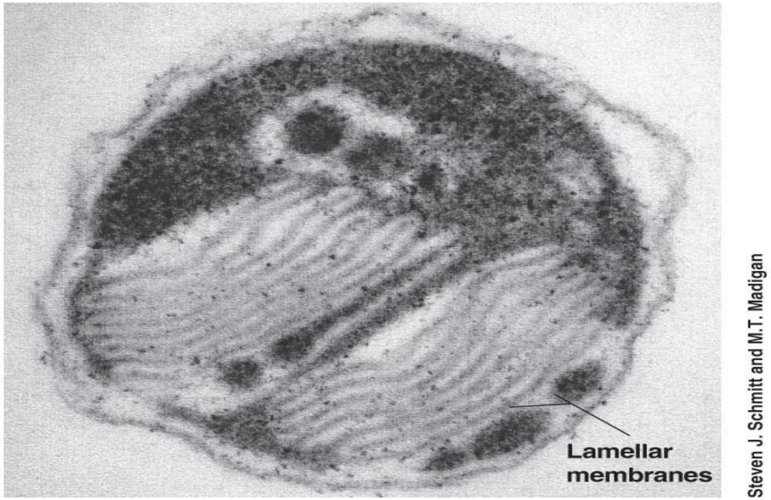


Membranes in Anoxygenic Photosynthesis



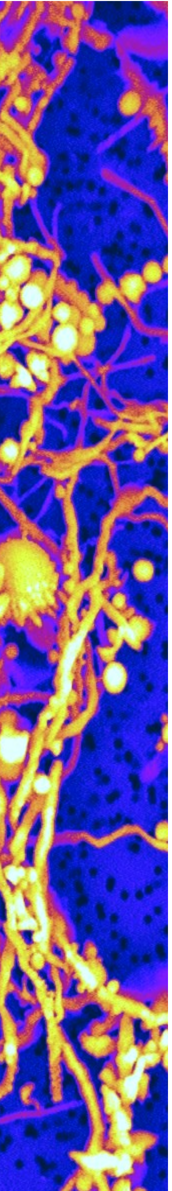
(a)

Chromatophores



(b)

Lamellar Membranes in the Purple Bacterium Ectothiorhodospira



Oxygenic Photosynthesis

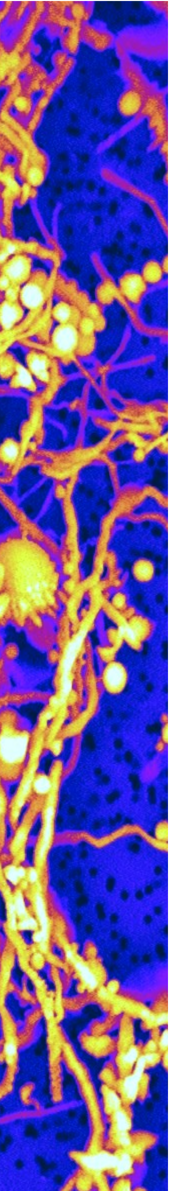
Oxygenic photosynthesis gets the name from the waste product of water oxidation, molecular oxygen

Within the prokaryotes, oxygenic photosynthesis is found exclusively in the Cyanobacteria (Bacteria), while it is present in algae and plants thanks to the endiosymbiosis of a proto-chloroplast

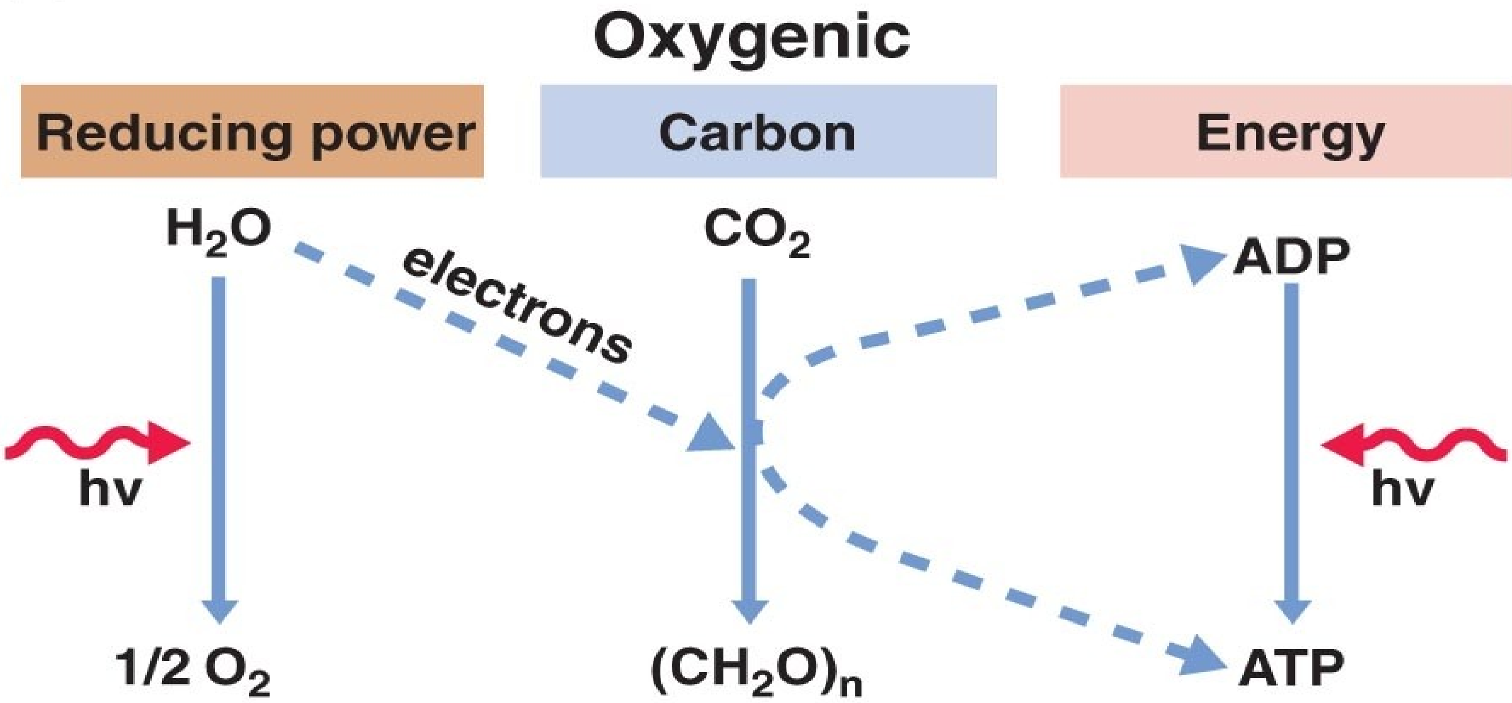
In term of evolution, oxygenic photosynthesis has founda ubiquitous electron donor to exploit: water

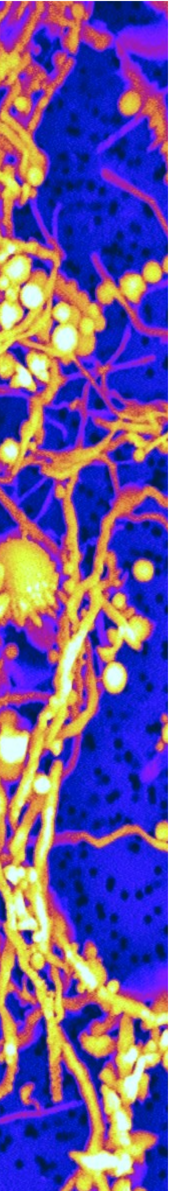
Two light reactions centers, called photosystem I and photosystem II, are involved in oxygenic photosynthesis, and use what is known as the "Z scheme" of photosynthesis (photosystem II transfers energy to photosystem I)

Under certain conditions, some oxygenic phototrophs can revert to anoxygenic photosynthesis

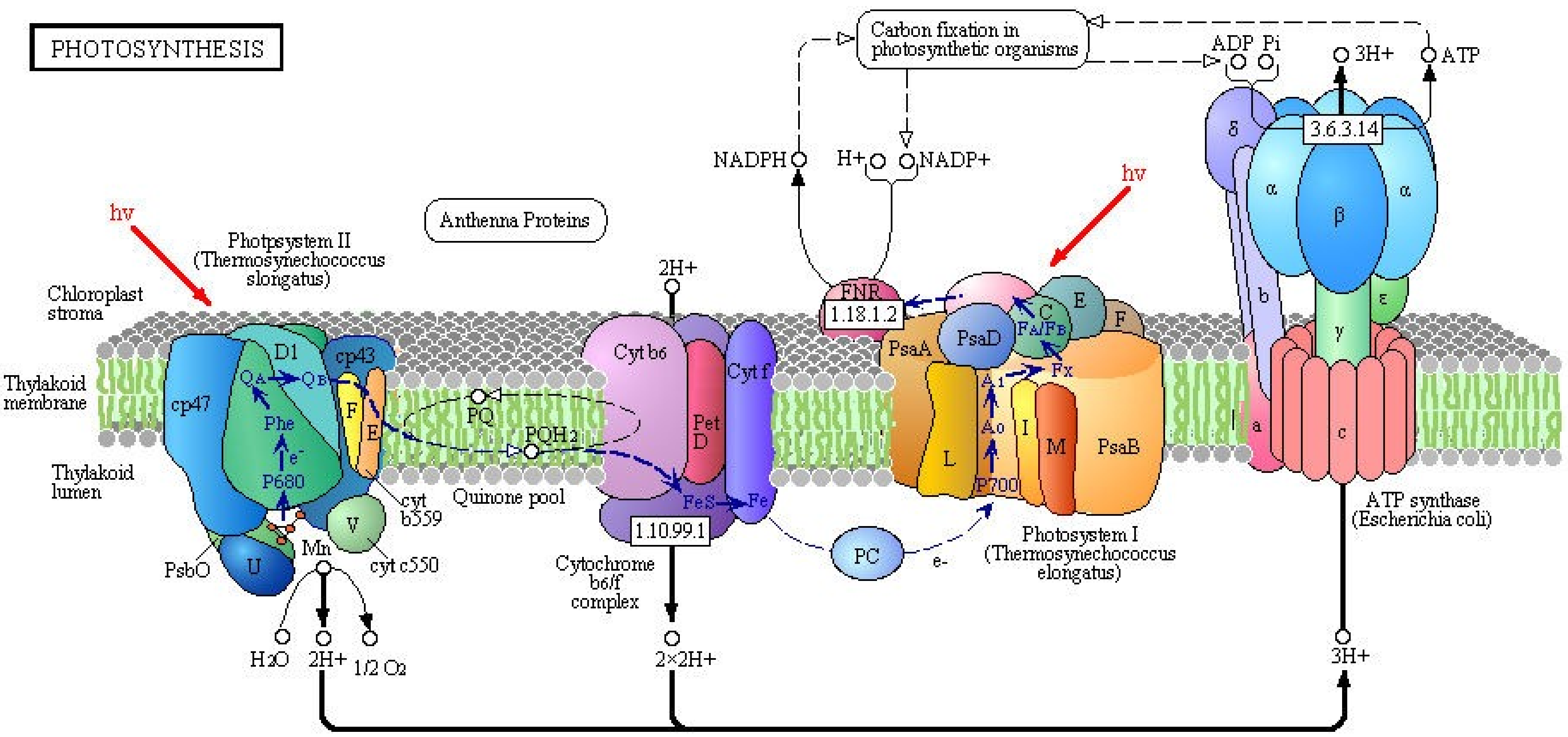


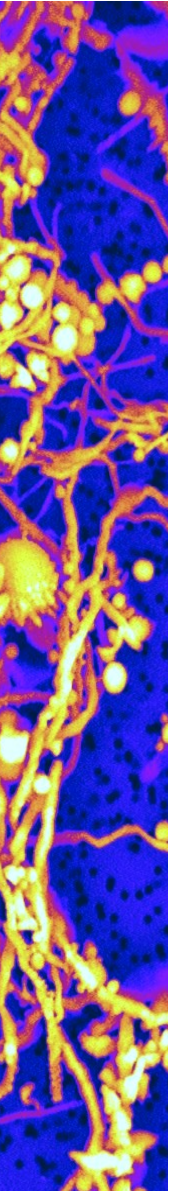
Oxygenic Photosynthesis



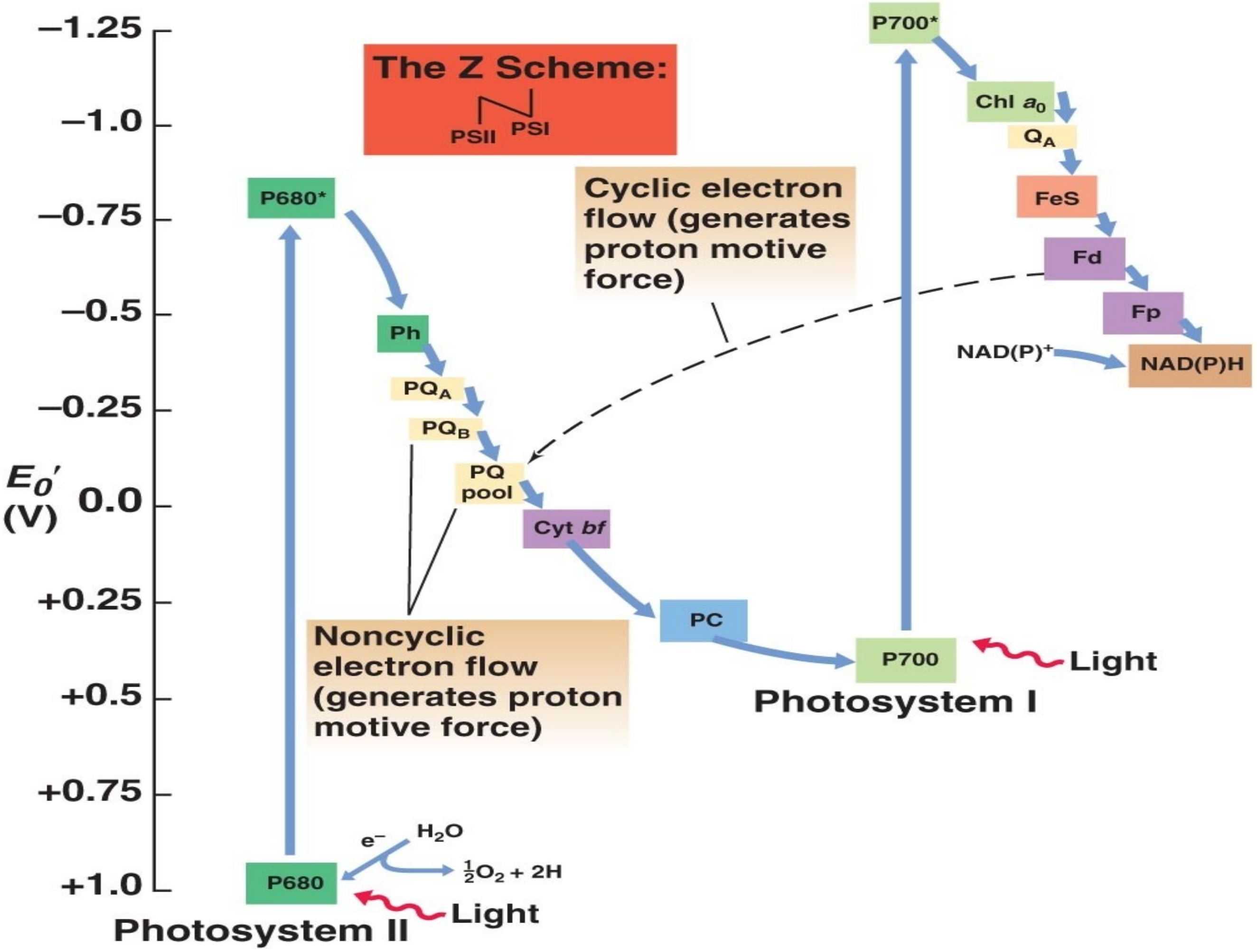


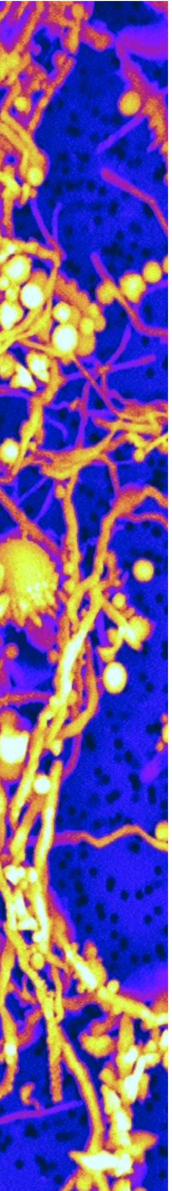
Arrangement of protein complexes



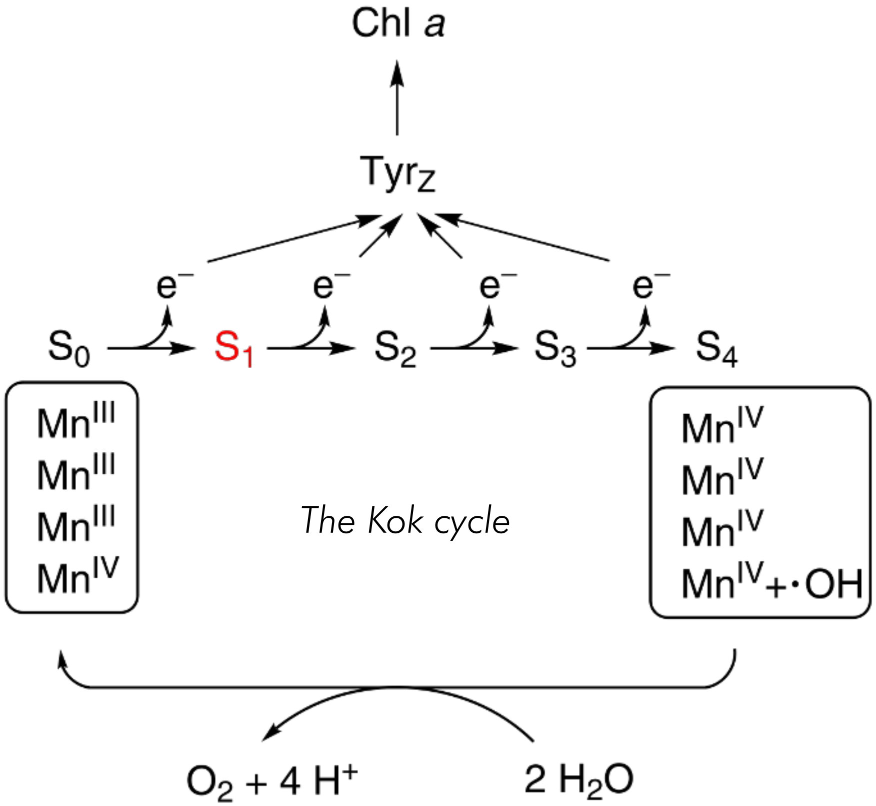


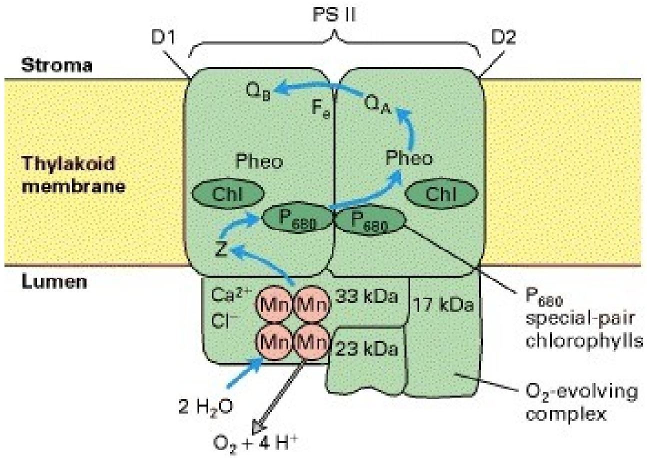
The Z Scheme of Oxygenic Photosynthesis

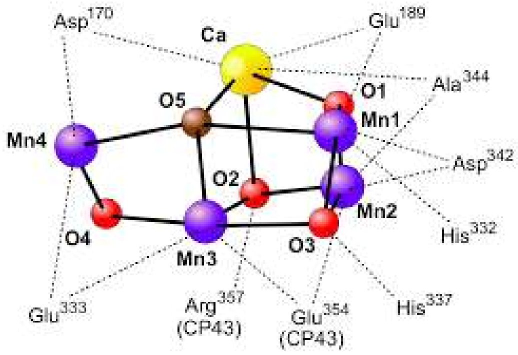




The Oxygen-evolving complex

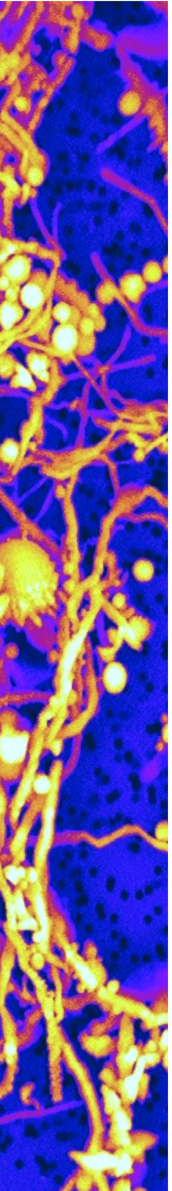


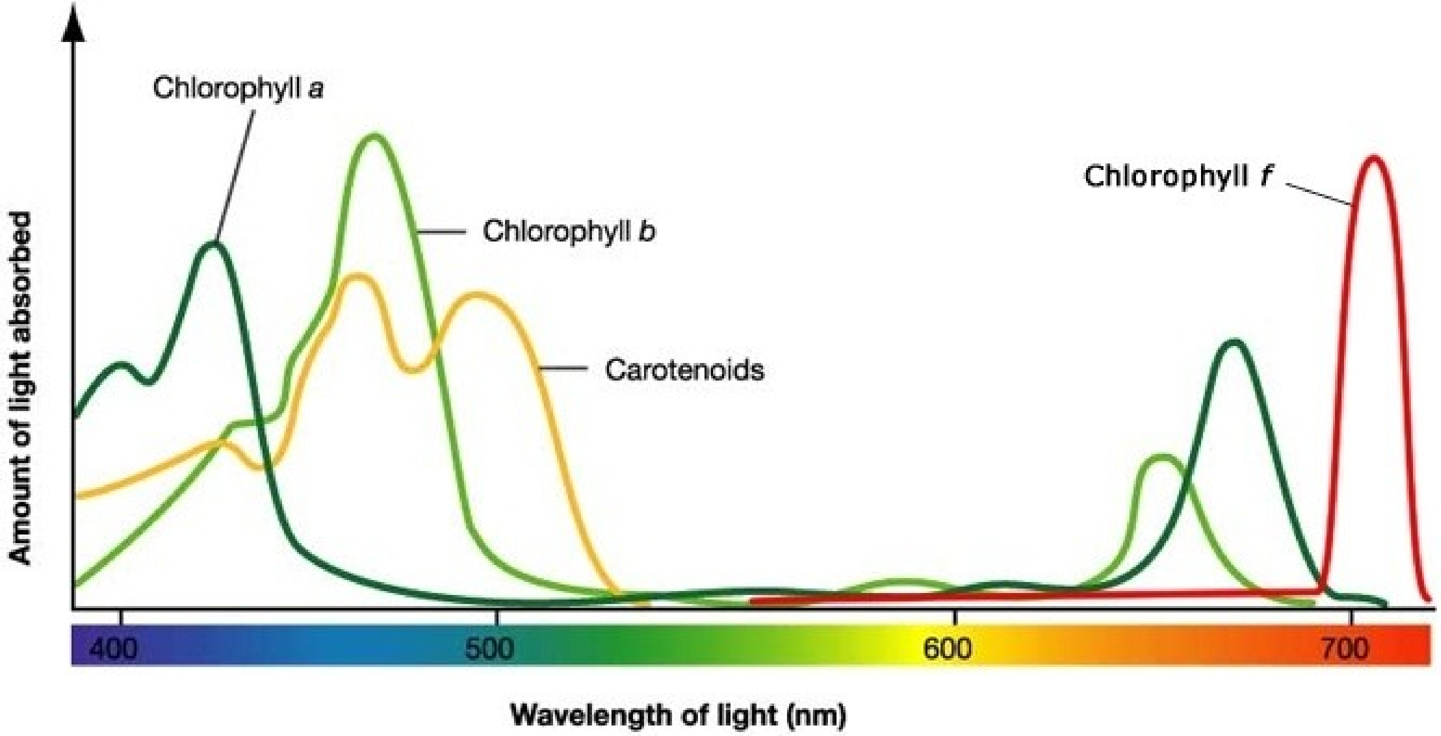


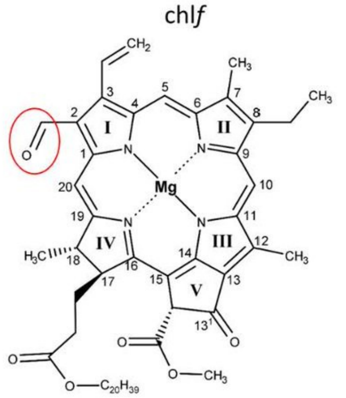


Mn_4CaO_5

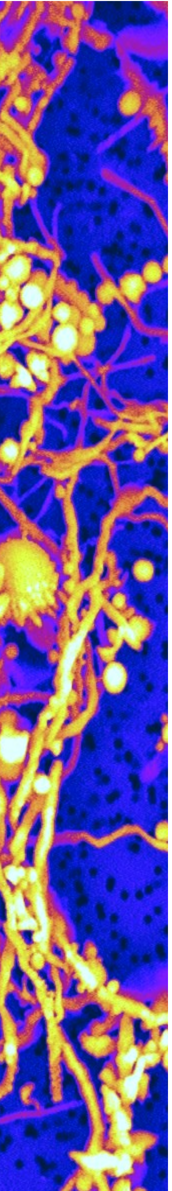
Far red light photosynthesis



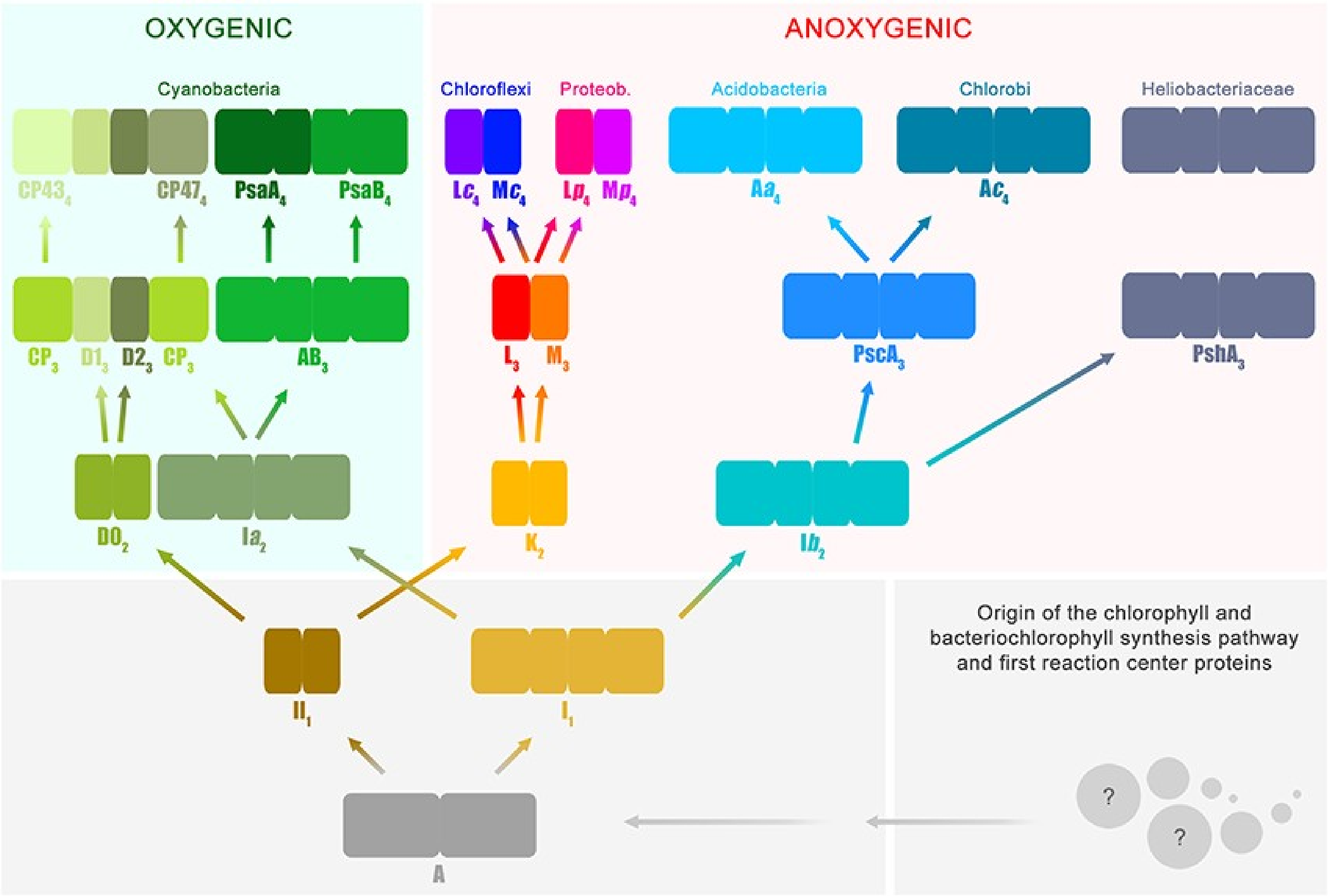




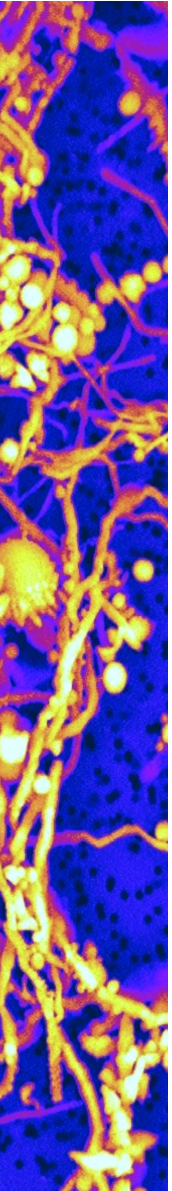
Chen et al. 2010 Science



On the origin of photosynthesis



Cadorna 2016 Front Plant Sci



This lecture reads

Jelen B, Giovannelli D, Falkowski PG . 2016. The Role of Microbial Electron Transfer in the Coevolution of the Geosphere and Biosphere. Annual Review of Microbiology, 70:45–62. DOI: 10.1146/annurev-micro-102215-095521

Cardona, T. (2016). Reconstructing the Origin of Oxygenic Photosynthesis: Do Assembly and Photoactivation Recapitulate Evolution? Frontiers in Plant Science 7, 257. doi:10.3389/fpls.2016.00257