Ch/APh2 Bioenergetics Section Lecture of May 14, 2009

Introduction to bioenergetics.

The thermodynamics of biological energy production.

Kinetic aspects of bioenergetic processes.

The molecular and cellular organization of bioenergetic systems.

Photosynthesis

Respiration and ATP synthesis

Haber-Bosch process and biological nitrogen fixation

Chemical potential:

the free energy change for a reaction forming a mole of products is given by:

$$\Delta G = \Delta G^\circ + RT \ln(\text{products})/(\text{reactants})$$

for n moles:

$$n\Delta G = n\Delta G^\circ + nRT \ln(\text{products})/(\text{reactants})$$

we introduce the chemical potential, μ_i, as the free energy per mole of compound i

$$\mu_i = \mu^\circ_i + RT \ln c_i$$

so, $\Delta G = \sum n_i\mu_i$ (products) - $\sum n_i\mu_i$ (reactants)

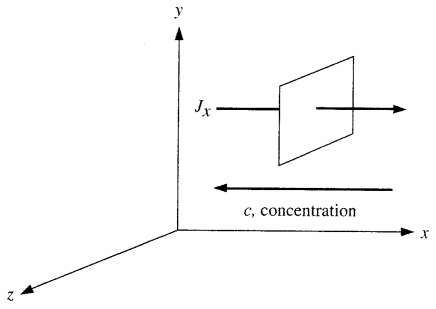
$$\mu_i = \mu^\circ_i + RT\ln c_i$$

the chemical potential is like other types of potential energy (say gravitational) in that it is <u>energetically favorable for the system to go from higher to lower μ</u>

so, the transport of molecules from regions of higher to lower concentration is the thermodynamically favorable direction

the gradient of a potential energy is a force; the force corresponding to the gradient of the chemical potential drives diffusive processes

$$J = L\nabla\mu$$



▶ FIGURE 6.4
Flux J_x is the net diffusional transport of material per unit time (mol cm^{-2} s^{-1}), in the x-direction, across a unit cross-sectional area perpendicular to x. The concentration c decreases with increasing x; the flux J_x is in the direction opposite to the concentration gradient.

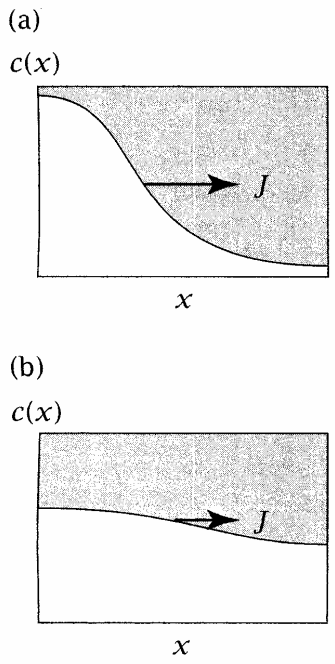


Figure 18.2 Flow results from concentration gradients. (a) A steep gradient causes large flux, while (b) a shallow gradient causes a small flux.

In addition to concentration, the energy of a charged species will also depend on the value of the electrostatic potential Φ at its location.

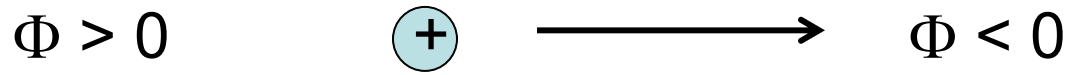
Φ is the electrostatic potential energy of a unit positive charge, i.e., the amount of work required to bring a unit (+) charge from zero potential, to a final potential Φ.

$$w = z F \Phi$$

z = ion charge (+1, -2); F = Faraday conversion factor = 96.5 kJ / Volt = 23.1 kcal / V

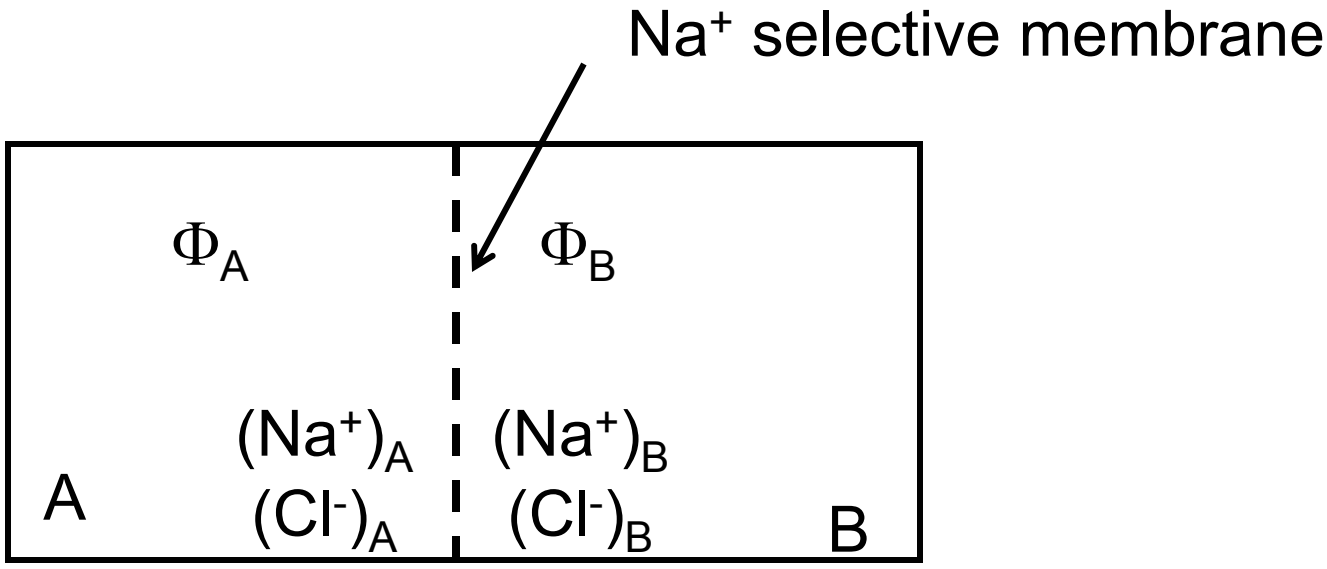
$\Phi > 0$	work needed to move (+) particle
$\Phi < 0$	work generated by moving (+) particle

units : J / coulombs = Volt
biological systems typically involve potentials between ±300 mV



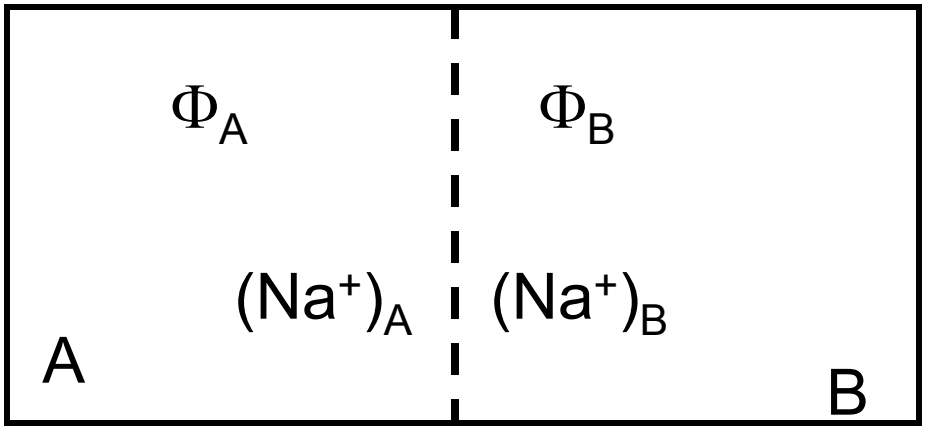
This term is added to our expression for μ for charged species to give:

$$\mu_i = \mu_i^\circ + RT \ln c_i + z_i F \Phi$$



If there is a potential difference across a membrane that is permeable to an ion, then at equilibrium, a concentration gradient will form to equalize the chemical potential in both compartments, and vice-versa

relationship between concentration and potential differences



at equilibrium, μ for Na^+ in two sides must be equal:

$$\mu_A = \mu_B$$

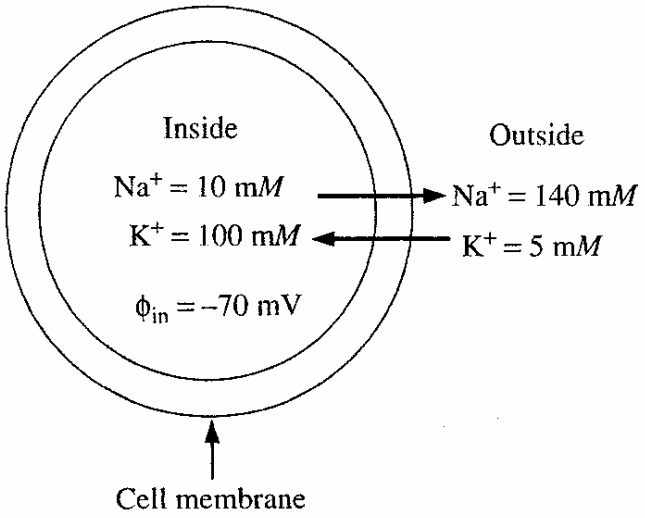
$$\mu^\circ + RT \ln(Na^+)_A + F\,\Phi_A = \mu^\circ + RT \ln(Na^+)_B + F\,\Phi_B$$

$$(\Phi_B - \Phi_A) = (RT/F) \ln (Na^+)_A / (Na^+)_B$$

$$= 0.059 \log (Na^+)_A / (Na^+)_B \qquad \text{(Volts)}$$

if $(Na^+)_A > (Na^+)_B$, then $(\Phi_B - \Phi_A) > 0$

the relationship between concentration and voltage gradients has significant bioenergetic consequences, and is responsible for the coupling of electron transfer and ATP synthesis, nerve conduction, metabolite transport, etc.



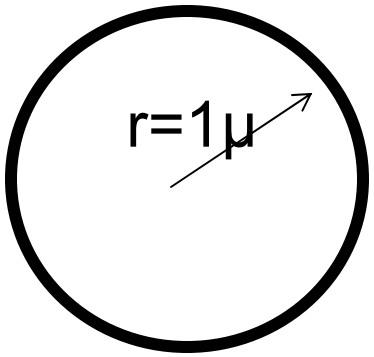
$\Phi_{in\text{-}out,\ K+} = 59 \log (5/100) = -77$ mV

$\Phi_{in\text{-}out,\ Na+} = 59 \log (140/10) = +67$ mV

resting cell membrane primarily permeable to K^+

◀ FIGURE 5.22
Ion and voltage gradients occur typically across cell membranes in plants and animals. These are maintained by active transport.

Charge movement associated with membrane potentials



E field across membrane
~50 MeV/meter

Capacitance C = Q/V (charge/voltage)
~ 1 μF cm^{-2} for cell membranes
V ~ 0.1 V

Q = CV = 10^{-7} Coulombs
~ 10^{-12} mole/cm^2 ~ 10^{12} charges/cm^2

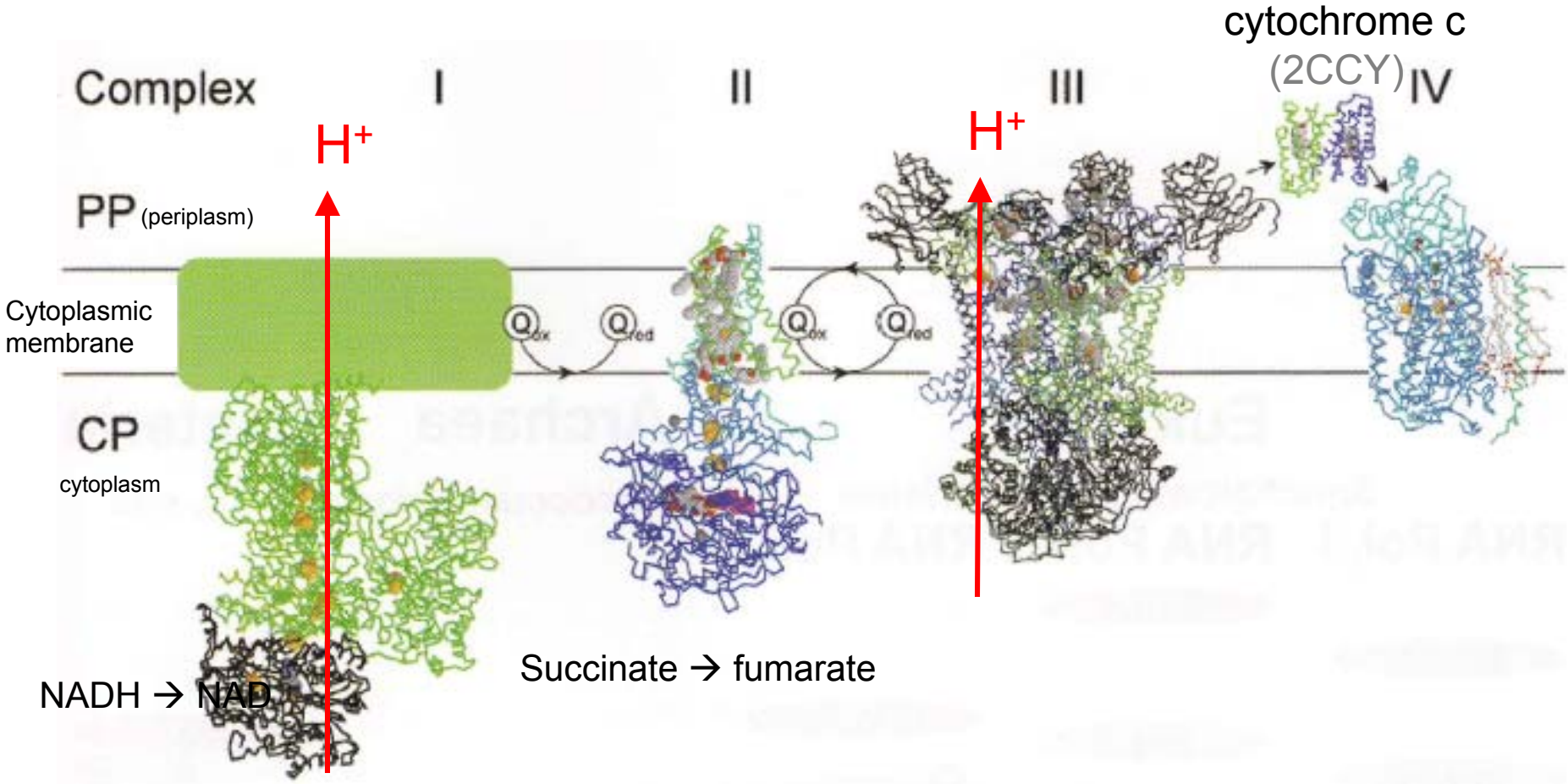
For r = 1 μ = 10^{-4} cm, A = ~10^{-7} cm^2
and V ~ 4 x 10^{-12} cm^3 = 4 x 10^{-15} l

Q = ~ 10^{-19} mole charge in volume V
~ 25 μM

1 charge = 1.602 x 10^{-19} Coulombs; 96487 Coulombs/mole (=F)

Respiratory Chain complexes

in bacteria and mitochondria



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When a proton goes from the outside of the cell to the inside, the change in chemical potential is given by:

$$\Delta\mu_{H^+} = \mu_{H^+_{in}} - \mu_{H^+_{out}}$$

$$= RT \ln \frac{a_{H^+,in}}{a_{H^+,out}} + F\left(\Phi_{in} - \Phi_{out}\right)$$

$$= 2.303\, RT \log \frac{a_{H^+,in}}{a_{H^+,out}} + F\left(\Phi_{in} - \Phi_{out}\right)$$

$$= -2.303\, RT\left(pH_{in} - pH_{out}\right) + F\left(\Phi_{in} - \Phi_{out}\right)$$

$$\equiv -2.303\, RT\Delta pH + F\Delta\Phi$$

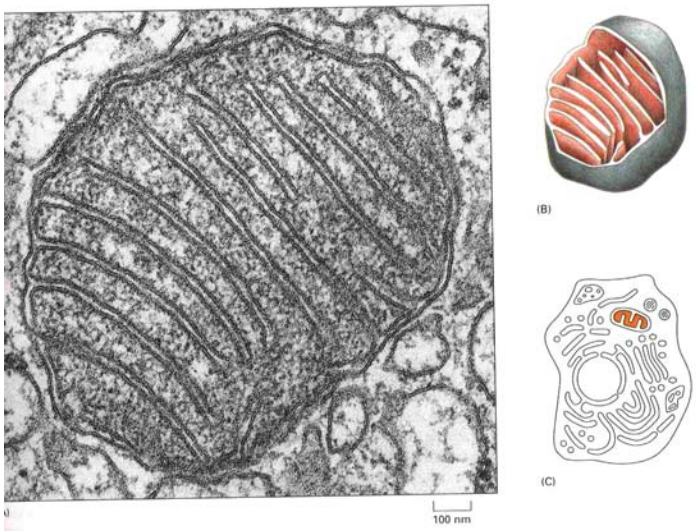
a = activities ~ concentration

pH = -loga_{H+} = -log[H^+]

At 298 K and with $\Delta\Phi$ in mV, this becomes:

$$\Delta\mu_{H^+} = -2.303\, RT\Delta pH + F\Delta\Phi$$

$$= -5.71\,\Delta pH + .0965\,\Delta\Phi \ \text{ kJ/mole}$$



mitochondria (Alberts et al. Essential Cell Biology)

A related quantity is the protonmotive force, $p = \Delta\mu/F$, and has units of (milli)volts

$$p = \Delta\Phi - 59\,\Delta pH \text{ mV}$$

In mitochondria, $\Delta pH \sim 1.4$ and $\Delta\Phi \sim -140$ mV (in – out), giving

$\Delta\mu \sim -21.2$ kJ/mole and $p \sim -220$ mV.

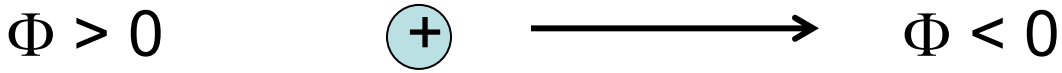
If 3 H^+ are translocated/ATP, then the free energy released is:

$$\begin{aligned}\Delta G &= 3\Delta\mu_{H^+} = 3\left(-5.71(1.4) + .0965(-140)\right) \text{ kJ/mole} \\ &= 3(-8.0 - 13.5) \\ &= -64.5 \text{ kJ/mole}\end{aligned}$$

which is about that required to synthesize ATP under physiological conditions

Electrochemical Equilibria: moving electrons

ion moving down potential gradient



redox reaction: $Zn^0 + Cu^{+2} \rightarrow Zn^{+2} + Cu^0$

An oxidation-reduction reaction is one in which different atoms gain or lose electrons during a reaction; that is, their oxidation state changes. The higher the oxidation state, the more oxidized the atom is, while the lower the oxidation state, the more reduced it is. Hence, **oxidation and reduction can be considered as the loss and gain of electrons, respectively** .

Respiratory processes are fundamentally oxidation-reduction reactions, just like electrochemical cells. The free energy change of an oxidation-reduction reaction is related to the electrical work done by the cell through the equation

$$\Delta G = W_{el} = -n \mathcal{F} \Delta E$$

which is the charge transferred (n $\mathcal{F}$) times the potential difference (ΔE).

The charge transferred is n $\mathcal{F}$ coulombs, where n is the number of moles of electrons and $\mathcal{F}$ is the Faraday, or 96,487 coulombs/mole or 96.49 kJ/V.

Since $\Delta G < 0$ for a spontaneous process, $\Delta E > 0$ for a spontaneous process.

So, electrons prefer to be transferred from donors of lower E values to acceptors of higher E values.

For the reaction: $A_{ox} + B_{red} \rightarrow A_{red} + B_{ox}$

Since: $$\Delta G = \Delta G^o + RT \ln \frac{[A_{red}][B_{ox}]}{[A_{ox}][B_{red}]}$$

with $\Delta G = -n \mathcal{F} \Delta E$

$$\Delta E = \Delta E^o - \frac{RT}{nF} \ln \frac{[A_{red}][B_{ox}]}{[A_{ox}][B_{red}]}$$ (the **Nernst equation**)

where $\Delta E^o = -\Delta G^o / n\mathcal{F}$ = standard reduction potential
= potential difference when all reactants are in the standard state.

at 25˚C, the Nernst equation becomes

$$\Delta E = \Delta E^o - \frac{0.059}{n} \log \frac{[A_{red}][B_{ox}]}{[A_{ox}][B_{red}]} \text{ Volts}$$

Important bioenergetic reactions:

H^+/H_2couple

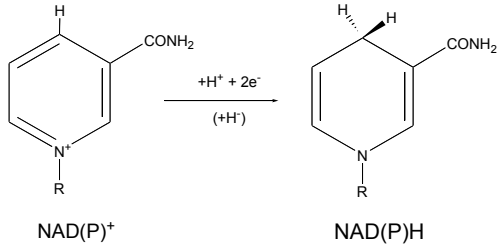
$2H^+ + 2e^- \leftrightarrow H_2$ $\quad E^\circ = 0V \quad E^{\circ\prime} = -0.414\ V$

O_2/H_2O couple:

$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$ $\quad E^\circ = +1.229\ V \quad E^{\circ\prime} = +0.816\ V$

NAD^+/NADH couple

$NAD^+ + 2e^- + H^+ \leftrightarrow NADH$ $\quad E^{\circ\prime} = -0.32\ V$



NAD(P)$^+$ NAD(P)H

Bioenergetic consequences:

- the availability of O_2 as an electron acceptor permitted effective energy metabolisms that could support the development of large, complex organisms.
- the H^+ and O_2 reduction potentials serve as general limits for the electrochemical reactions in biological systems:

-0.414 V to + 0.816 V (at pH 7)

hydrogen is a good reductant; oxygen is a good oxidant

Thermodynamics of bioenergetics: Summary

- Chemical potential u (free energy per mole) of a charged species equals:

$$\mu_i = \mu_i^\circ + RT \ln c_i + z F \Phi$$

where F = Faraday =9.5 kJ/V

- The change in chemical potential of a proton going from outside to inside across a pH gradient and membrane potential difference ΔF (in mV) at 298 K:

$$\Delta\mu_{H^+} = -2.303\ RT\ \Delta pH + F\ \Delta\Phi$$
$$= -5.71\ \Delta pH + 0.0965\ \Delta\Phi \quad \text{(kJ/mole)}$$

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convention - thermodynamically favored direction - electrons transferred from groups with lower E to higher E

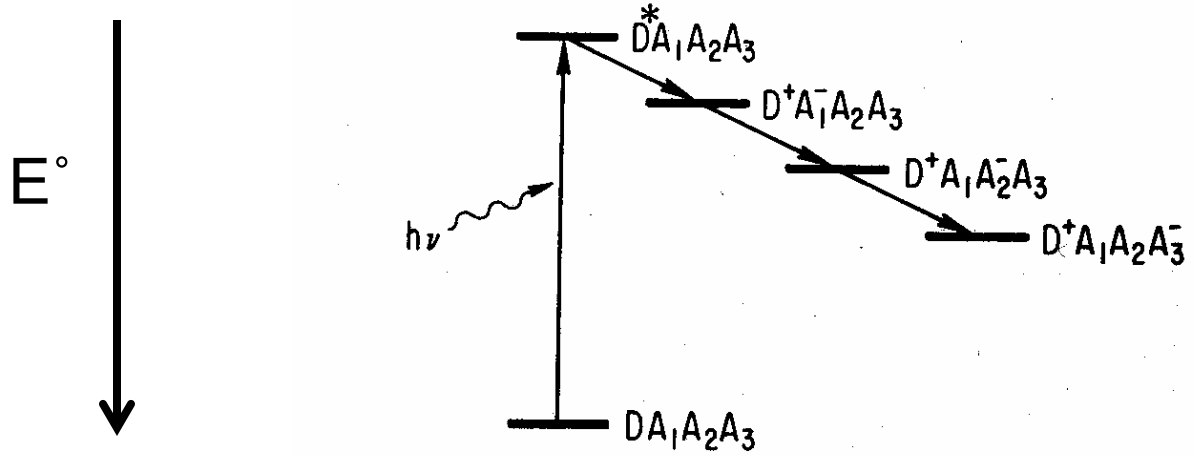
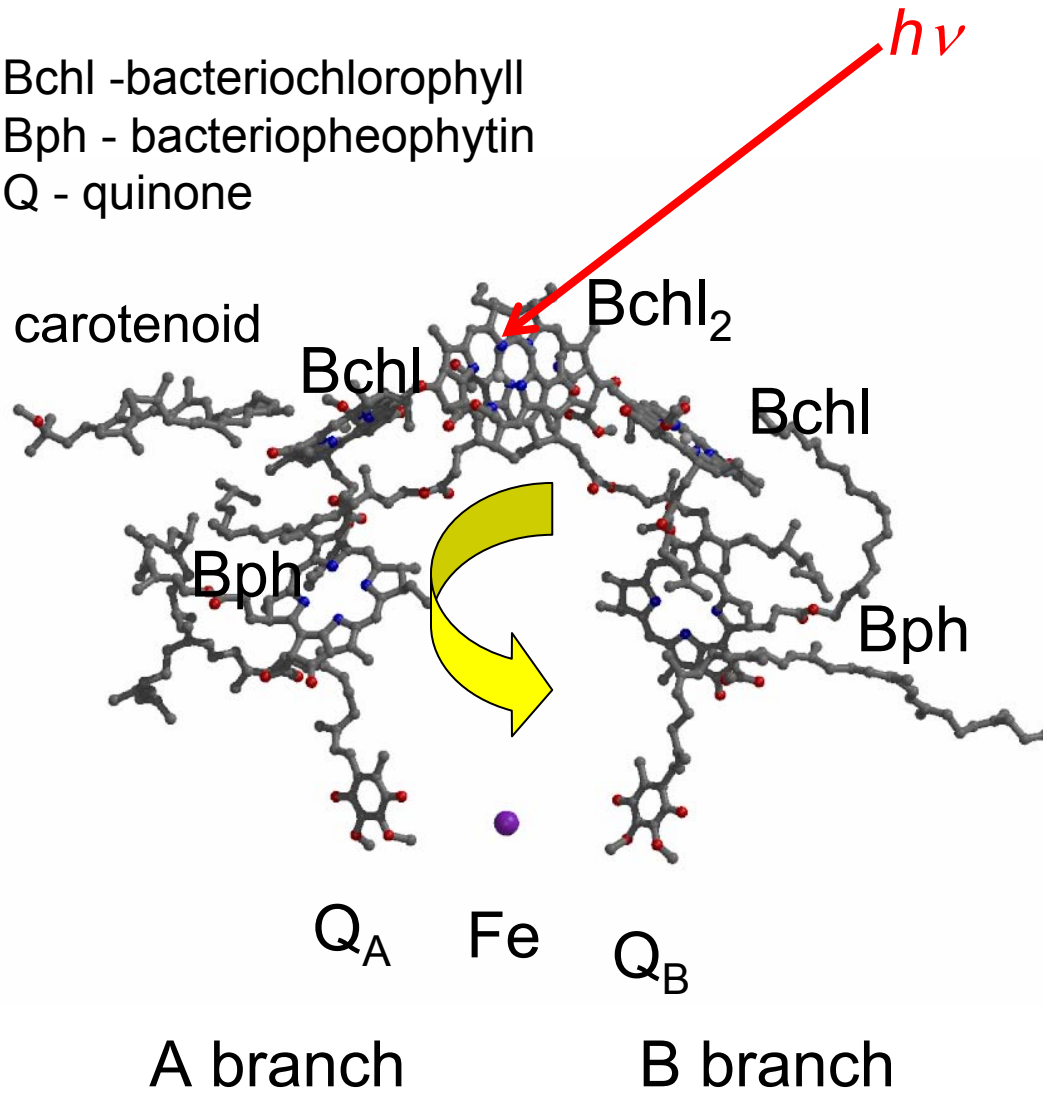
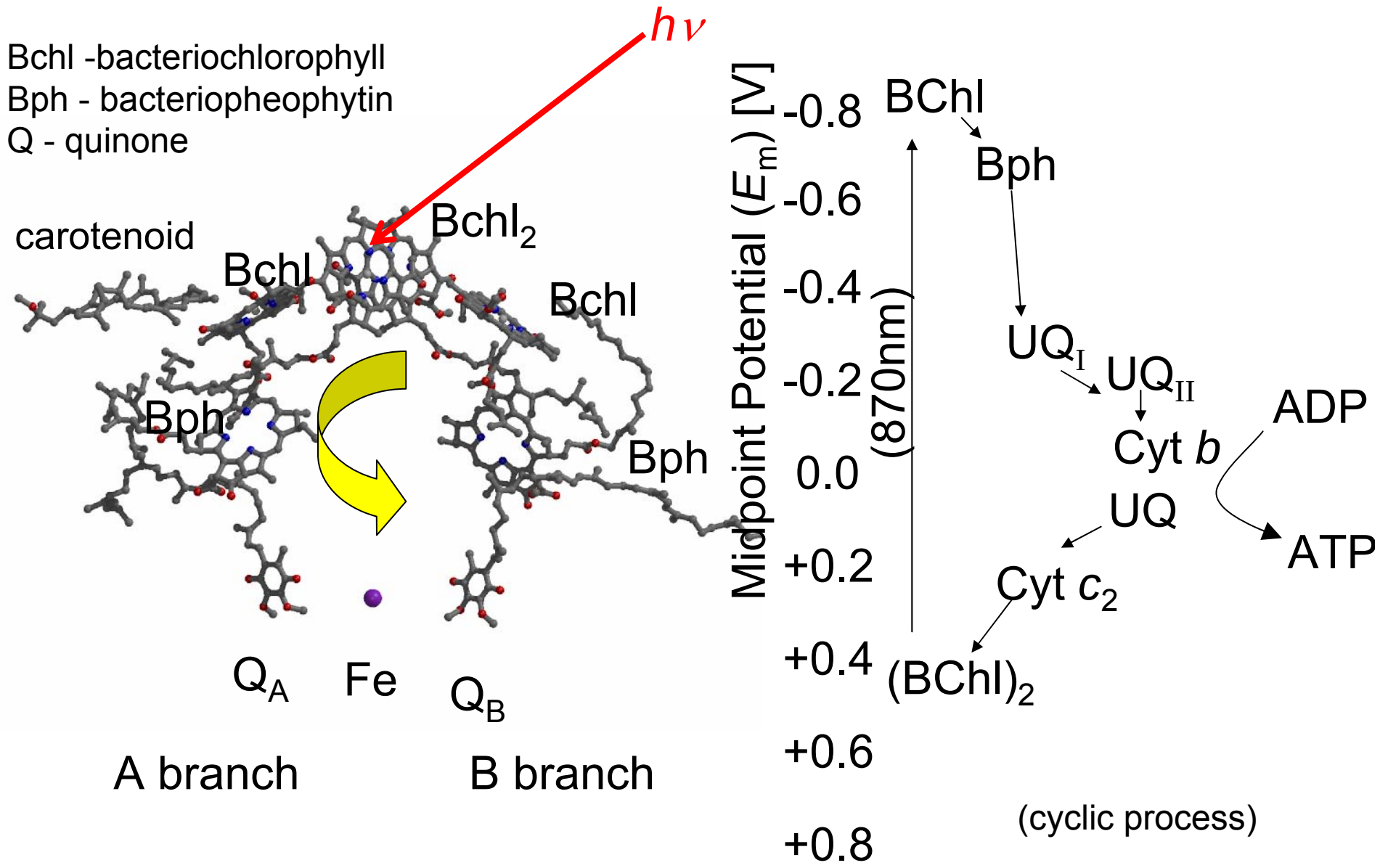


FIG. 1 Schematic representation of the initial charge separation in bacterial photosynthesis. The absorption of a photon is followed by ejection of an electron from the excited donor D*. The electron is transferred to the acceptor A_1 and then shuttled through a series of acceptors $A_2, A_3, \ldots$ This is shown in more detail in Fig. 2. The minimum unit capable of producing the charge separation is called the reaction centre.

Bchl -bacteriochlorophyll
Bph - bacteriopheophytin
Q - quinone

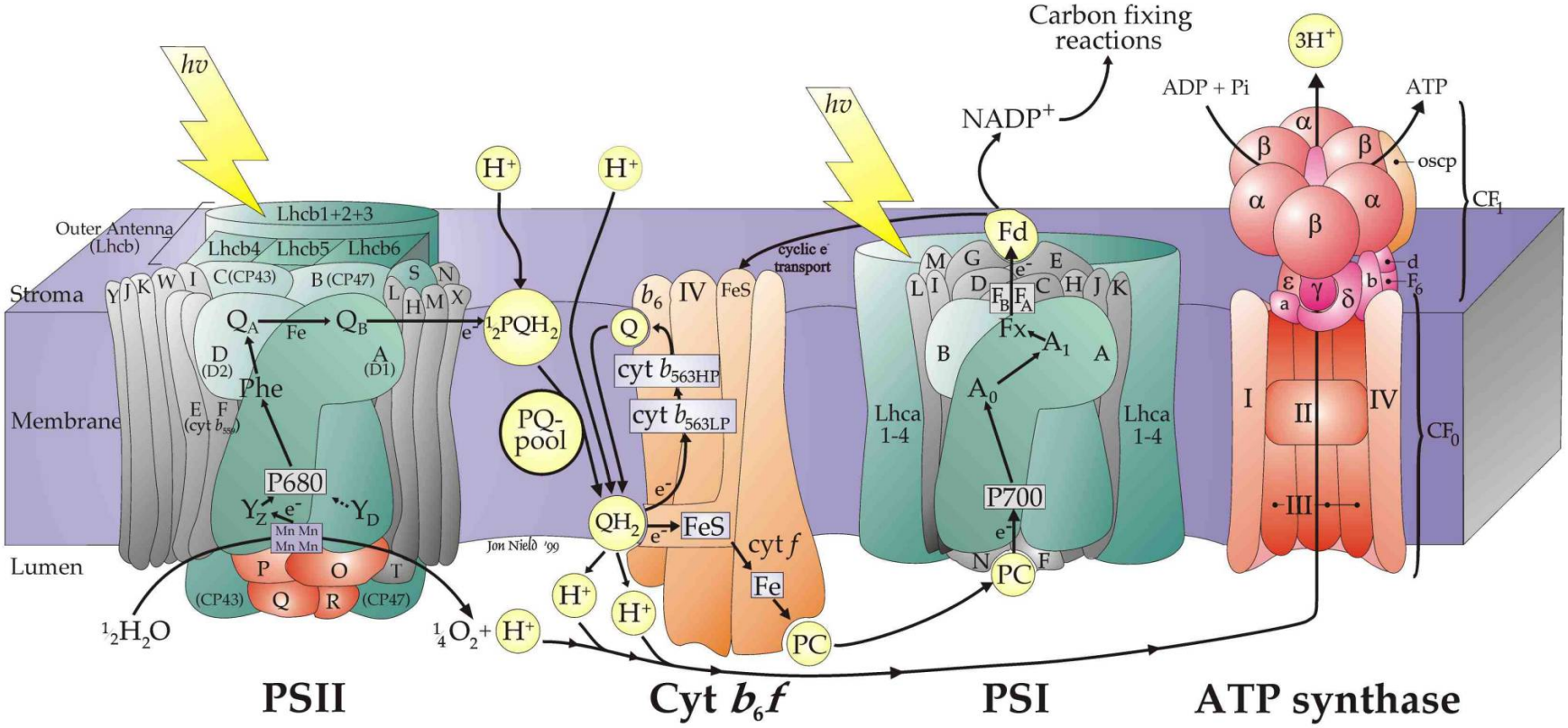


Feher *et al. Nature* **339**, 111 (1989)



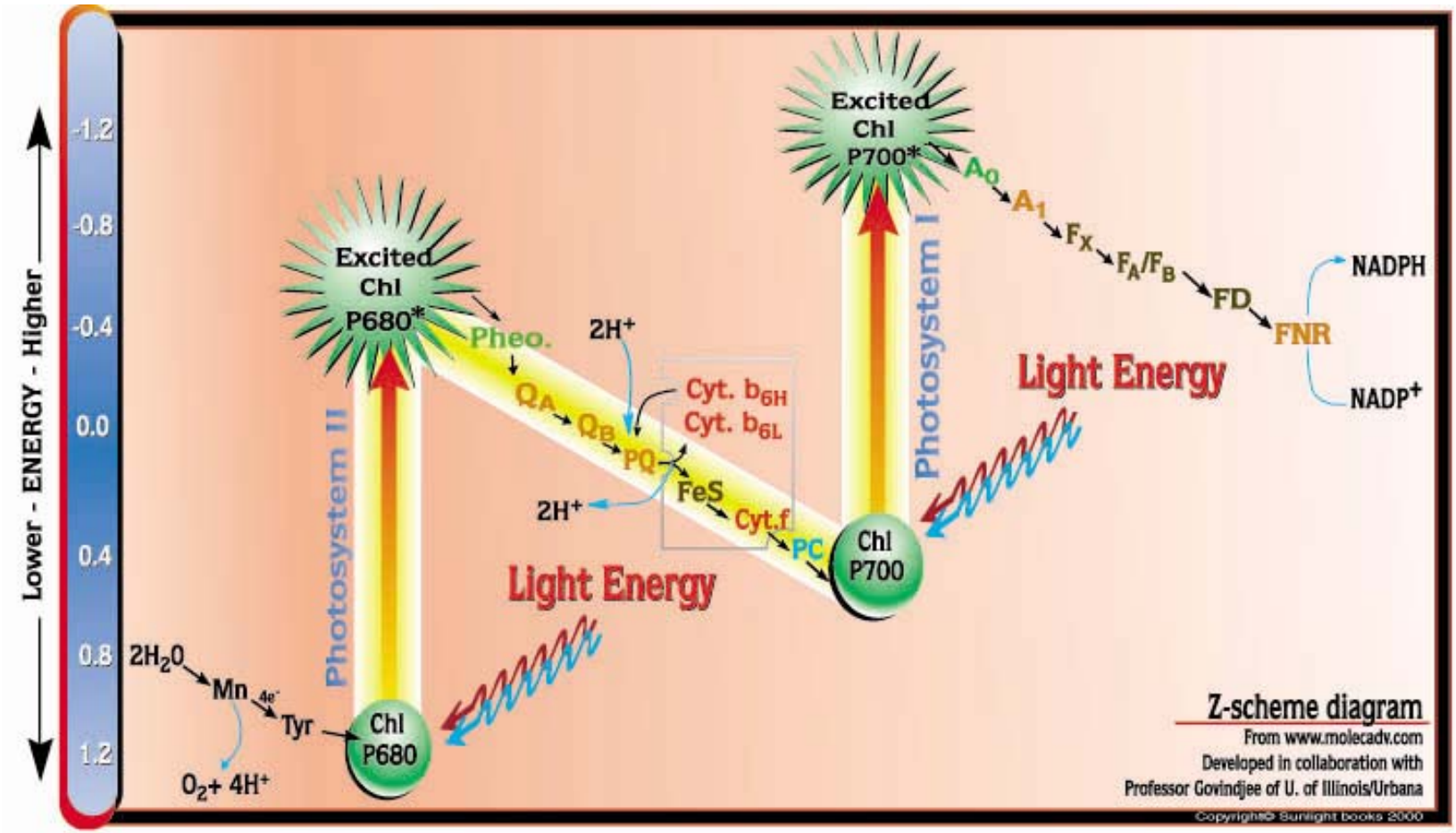
Feher *et al. Nature* **339**, 111 (1989)

organization of photosynthetic electron transfer assembly

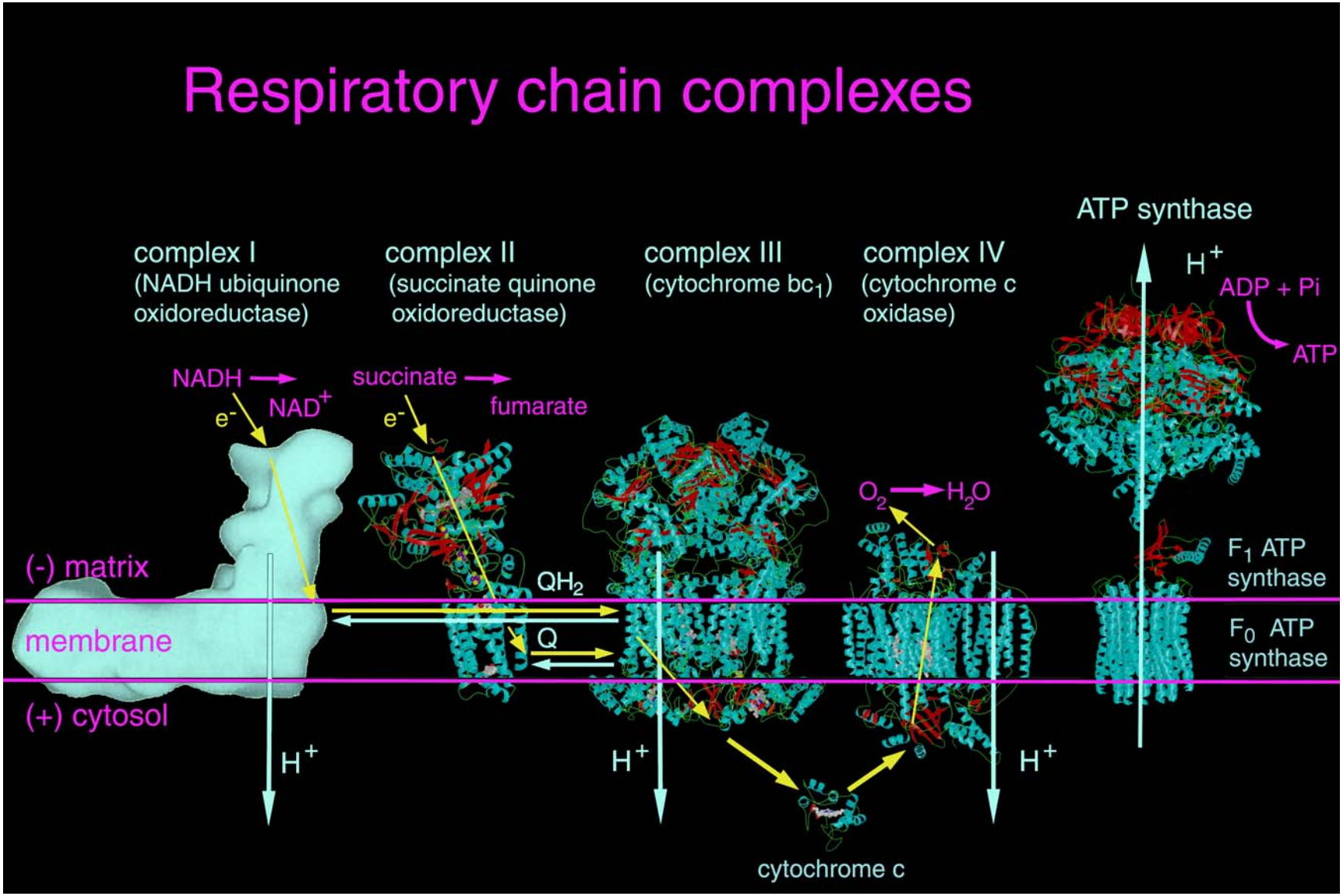


ATP

http://www.bio.ic.ac.uk/research/barber/photosystemII.html

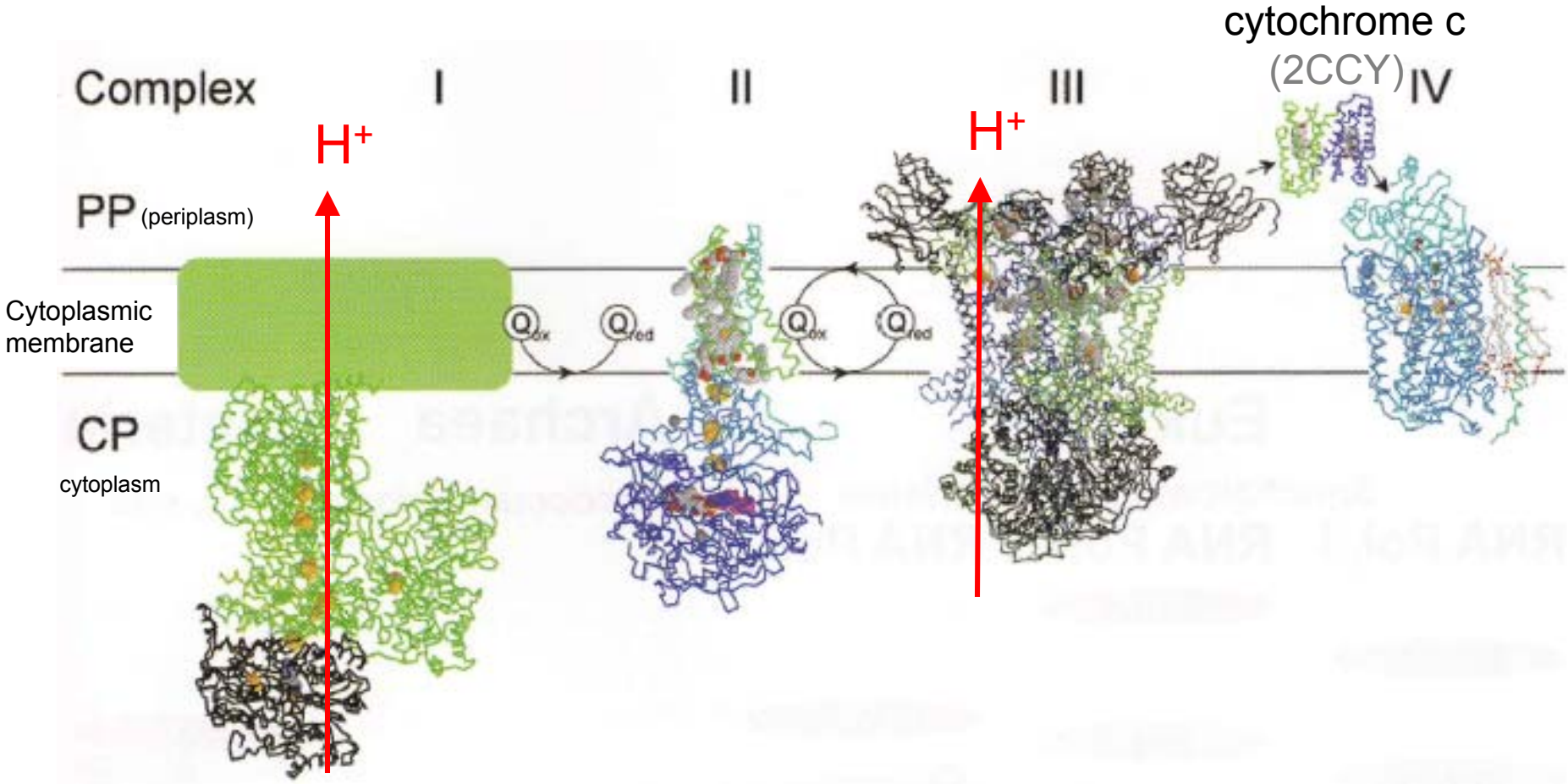


noncyclic process


Respiratory chain complexes
complex I
(NADH ubiquinone
oxidoreductase)
complex II
(succinate quinone
oxidoreductase)
complex III
(cytochrome bc1)
complex IV
(cytochrome c
oxidase)
ATP synthase
NADH
NAD+
e-
succinate
fumarate
e-
H+
ADP + Pi
ATP
O2
H2O
(-) matrix
membrane
(+) cytosol
QH2
Q
F1 ATP
synthase
F0 ATP
synthase
H+
H+
H+
cytochrome c

Respiratory Chain complexes

in bacteria and mitochondria



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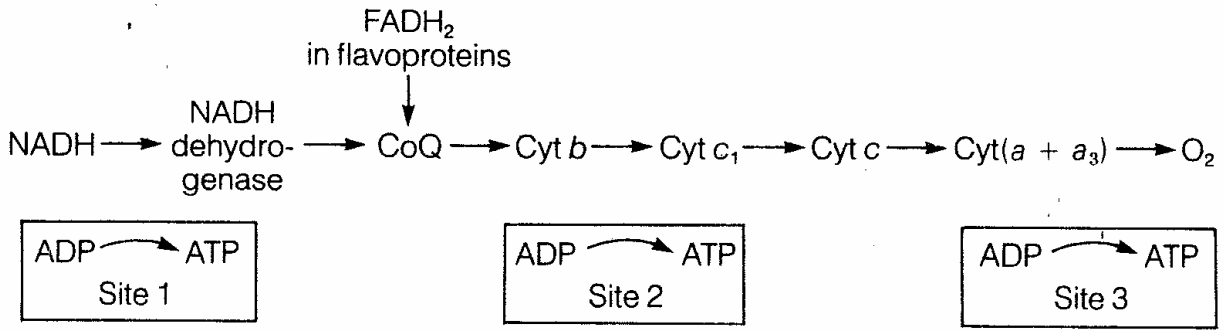
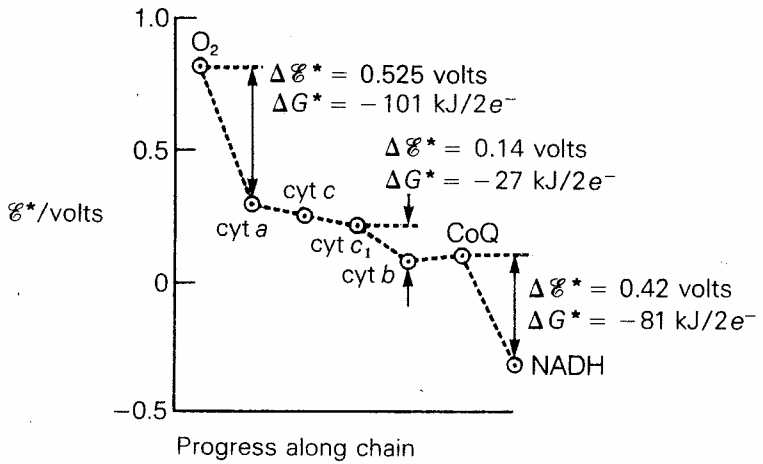


FIGURE 9-3
Sequence of electron carriers in the respiratory chain. Electrons are transferred either from NADH or from the coenzyme $FADH_2$ in flavoproteins through coenzyme Q and the cytochromes to molecular oxygen. NADH is oxidized to NAD^+, and O_2 is reduced to H_2O, while the intermediates undergo cyclic oxidation and reduction. (From *Biochemistry* by Lubert Stryer. W. H. Freeman and Company. Copyright © 1975.)



thermodynamics of ATP

$\Delta G^{\circ\prime} = -30$ kJ/mole

$\Delta G = -53$ kJ/mole

$\Delta E^{\circ\prime} = -\Delta G^{\circ\prime}/2F = 0.16$ V

$\Delta E = -\Delta G/2F = 0.27$ V

FIGURE 9-4
Variation of standard electrode potentials in the electron transport chain. The standard potential differences and free-energy changes per two electrons transferred are shown for the three sites of ATP formation.

Aerobic respiration is favorable because oxygen is a strong oxidant

ΔE for respiratory chain is 1.1V (NADH to O_2)

$NAD^+/NADH \rightarrow$ nitrate/nitrite	E° from -32 to +42 mV
$NADH \rightarrow$ fumarate	E° from -0.32 to 0.033 mV
$H^+/H_2 \rightarrow$ fumarate	E° from -0.42 to 0.033 mV

		$\Delta G^{\circ\prime}$
(acetogens)	$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$	-107.1 kJ/mol
	$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	-484.4
(methanogens)	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-131 kJ/mol
	$CH_3COOH \rightarrow CH_4 + CO_2$	-37

Methane biosynthesis

- methane(CH_4), the most reduced organic compound, is a major energy source and a greenhouse gas
- synthesized biologically from CO_2 and H_2 by **methanogens**

methane formation is terminal step of an anaerobic food chain -

abundant in marshes (swamp gas), sediments, and sites of waste material biodegradation (landfills)

this process does not involve a respiratory chain coupled to reduction

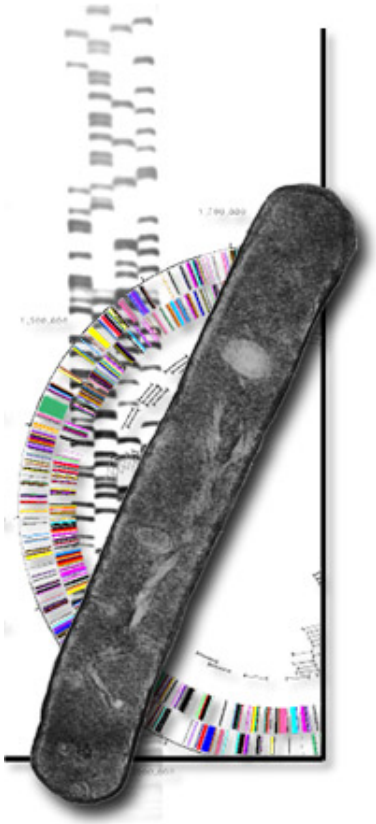
of O_2 or nitrate;

hence the energy yields (ATP formation) are poor

Methane biosynthesis in

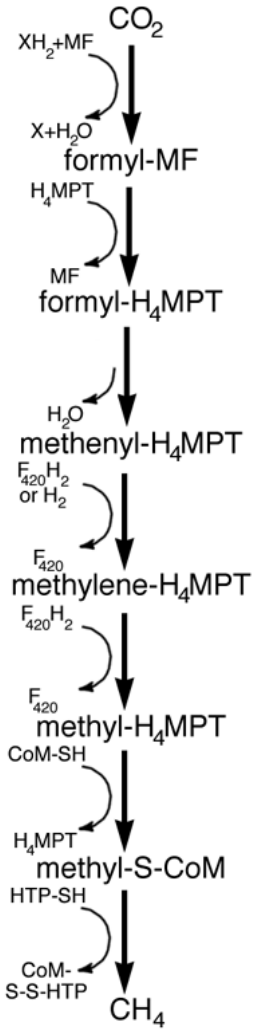
Methanobacterium thermoautotrophicum

(homepage: www.biosci.ohio-state.edu/~genomes/mthermo/)



M. thermoautotrophicum is an obligate chemolithotrophic methanogen - uses an inorganic electron donor (H_2) and CO_2 as the carbon source - does not require organic substrates.

CH_4 synthesized from CO_2 and H_2 (ultimately) in a series of seven chemically remarkable steps



Original Volta experiment (1776)

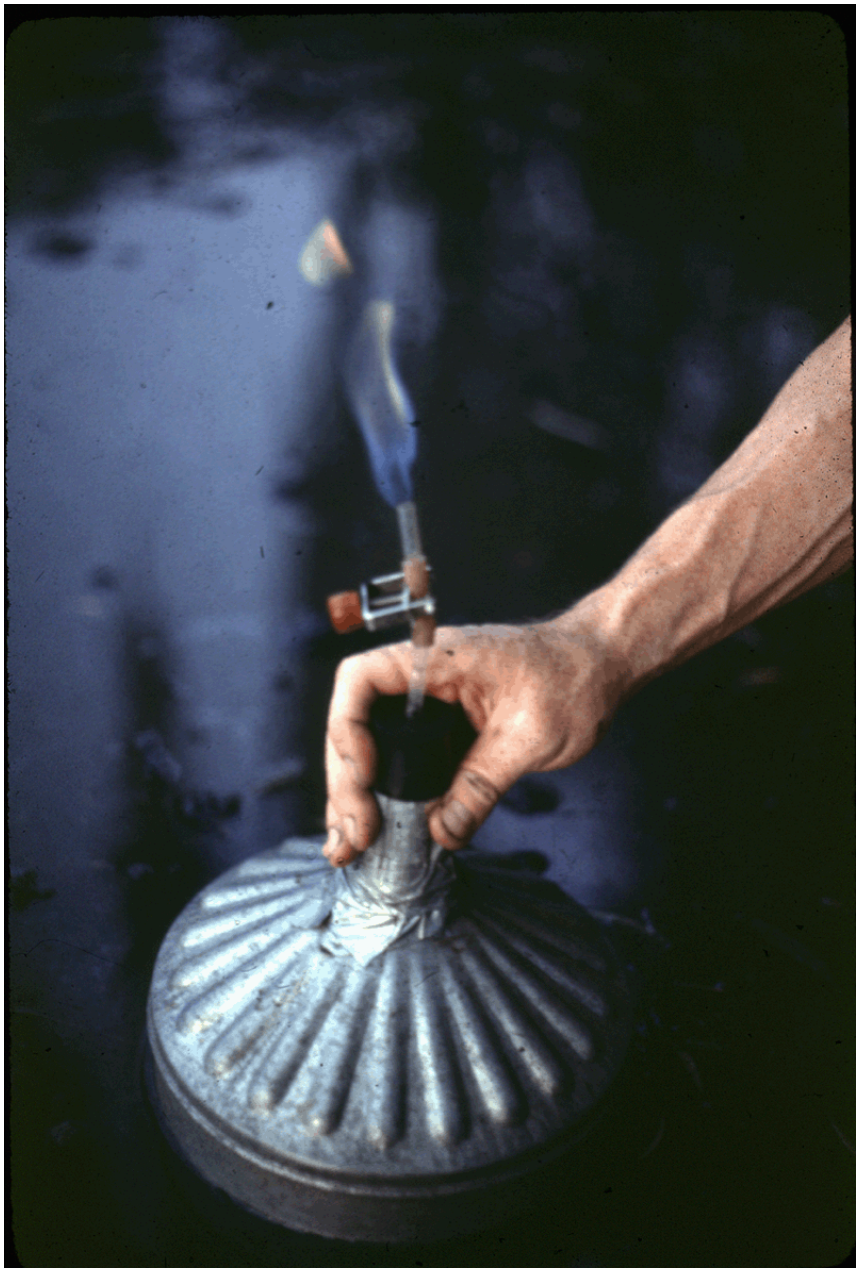
Alessandro Volta

Lake Como (freshwater)

courtesy of J.G. Ferry, Penn State

Modern Volta experiment (Part I)

courtesy of J.G. Ferry, Penn State



Modern Volta experiment (Part II)

one-shot Volta-funnel experiments

courtesy of J.G. Ferry, Penn State

courtesy of Dianne Newman, MIT

CH_4 formed by sequential reduction of CO_2

$CO_2 + H^+ + 2e^- \leftrightarrow HCOO^-$ $E^{\circ\prime} = -432$ mV

$HCOO^- + 3H^+ + 2e^- \leftrightarrow H_2CO + H_2O$ $E^{\circ\prime} = -535$ mV

$H_2CO + 2H^+ + 2e^- \leftrightarrow CH_3OH$ $E^{\circ\prime} = -182$ mV

$CH_3OH + 2H^+ + 2e^- \leftrightarrow \mathbf{CH_4} + H_2O$ $E^{\circ\prime} = +169$ mV

coupled to oxidation of H_2

$2H^+ + 2e^- \leftrightarrow H_2$ $E^{\circ\prime} = -414$ mV

overall reaction

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ $\Delta G^{\circ\prime} = -131$ kJ/mole

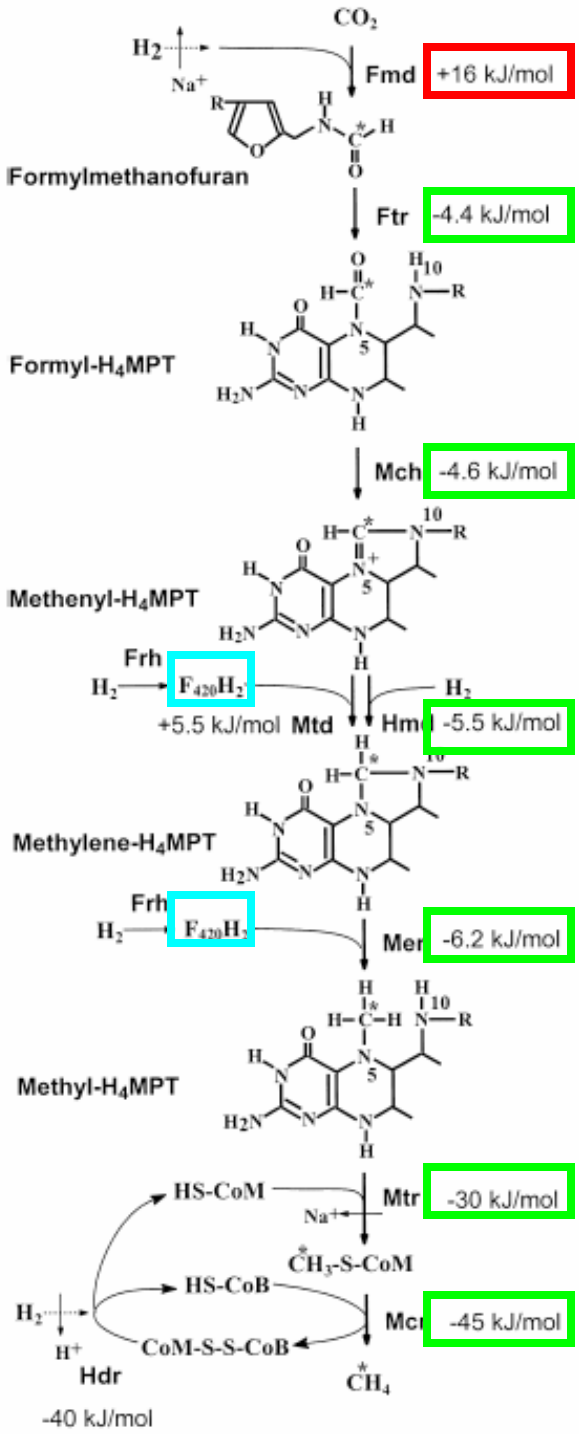
where does H_2 come from? anaerobic fermentation of reduced compounds:

$CH_3CH_2OH + H_2O \leftrightarrow CH_3COO^- + H^+ + 2H_2$ $\Delta G^{\circ\prime} = +9.6$ kJ/mole

(obligate syntrophy)

- overall $\Delta G^{\circ\prime} = -131$ kJ/mole (but, $\Delta G \sim -30$ kJ/mole since $(H_2) \sim 10$ μM);
  $\therefore$ expect ~ 0.6 ATP / CH_4 produced

Energetics of biological methanogenesis



Shima *et al. J. Biosci. Bioeng*. **93**, 519 (2002)

methylotrophic methanogens ferment acetate

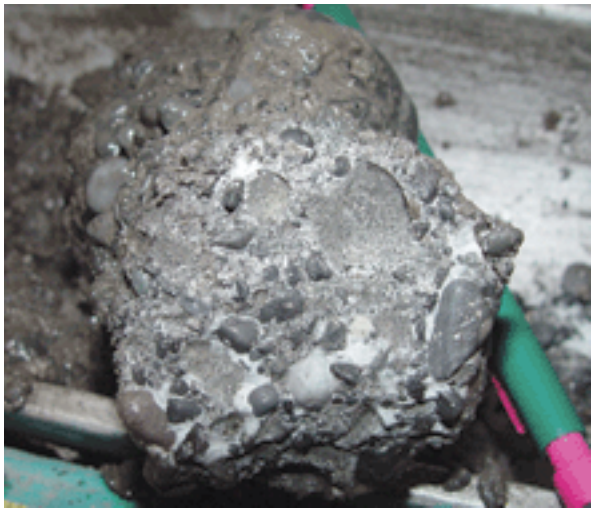
$CH_3COOH \rightarrow CH_4 + CO_2$ ($\Delta G^{\circ\prime} = -37$ kJ/mole)

culture of *Methanosarcina thermophila* metabolizing acetate to CO_2 and CH_4

courtesy of J.G. Ferry, Penn State

Methane - hydrates

formed when methane from organic decomposition comes together with water at low enough temperatures and high enough pressures to form clathrate - type structures

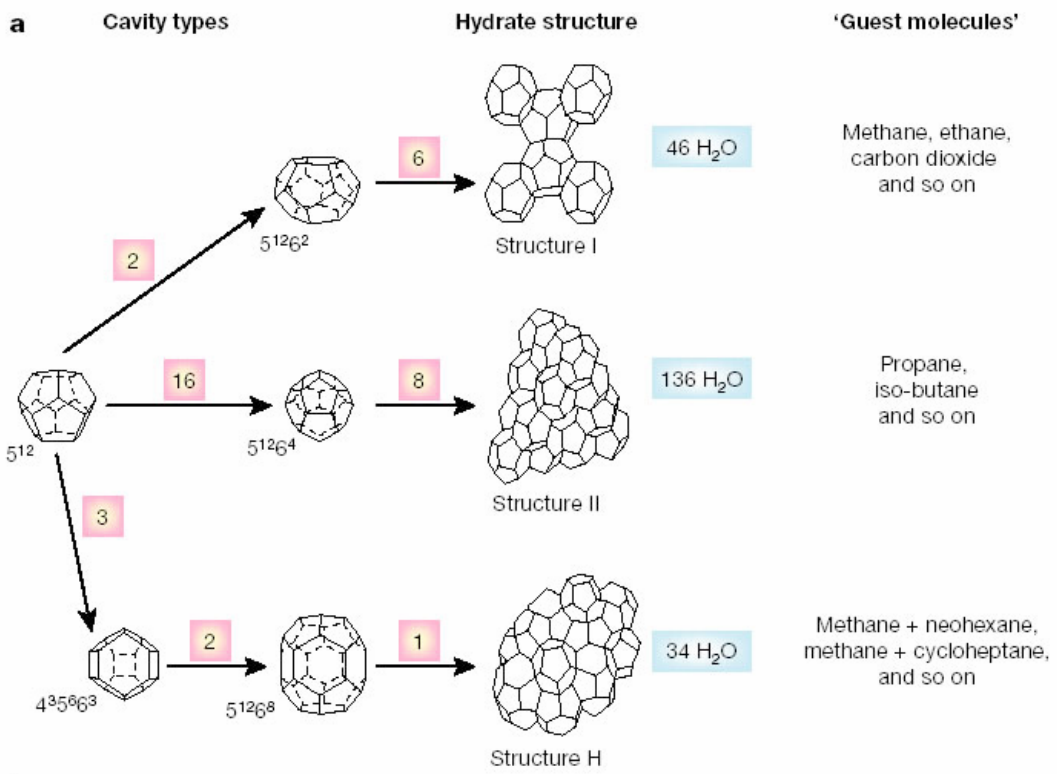


The mother lode. Sand and gravel from a kilometer beneath the Arctic surface is laced with gas-charged hydrate (white infilling).

estimated reserves up to perhaps 100x those recoverable from all the world's natural gas deposits

Kerr, *Science* **303**, 946 (2004)

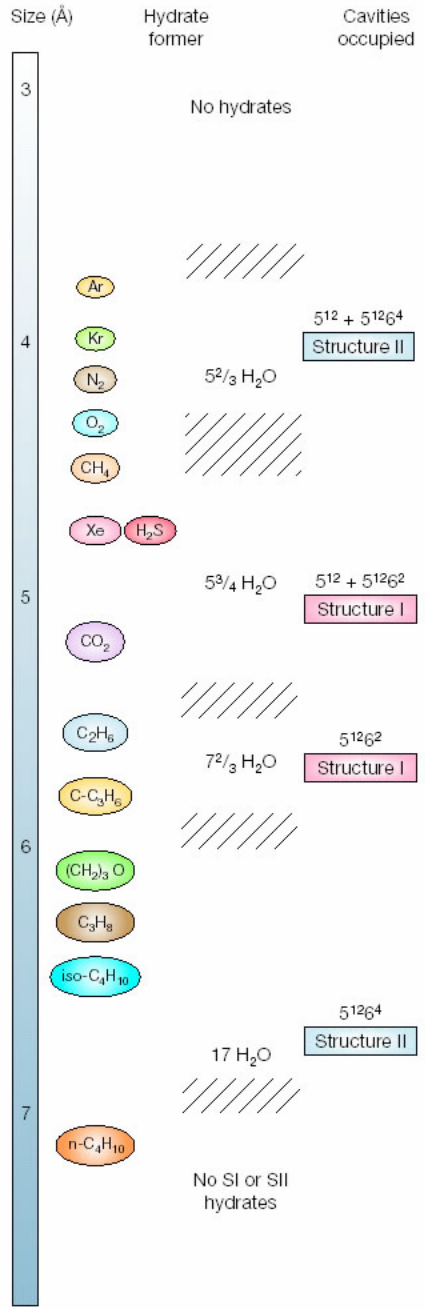
Hydrate structures



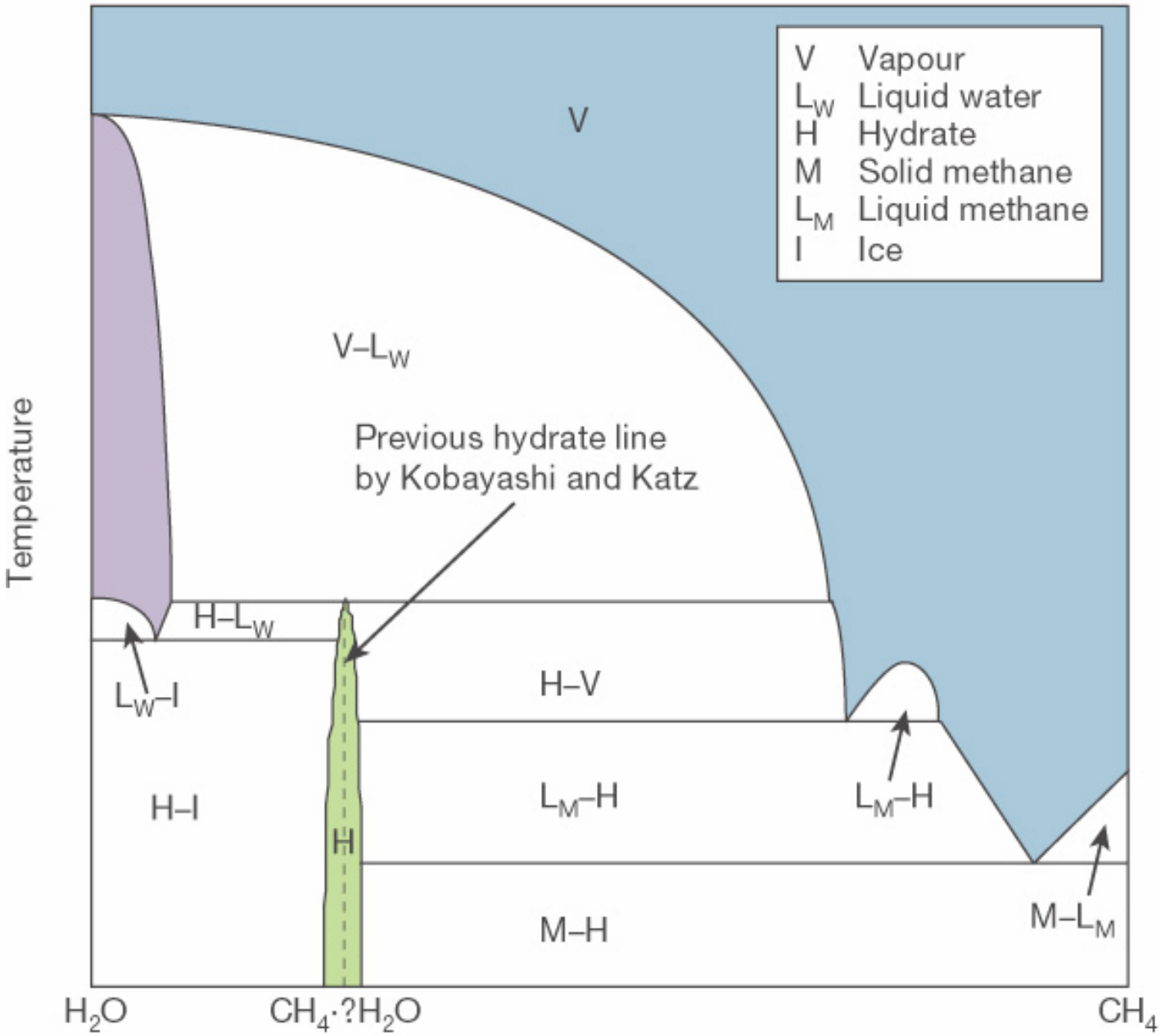
b

Hydrate crystal structure	I		II		H		
Cavity	Small	Large	Small	Large	Small	Medium	Large
Description	5^{12}	$5^{12}6^2$	5^{12}	$5^{12}6^4$	5^{12}	$4^35^66^3$	$5^{12}6^8$
Number of cavities per unit cell	2	6	16	8	3	2	1
Average cavity radius (Å)	3.95	4.33	3.91	4.73	3.91†	4.06†	5.71†
Coordination number*	20	24	20	28	20	20	36
Number of waters per unit cell	46		136		34		

*Number of oxygens at the periphery of each cavity.
†Estimates of structure H cavities from geometric models.



Sloan, *Nature* **426**, 353 (2003)



isobaric methane-water phase diagram

Sloan, *Nature* **426**, 353 (2003)

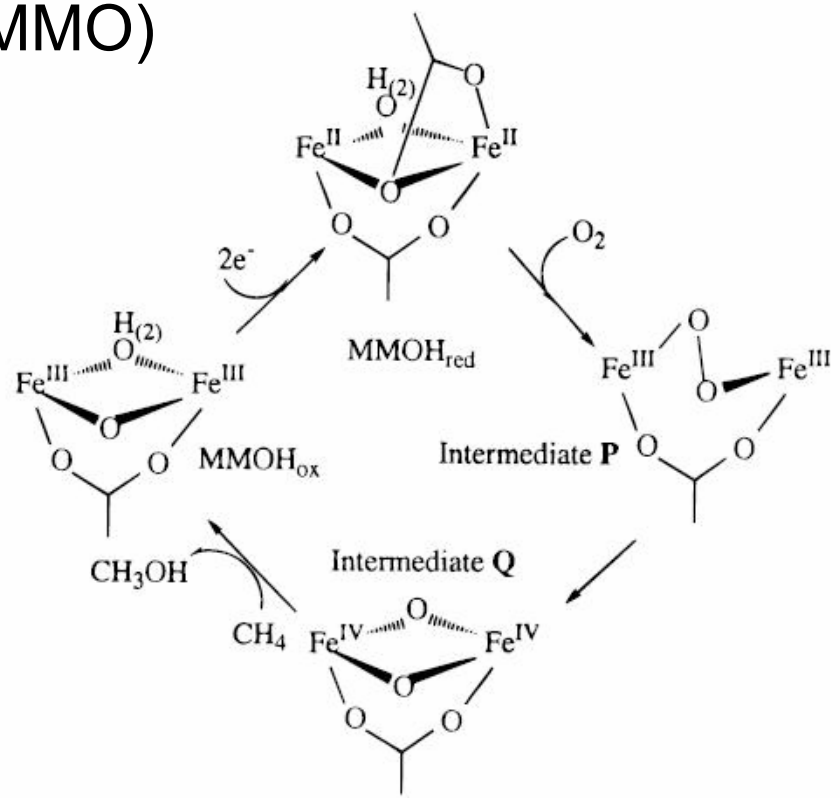
Methanotrophs

$CH_4 + O_2 \rightarrow CH_3OH$

Catalysts for this reaction would be very valuable!

- Methanotrophs use methane, and other 1 carbon molecules as sole carbon source.
- Methane monooxygenase (MMO)
  - sMMO
  - pMMO
- Three subunits, contains a di-iron center
- Enzyme is slow!!

  Rate is on the order of seconds



Global Carbon Cycle