Microbial Metabolism

What constrains
microbial metabolism?

- Thermodynamics
- Kinetics
- Diversity and paucity of reaction mechanisms
- Flux of energy, substrate & competition (rate-yield)
- Speciation of pathways
- At high flux and under competition it is advantageous to use a substrate inefficiently (and by short pathways)
- For a given n_{ATP}, there is an optimal pathway length

Last Thursday

- Metabolism in Cellular Context
- Types of Microbial Metabolism (Catabolism)
- How Microbes use Energy
- Link between Thermodynamics and Kinetics
- Speciation of Metabolic pathways
- Metabolism in Natural Selection and Isolation of Microbes

Microbial Metabolism

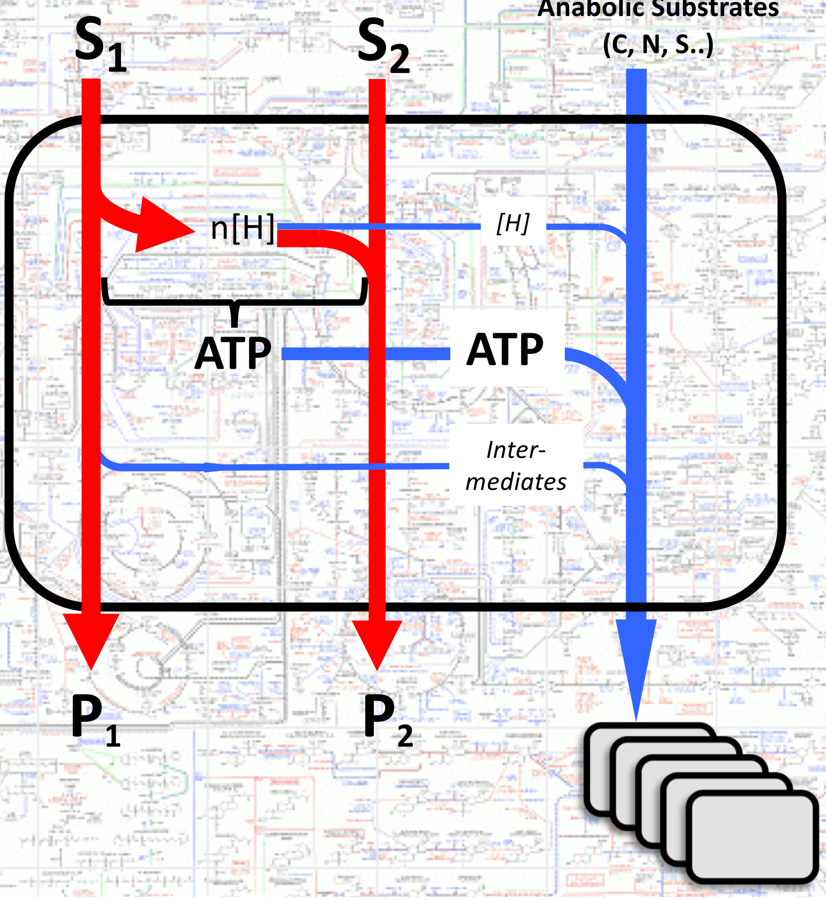
Today

Chemical constraints
microbial metabolism

- Principles of Metabolic Transformations
- Patterns of Metabolism

Catabolic and Anabolic Pathways

General



S_1

S_2

Anabolic Substrates
(C, N, S..)

n[H]

[H]

ATP

ATP

Inter-
mediates

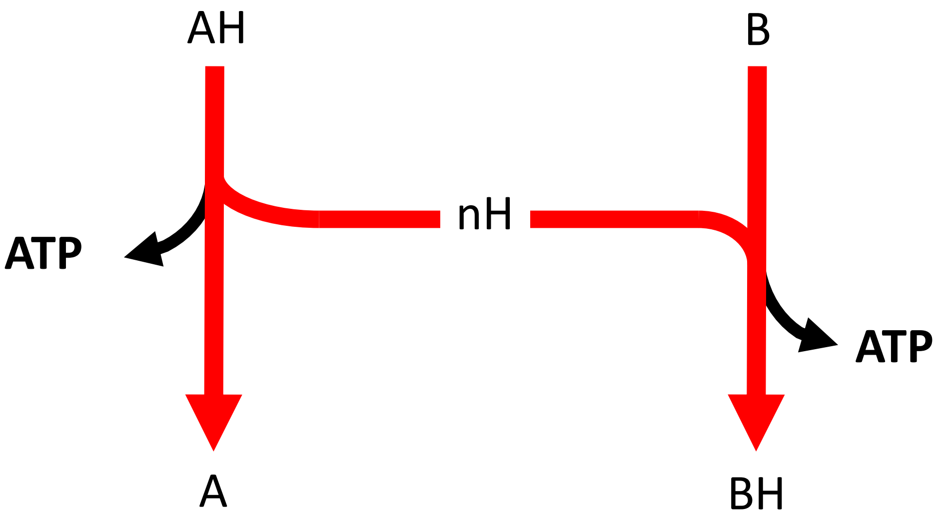
P_1

P_2

Life is redox chemistry

(remember, Albert Szent–Gyorgi was here)

$$AH + B \longrightarrow A + BH \qquad \Delta G<0$$



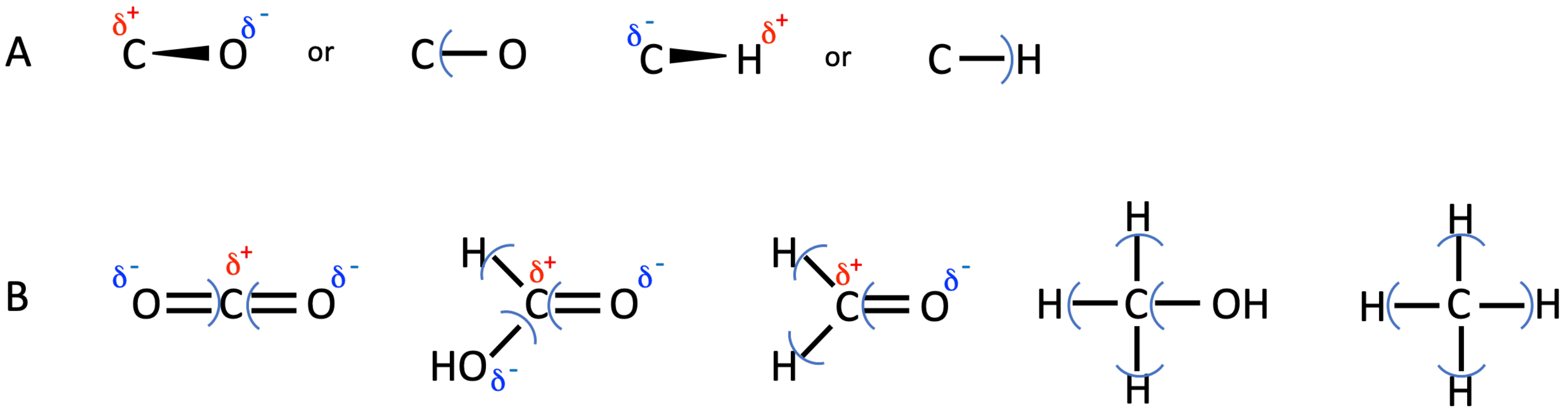
Substrate Level Phosphorylation
(energy-rich bond as intermediate)
-50kJ/rxn

Transport Coupled Phosphorylation
(electrochemical gradient as intermediate)
-20kJ/rxn

Electrons and energy-conserving metabolism

Electronegativities and oxidation states

O>N>S>C>H



CO_2	HCO_3H	CH_2O	CH_3OH	CH_4
Carbon dioxide	Formic acid	Formaldehyde	Methanol	Methane
+4	+2	0	-2	-4

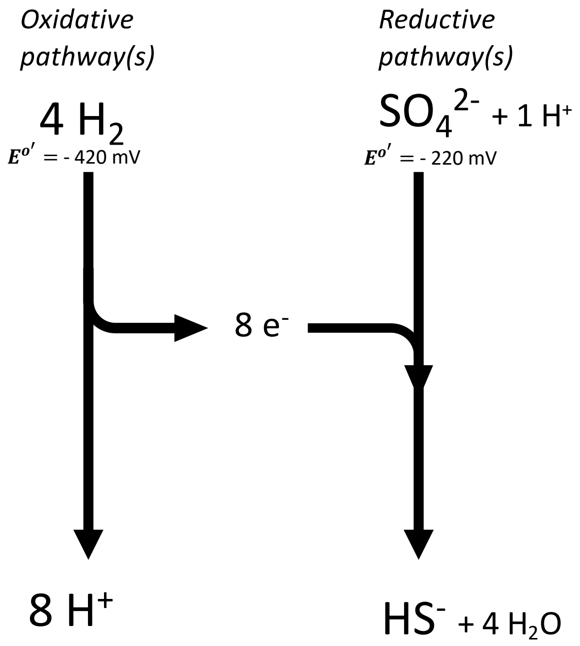
Electrons and energy-conserving metabolism

Conjugated redox couples

$$4\ H_2 + H^+ + SO_4^{2-} \longrightarrow HS^- + 4\ H_2O \qquad \Delta G^{o\prime} = -154 kJ/mol$$

oxidative half reaction
(electron donor)

$$\underbrace{4\ H_2 \longrightarrow 8\ e^- + 8\ H^+}$$



Oxidative pathway(s)

$4\ H_2$

$E^{o\prime}$ = - 420 mV

Reductive pathway(s)

$SO_4^{2-} + 1\ H^+$

$E^{o\prime}$ = - 220 mV

$8\ e^-$

$8\ H^+$

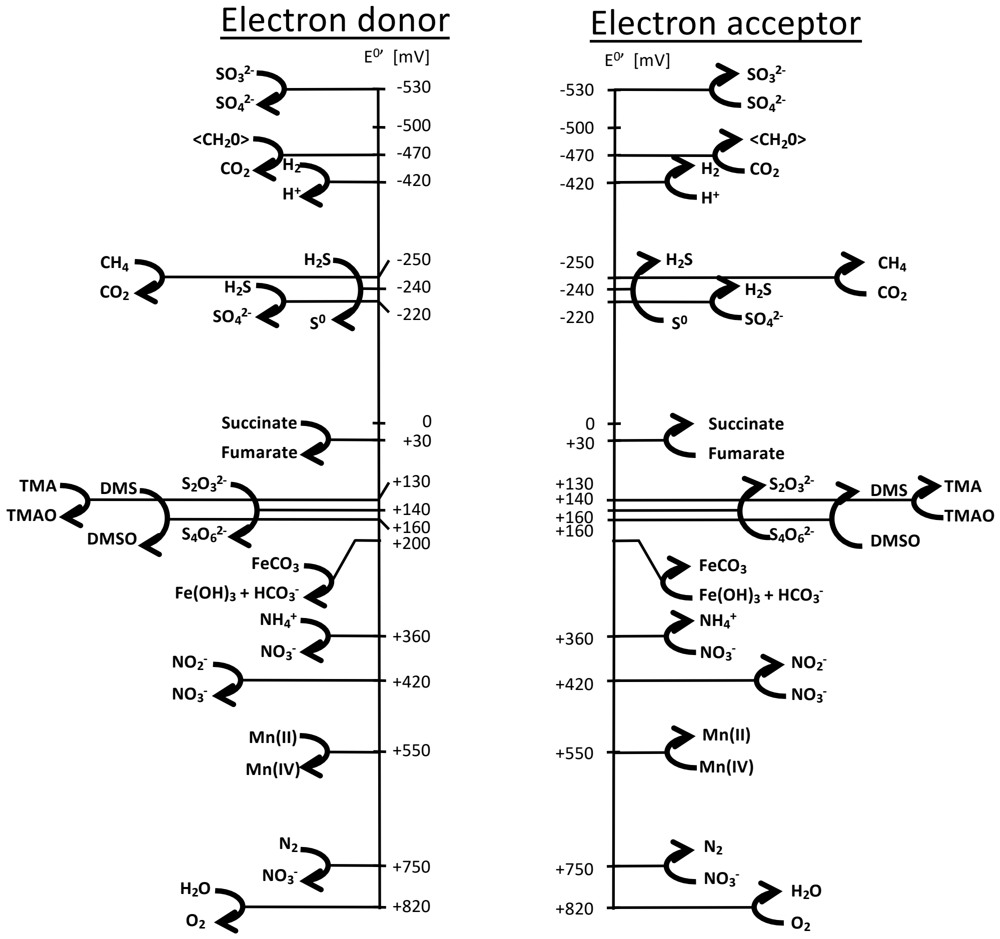
$HS^- + 4\ H_2O$

reductive half reaction
(electron acceptor)

$$\underbrace{SO_4^{2-} + 8\ e^- + 9\ H^+ \longrightarrow HS^- + 4\ H_2O}$$

Electrons and energy-conserving metabolism

Redox potentials of microbe-mediated redox processes
(Standard-state conditions, pH7)



$$\Delta G^{o\prime} = -nF\Delta E^{o\prime}$$

$$\Delta E^{0\prime} = (E^{0\prime}{}_{Acceptor} - E^{0\prime}{}_{Donor})$$

$$E' = E^{o\prime} + \frac{RT}{nF}\ln\frac{[C_{ox}]}{[C_{red}]}$$

Electrons and energy-conserving metabolism

The redox potential of H^+/H_2

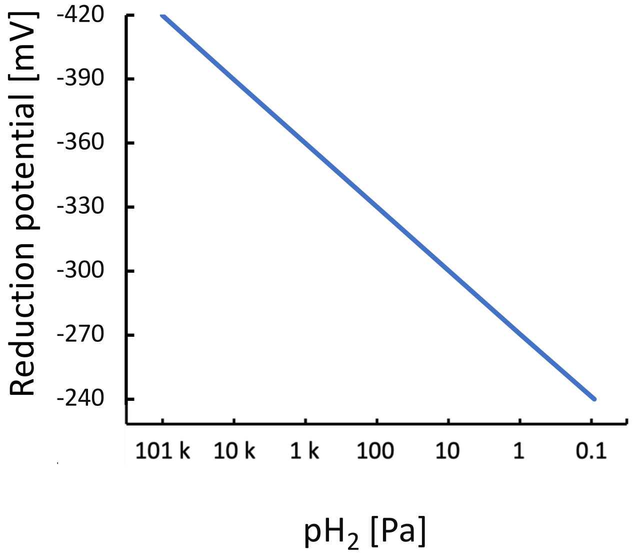
$$H_2 \leftrightarrows 2\,H^+ + 2\,e^-$$

$$\boldsymbol{E \quad = E^o + \frac{RT}{nF} ln \frac{[C_{ox}]}{[C_{red}]}}$$

$$E = E^o + \frac{0.06}{2} lg \frac{[H^+]^2}{[H_2]} = E^o + 0.03 lg \frac{[H^+]^2}{[H_2]}$$

At pH 7:

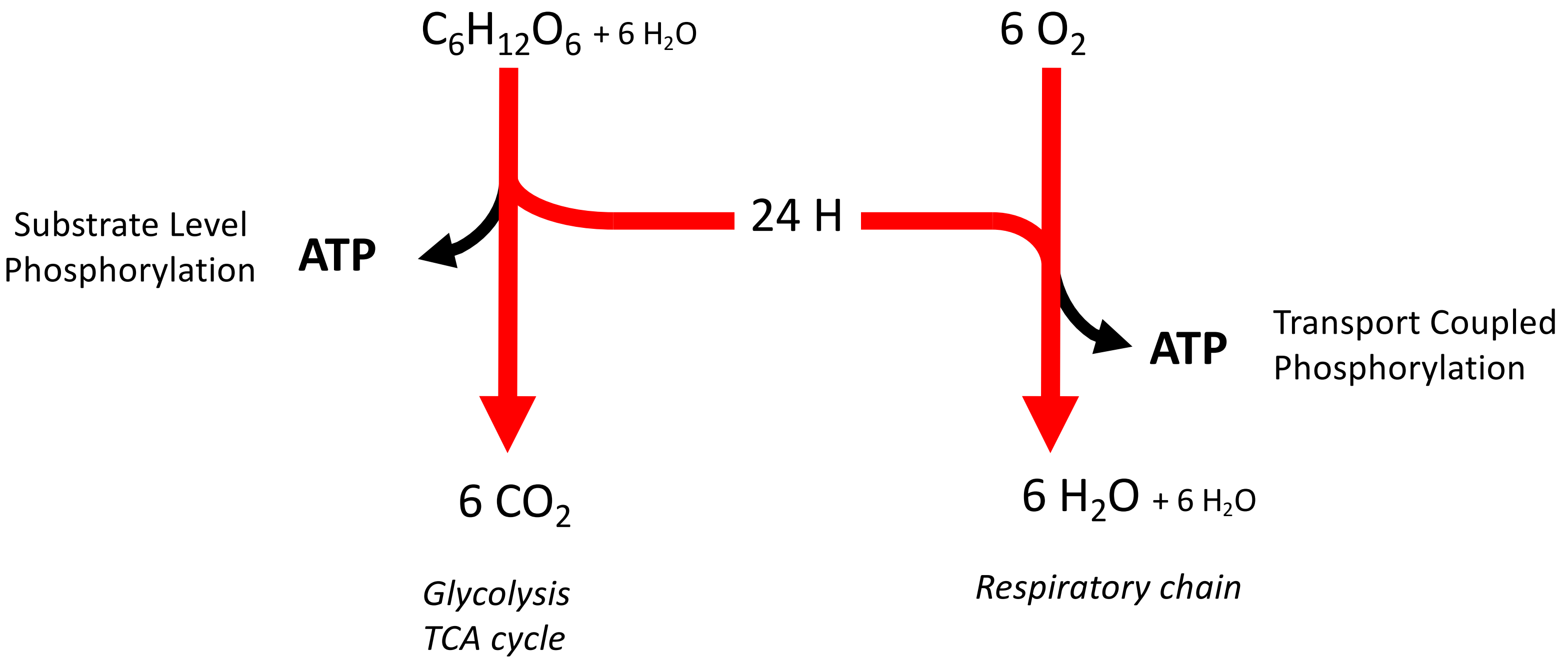
$E' = 0 + 0.03 \times lg(10^{-7})^2 = -420$ mV



Electrons and energy-conserving metabolism

Aerobic glucose metabolism by *E. coli*

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$



$C_6H_{12}O_6 + 6\ H_2O$

$6\ O_2$

24 H

Substrate Level Phosphorylation

ATP

ATP

Transport Coupled Phosphorylation

$6\ CO_2$

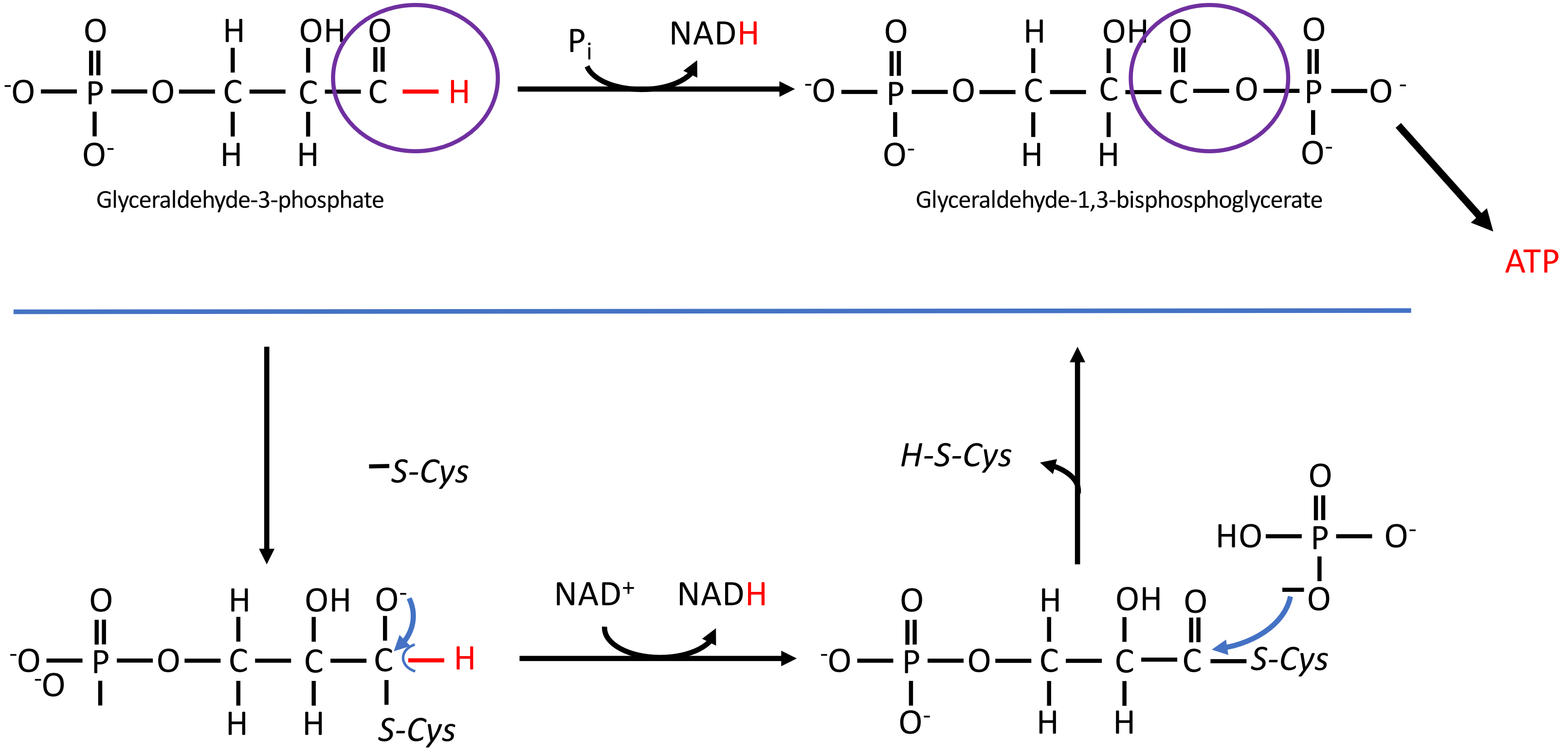
$6\ H_2O + 6\ H_2O$

Glycolysis
TCA cycle

Respiratory chain

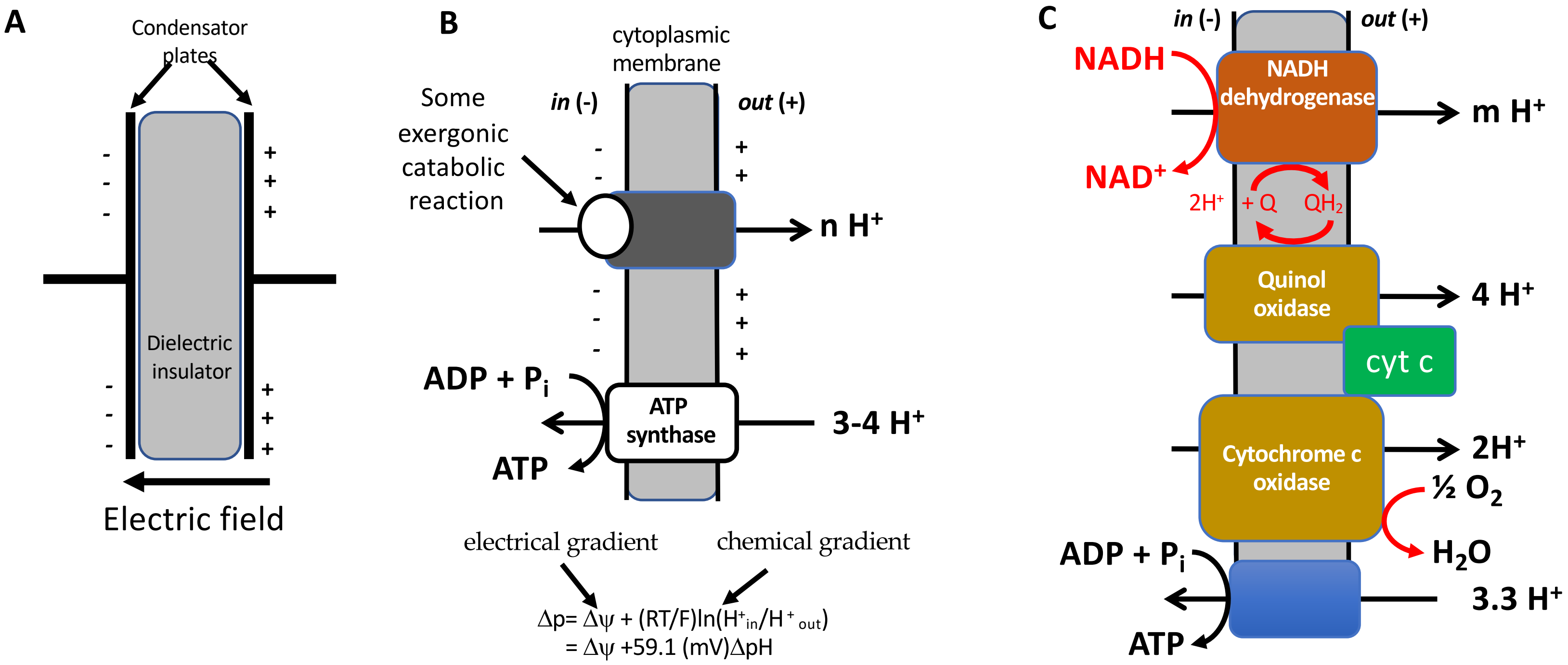
Electrons and energy-conserving metabolism

Substrate Level Phosphorylation



Electrons and energy-conserving metabolism

(Electron) Transport Coupled Phosphorylation



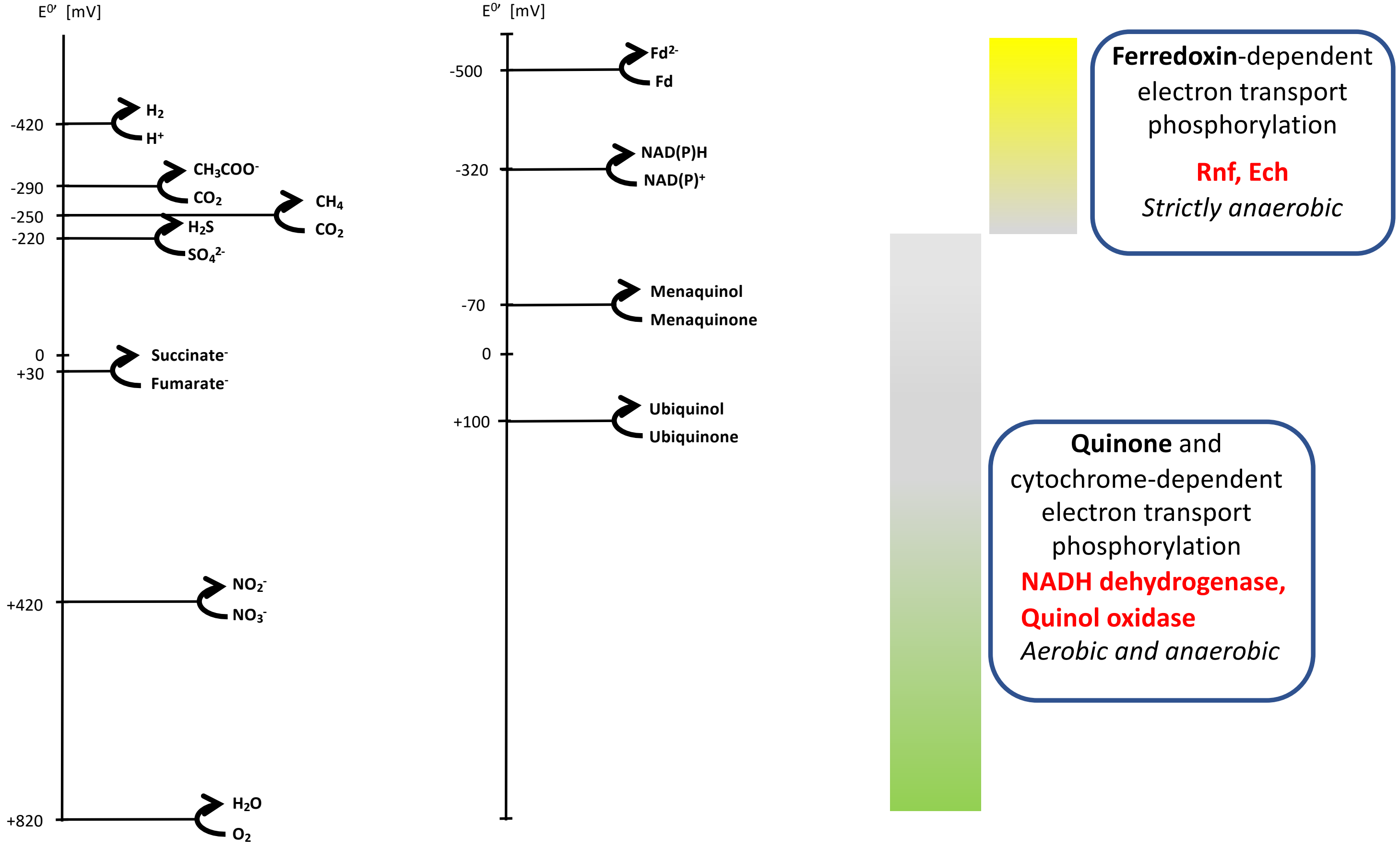
$$\Delta p = \Delta\psi + (RT/F)\ln(H^+_{in}/H^+_{out})$$
$$= \Delta\psi + 59.1\ (mV)\Delta pH$$

Electrons and energy-conserving metabolism

Environmental electron acceptor

Cellular electron carrier

Major routes for electron transport phosphorylation



Ferredoxin-dependent electron transport phosphorylation

Rnf, Ech

Strictly anaerobic

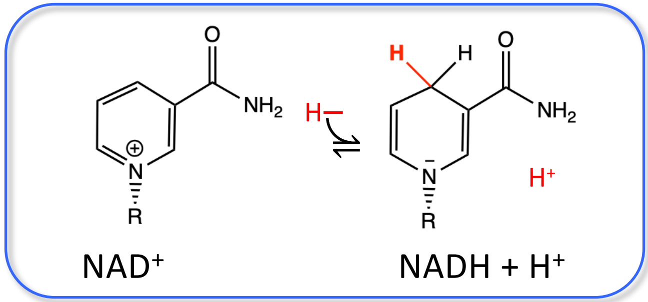
Quinone and cytochrome-dependent electron transport phosphorylation

NADH dehydrogenase, Quinol oxidase

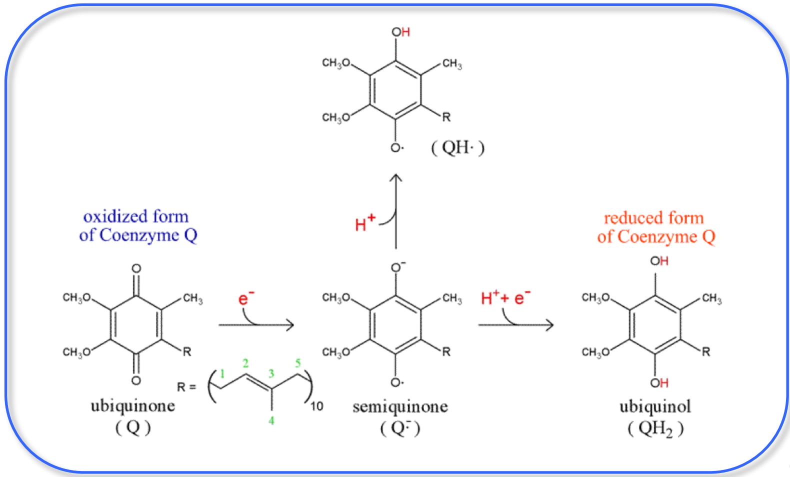
Aerobic and anaerobic

Electrons and energy-conserving metabolism

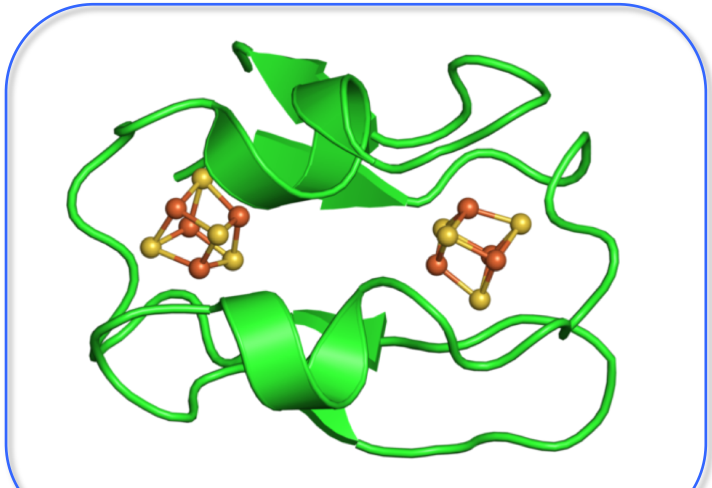
Major catabolic electron carriers



NAD$^+$/NADH
$E^{0\prime}$ = -320 mV



Quinone/quinol
$E^{0\prime}_{MQ}$ = - 70 mV $E^{0\prime}_{MQ}$ = + 100 mV



Ferredoxin from *C. acidiurici*

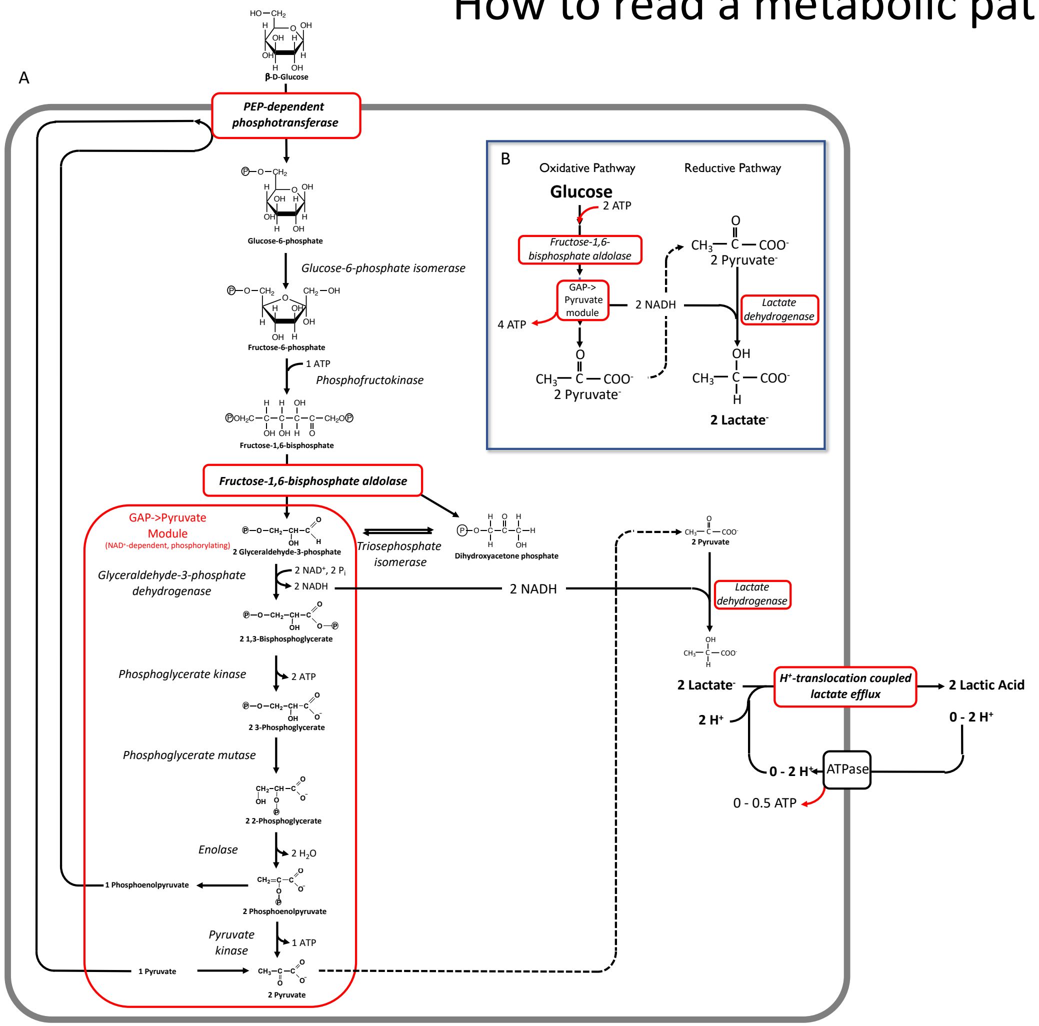
Fd/Fd^{2-}
$E^{0\prime}$ = -500 mV

$$Fd_{(4Fe4S)} + 2e^- \rightleftharpoons Fd^{2-}$$

Electrons and energy-conserving metabolism

- Oxidation and reduction reactions are intrinsic to the main modes of energy conservation
- Identify reduced and oxidized substrates (i.e. electron donor and electron acceptor) (= oxidation states)
- Determine difference in redox potential ($\triangleq \Delta G$)
- Focus on electrons, electron carriers and number of oxidation/reduction reactions (= balancing catabolic equation, flux of energy & substrates)

How to read a metabolic pathway



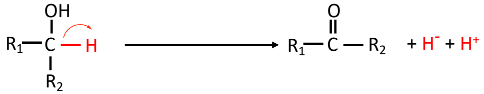
Glycolytic pathway of glucose fermentation to lactate

$$\text{Glucose} \rightarrow 2\ \text{Lactate}^- + 2\ \text{H}^+$$

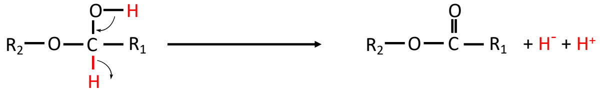
$$\Delta G^{0\prime} = -180\ \text{kJ/mol glucose}$$

Oxidations/reductions

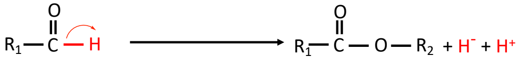
Alcohol oxidation



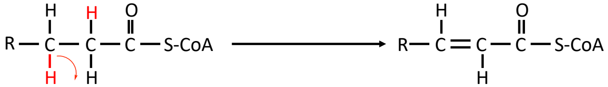
Hemiacetal oxidation



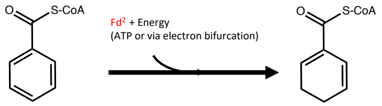
Aldehyde oxidation



Acyl-CoA oxidation



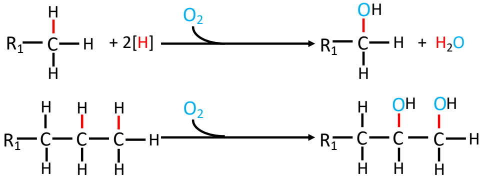
Benzoyl-CoA reduction (dearomatizing)



Methyl-CoM reduction

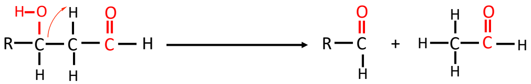
$\mathbf{CH_3}\text{-S-CoM} + \text{H-S-CoB} \rightleftharpoons \text{CoM-S-S-CoB} + \mathbf{CH_4}$

Oxygenation

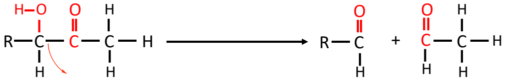


C-C cleavage/ condensations

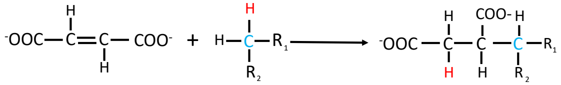
Aldol cleavage



Ketol cleavage



Fumarate addition



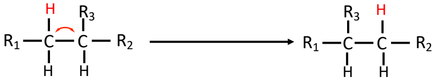
De/carbonylation

$CO + HS\text{-}CoA + CH_3\text{-}H_4F \rightleftharpoons CH_3CO\text{-}S\text{-}CoA + H_4F$

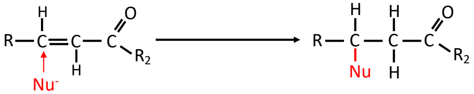
$CO_2 + Fd^{2-} + 2H^+ \rightleftharpoons Fd + H_2O + CO$

Auxiliary reactions

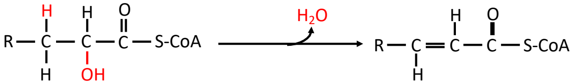
Rearrangement



Nucleophilic addition/elimination



α hydroxy elimination



Hydrogen oxidation

$H_2 \rightleftharpoons 2\,e^- + 2\,H^+$

How to read a metabolic pathway

- Focus on the electrons! ….for now.

Architecture of pathways and n_{ATP}

Propionibacterium freudenreichii

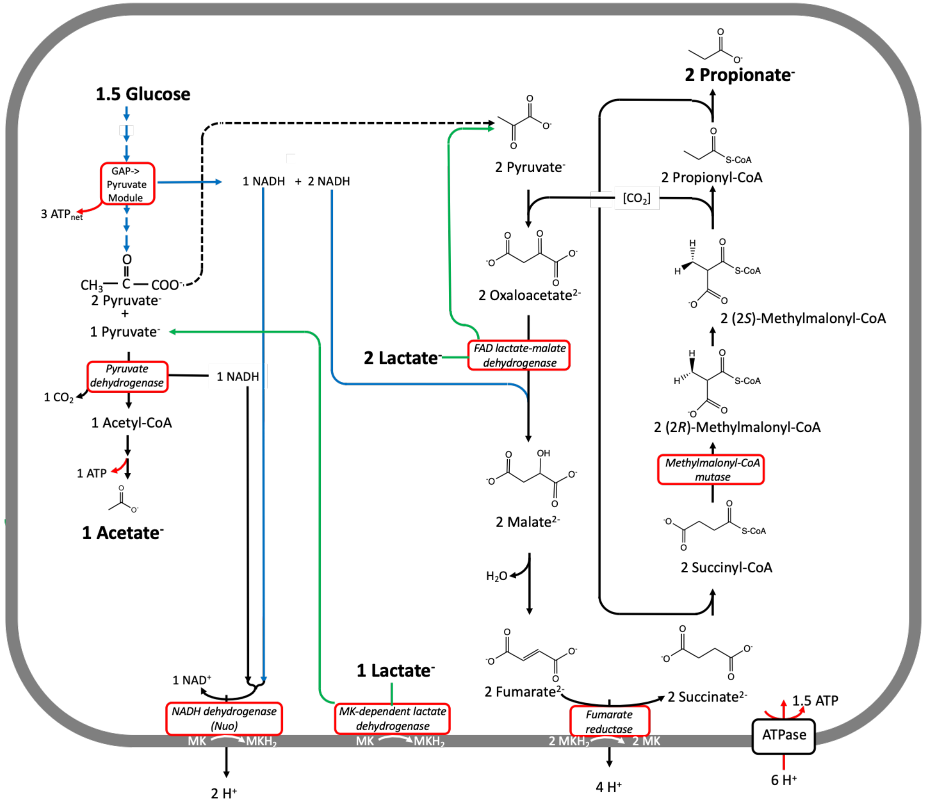
$1.5\ \text{Glucose} \rightarrow 1\ \text{Acetate}^- + 2\ \text{Propionate} + 1\ CO_2 + 3\ H^+ + 1\ H_2O$ $\Delta G^{0\prime} = -465$ kJ/mol acetate

2.75 ATP/Propionate

$3\ \text{Lactate} \rightarrow 1\ \text{Acetate}^- + 2\ \text{Propionate} + 1\ CO_2 + 2\ H^+ + 1\ H_2O$ $\Delta G^{0\prime} = -170$ kJ/mol acetate

0.8 ATP/Propionate

$n_{ATP} = 0.8$



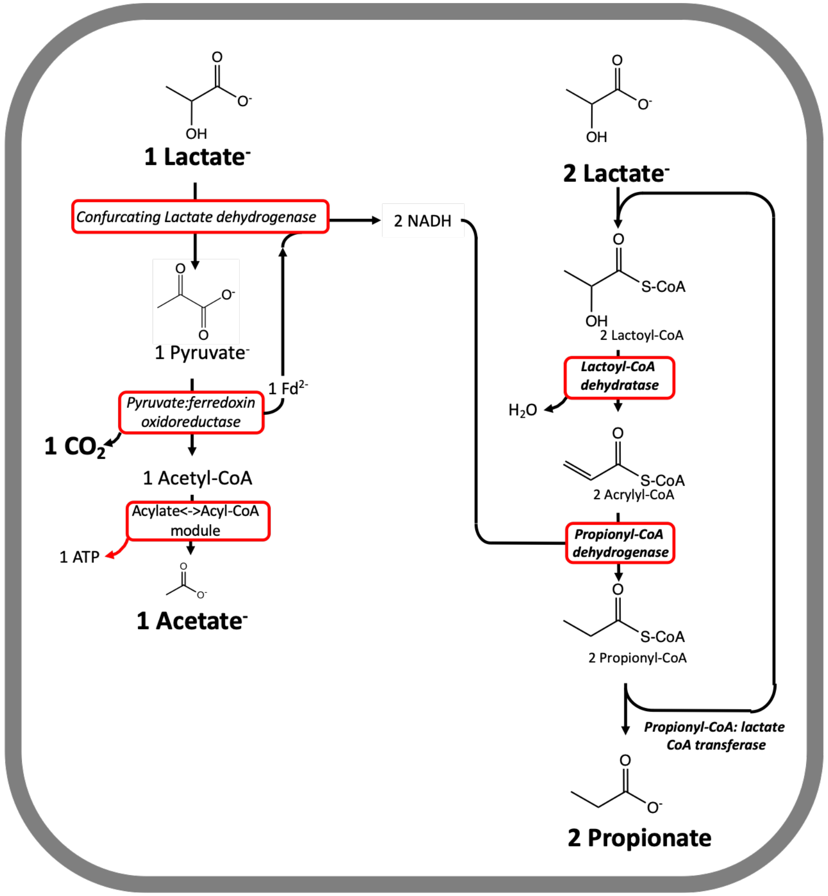
Architecture of pathways and n_{ATP}

Clostridium homopropionicum

$$3\ \text{Lactate} \xrightarrow[0.3\ \text{ATP/Propionate}]{} 1\ \text{Acetate}^- + 2\ \text{Propionate} + 1\ CO_2 + 2\ H^+ + 1\ H_2O$$

$\Delta G^{0\prime} = -170$ kJ/mol acetate

$n_{ATP} = 0.3$



Architecture of pathways and n_{ATP}

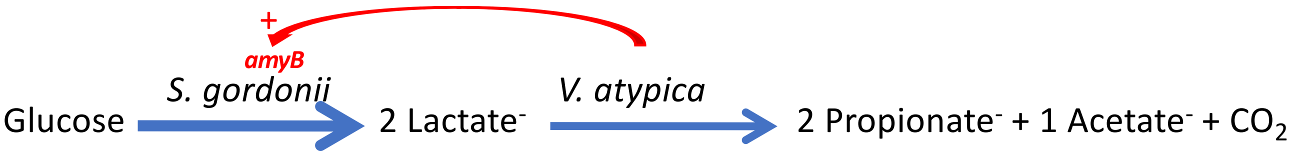
Anoxic organic-rich environments:

Low flux, low competition: $\textbf{Glucose} \xrightarrow{\textit{Propionic acid bacterium}} \textbf{Propionate}^-\textbf{, Acetate}^-$

High flux, high competition: $\textbf{Glucose} \xrightarrow[\textit{bacterium}]{\textit{Lactic acid}} \textbf{Lactate}^- \xrightarrow[\textit{bacterium}]{\textit{Propionic acid}} \textbf{Propionate}^-\textbf{, Acetate}^-$

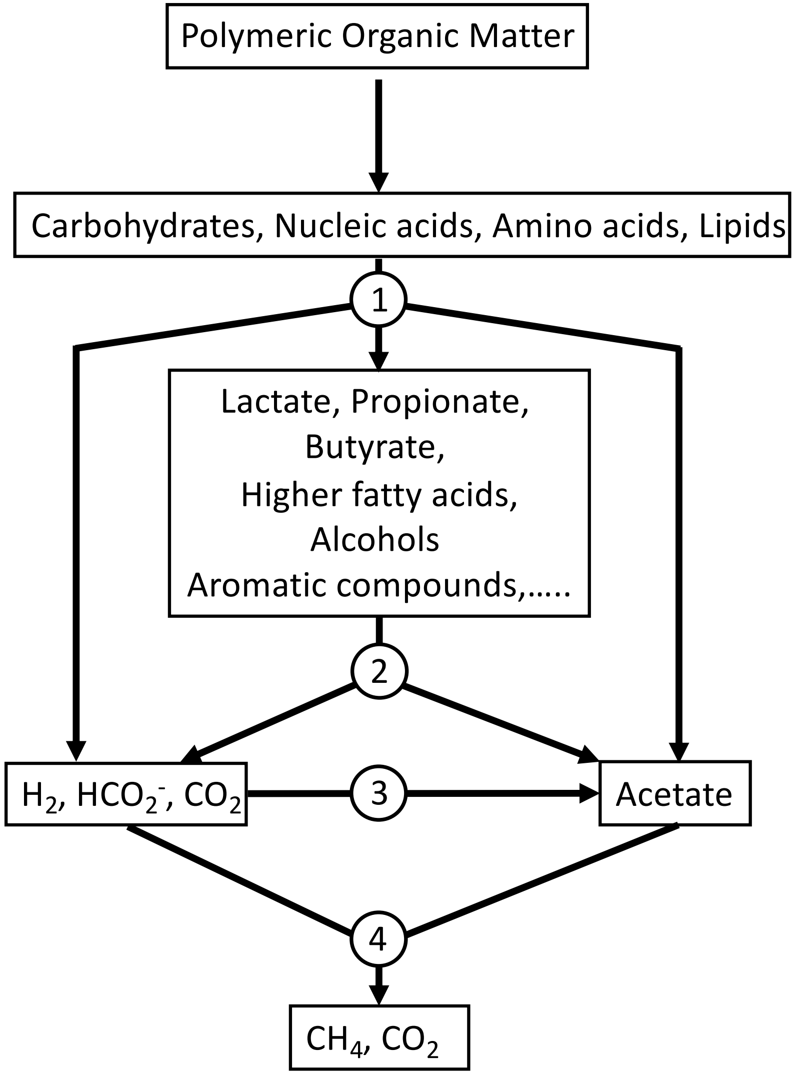
$$\text{Glucose} \xrightarrow{\textit{S. gordonii}} 2\ \text{Lactate}^- + 2\ \text{H}^+$$

$$2\ \text{Lactate}^- \xrightarrow{\textit{V. atypica}} 2\ \text{Propionate}^- + 1\ \text{Acetate}^- + \text{CO}_2$$



$$\text{Glucose} \xrightarrow{\textit{S. gordonii}} 2\ \text{Lactate}^- \xrightarrow{\textit{V. atypica}} 2\ \text{Propionate}^- + 1\ \text{Acetate}^- + \text{CO}_2$$

Flow of Electrons and Carbon under Methanogenic Conditions



Polymeric Organic Matter

Carbohydrates, Nucleic acids, Amino acids, Lipids

①

Lactate, Propionate,
Butyrate,
Higher fatty acids,
Alcohols
Aromatic compounds,…..

②

H_2, HCO_2^-, CO_2

③

Acetate

④

CH_4, CO_2

Architecture of pathways and n_{ATP}

Ruminococcus albus

Glucose + 2 H_2O → 2 $Acetate^-$ + 2 H^+ + 2 CO_2 + 4 H_2

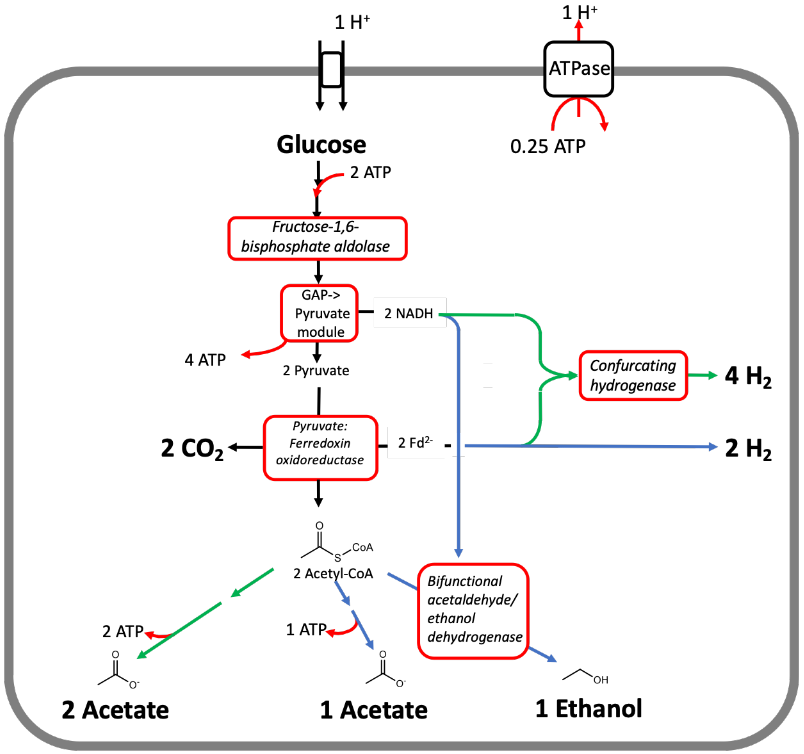
3.75 ATP/Glucose

$\Delta G^{0\prime}$ = - 197 kJ/mol glucose

Glucose + 1 H_2O → 1 $Acetate^-$ + 1 Ethanol + 1 H^+ + 2 CO_2 + 2 H_2

2.75 ATP/Glucose

$\Delta G^{0\prime}$ = - 207 kJ/mol glucose



Low pH_2 (10 Pa)

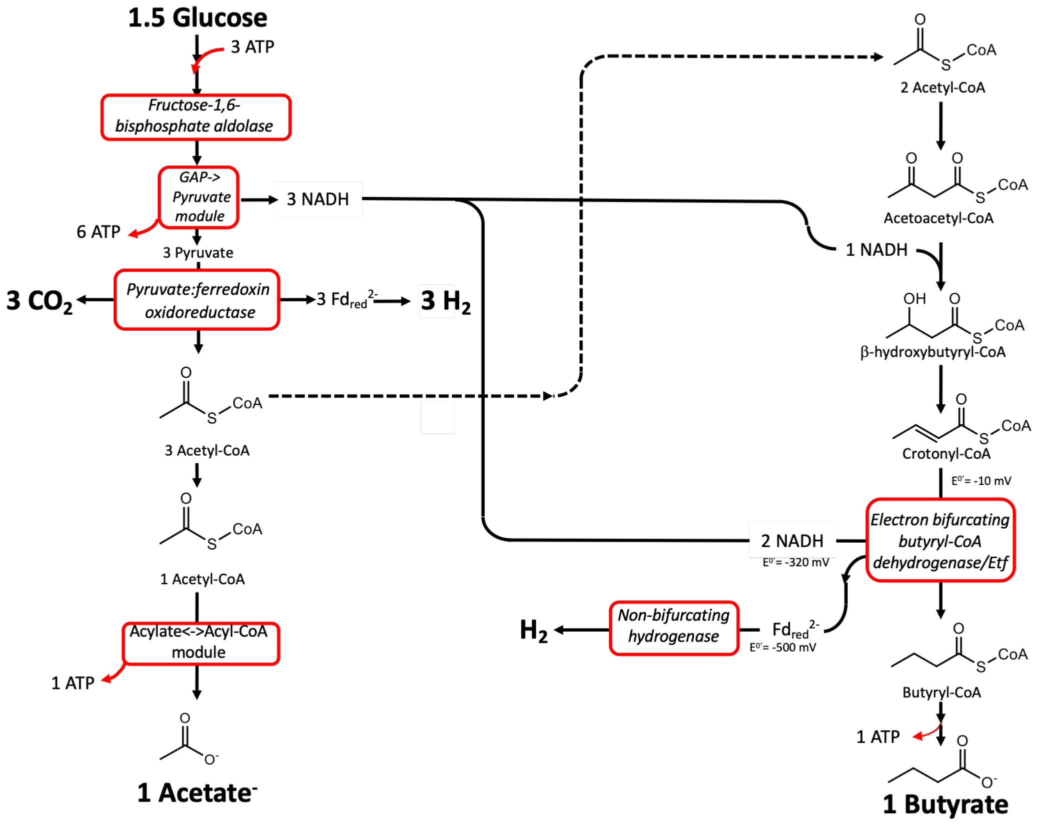
High pH_2 (101 kPa)

Architecture of pathways and n_{ATP}

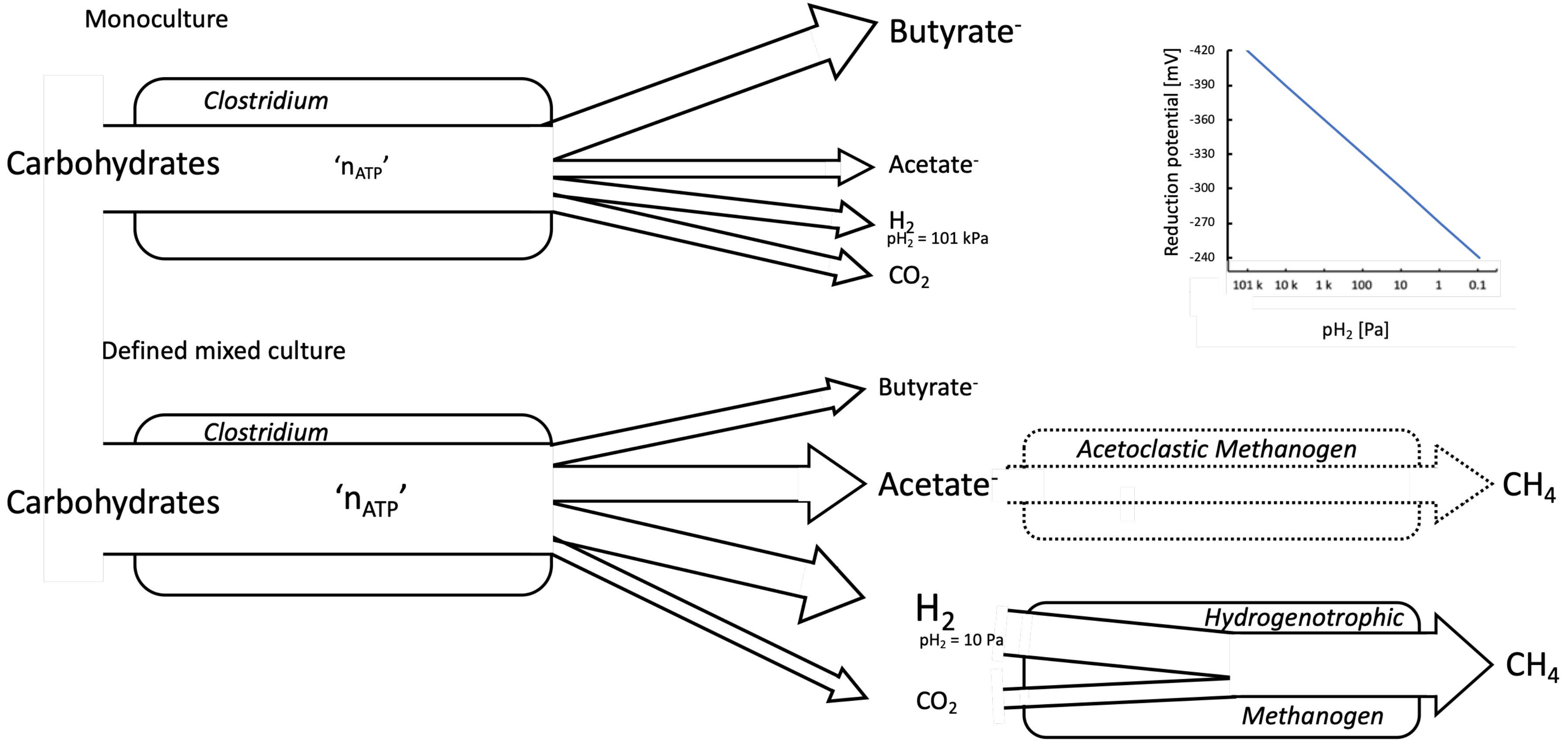
Clostridium pasteurianum

$$1.5\ \text{Glucose} + H_2O \xrightarrow[3.3\ \text{ATP/Glucose}]{} 1\ \text{Acetate}^- + 1\ \text{Butyrate}^- + 3\ CO_2 + 4\ H_2 + H^+$$

$\Delta G^{0\prime}$=-250kJ/mol glucose



Architecture of pathways and n_{ATP}



Monoculture

Clostridium

Carbohydrates

'n_{ATP}'

Butyrate$^-$

Acetate$^-$

H_2

pH_2 = 101 kPa

CO_2

Defined mixed culture

Clostridium

Carbohydrates

'n_{ATP}'

Butyrate$^-$

Acetate$^-$

Acetoclastic Methanogen

CH_4

H_2

pH_2 = 10 Pa

CO_2

Hydrogenotrophic Methanogen

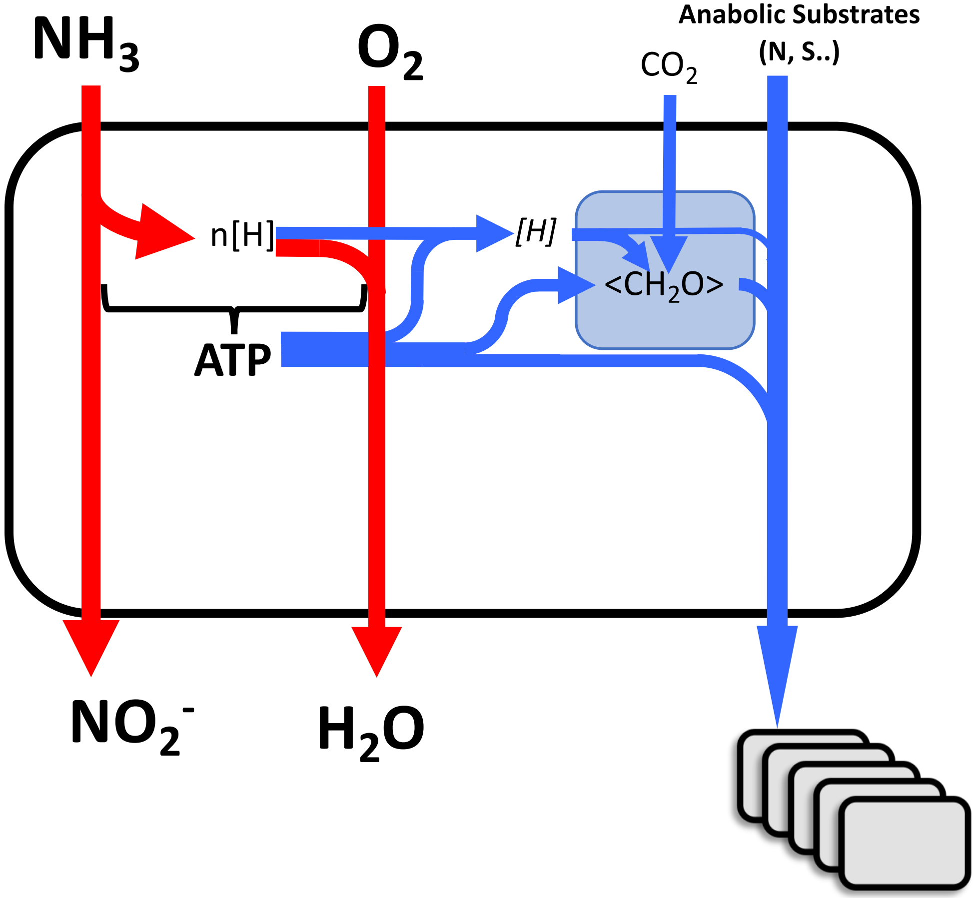
CH_4

Architecture of pathways and n_{ATP}

- Flux of substrate and energy drives speciation and metabolic interactions
- Multiple pathways with different n_{ATP} can exist in diverse microbes for a single catabolic reaction. Implications for rate-yield and low substrate utilization.

The cost of autotrophy

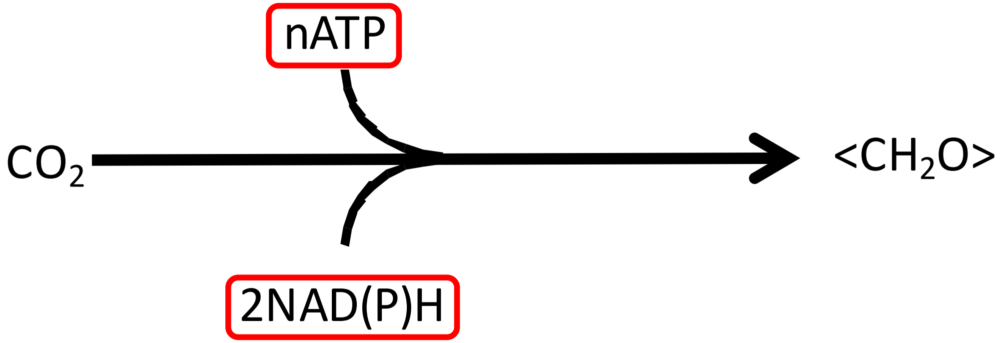
Lithoautotrophic



***Use of inorganic compounds both as electron donor (S_1) and electron acceptor** (S_2)*

The cost of autotrophy

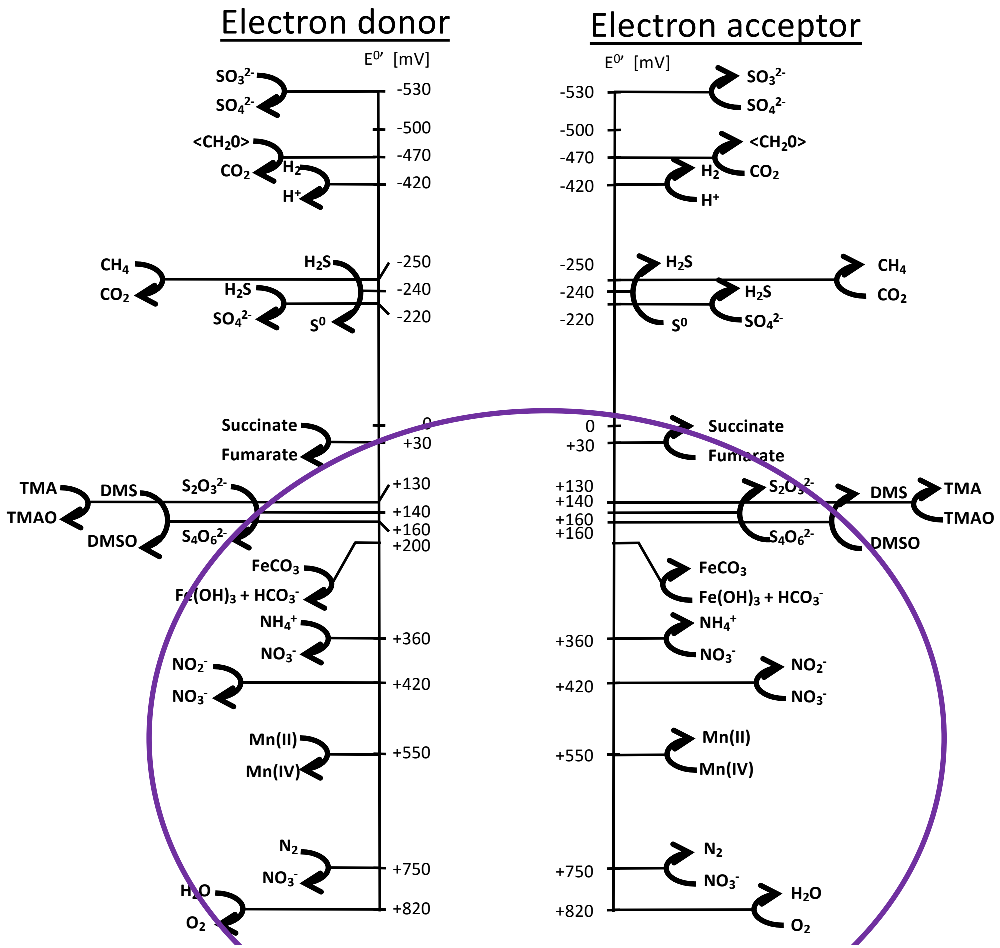
Autotrophs: Microbes that derive more than 50% of the cell carbon from CO_2.

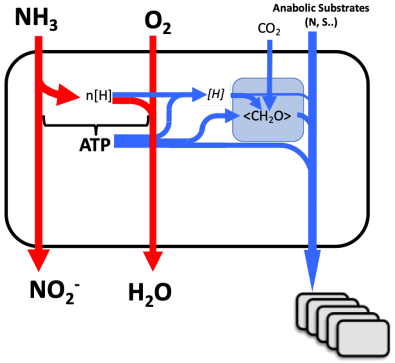


$$CO_2 \xrightarrow[\boxed{2NAD(P)H}]{\boxed{nATP}} <CH_2O>$$

The cost of autotrophy

Redox potentials of microbe-mediated redox processes
(Standard-state conditions, pH7)

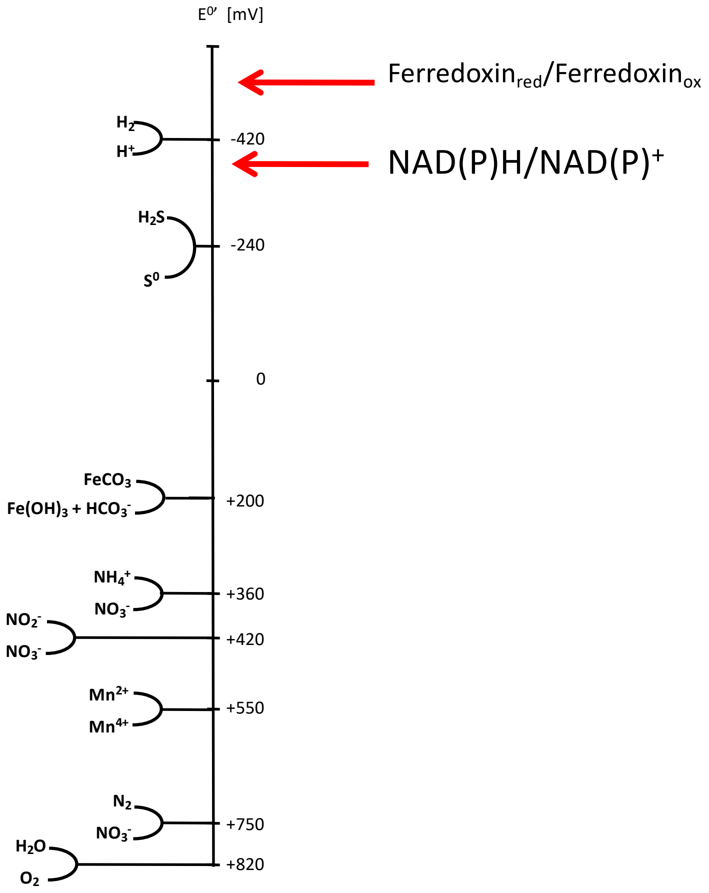


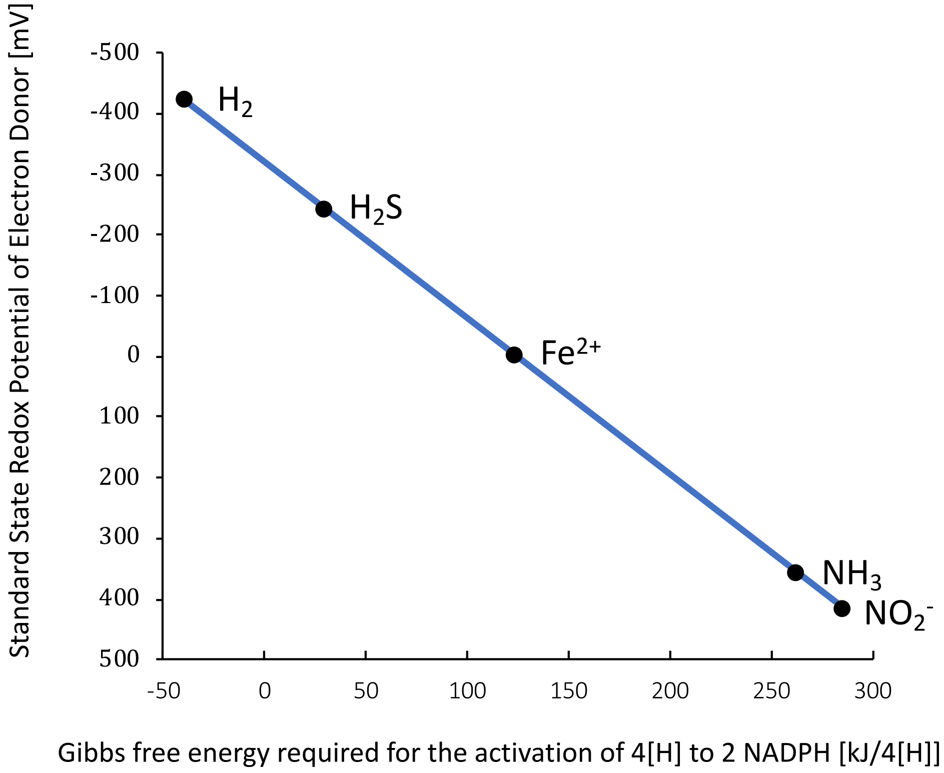


KITP Forum Microbial Metabolism 2021

The cost of autotrophy

Electron donor



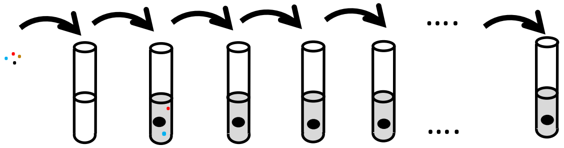


The cost of autotrophy

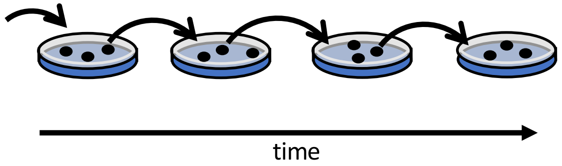
- Not all electrons are equal
- Being an aerobe does not mean, the microbe has a lot of energy available

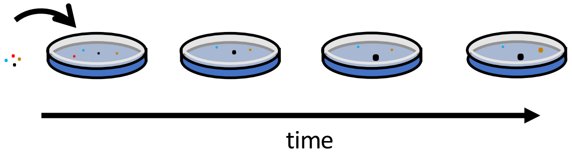
Isolation of Microbes and Selection

Liquid enrichments



Direct isolations

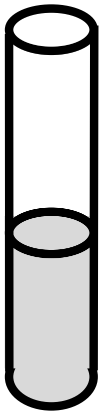


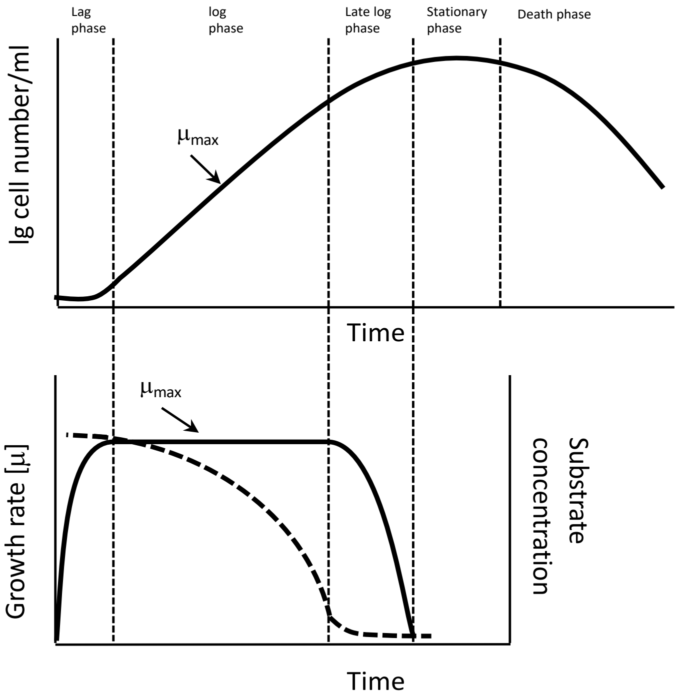


Competition is global;
Selection under competition favors fast growing microbes (high $J_{S,}$ high J_{ATP})

Competition is local (initially)
Effect of Drift

Growth in Batch

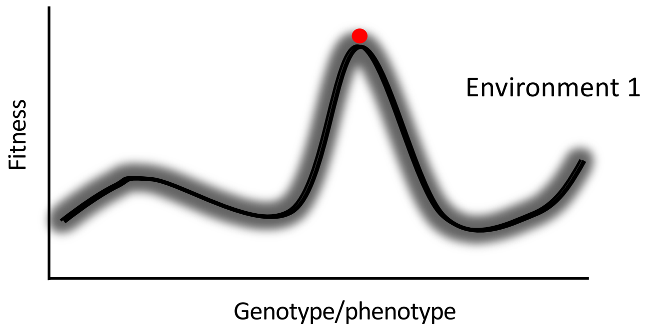




Growth in batch selects for the fastest microbes (dATP/dt)

KITP Forum Microbial Metabolism 2021

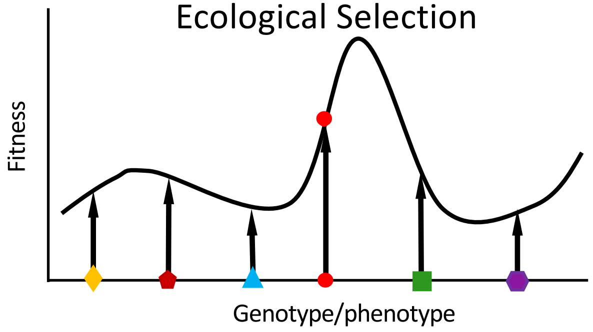
Fitness landscape



Selection

KITP Forum Microbial Metabolism 2021

Ecological Selection



Evolutionary Selection