Fotosyntéza

②

Ondřej Prášil

prasil@alga.cz
384-340430

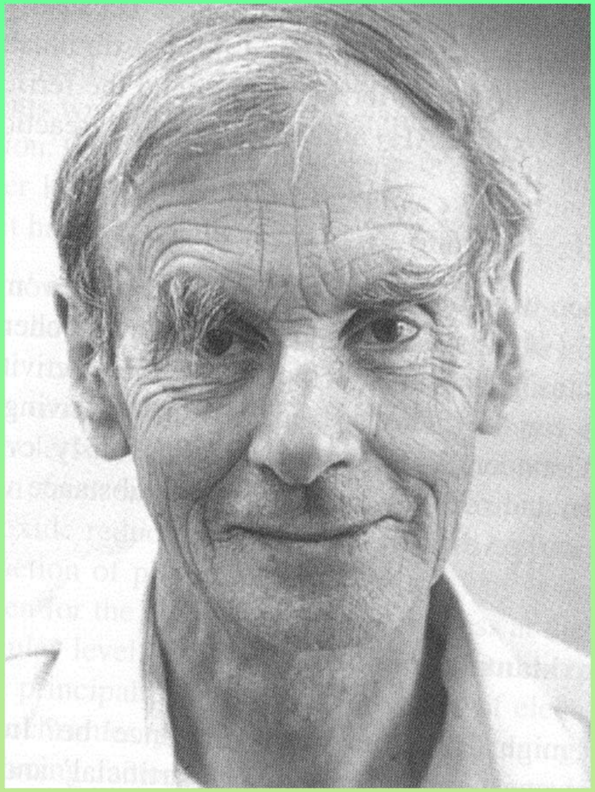
Fotosyntéza: světlem indukované oxidačně redukční reakce

Cornelius van Niel, 30.léta 20.století
Holandský mikrobiolog, Stanford University, Kalifornie

$CO_2 + 2H_2A \rightarrow (CH_2O) + 2A + H_2A$

2 oddělené redoxní reakce
$2H_2A \rightarrow 2A + 4e^- + 4H^+$
$CO_2 + 4e^- + 4H^+ \rightarrow (CH_2O) + H_2A$

Robert Hill, Cambridge 1930



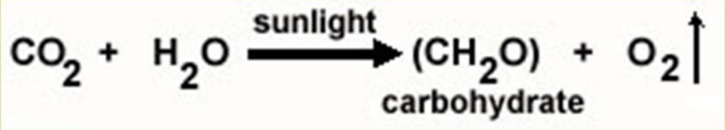
Obnovení fotosyntetických aktivit v izolovaných chloroplastech po přidání akceptorů elektronů

Fotosyntéza je **redoxní** reakcí

Kyslík pochází z oxidace vody, ne z CO_2

$2H_2O + 4Fe^{3+} \rightarrow O_2 + 4Fe^{2+} + 4H^+$

Měřil kyslík změnou barvy hemoglobinu



$$CO_2 + H_2O \xrightarrow{\text{sunlight}} \underset{\text{carbohydrate}}{(CH_2O)} + O_2\uparrow$$

Maximální kvantový výtěžek vývoje kyslíku

Otto Warburg

Proč je důležitý?
-stanovuje maximální možnosti
-ukazuje na omezení ve využití světla

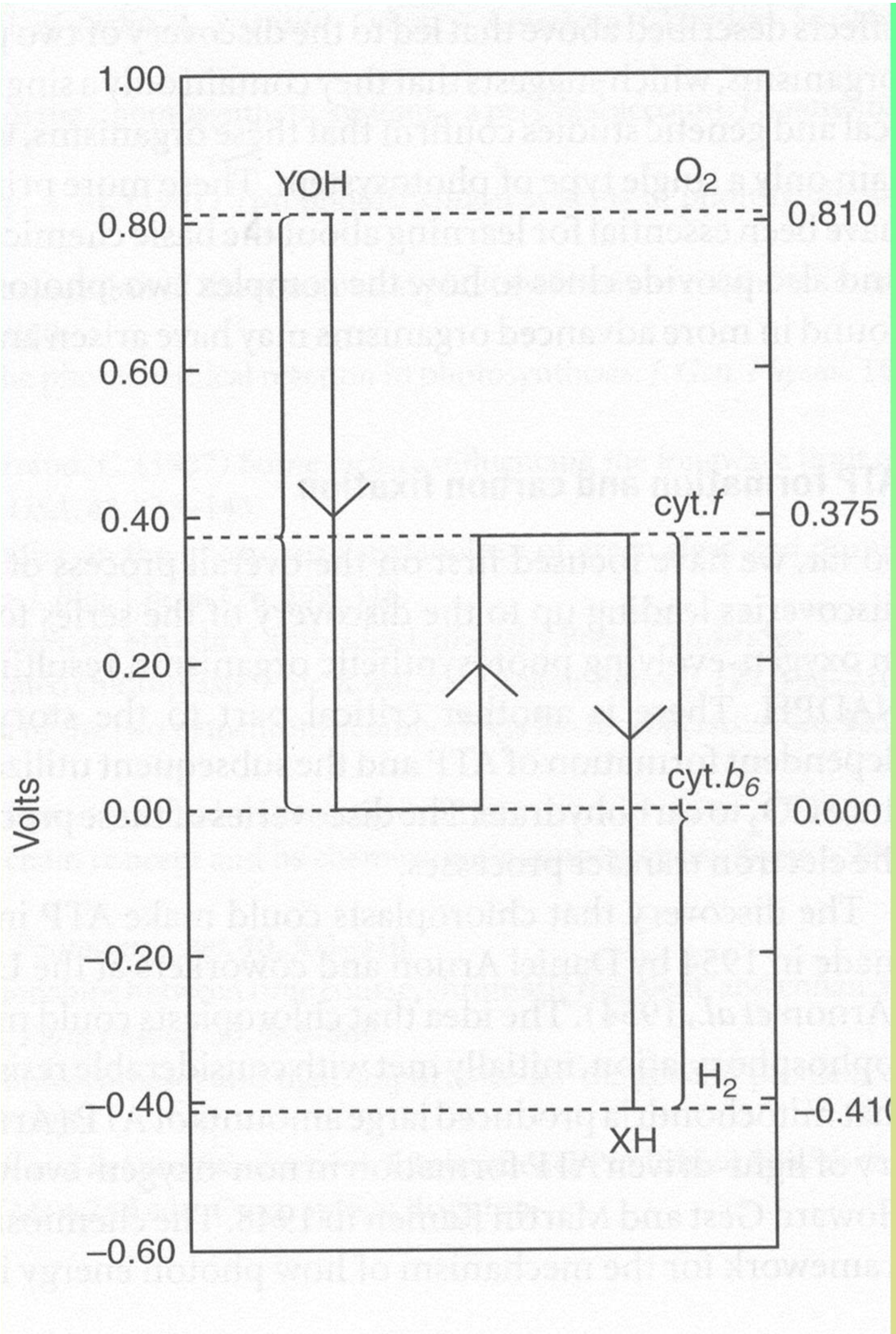
definice Φ = moly produktu / moly absorbovaných fotonů
kvantový požadavek = $1/\Phi$
kvantová účinnost (λ) : energie uložená v produktu / energie absorbovaná

Maximální kvantový výtěžek

Otto Warburg, 20.léta: $\Phi = 0.25$ q/CO_2 (4 fotony, jedna fotoreakce)

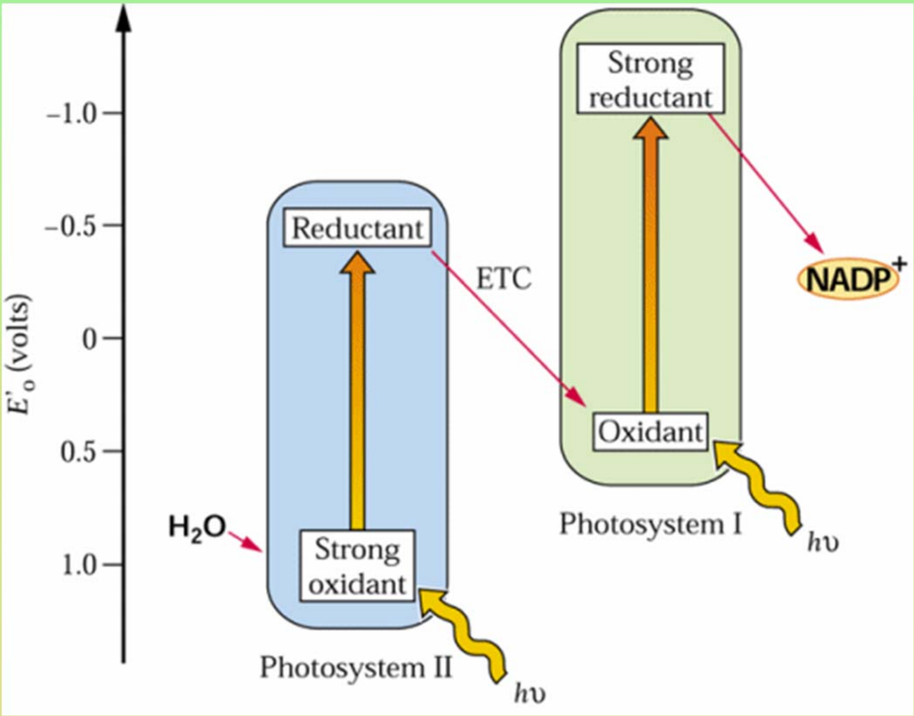
Emerson, 50.léta: $\Phi = 0.12 - 0.10$ q/CO2 (8 fotonů, 2 fotosystémy)

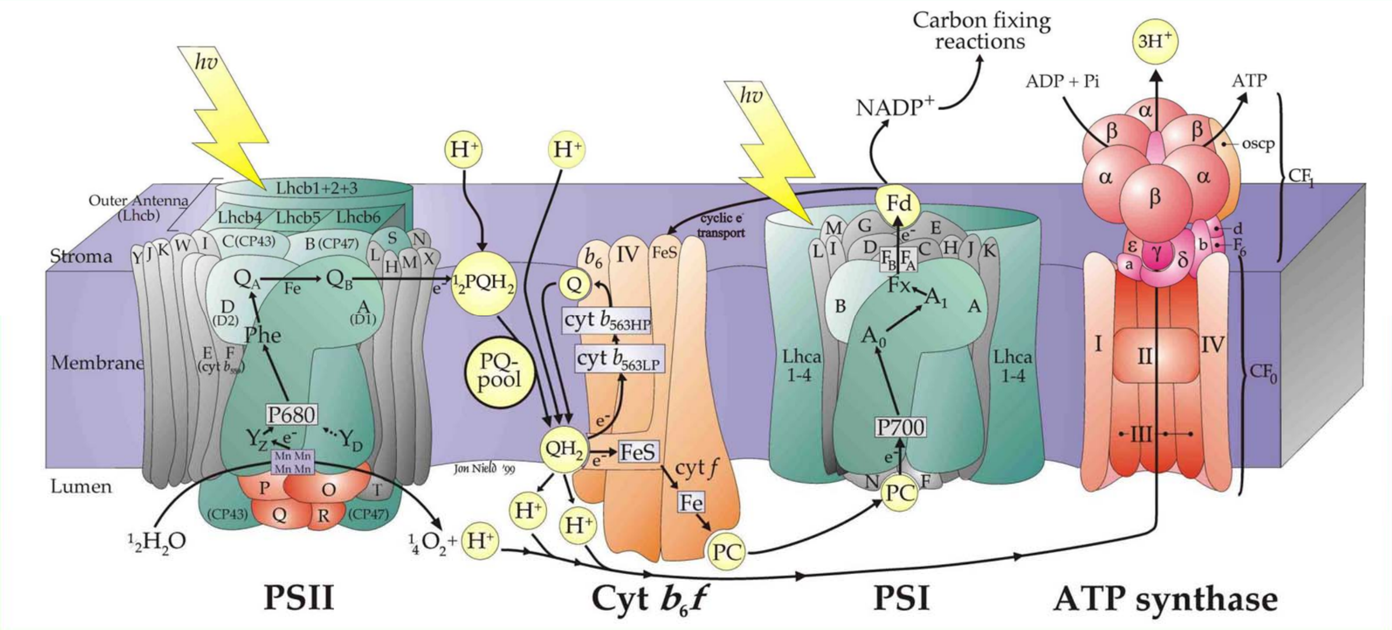
Z (zig-zag) schéma fotosyntézy

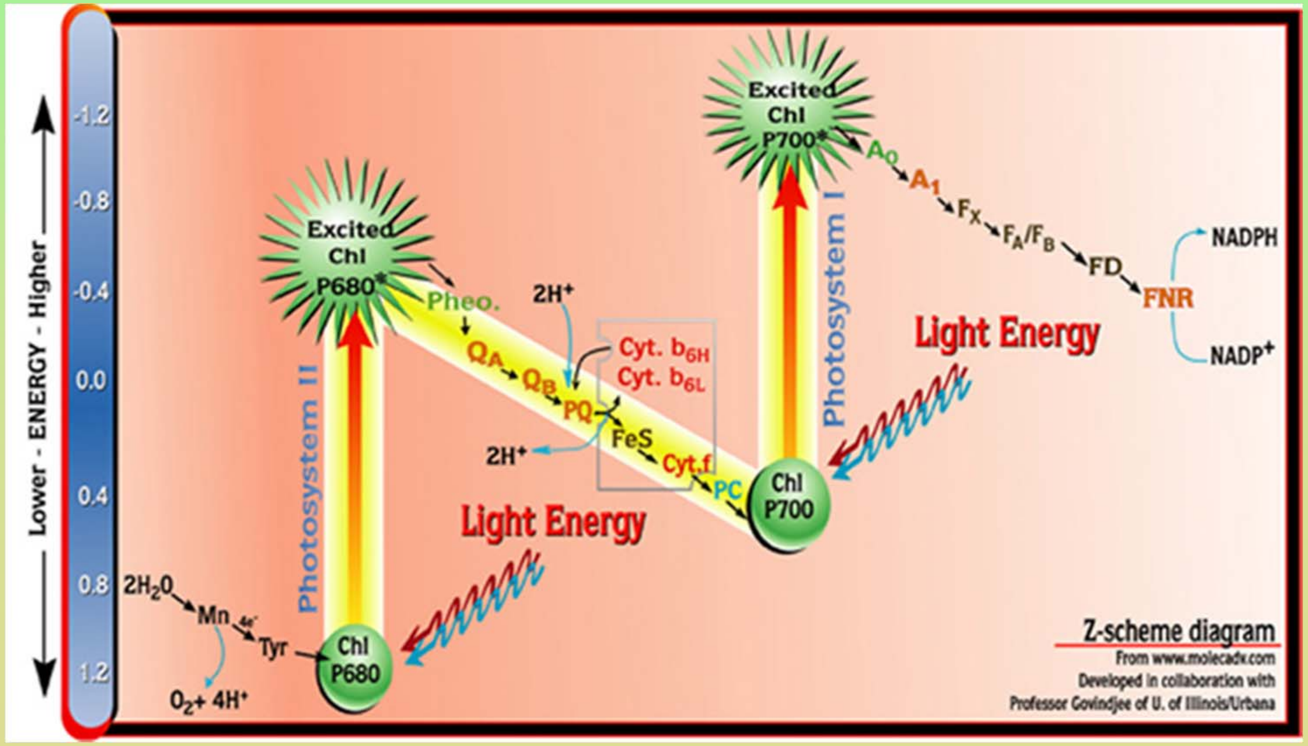


Robert Hill a Fay Bendall, 1960
Duysens 1961

2 reakce v tandemu



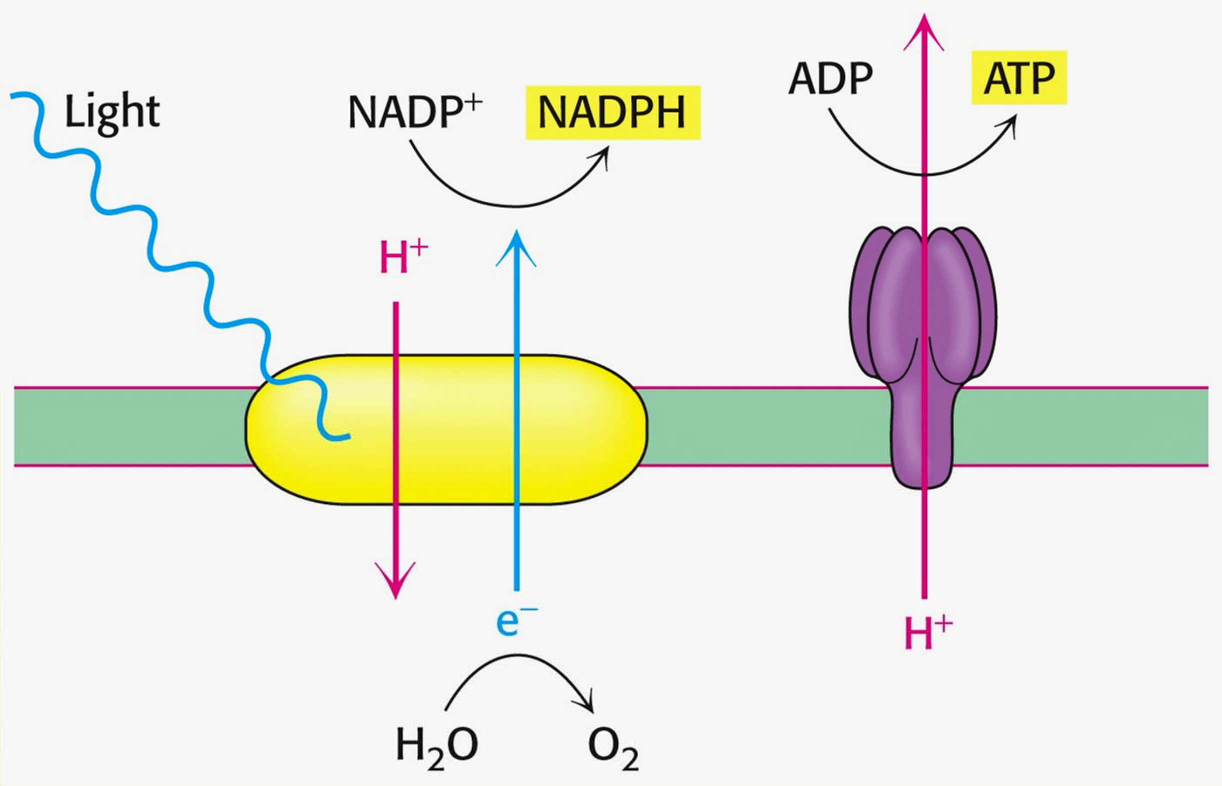




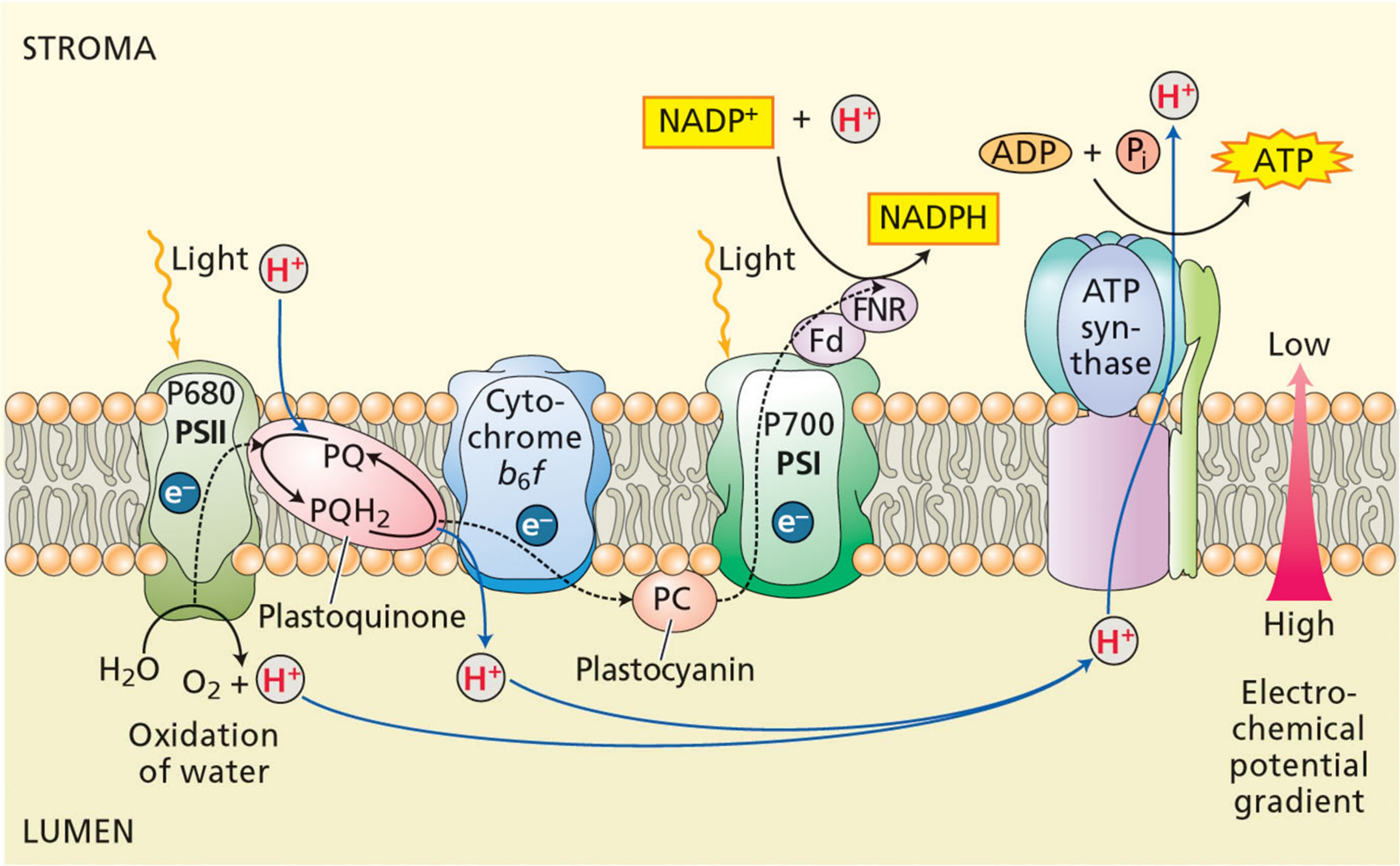
Z-scheme diagram

From www.molecadv.com

Developed in collaboration with Professor Govindjee of U. of Illinois/Urbana během „světelných“ reakcí fotosyntézy je energie pohlcená pigmenty transformovaná do energie elektronů a využita k redukci universálního nosiče redukčních ekvivalentů NADP a k tvorbě univerzálního buněčného paliva ATP



Vektoriální přenos elektronů a protonů



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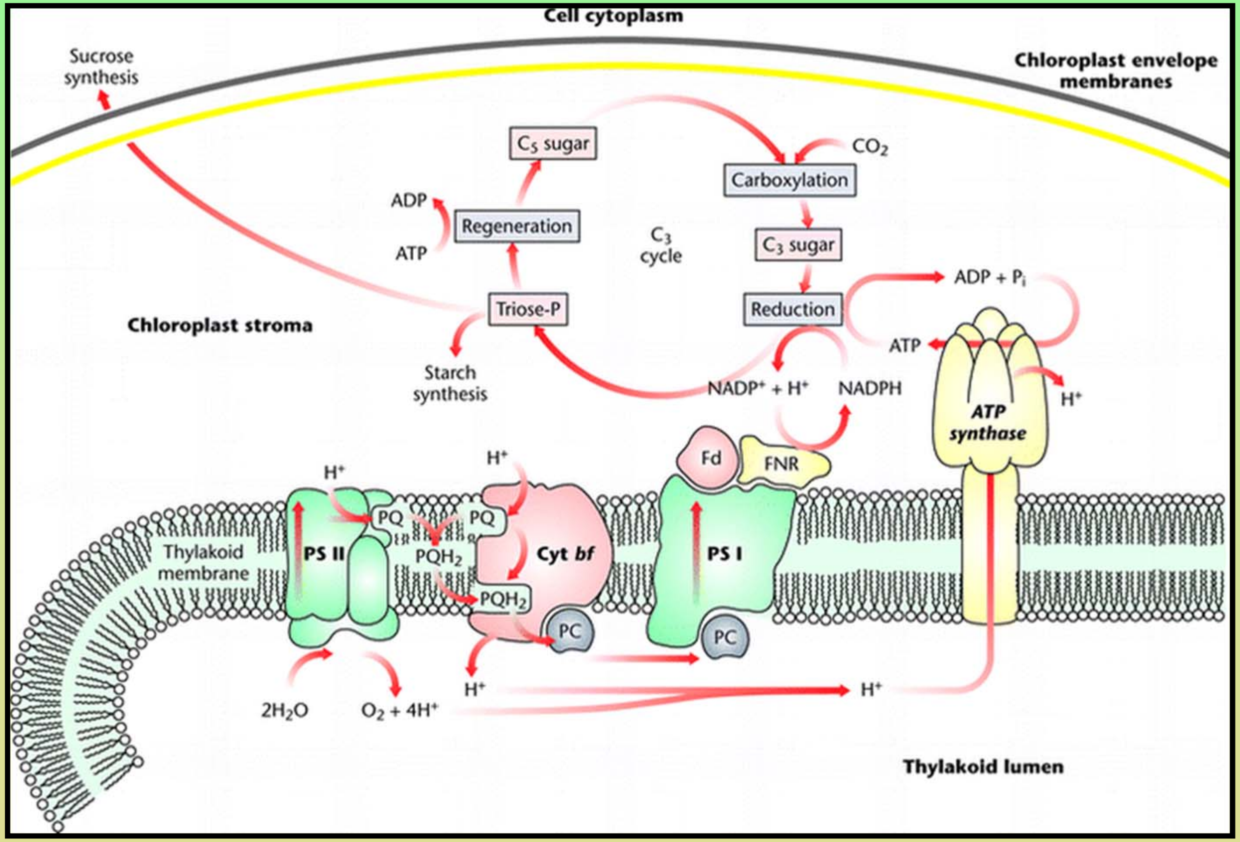
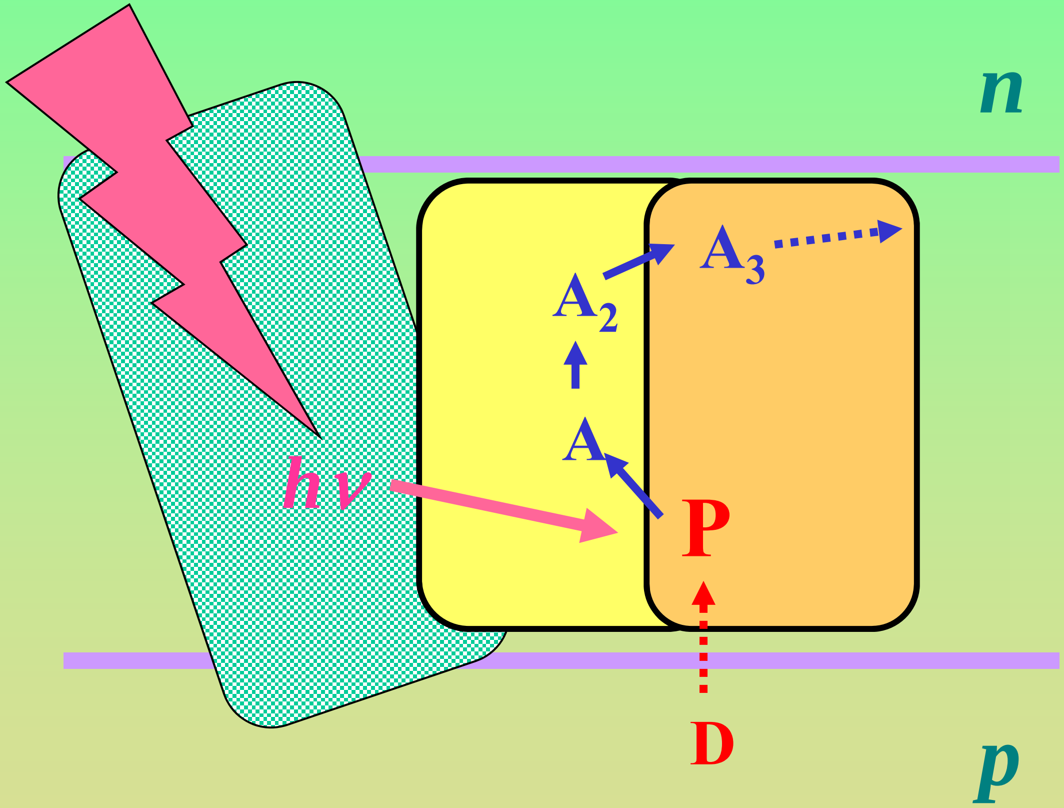
Čtyři fáze procesu přeměny energie ve fotosyntéze

1. absorbce světla a přenos energie v anténních systémech
2. primární rozdělení nábojů a přenos elektronů v reakčních centrech
3. stabilizace energie v sekundárních procesech
4. syntéza a export stabilních produktů



Reakční centra



$$\begin{array}{c} PA \\ \Big\downarrow\ h\nu \\ P^{*}A \\ \Big\downarrow \\ P^{+}A^{-} \\ \Big\downarrow \\ D^{+}PAA_2^{-} \end{array}$$

P primární donor, D sekundární donor, A akceptor elektronů

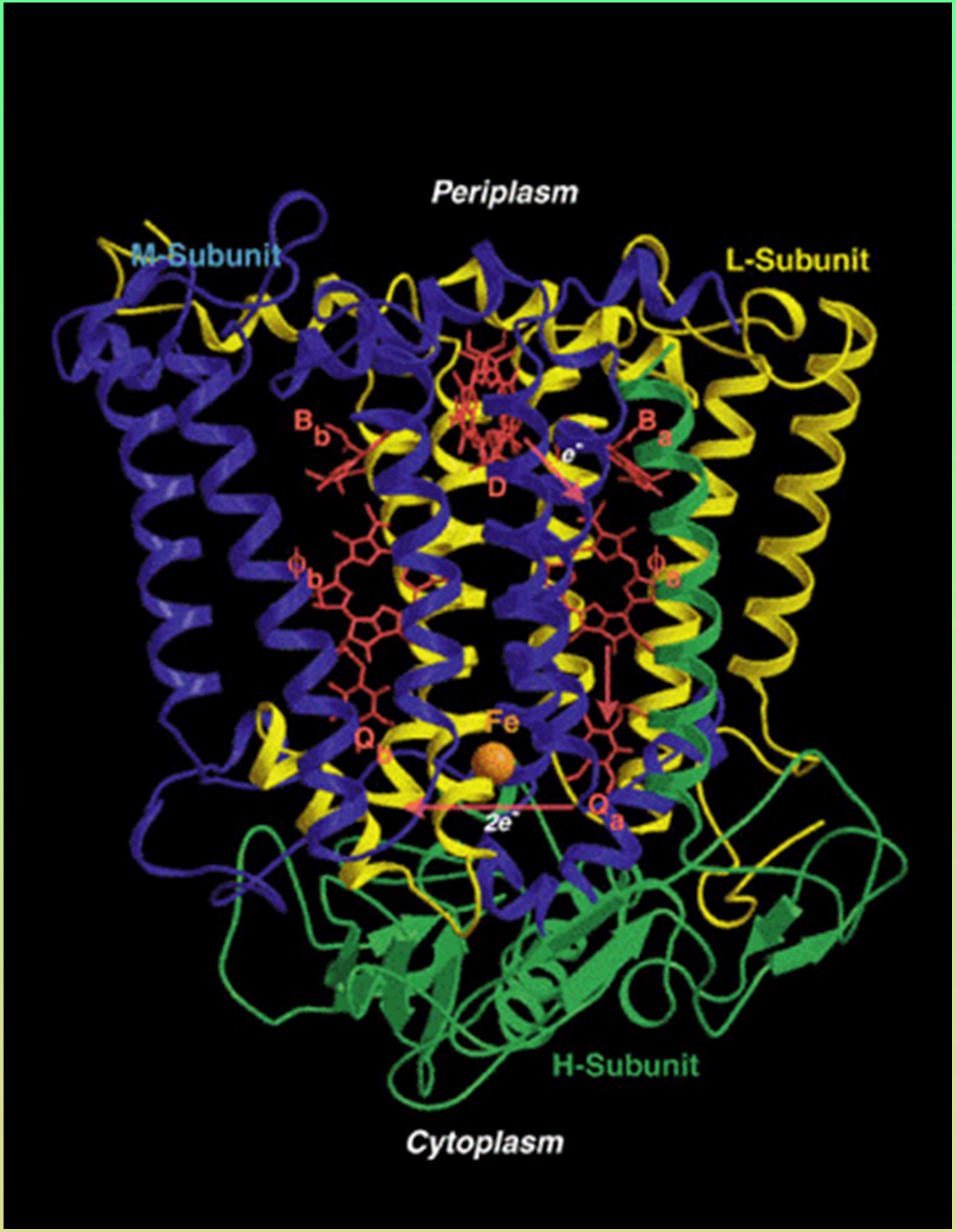
Obecné vlastnosti reakčních center

integrální membránové
pigment-proteinové
komplexy

vektoriální orientace v
membráně

světlem indukované
přenosy elektronů,
rozdělení nábojů

některá RC pumpují protony



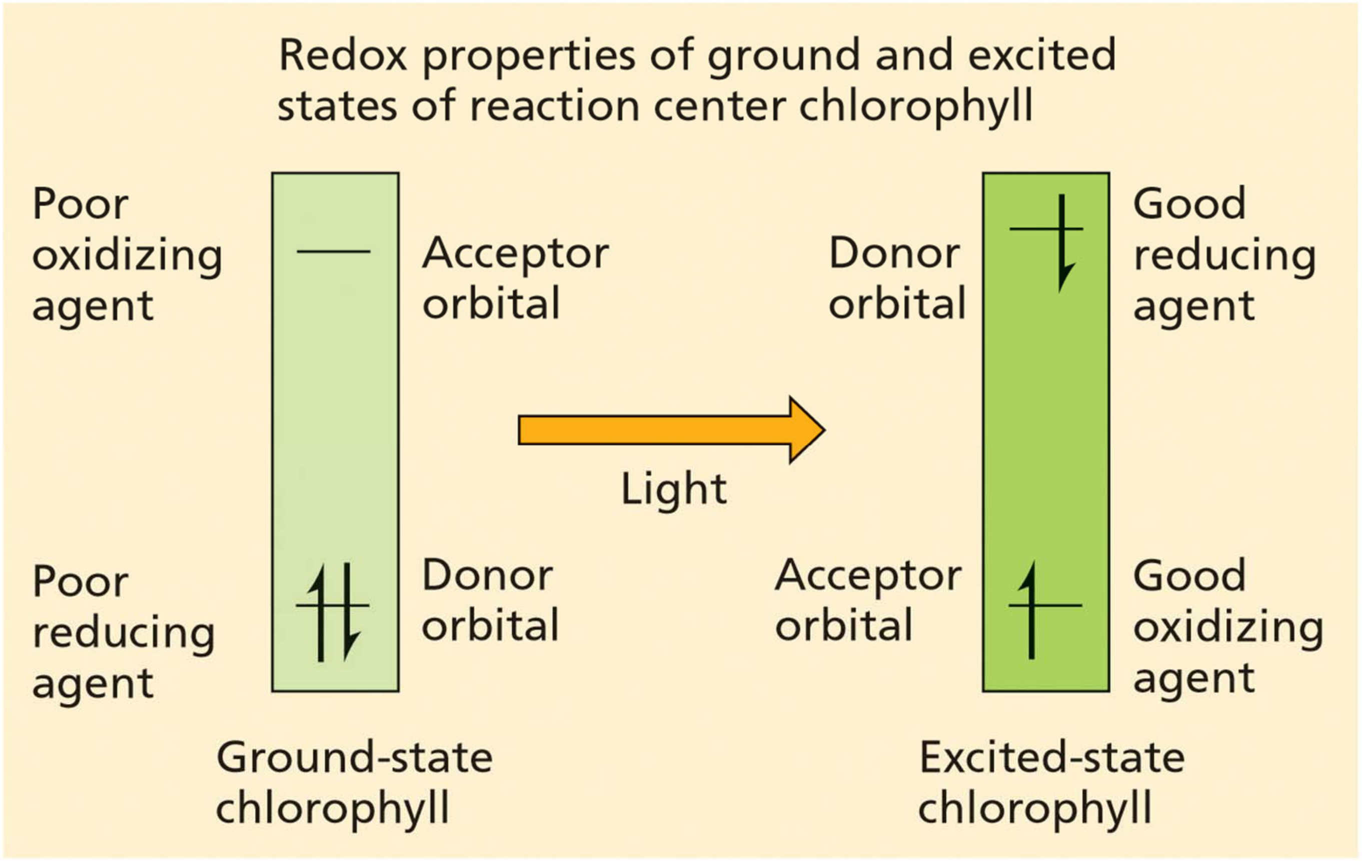
Chemické vlastnosti pigmentů v excitovaném stavu

jsou odlišné od molekuly v základním stavu

redoxní potenciál excitovaného stavu je jiný než stavu základního

LUMO

HOMO



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Střední redoxní potenciál excitovaného stavu

$E_m(P^+/P^*) = E_m(P^+/P) - E(P/P^*)$

$E(P/P^*)$ je rozdíl energie mezi excitovaným a základním stavem

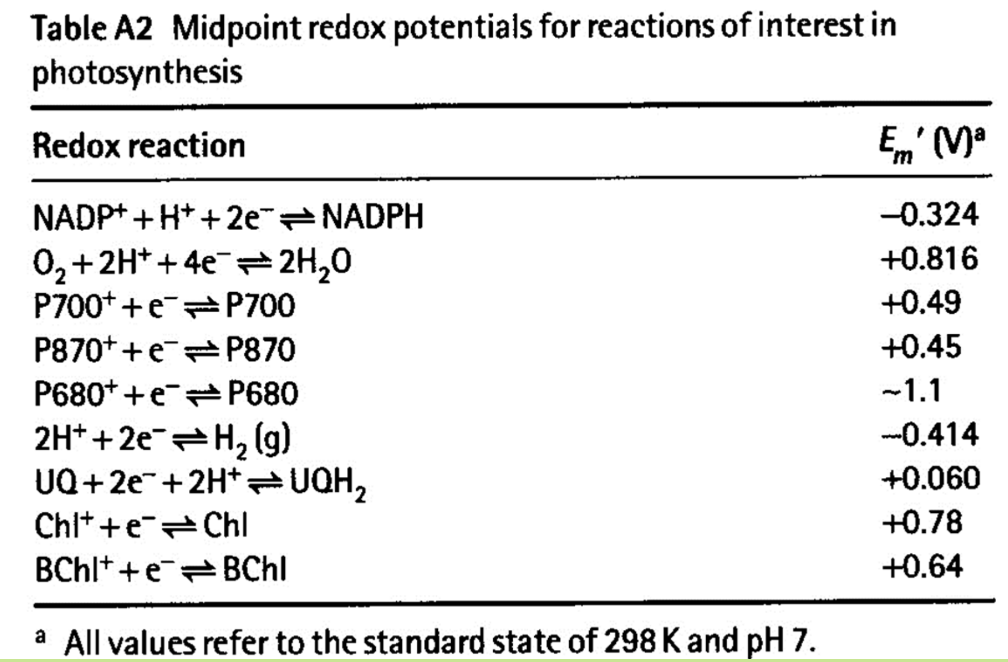
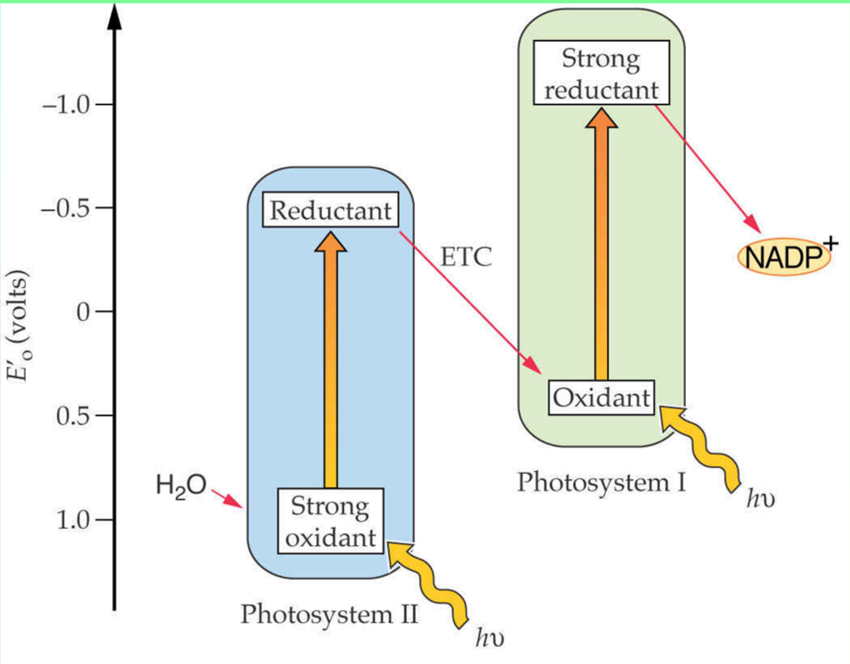


Table A2 Midpoint redox potentials for reactions of interest in photosynthesis

Redox reaction	E_m' (V)[a]
$NADP^+ + H^+ + 2e^- \rightleftharpoons NADPH$	−0.324
$O_2 + 2H^+ + 4e^- \rightleftharpoons 2H_2O$	+0.816
$P700^+ + e^- \rightleftharpoons P700$	+0.49
$P870^+ + e^- \rightleftharpoons P870$	+0.45
$P680^+ + e^- \rightleftharpoons P680$	~1.1
$2H^+ + 2e^- \rightleftharpoons H_2$ (g)	−0.414
$UQ + 2e^- + 2H^+ \rightleftharpoons UQH_2$	+0.060
$Chl^+ + e^- \rightleftharpoons Chl$	+0.78
$BChl^+ + e^- \rightleftharpoons BChl$	+0.64

[a] All values refer to the standard state of 298 K and pH 7.



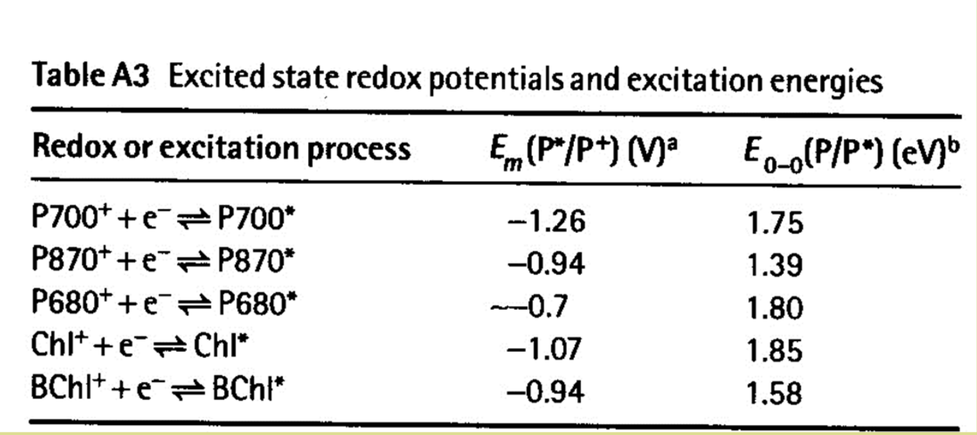
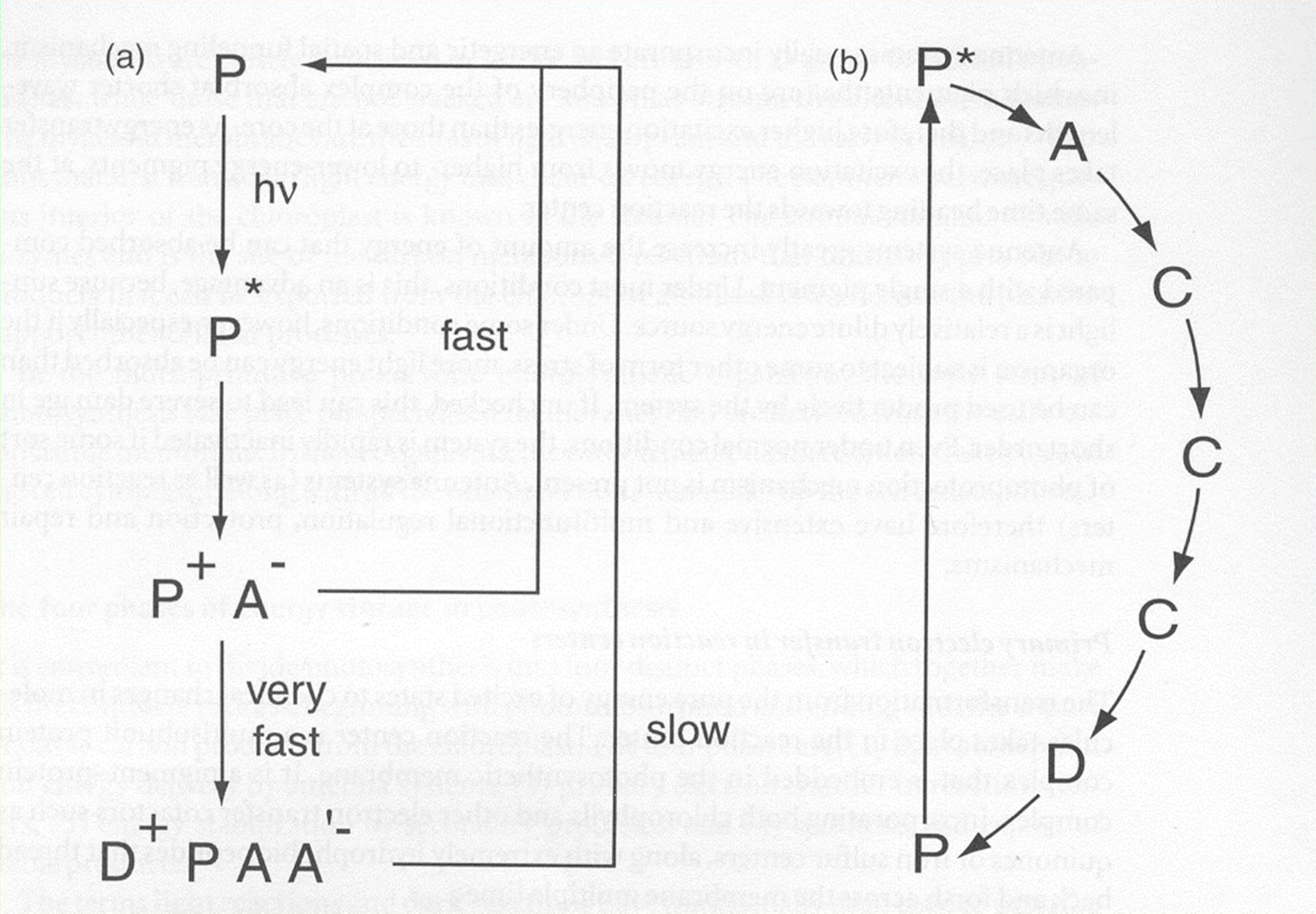


Table A3 Excited state redox potentials and excitation energies

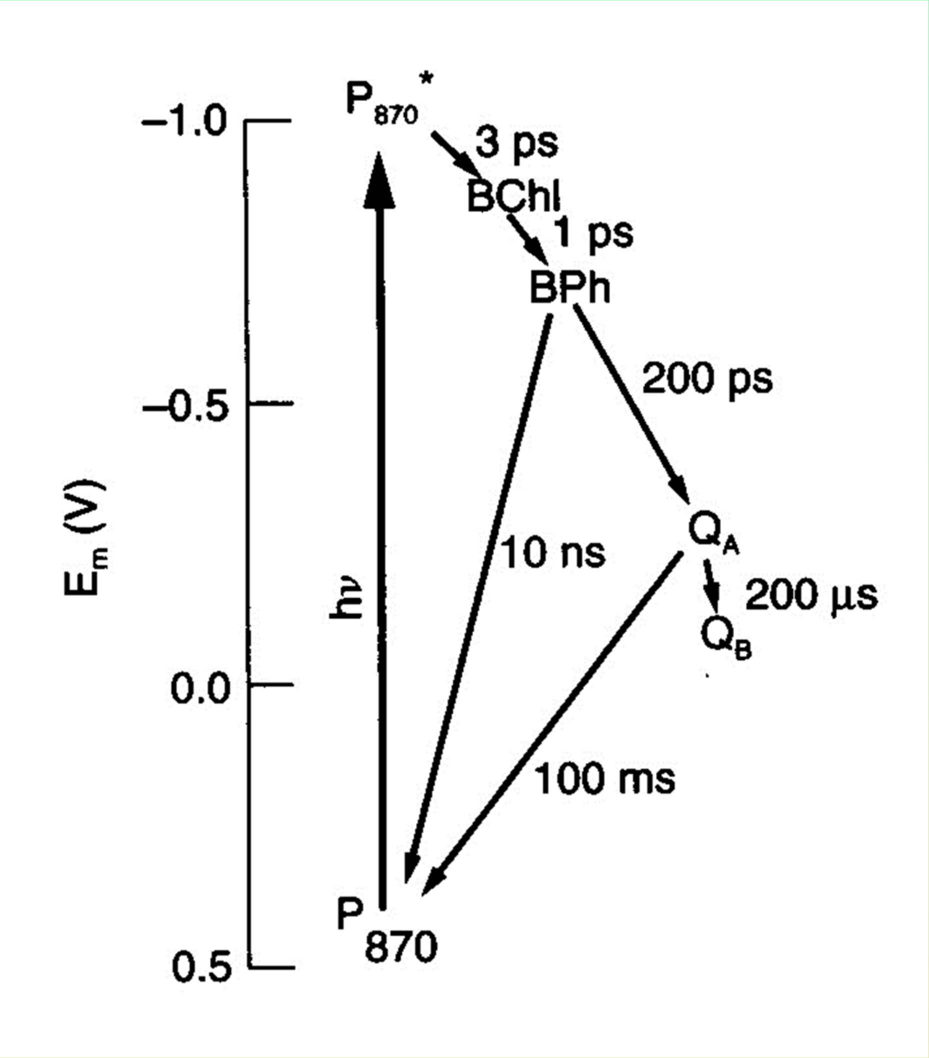
Redox or excitation process	$E_m(P^*/P^+)$ (V)[a]	$E_{0-0}(P/P^*)$ (eV)[b]
$P700^+ + e^- \rightleftharpoons P700^*$	−1.26	1.75
$P870^+ + e^- \rightleftharpoons P870^*$	−0.94	1.39
$P680^+ + e^- \rightleftharpoons P680^*$	~−0.7	1.80
$Chl^+ + e^- \rightleftharpoons Chl^*$	−1.07	1.85
$BChl^+ + e^- \rightleftharpoons BChl^*$	−0.94	1.58

Principy stabilizace nábojů v RC



Kinetika přenosu elektronů a rekombinace v RC

rekombinace je >50x pomalejší než přenos elektronů



Popis mechanismu přenosu elektronů v biologických systémech

Rudolph Marcus
Nobelova cena za chemii 1992
přenos elektronů jako nezářivý relaxační proces
$D_{red} + A_{ox} \rightarrow D_{ox} + A_{red}$
Fermiho zlaté pravidlo – rychlostní konstanta k_{et}

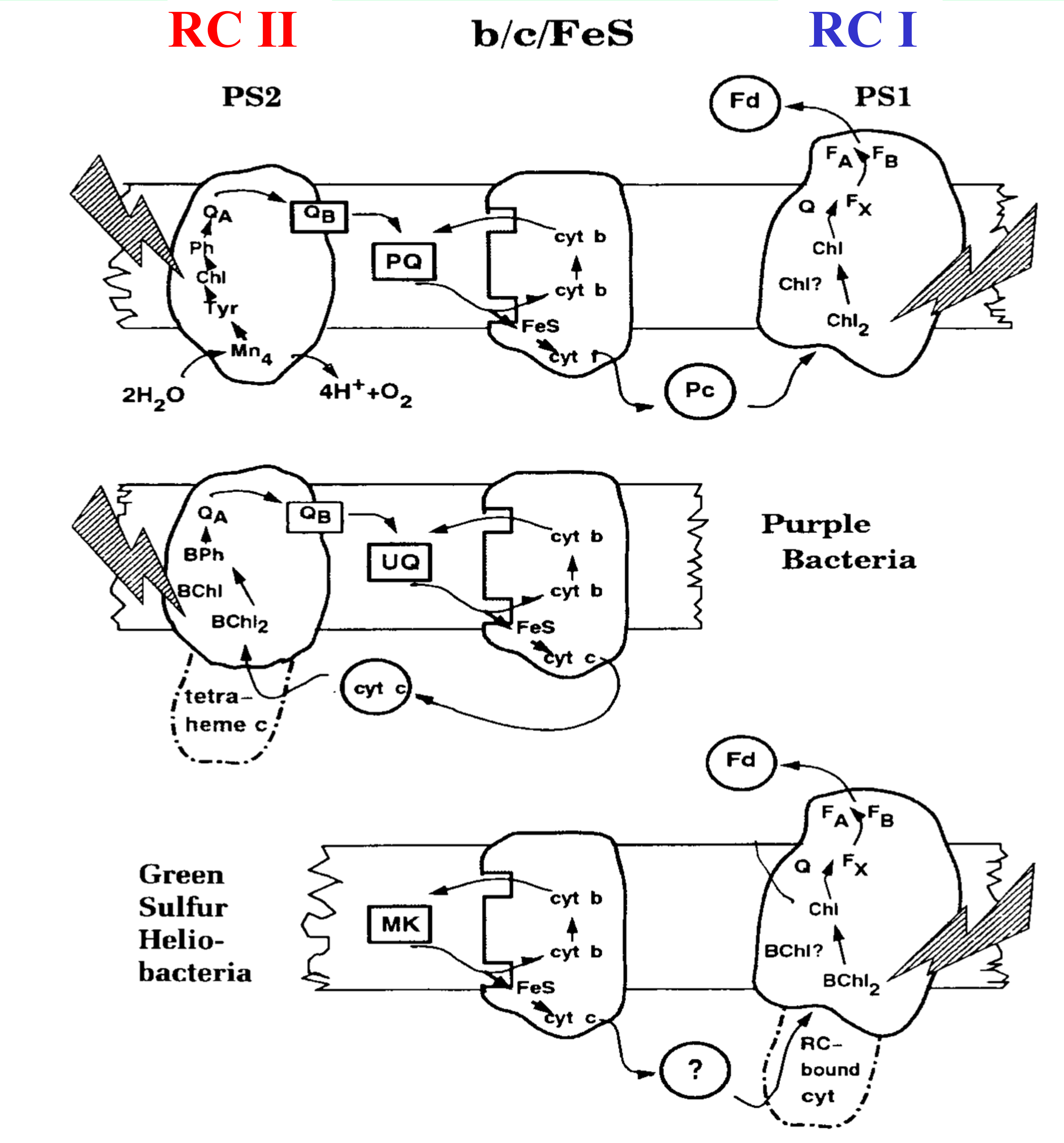
$$k_{et} = 2\pi/\hbar \, | < \Psi_i | V | \Psi_f > |^2 \, \delta(E_i - E_f)$$

Vychází z Franck-Condonova principu. V je energie
$k_{et} \sim V_e^2 \, FC$
V_e^2 závisí na vzdálenosti a orientaci A a D
$V_e^2 \sim \exp(-d)$ klesá exponenciálně se vzdáleností

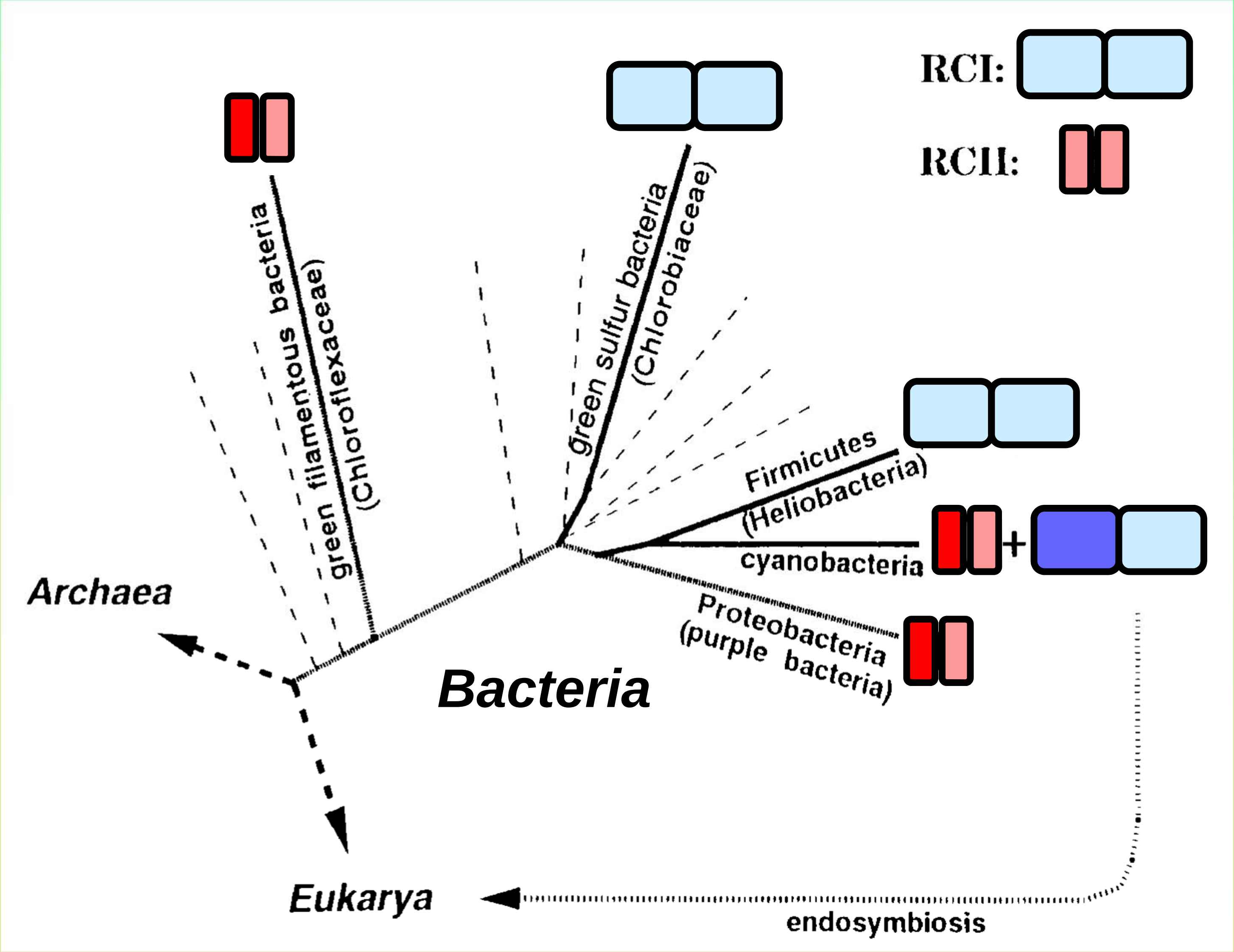
Dva typy RC:

chinonové
(vysokopotenciálové)

FeS
(redukční)

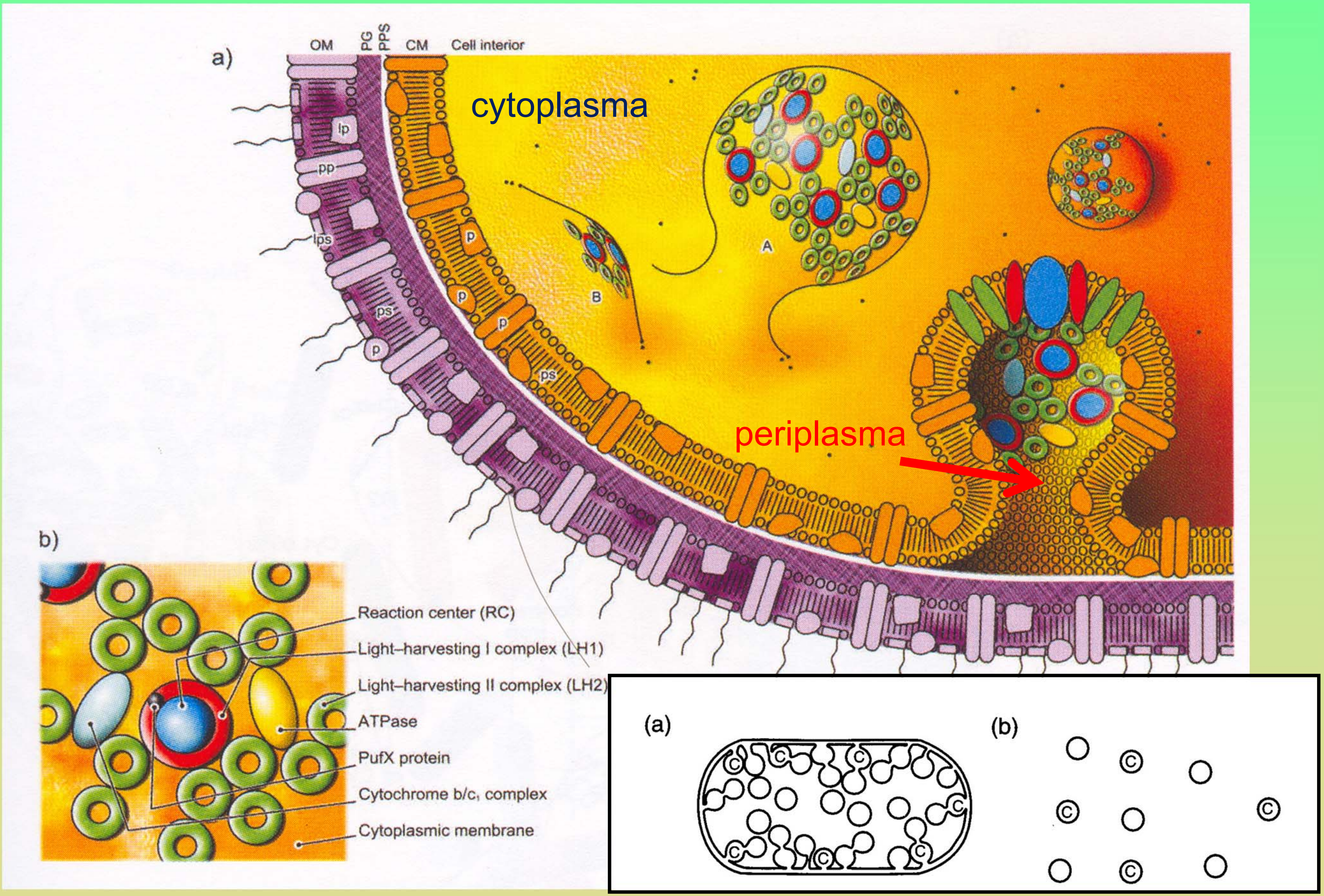


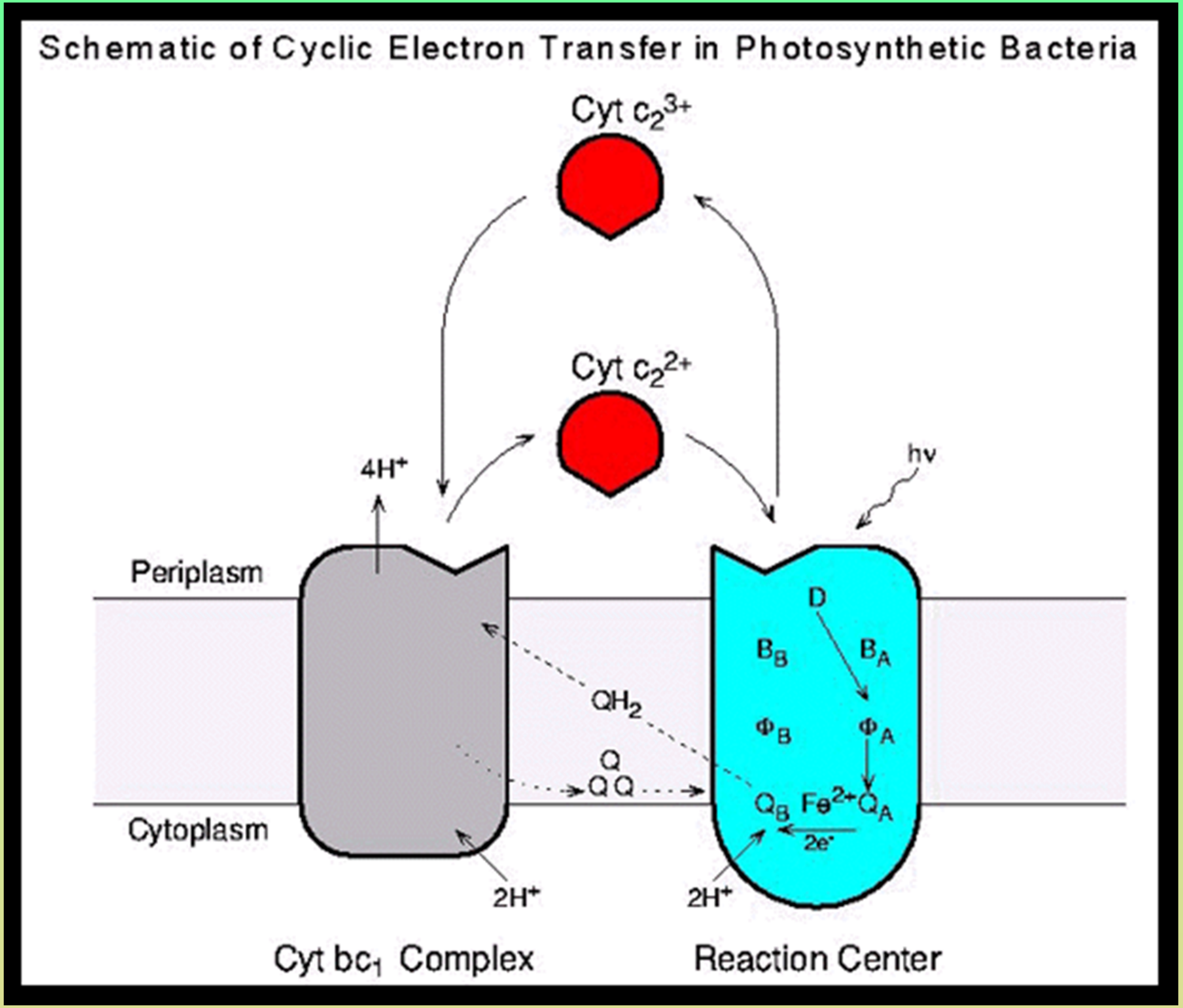
Zastoupení typů RC u prokaryot



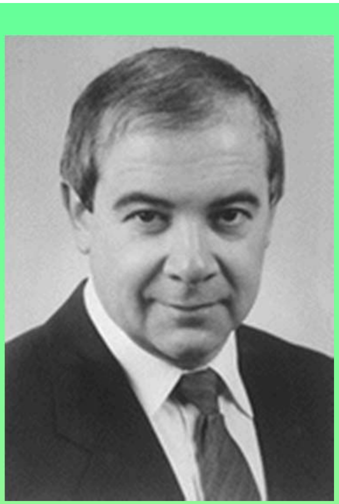
Fotosyntetická membrána purpurových bakterií

chromatofory ve vnitřní cytoplasmatické membráně

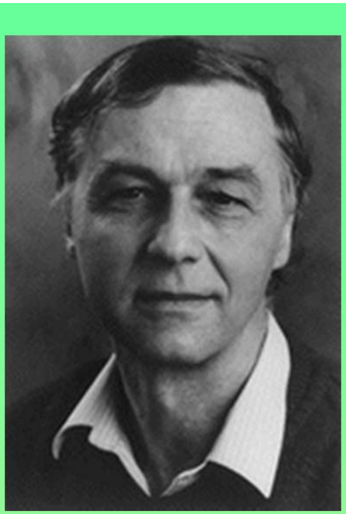




Schematic of Cyclic Electron Transfer in Photosynthetic Bacteria

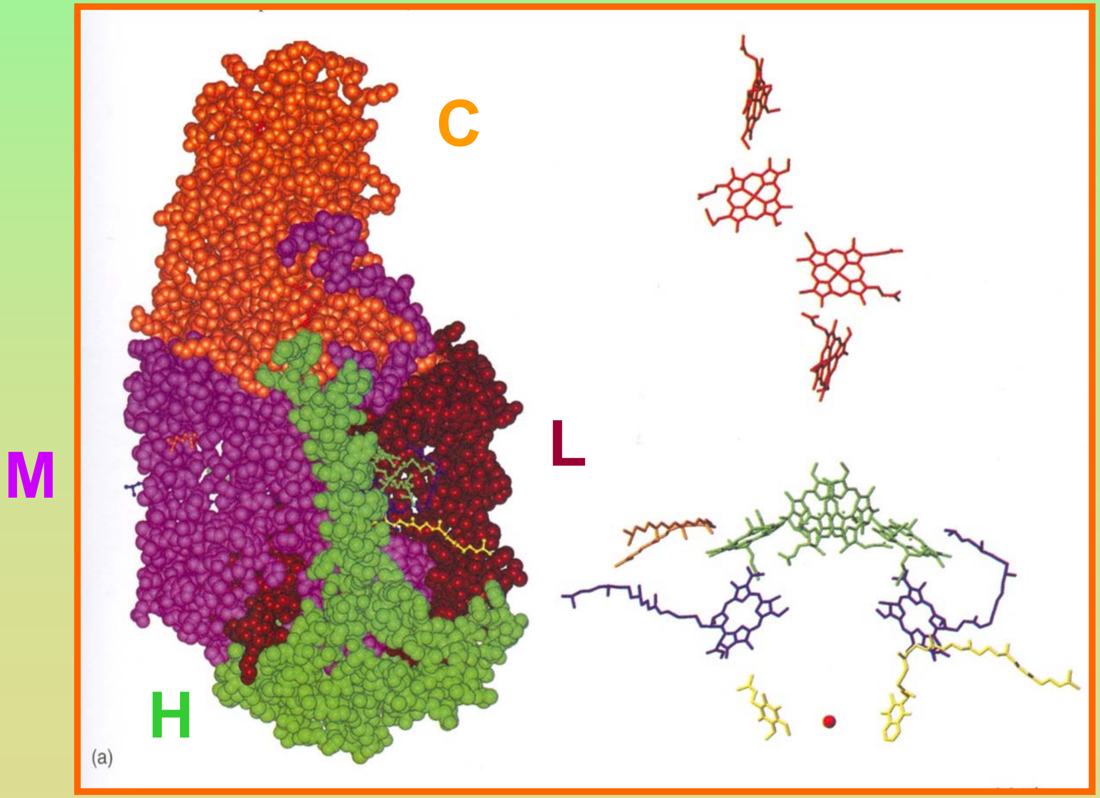


Hans Deisenhofer



Robert Huber

Hartmut Michel

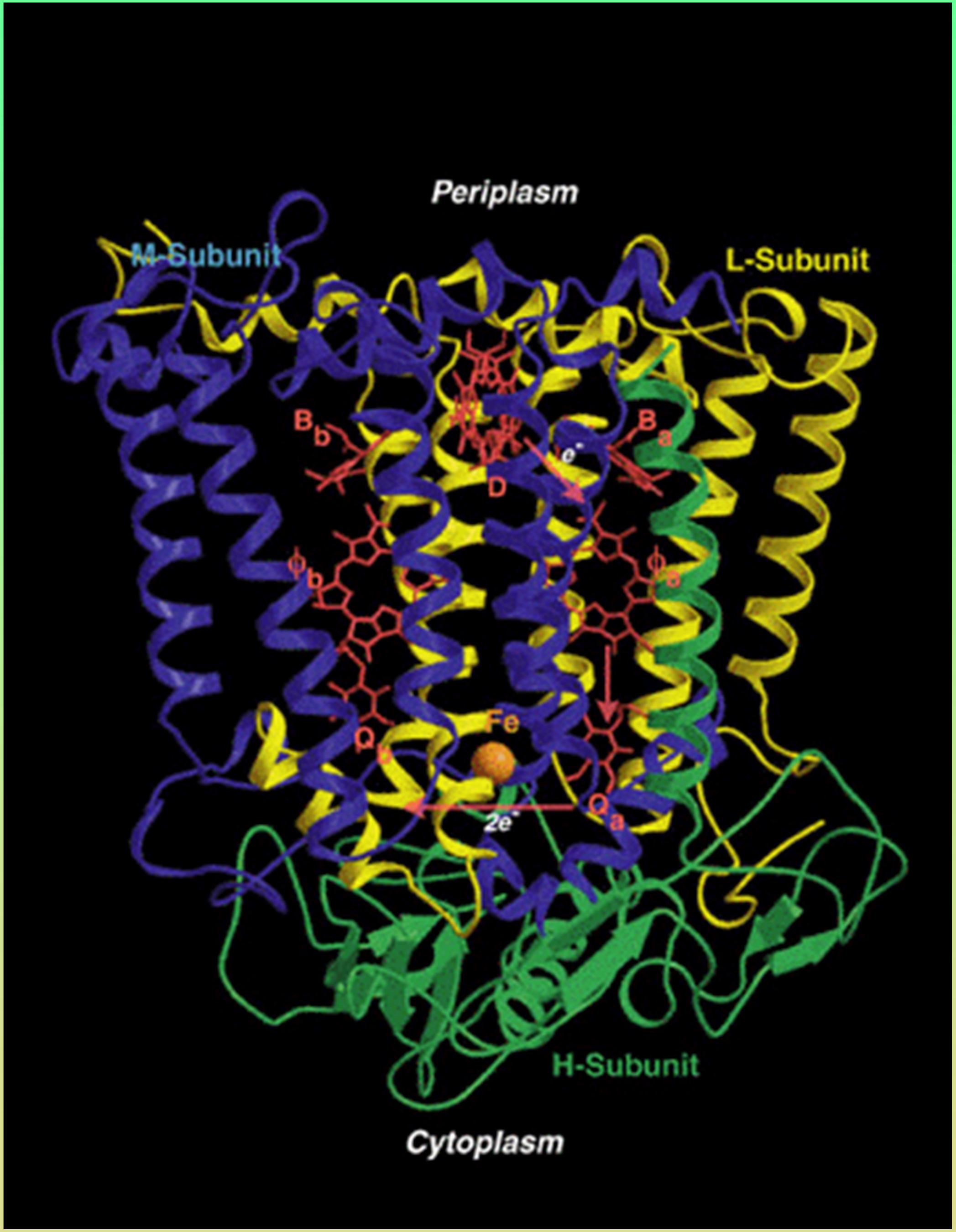


Nobelova cena
1988

L & M podjednotky:

5 transmembránových α šroubovic, které tvoří hydrofóbní aminokyseliny

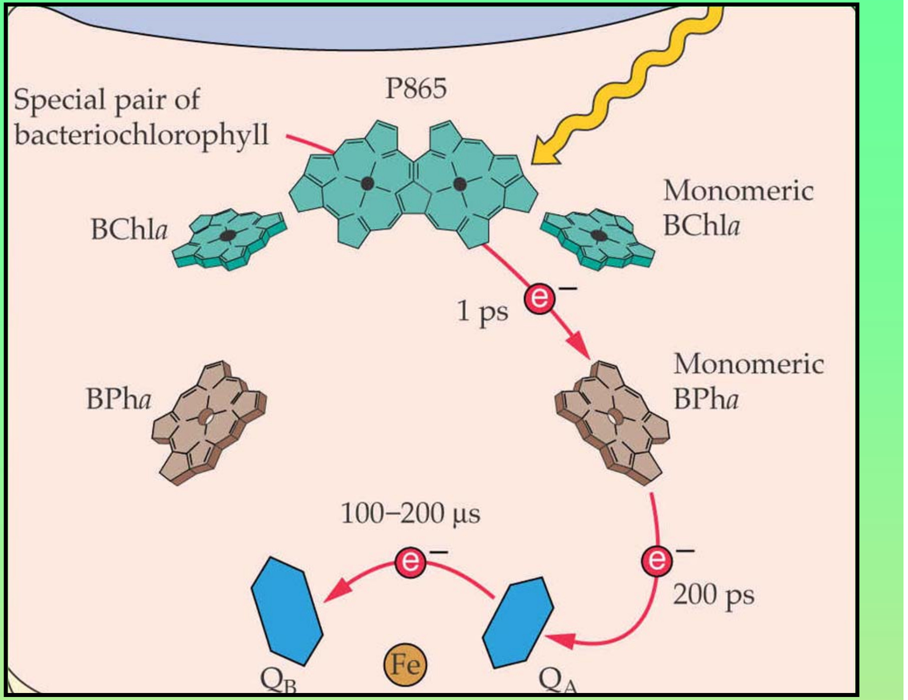
Pseudo-dvoučetná symetrie
L a M podjednotky jen 30% sekvenční identita

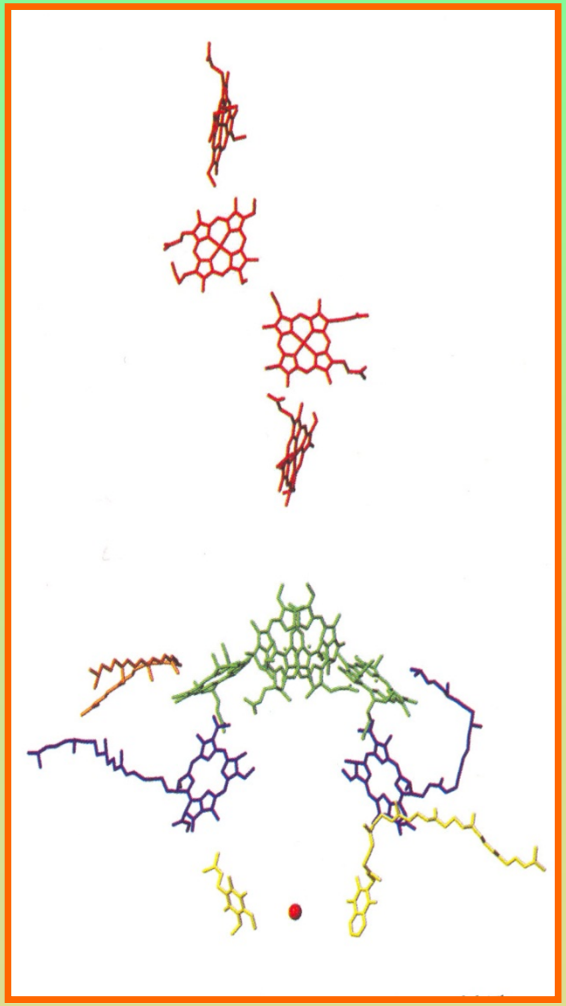


Redoxní kofaktory BRC

elektronový transport jen po větvi A

P865 – λ maxima absorbce bakteriochlorofylu a





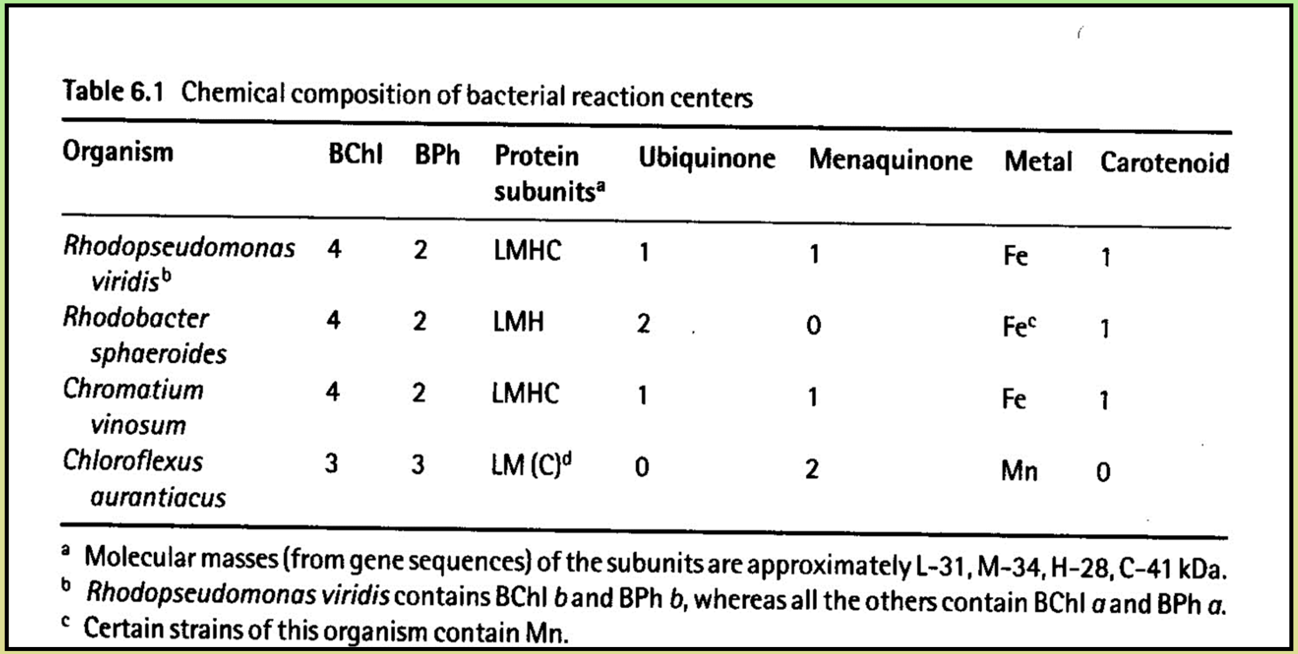


Table 6.1 Chemical composition of bacterial reaction centers

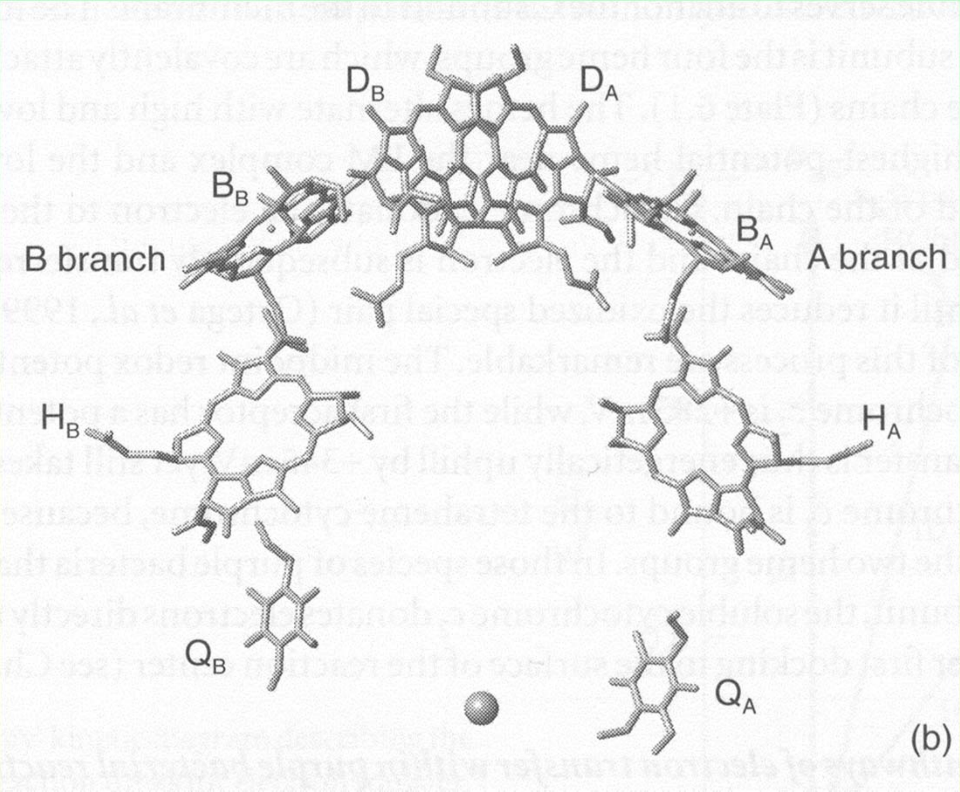
Organism	BChl	BPh	Protein subunits[a]	Ubiquinone	Menaquinone	Metal	Carotenoid
Rhodopseudomonas viridis[b]	4	2	LMHC	1	1	Fe	1
Rhodobacter sphaeroides	4	2	LMH	2	0	Fe[c]	1
Chromatium vinosum	4	2	LMHC	1	1	Fe	1
Chloroflexus aurantiacus	3	3	LM (C)[d]	0	2	Mn	0

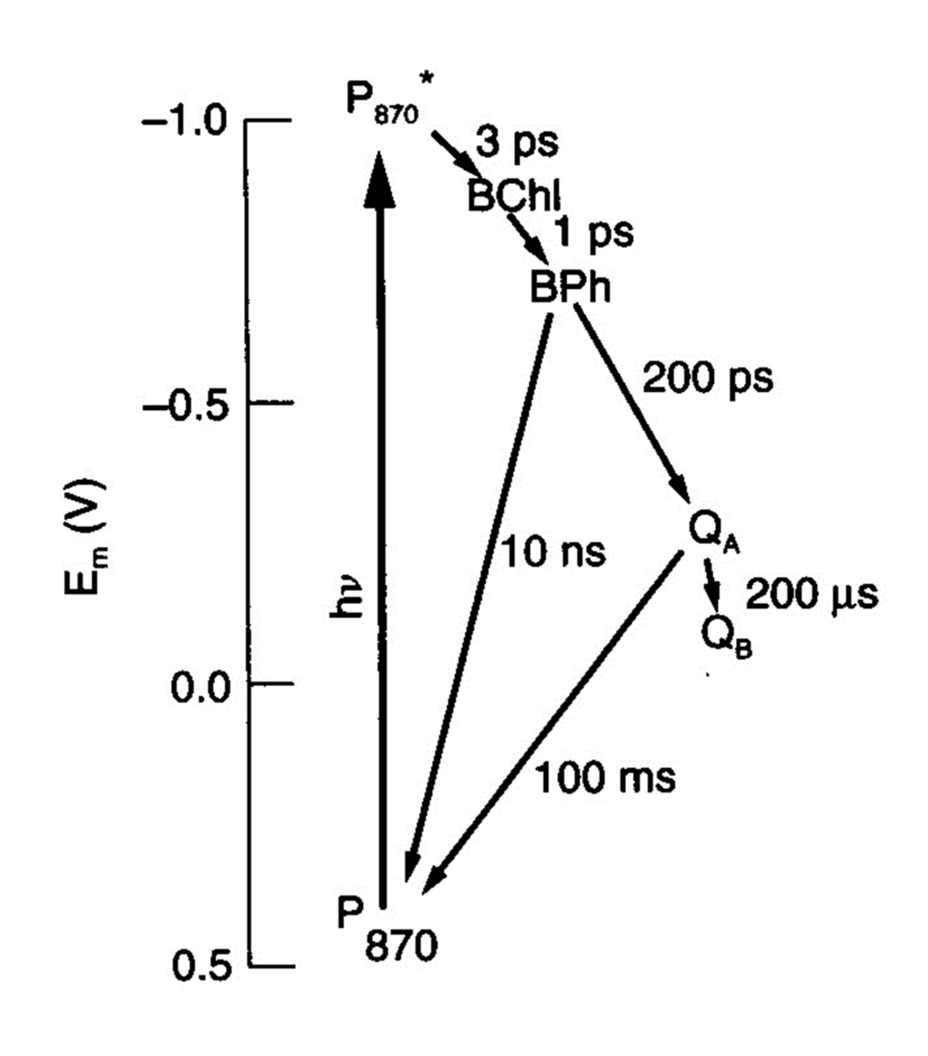
[a] Molecular masses (from gene sequences) of the subunits are approximately L-31, M-34, H-28, C-41 kDa.

[b] *Rhodopseudomonas viridis* contains BChl *b* and BPh *b*, whereas all the others contain BChl *a* and BPh *a*.

[c] Certain strains of this organism contain Mn.

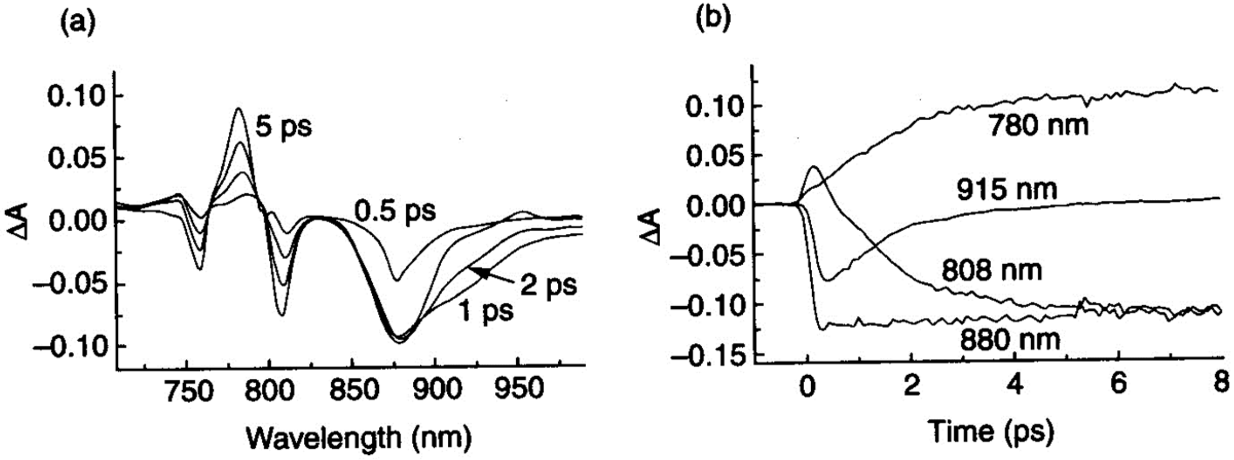
Primární procesy v BRC

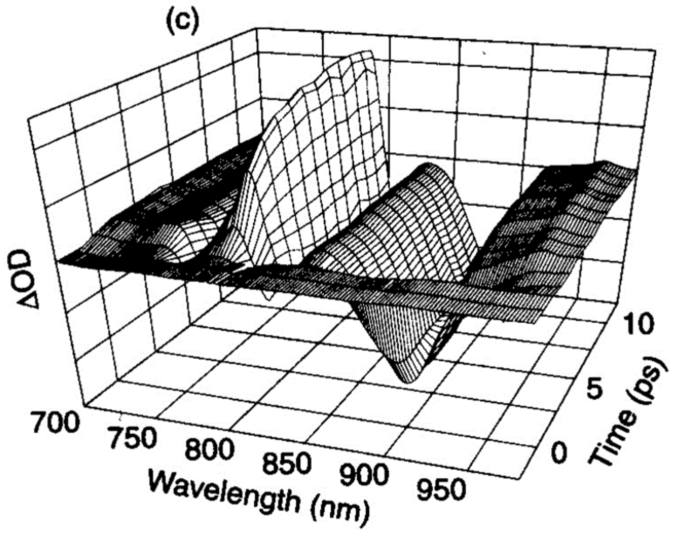


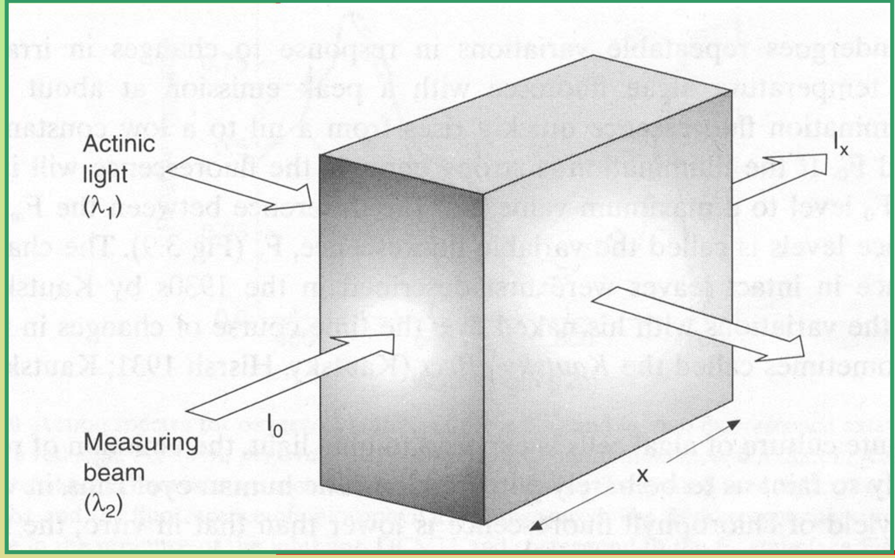


Elektron oxidovanému $P870^+$ je dodán cytochromem ~ 100 μsec

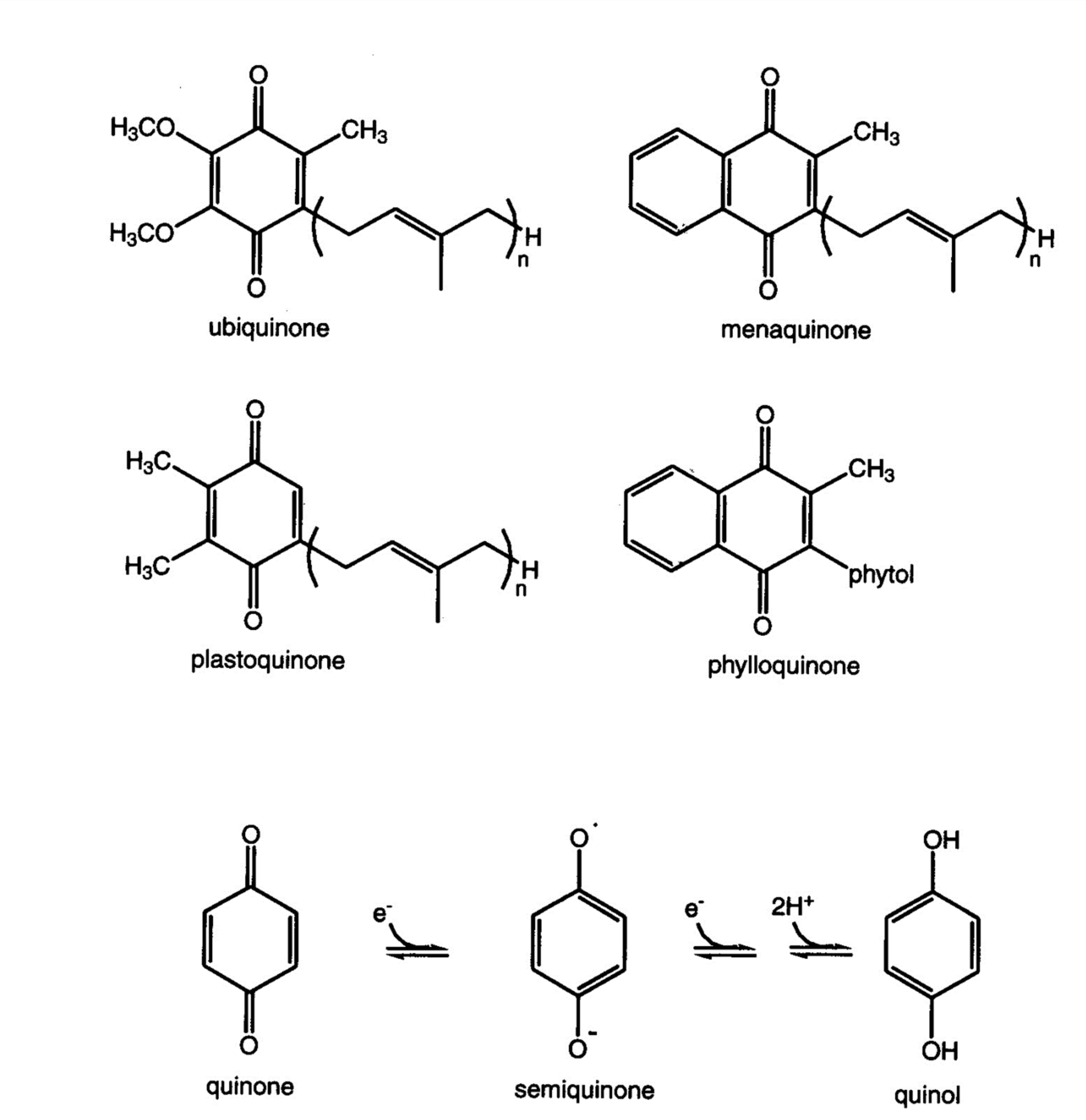
Kinetická absorbce – procesy s rychlostí od ps do ms



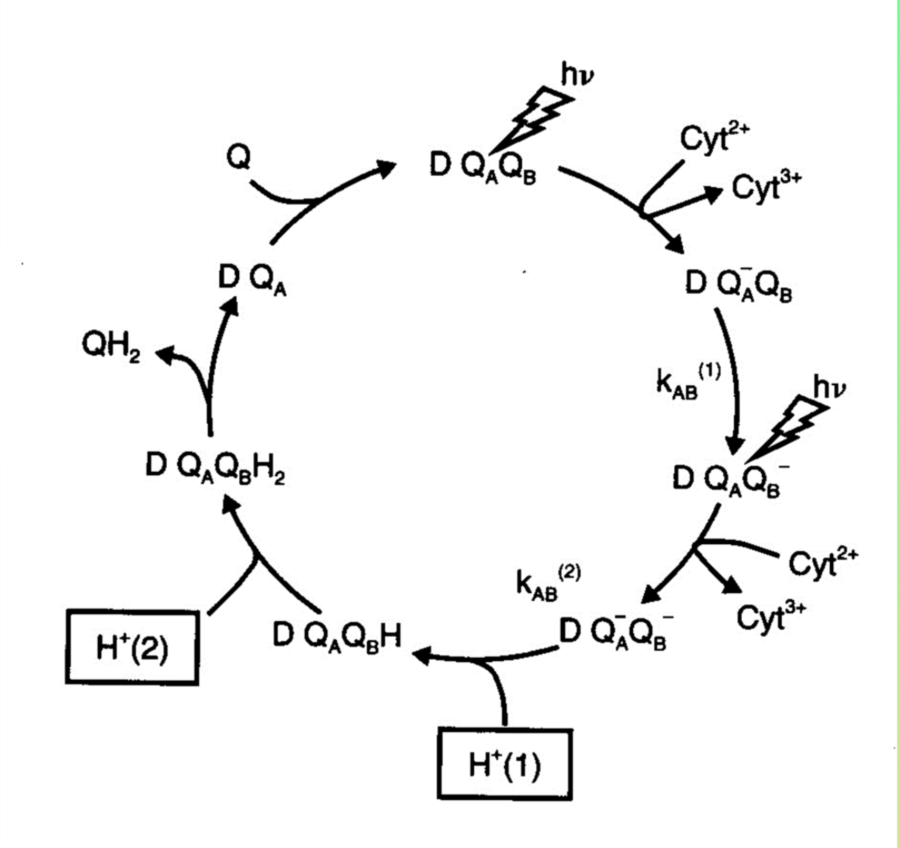


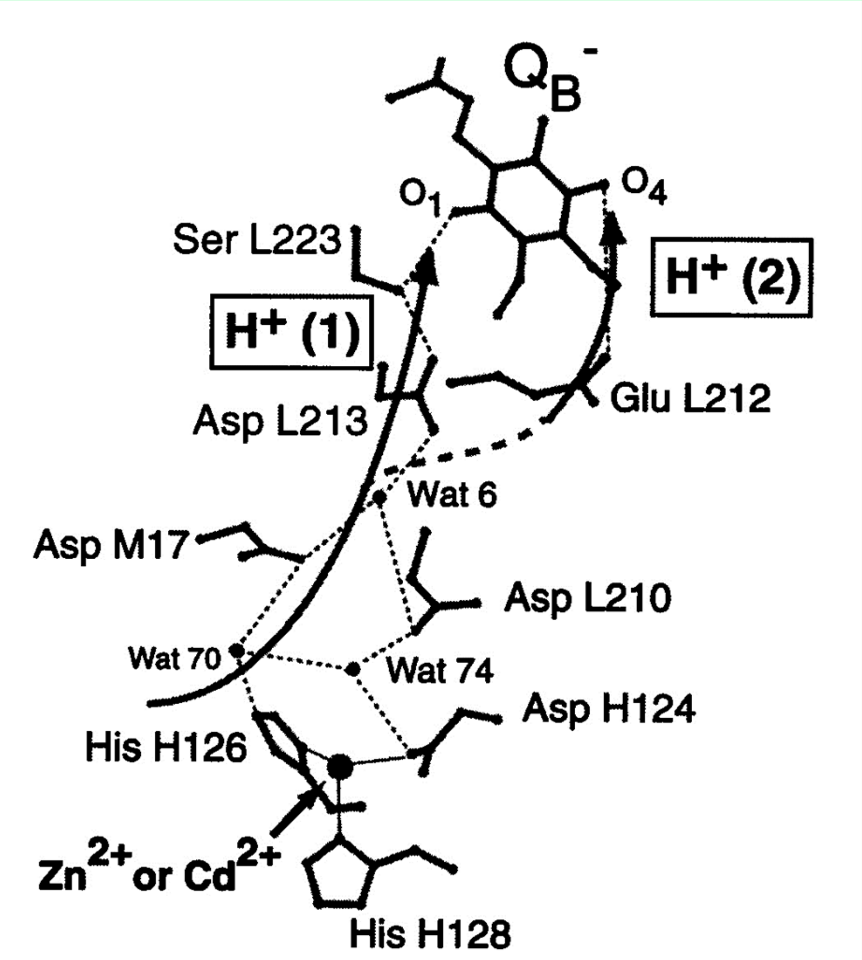


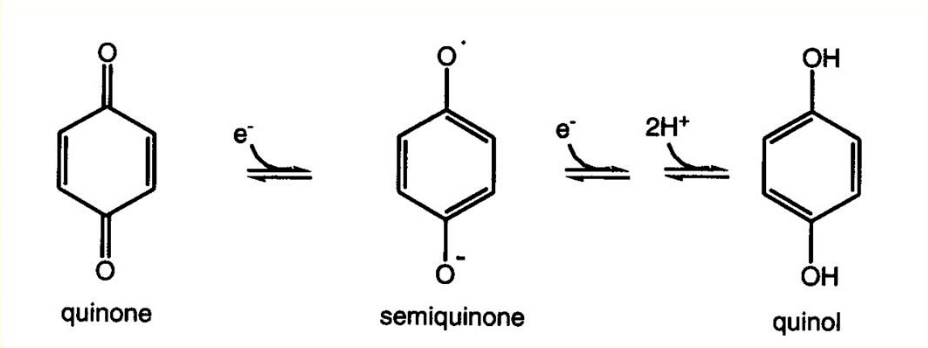
Chinony

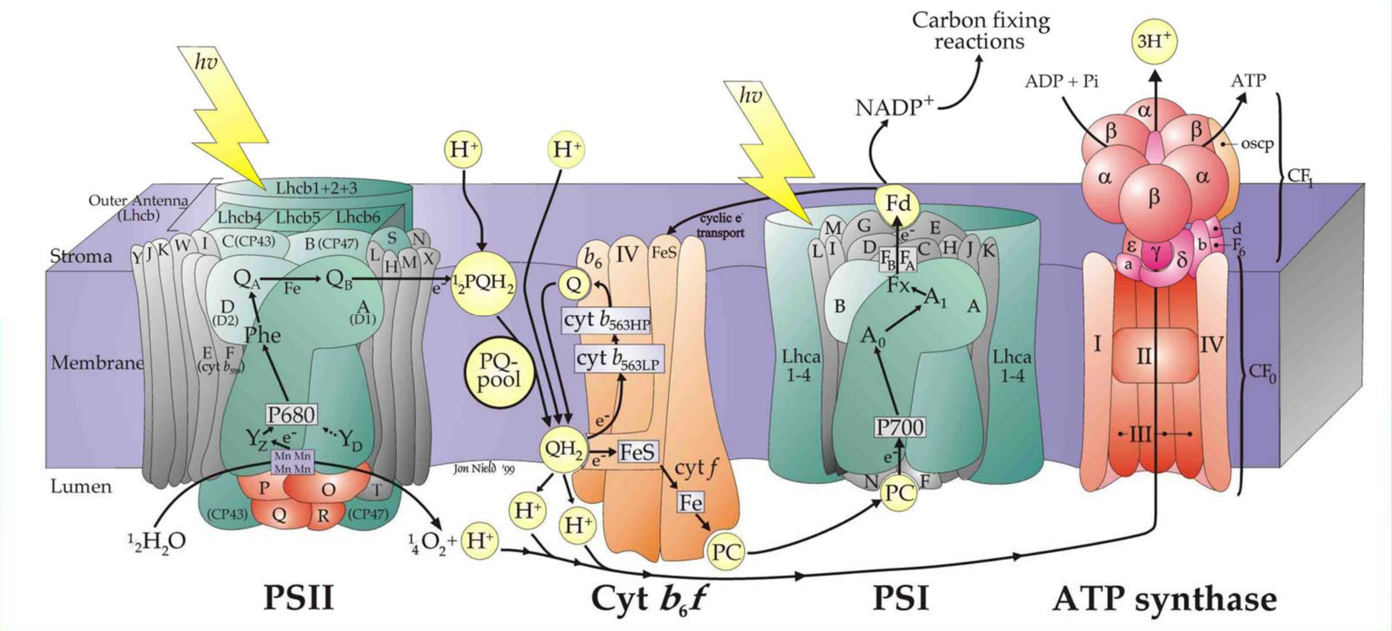

O
H3CO
CH3
H3CO
H
n
O
ubiquinone
O
CH3
H
n
O
menaquinone
O
H3C
H3C
H
n
O
plastoquinone
O
CH3
phytol
O
phylloquinone
O
O
quinone
e-
O·
O-
semiquinone
e-
2H+
OH
OH
quinol

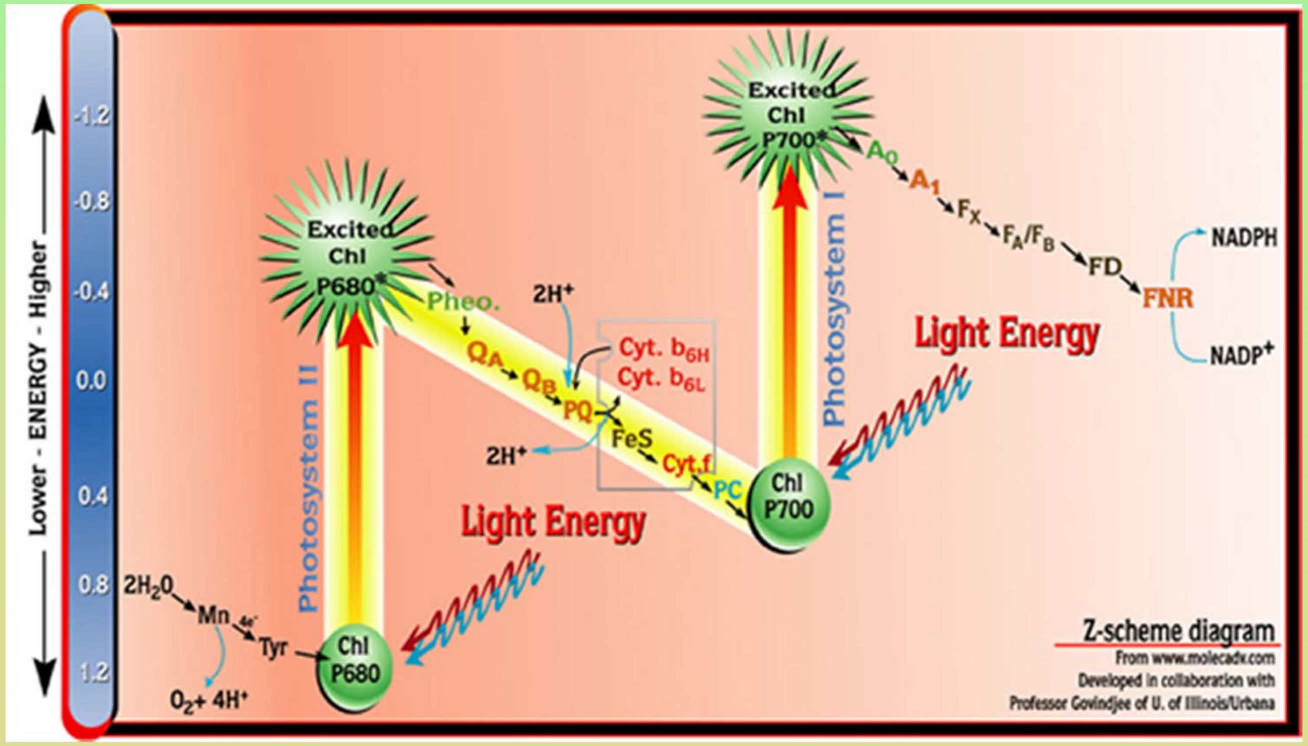
„Dvouelektronová brána“ a protonace chinonů



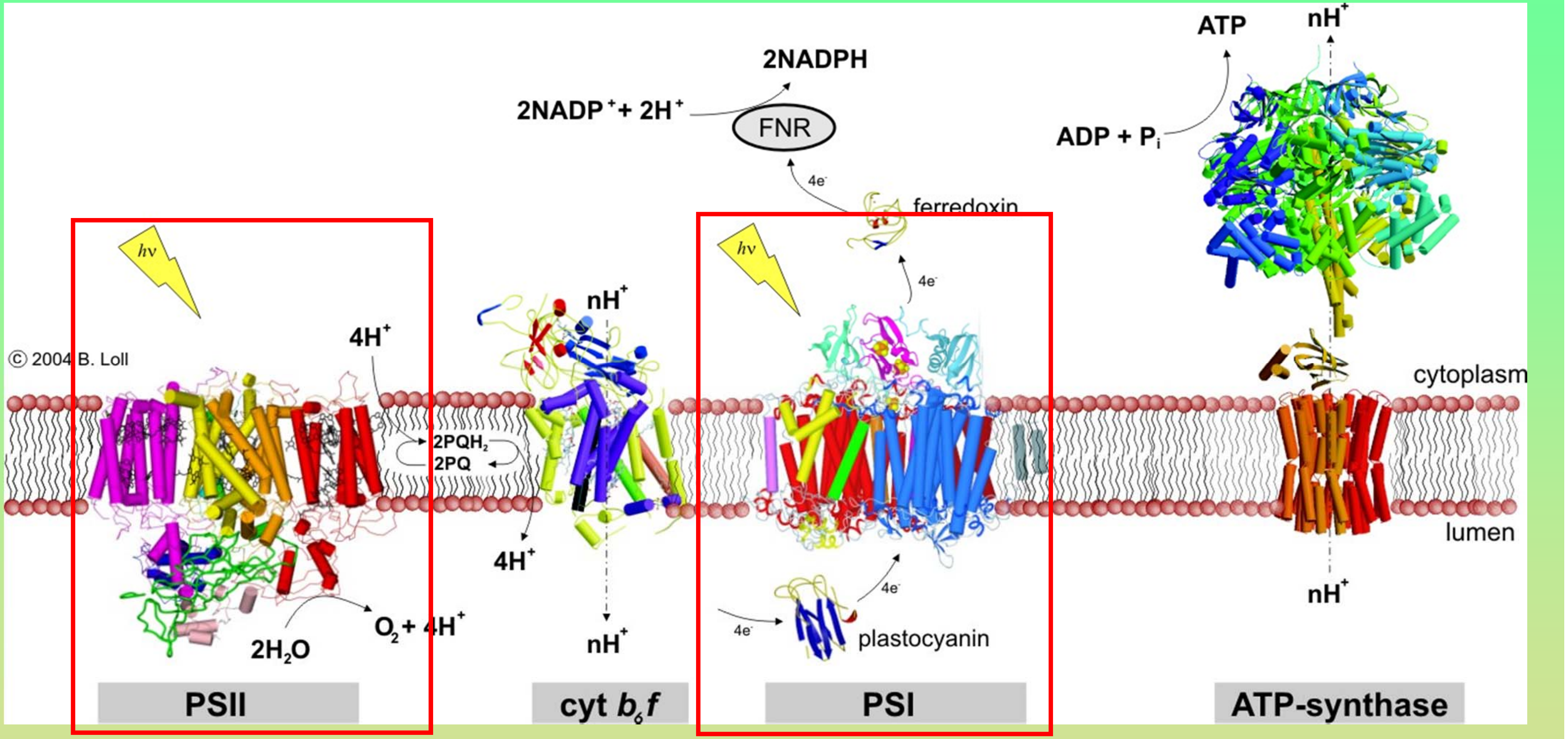





hv
Outer Antenna (Lhcb)
Lhcb1+2+3
Lhcb4 Lhcb5 Lhcb6
Stroma
Membrane
Lumen
Y J K W I C(CP43) B(CP47) L H M X S N
QA Fe QB
D (D2)
A (D1)
Phe
E F (cyt b559)
P680
YZ e- YD
Mn Mn Mn Mn
P O T
(CP43) Q R (CP47)
1/2 H2O
1/4 O2 + H+
H+
e- 1/2 PQH2
PQ-pool
H+
b6 IV FeS
Q
cyt b563HP
cyt b563LP
e-
QH2
FeS
cyt f
Fe
PC
H+
H+
Jon Nield '99
cyclic e- transport
hv
Carbon fixing reactions
NADP+
Fd
M G E
L I D C H J K
FB FA
Fx A1
B A
A0
Lhca 1-4
Lhca 1-4
P700
e-
N F
PC
3H+
ADP + Pi
ATP
α β β α β α
oscp
CF1
ε γ δ
a b d F6
I II IV
III
CF0
PSII
Cyt b6f
PSI
ATP synthase


Lower - ENERGY - Higher
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Excited Chl P700*
A0
A1
Fx
FA/FB
FD
FNR
NADPH
NADP+
Excited Chl P680*
Pheo.
2H+
QA
QB
PQ
Cyt. b6H
Cyt. b6L
FeS
Cyt.f
PC
2H+
Chl P700
Photosystem I
Light Energy
Photosystem II
Light Energy
2H2O
Mn
4e-
Tyr
Chl P680
O2+ 4H+
Z-scheme diagram
From www.molecadv.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana

Detailní struktury obou fotosystémů u sinic jsou známy s rozlišením 2.5- 3 Å, u rostlin pouze PSI s rozlišením 3.4 Å



T. elongatus, Dimer, 3.8 Å, Zouni et al, *Nature* **409**, 739-43 (2001), 1FE1

3.5 Å, Ferreira et al, *Science* **303**, 1831-8 (2004),1S5L

3.0 Å, Loll et al, *Nature* **438**, 1040-44 (2005), 2AXT

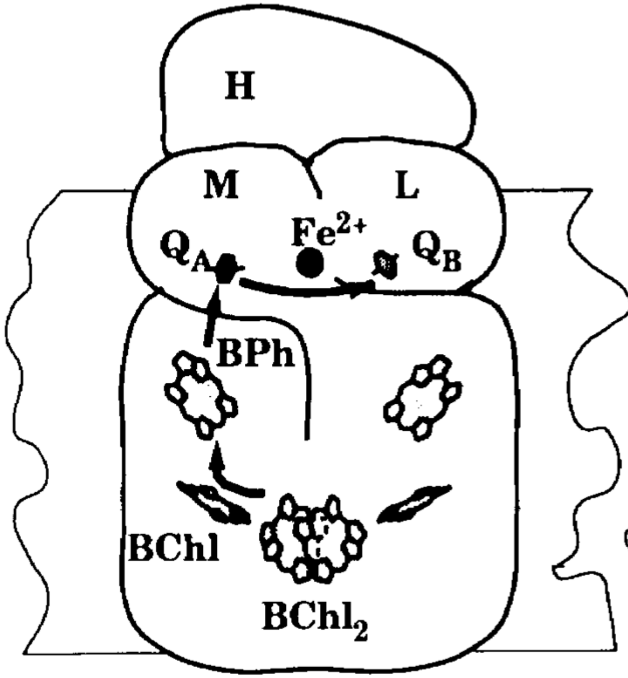
P. laminosus: Dimer, 3.0 Å, Kurisu et al, *Science* **302**, 1009-14 (2003), 1VF5

C. reinhardtii: Dimer, 3.1 Å, Stroebel et al, *Nature* **426**, 413-8 (2003), 1Q90

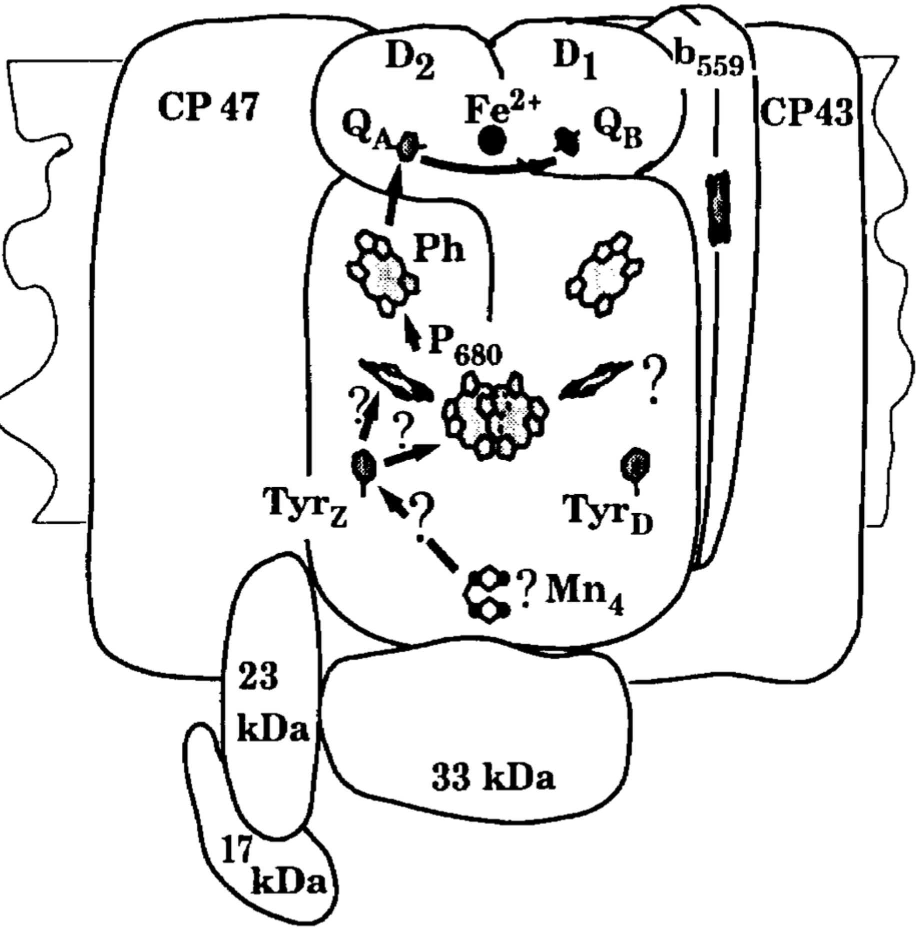
T. elongatus Trimer,2.5 Å, Jordan et al, *Nature* **411**, 909-17 (2001), 1JBO

Pea, Monomer, 4.4 Å, Ben-Shem, et al. *Nature* **426**, 630-5 (2003), 1QZV; 3.4 Å *Nature* (May 2007)

Purple bacterial reaction center



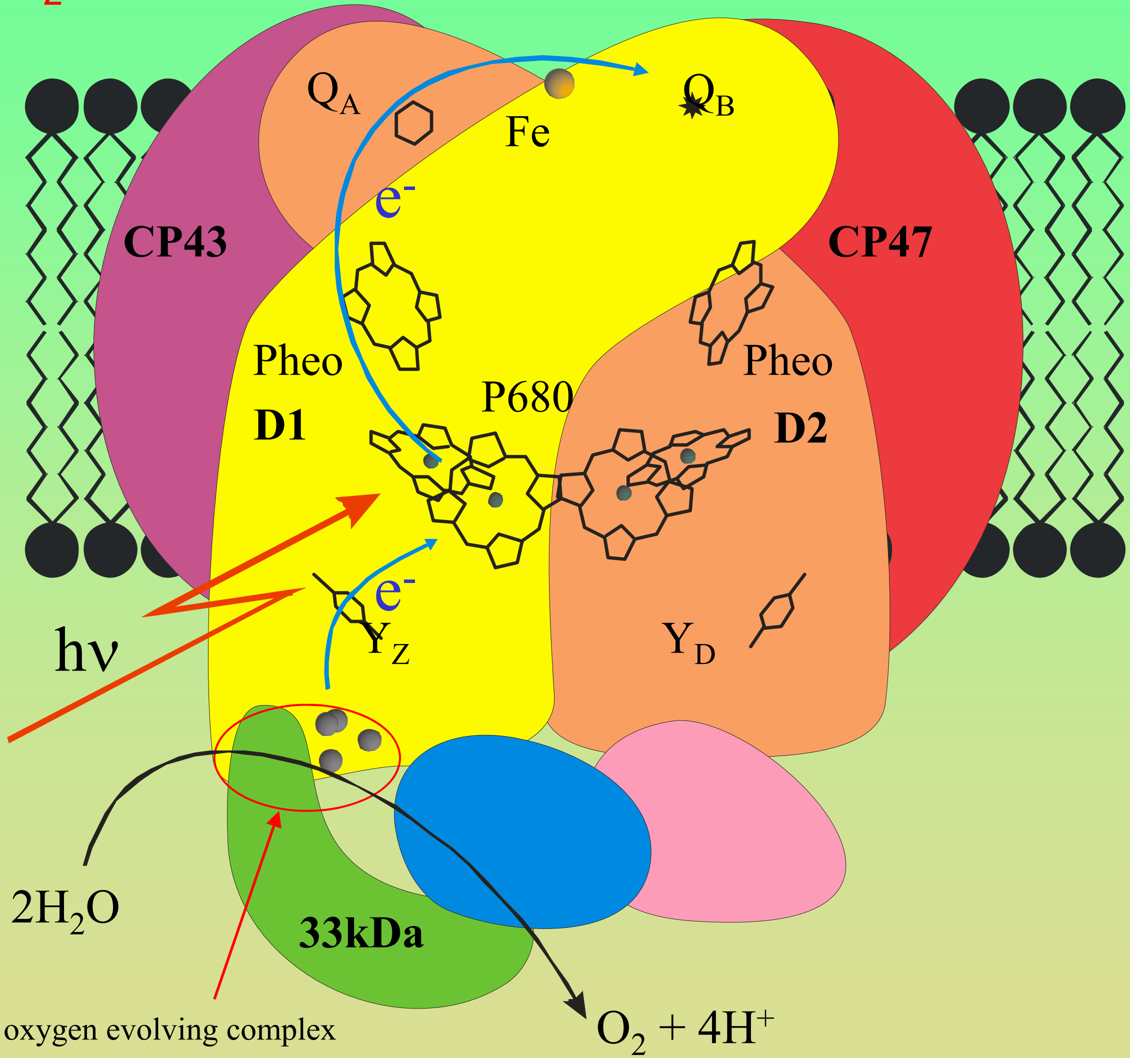
Photosystem 2 reaction center



Fotosystém II

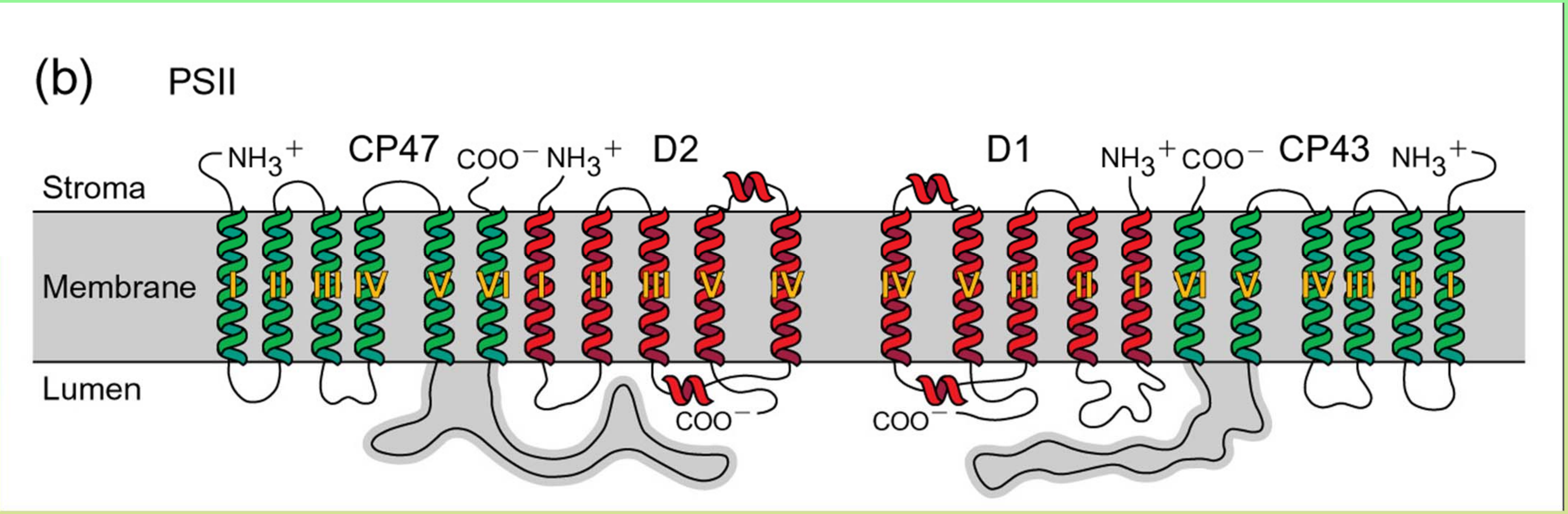
$2H_2O + 2PQ \rightarrow O_2 + 2PQH_2$

- z hlediska enzymové aktivity je to voda:plastochinon oxidoreduktáza
- složitější než BRC, PSII má až 30 podjednotek (nutnost regulace a ochrany před kyslíkem?)

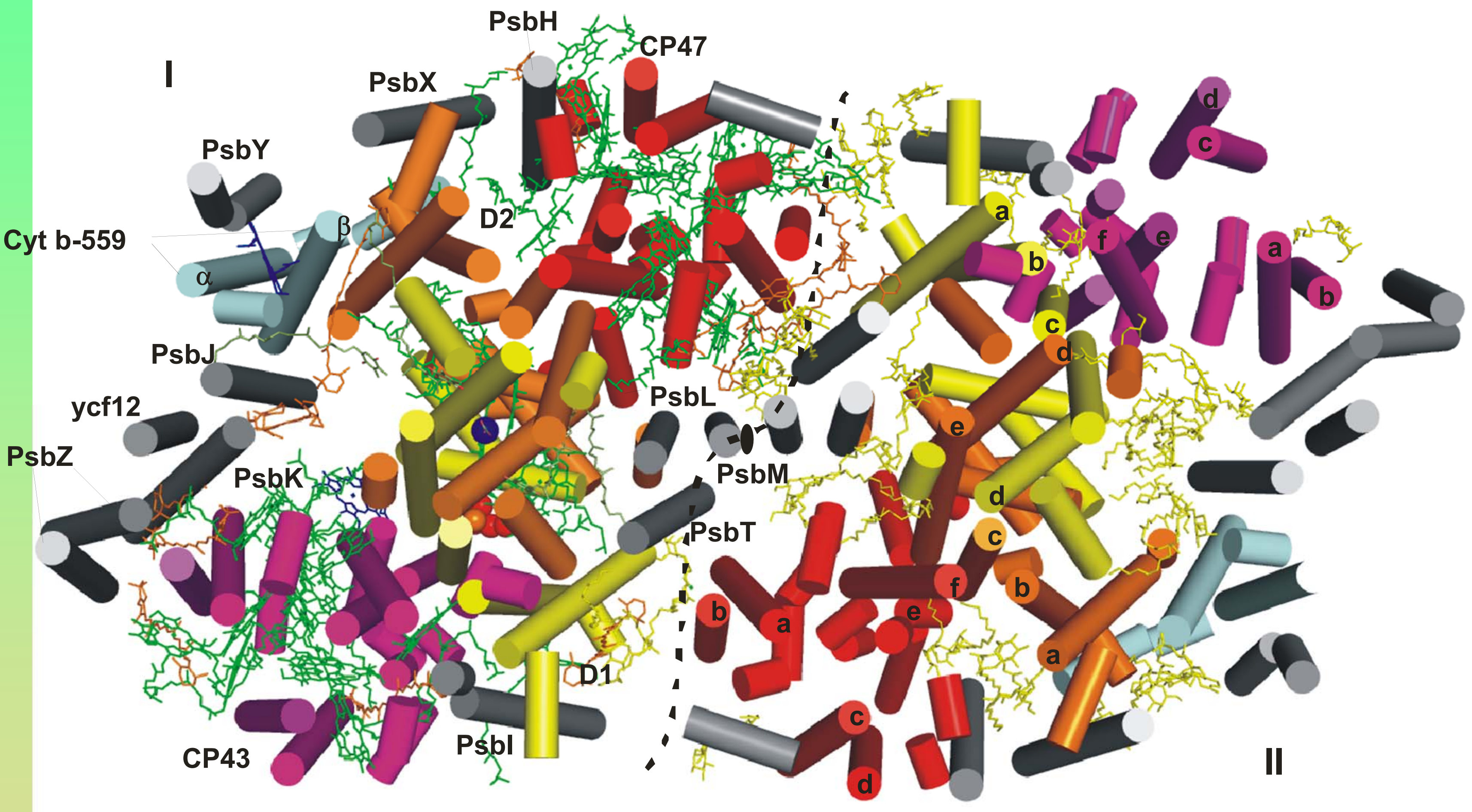


Velké proteinové podjednotky fotosystému II

- u fotosystému II váží pigmenty reakčního centra homologní membránové podjednotky D1 a D2, zatímco anténní pigmenty jsou vázány symetricky vázanými proteiny CP47 a CP43



Struktura fotosystému II z *Thermosynechococcus elongatus* s rozlišením 3.0 Å (Kern et al., Nature 2005)



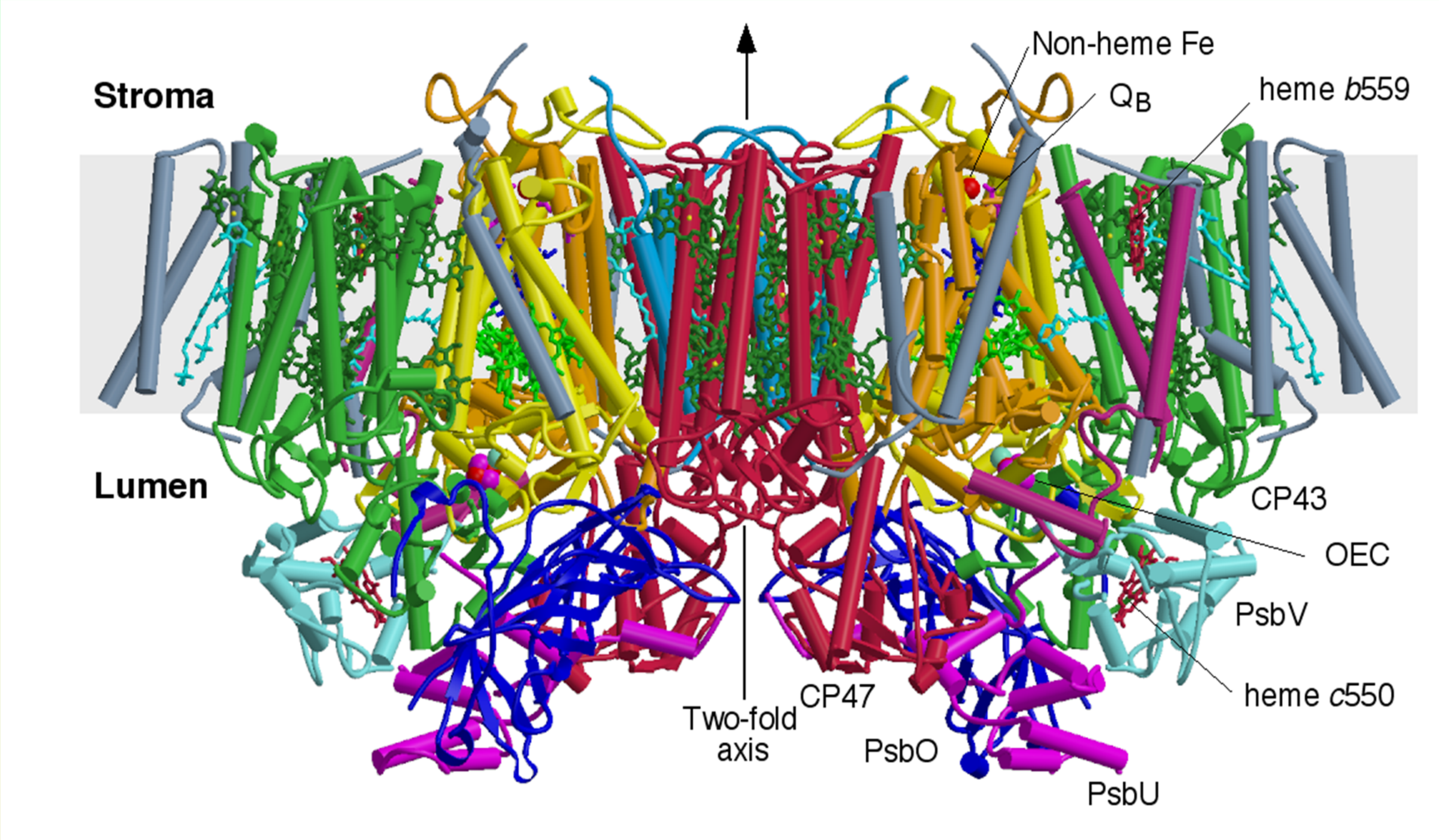
Oligomerní stav, velikost: dimer, 750 kDa

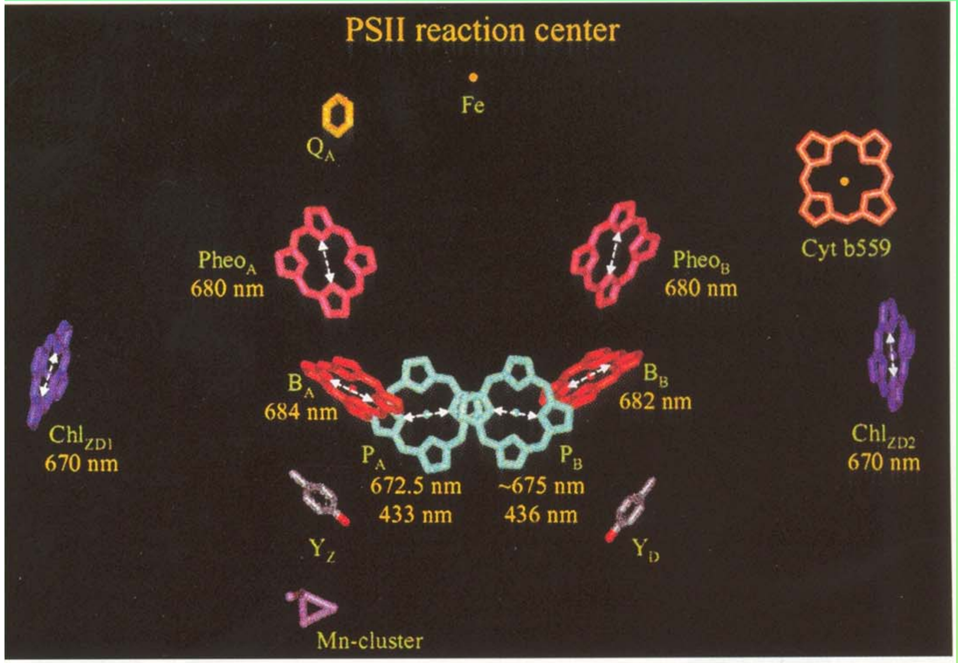
Počet podjednotek na monomer : 20 obsahující 36 TMH

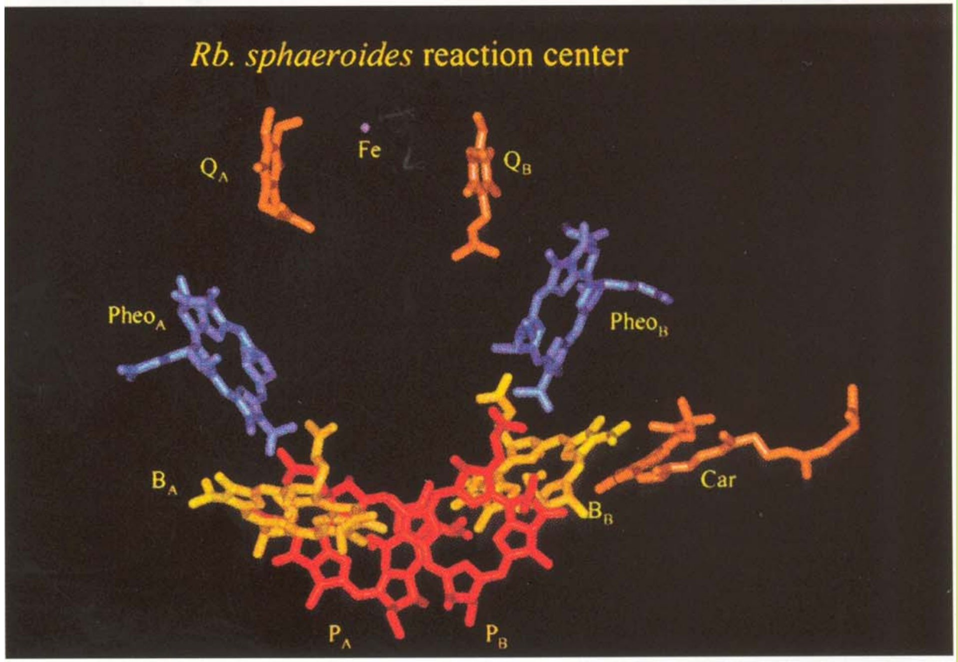
Počet kofaktorů na monomer : 89 z toho:

35 Chl*a*, 12 Car, 25 lipidů, 3 PQ, 2 hem, 2 Pheo, 1 bicarbonát, Fe^{2+}, Mn_4Ca, Cl^-, 2 Ca^{+2} (a 7 DM)

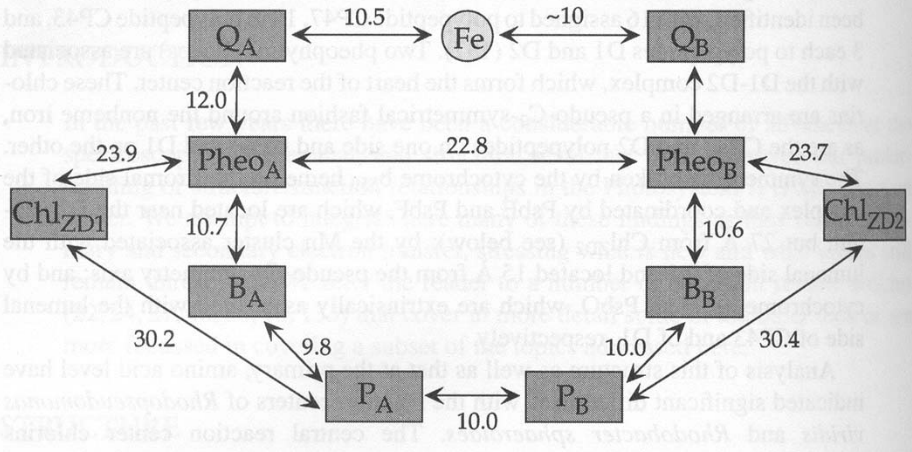
Na lumenální straně fotosystému II je vázán kyslík vyvíjející Mn clastr s 3 stabilizujícími proteiny PsbO, PsbV a PsbU



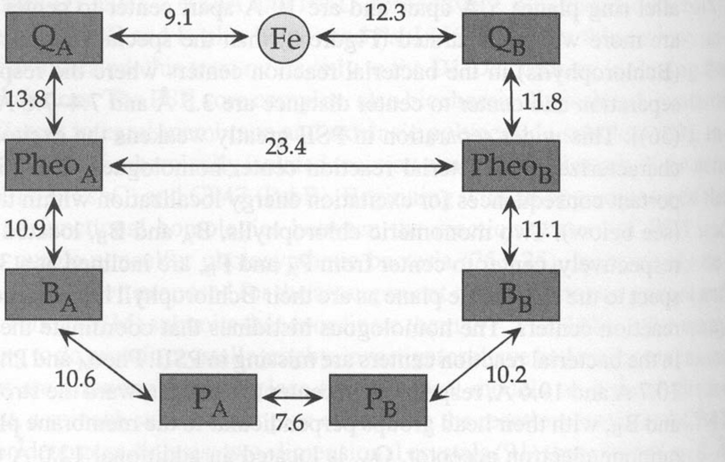

PSII reaction center


Rb. sphaeroides reaction center

Center-to-center distances (Å)

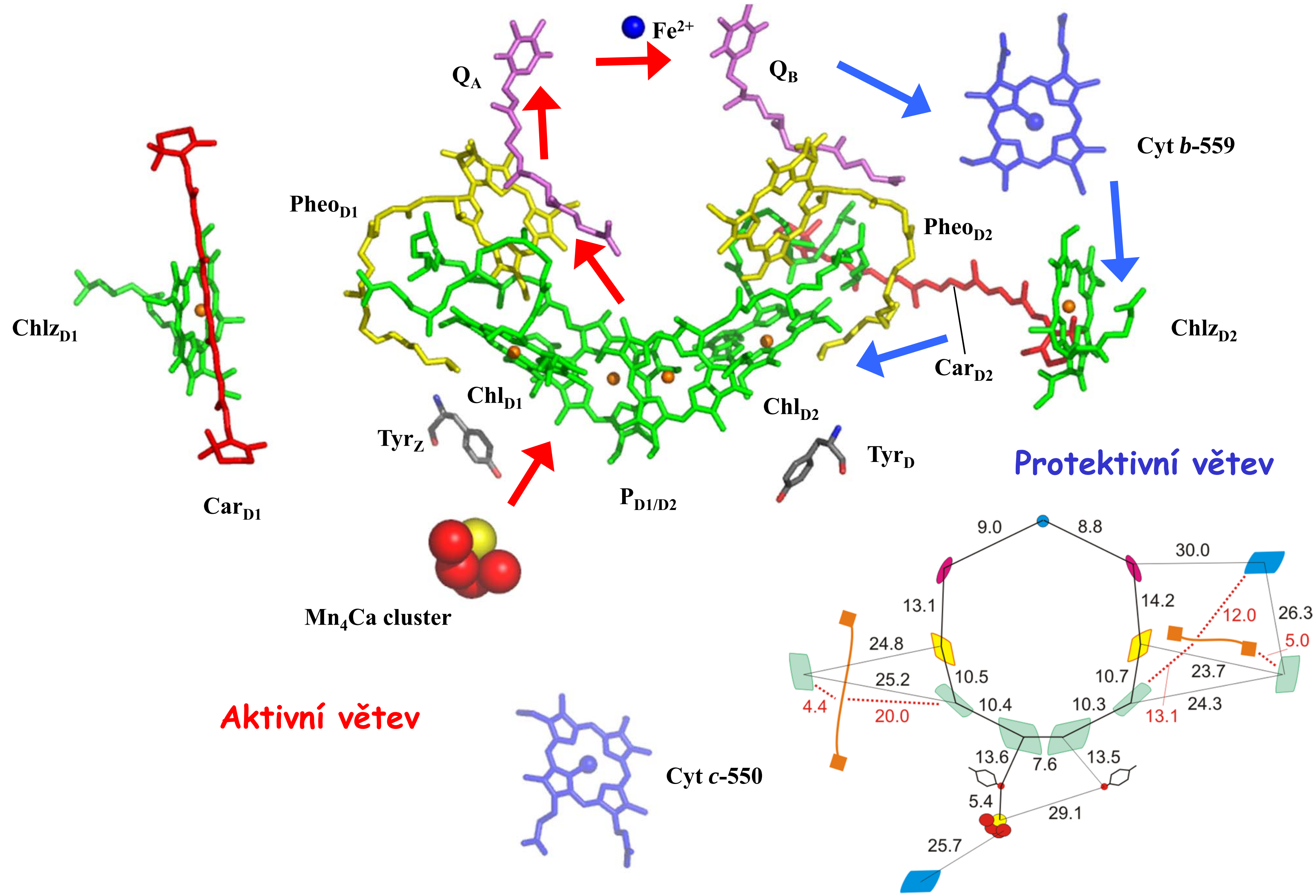


PSII - *Synechococcus elongatus* (149)



BRC - *Rhodobacter sphaeroides* (36)

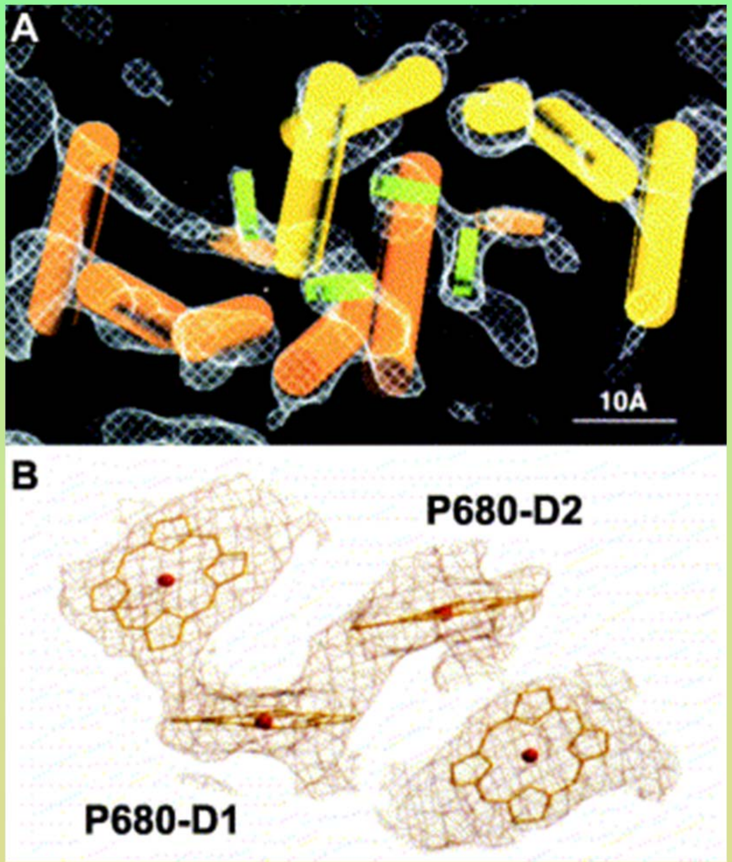
Elektron-transportní řetězec fotosystému II (Kern et al., Nature 2005)

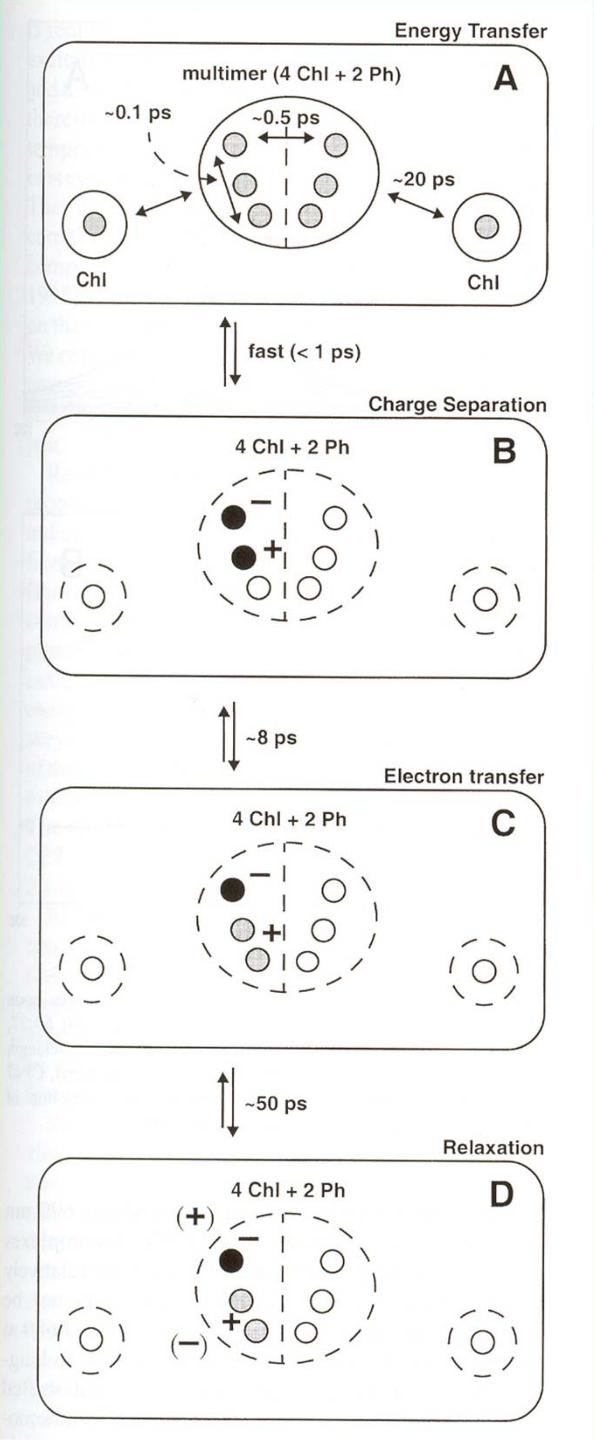


Protektivní větev

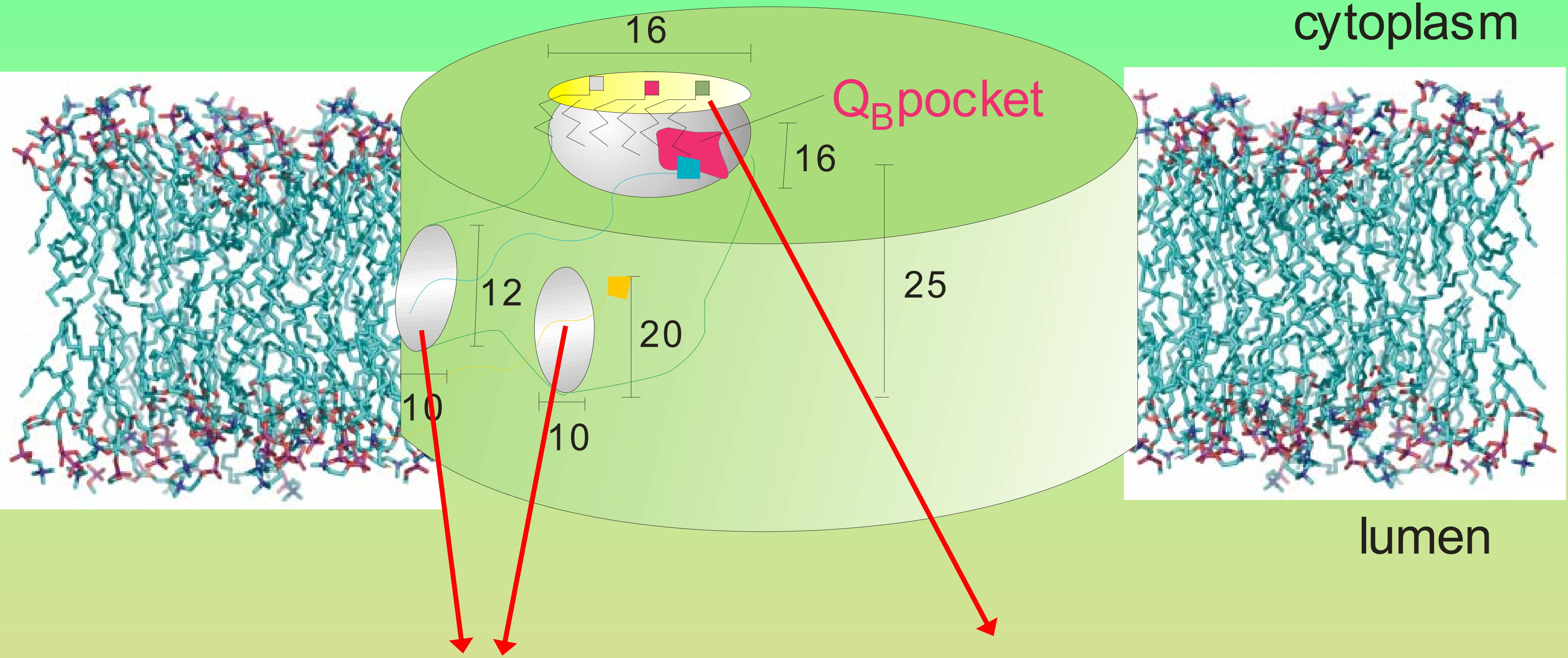
Aktivní větev

Na separaci nábojů se podílí multimer molekul chlorofylu P680





Struktura Q_B kapsy: kromě chinonu Q_B i vazba dalšího chinonu Q_C



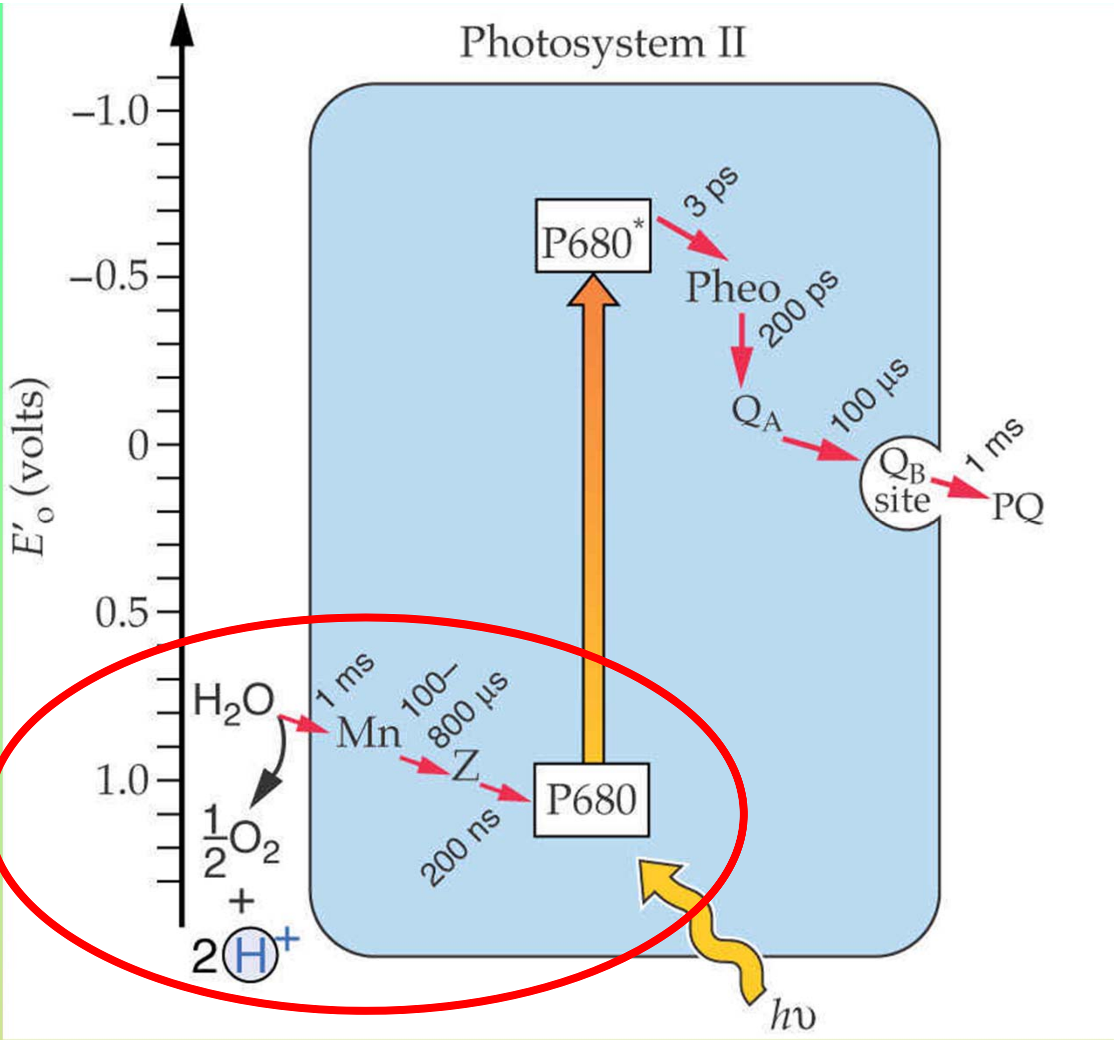
2 kanály spojují Q_B kapsu s membránou, v jednom je Q_B, v druhém Q_C

přístup ke Q_B kapse z cytoplazmy je blokován lipidy

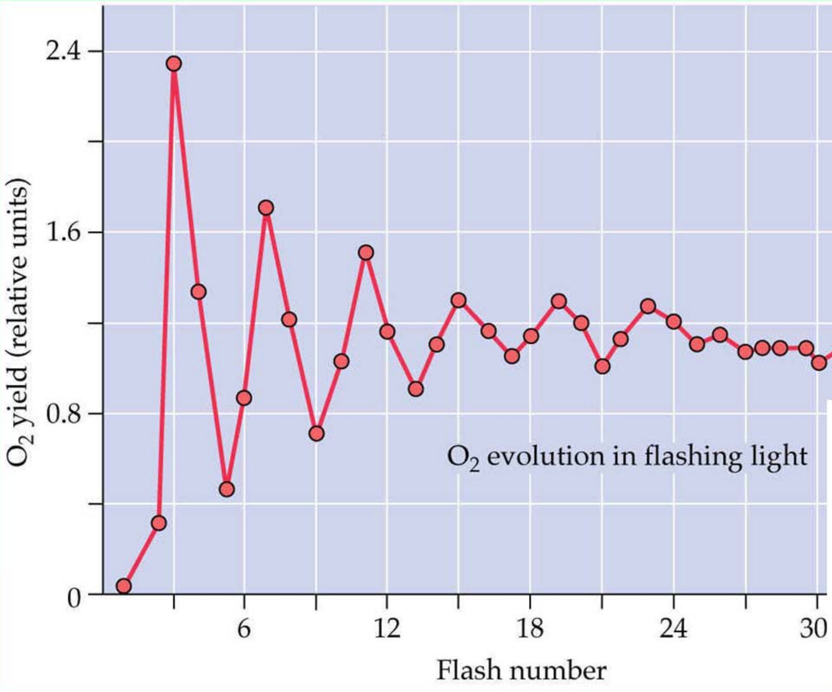
Výwoj kyslíku v PSII

$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$

$E_m = +\ 0.82\ V$

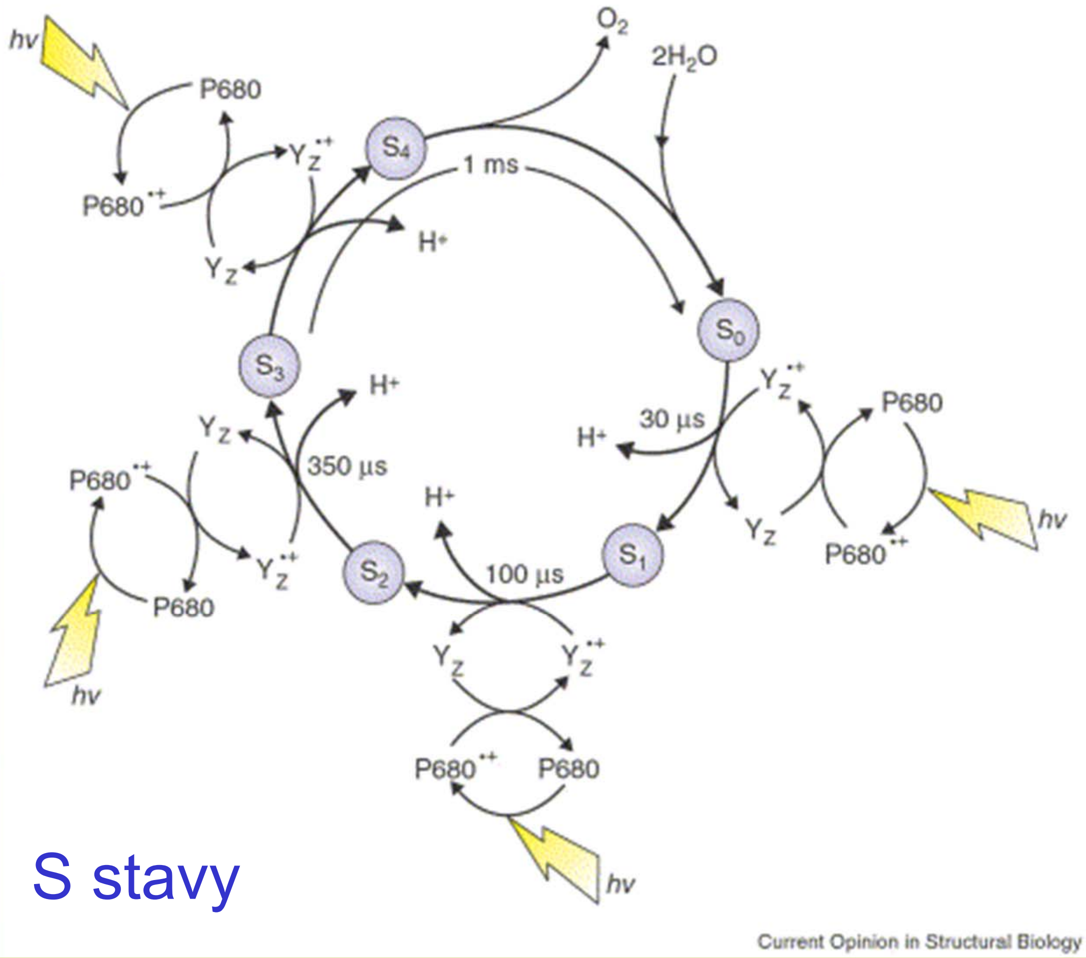


P680$^+$
$E_m = +\ 1.2\ V$



4 e$^-$ ale jen 1 foton ??

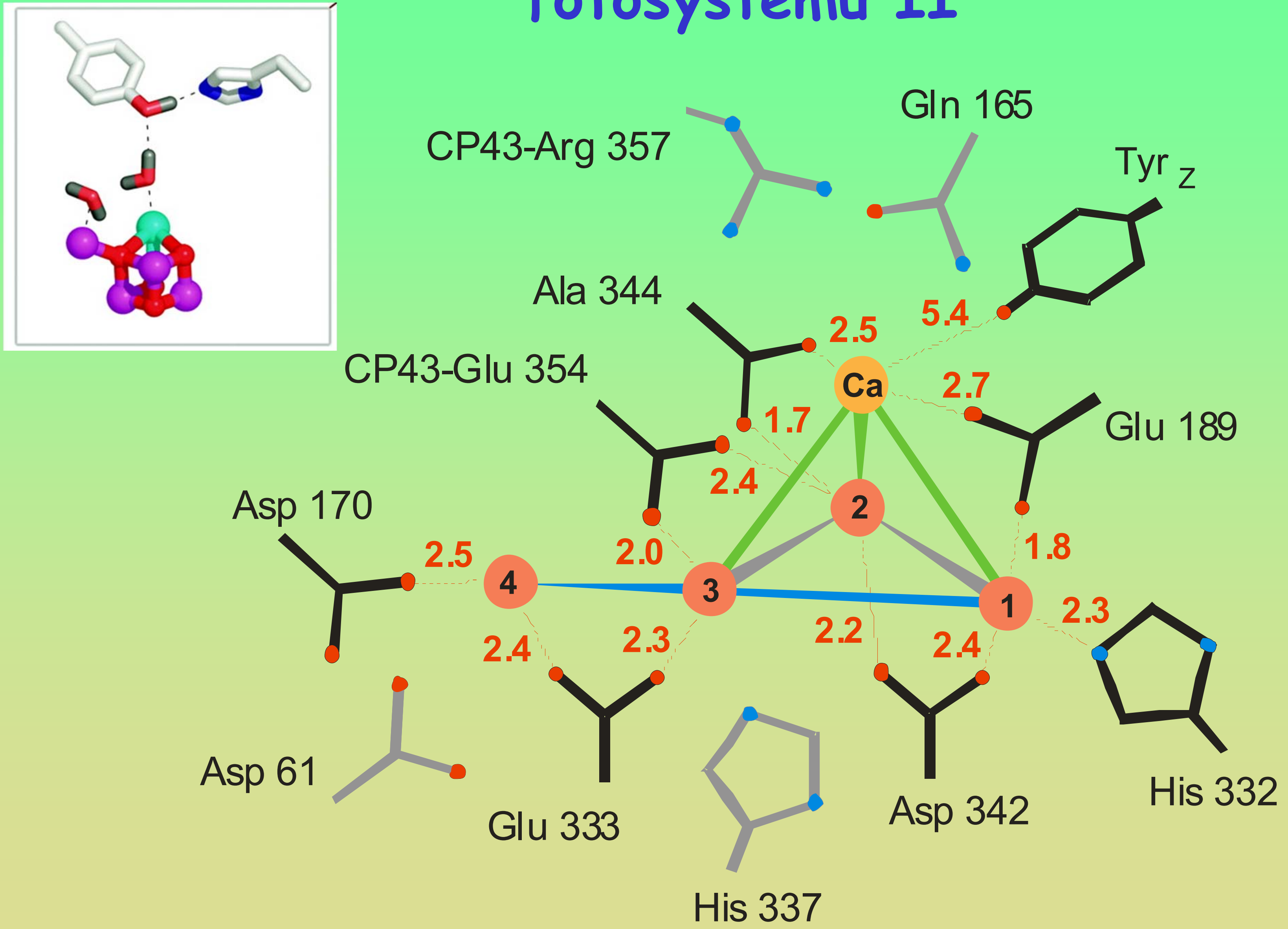
P.Joliot a B.Kok, 1970



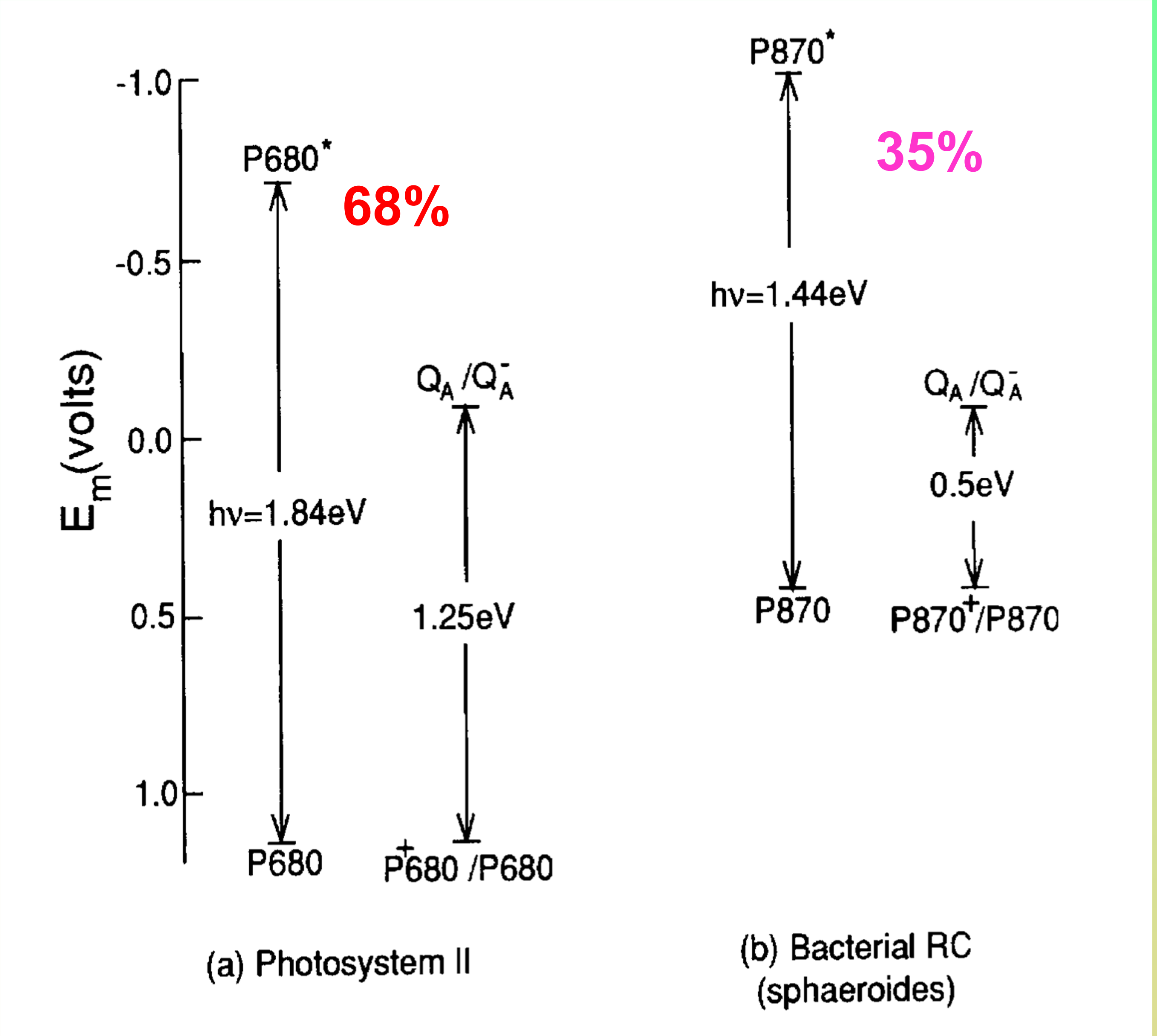
S stavy

Current Opinion in Structural Biology

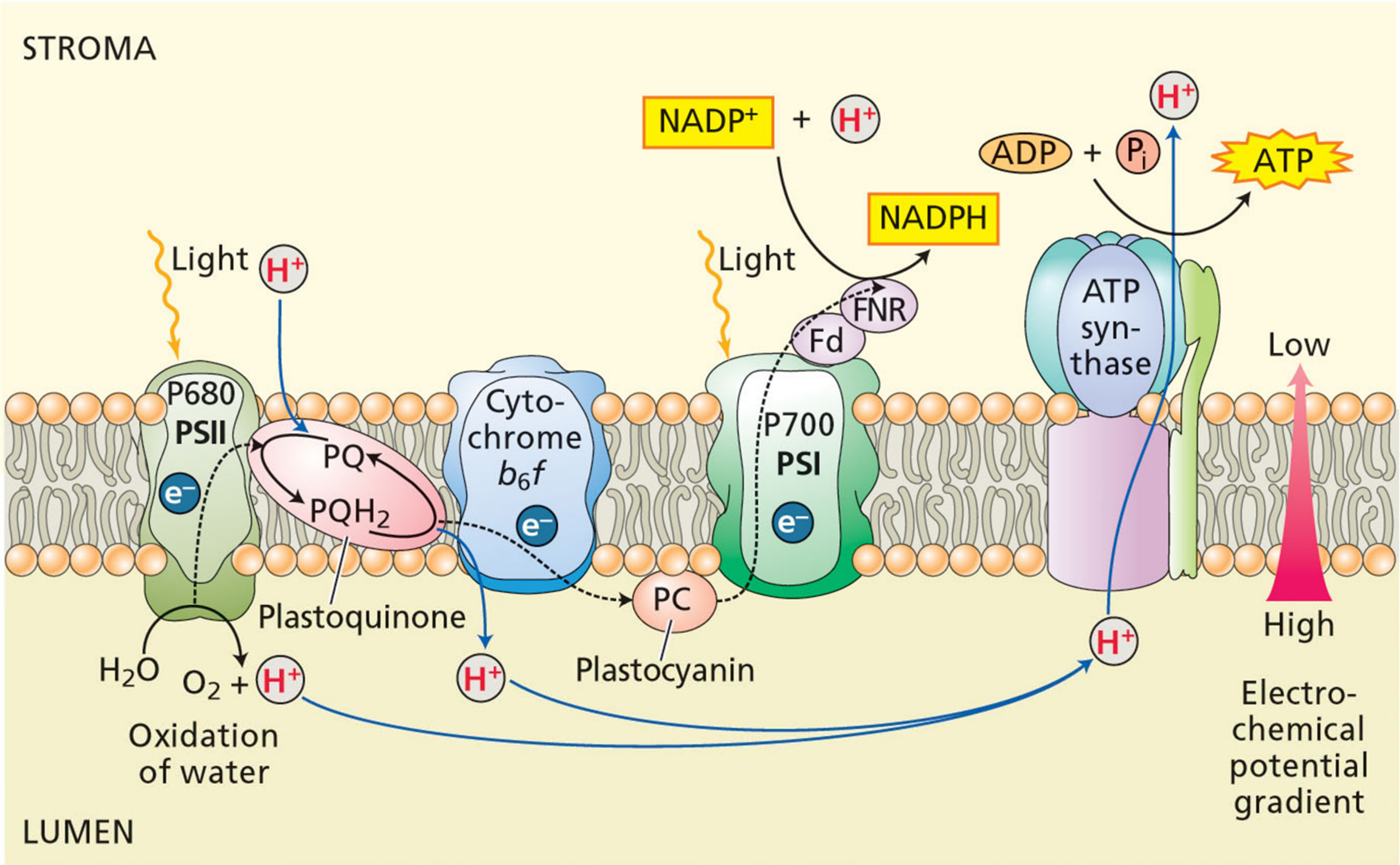
Struktura klastru Mn_4Ca a jeho vazba ve fotosystému II



Srovnání termodynamické účinnosti BRC a PSII



Vektoriální přenos elektronů a protonů



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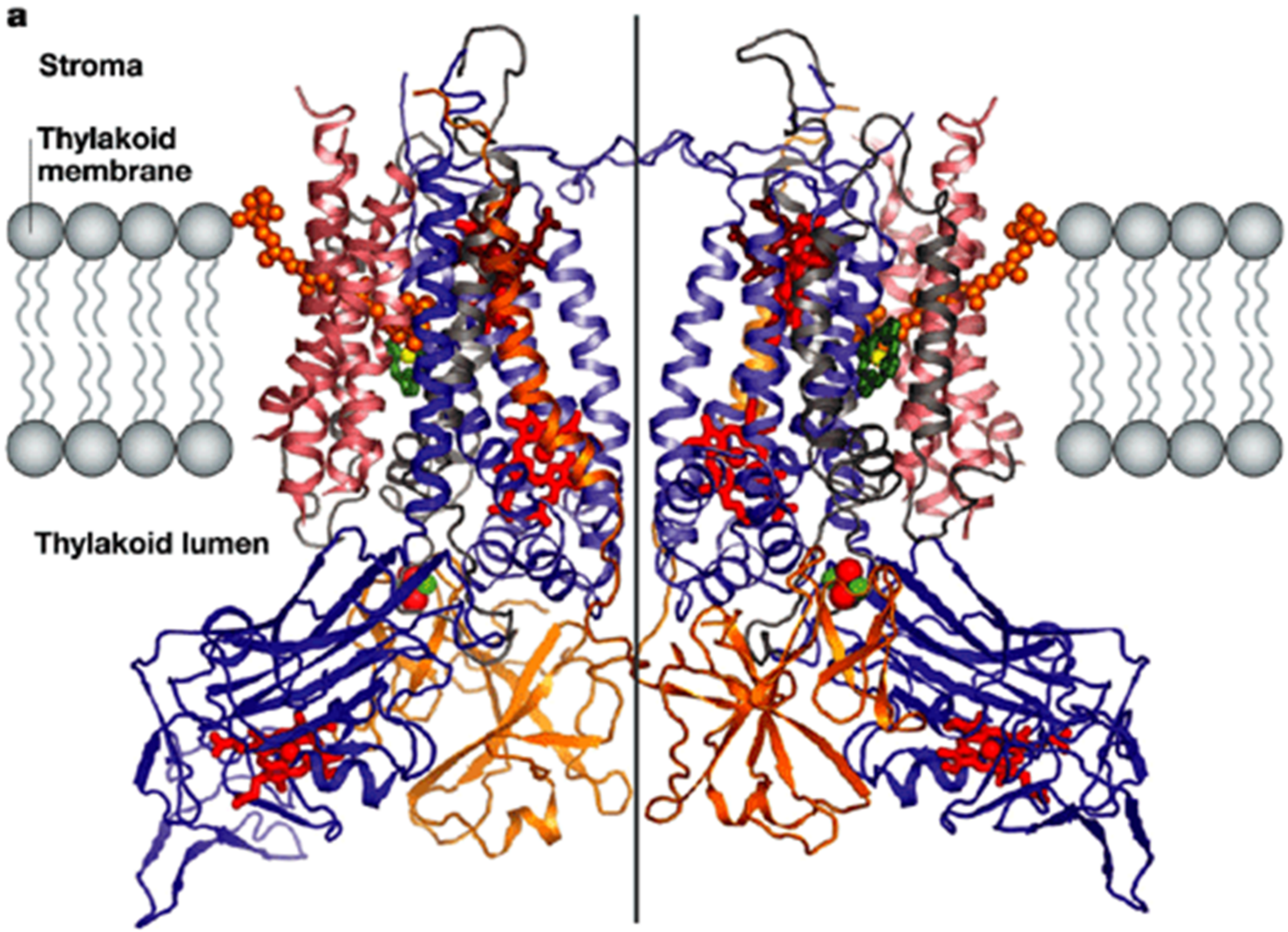
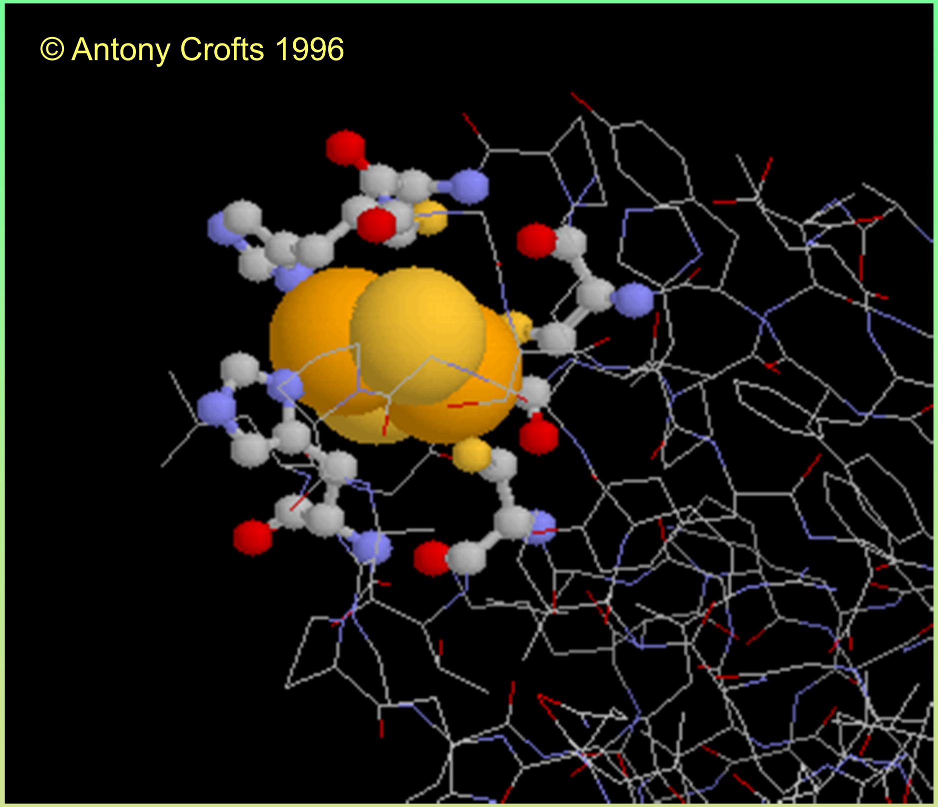
Komplex cyt b6/f
dimer: cyt bf, Rieske Fe-S + ~5 proteins



Rieske 2Fe-2S protein

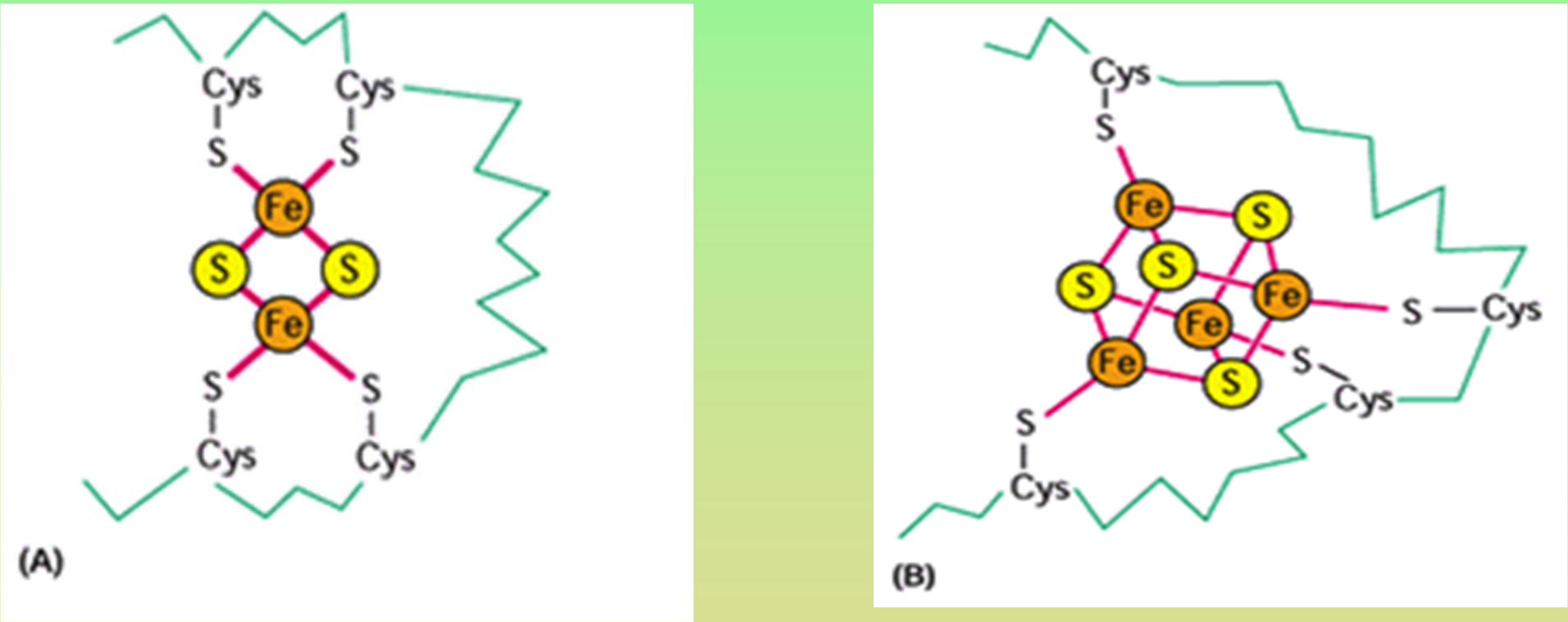


2Fe.2S iron sulfur center from Rieske protein of the bc_1-complex

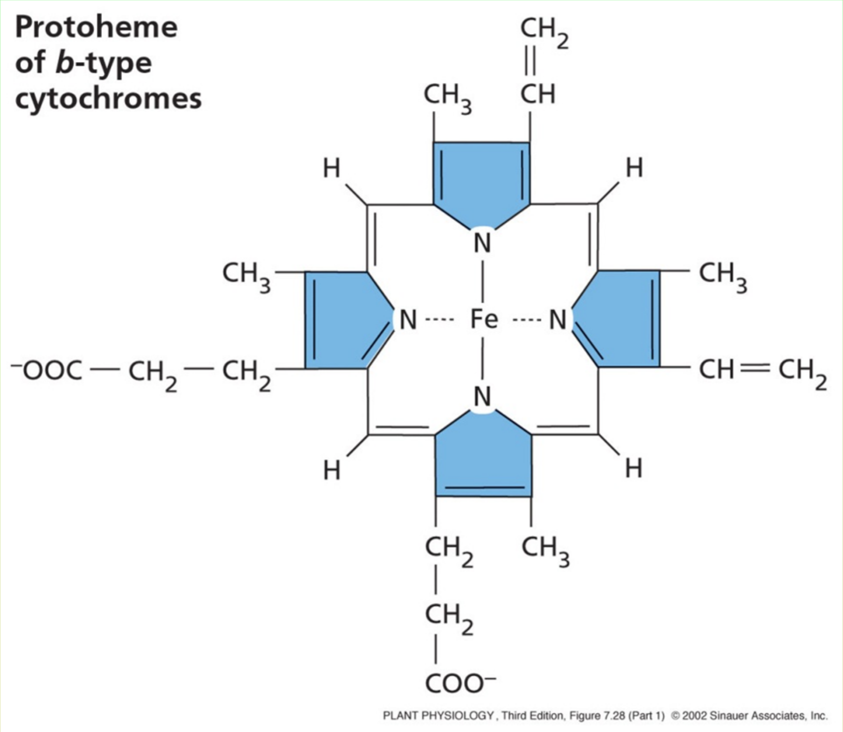
$2Fe2S \Longrightarrow 2Fe2S^+ + e^-$

Midpoint redox potential: $E_{m,\ pH\ 7}$ = 290 mV

The structures of two types of iron-sulfur centers. (A) A center of the 2Fe2S type. (B) A center of the 4Fe4S type. Although they contain multiple iron atoms, each iron-sulfur center can carry only one electron at a time. There are more than six different iron-sulfur centers in the respiratory chain.



Hemy – integrální součást cytochromů



Protoheme of *b*-type cytochromes

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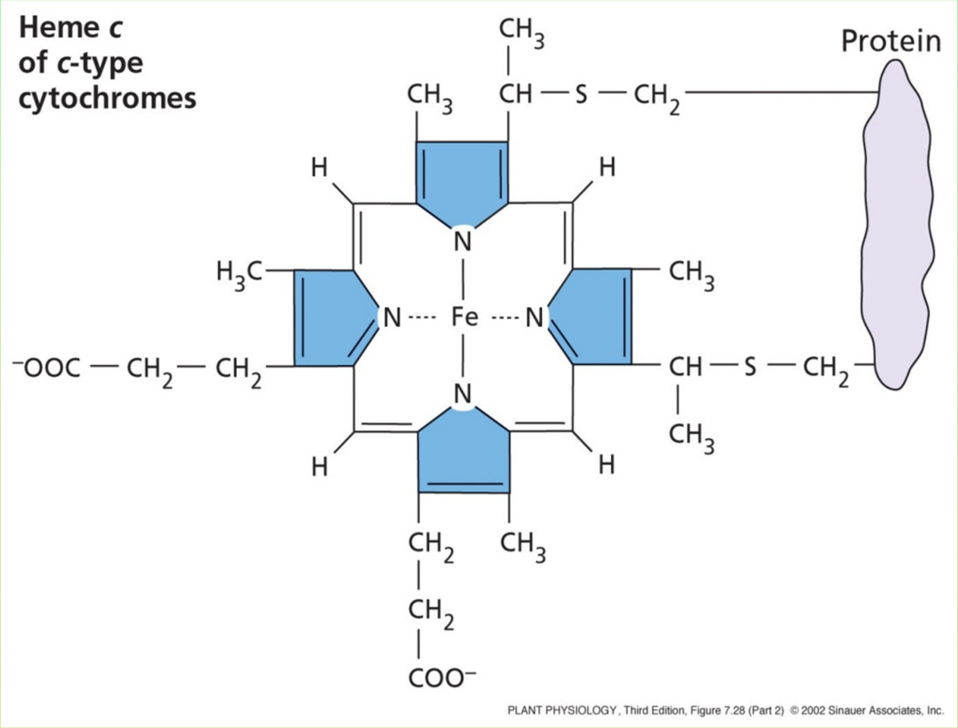

Heme *c* of *c*-type cytochromes

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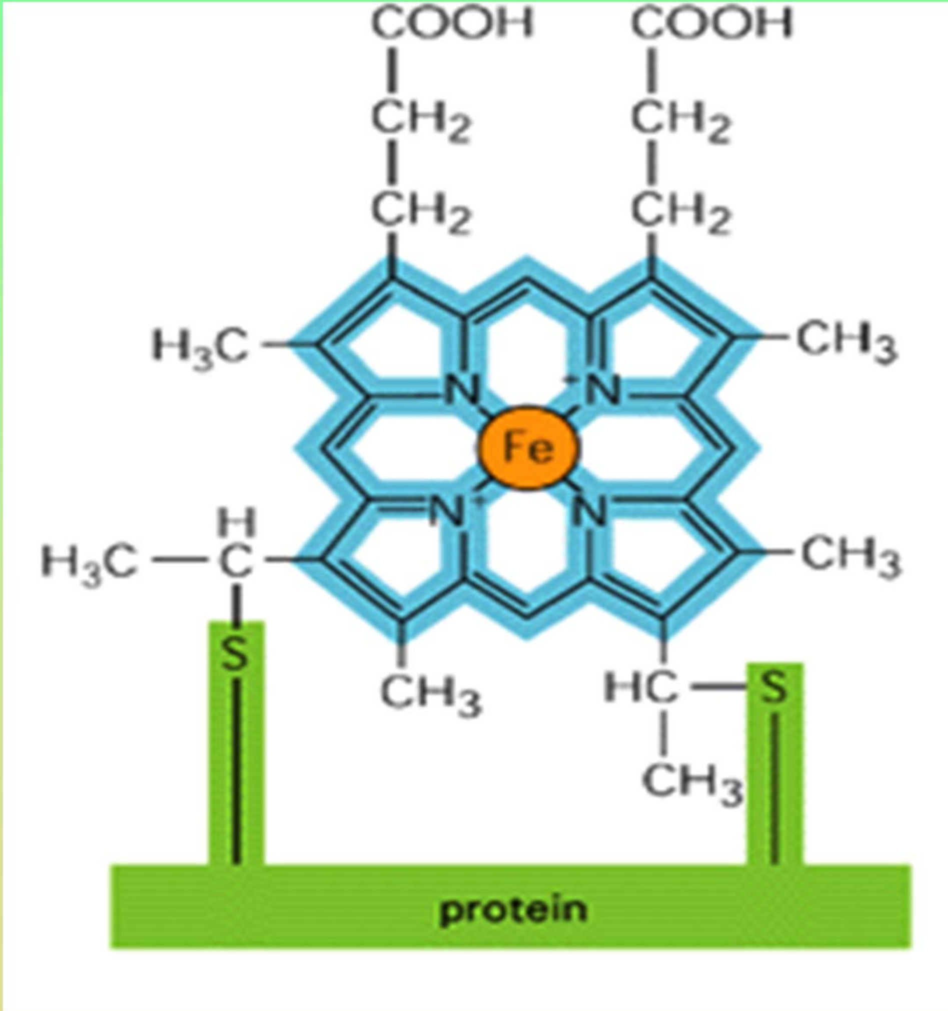
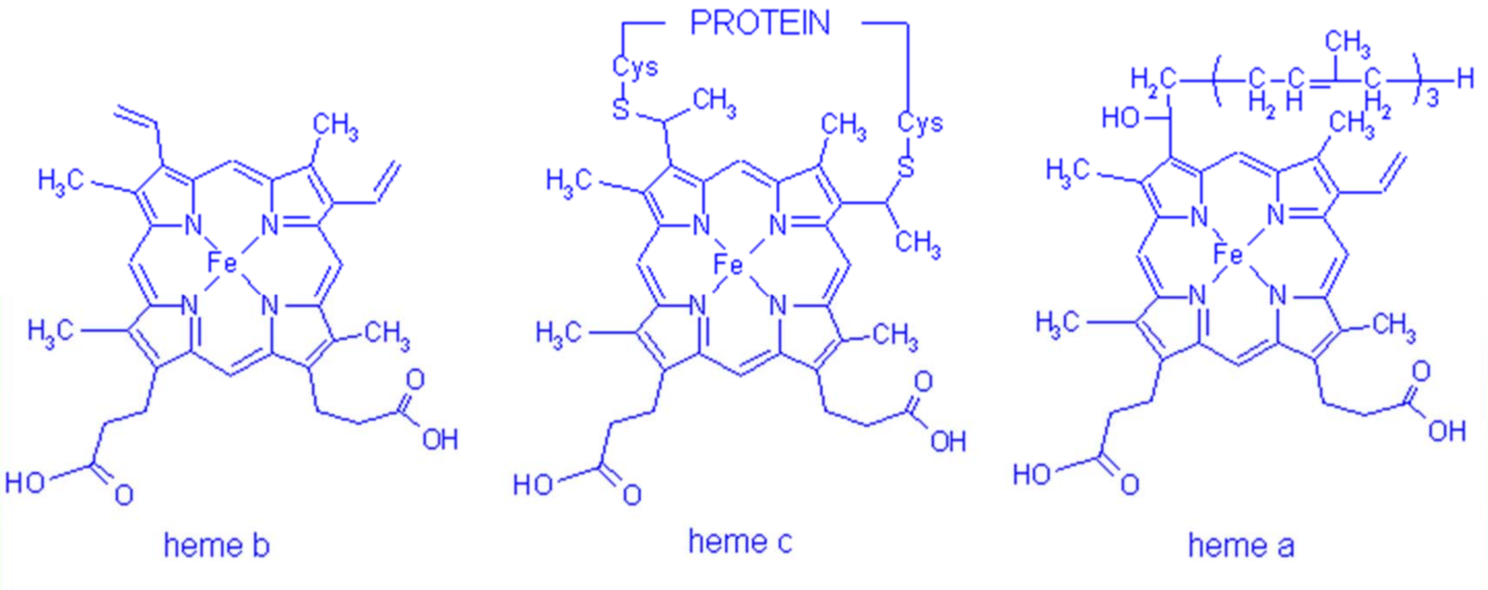
The structure of the heme group attached covalently to cytochrome c. The porphyrin ring is shown in blue. There are five different cytochromes in the respiratory chain. Because the hemes in different cytochromes have slightly different structures and are held by their respective proteins in different ways, each of the cytochromes has a different affinity for an electron.



Cytochromes



Position of α - band

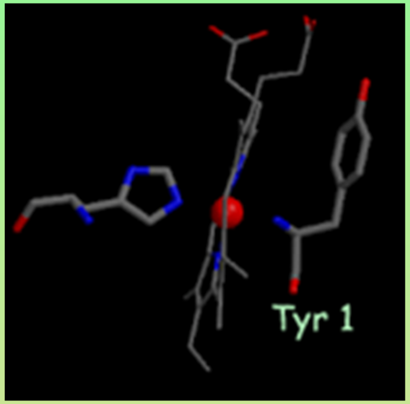
cyt *a* ~ 600 nm

cyt *b* ~ 560 nm

cyt *c* ~ 550 nm

Cytochrome *f*

c - type cytochrome, transfers electrons to plastocyanin

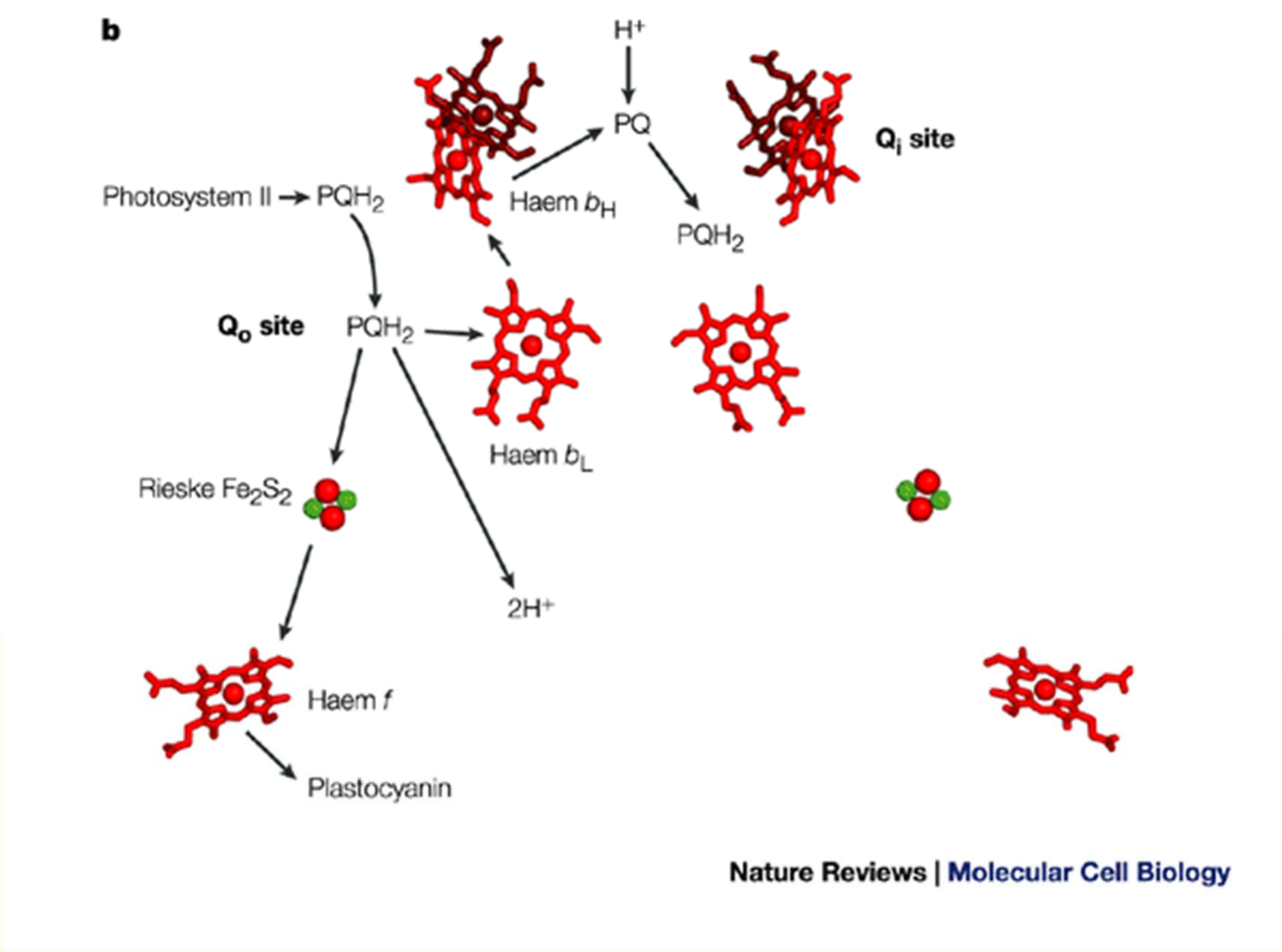


Heme *c*, with two cysteine ligands

ferrocytochrome c ==> ferricytochrome c^+ + e-

Midpoint redox potential: $E_{m,\ pH\ 7}$ = 350mV

Komplex cyt b6/f
dimer: cyt bf, Rieske Fe-S + ~5 bílkovin



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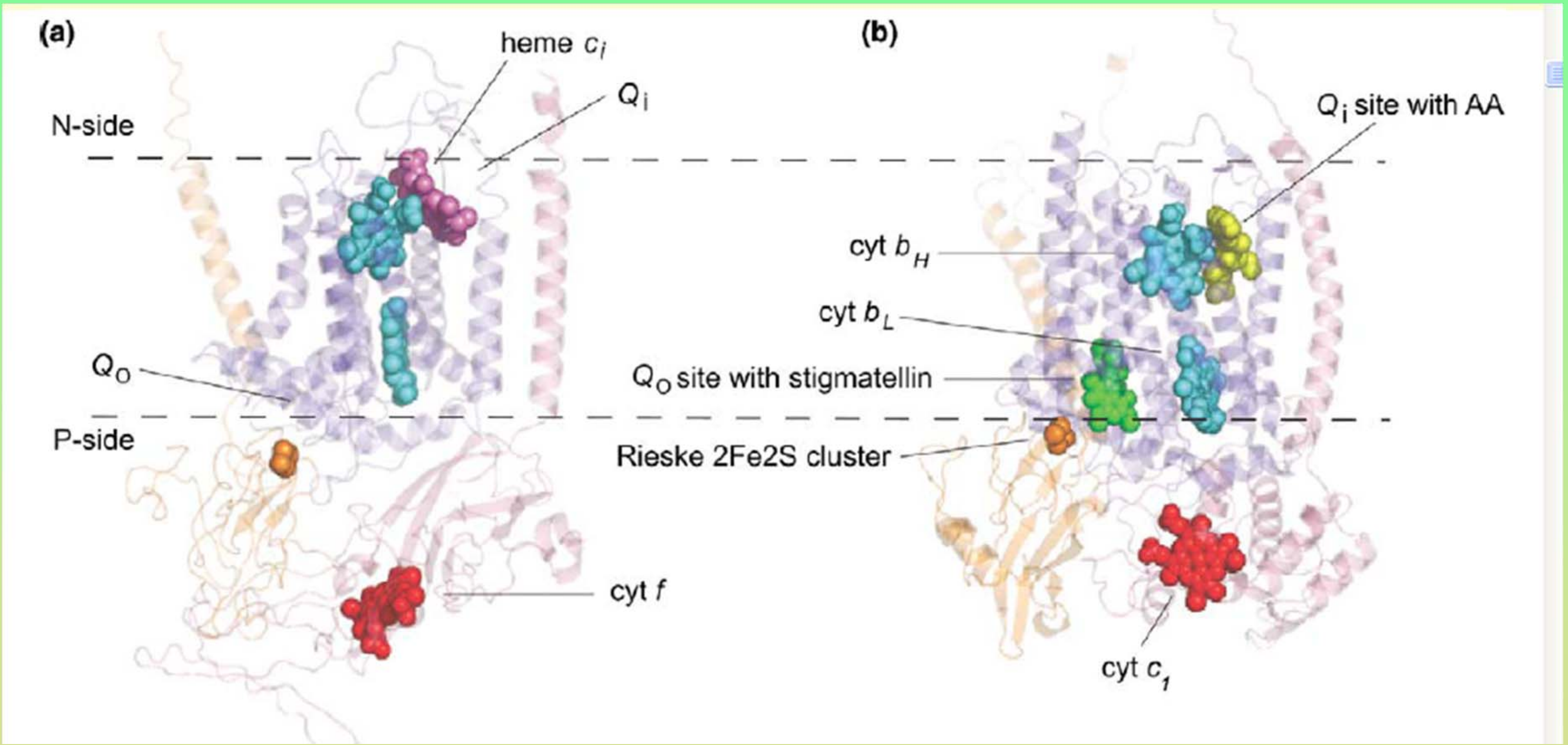
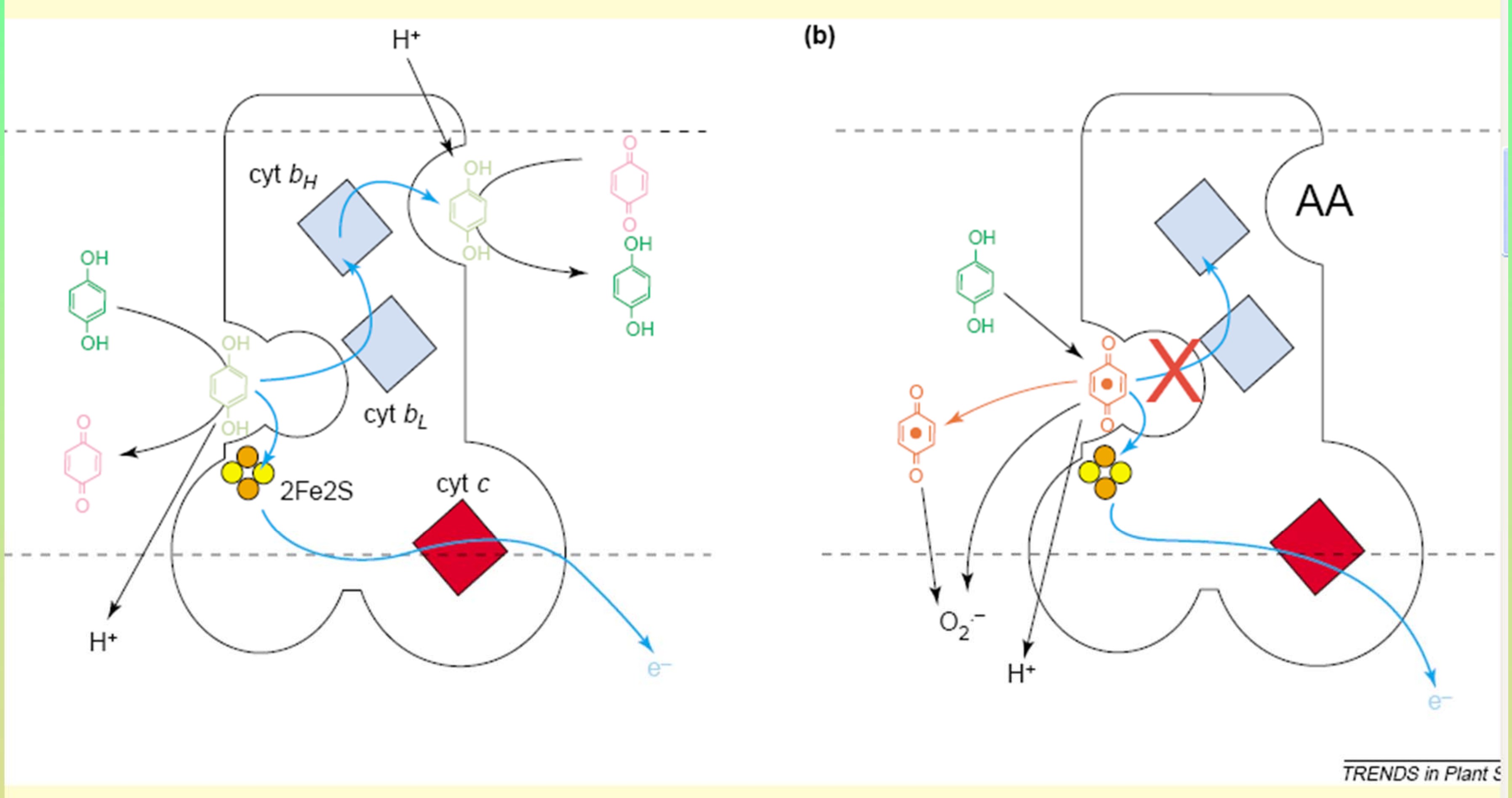
cyt b6/f cyt bc

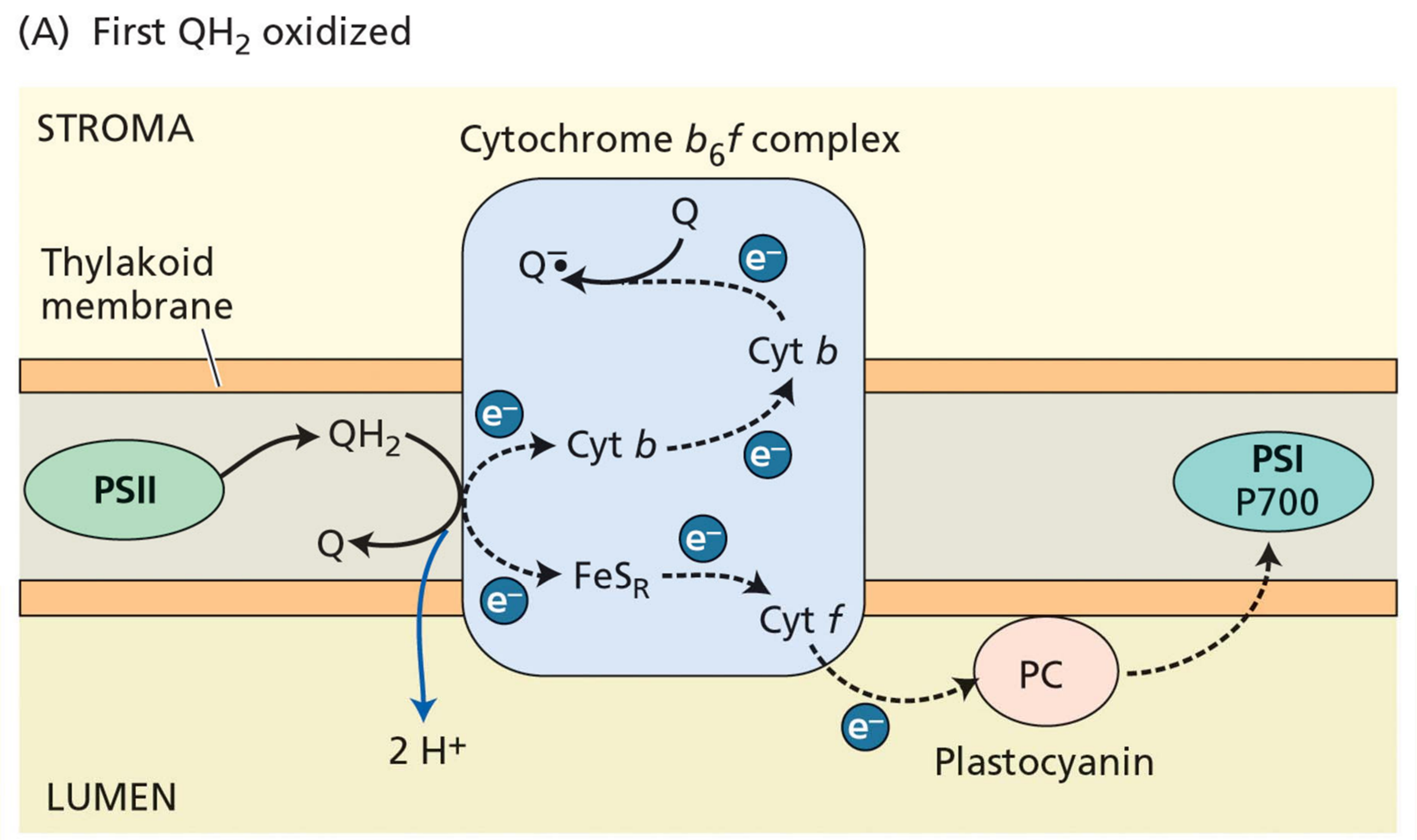


Q cyklus



TRENDS in Plant S

Q cyklus přenosu elektronů v cytb$_6$/f



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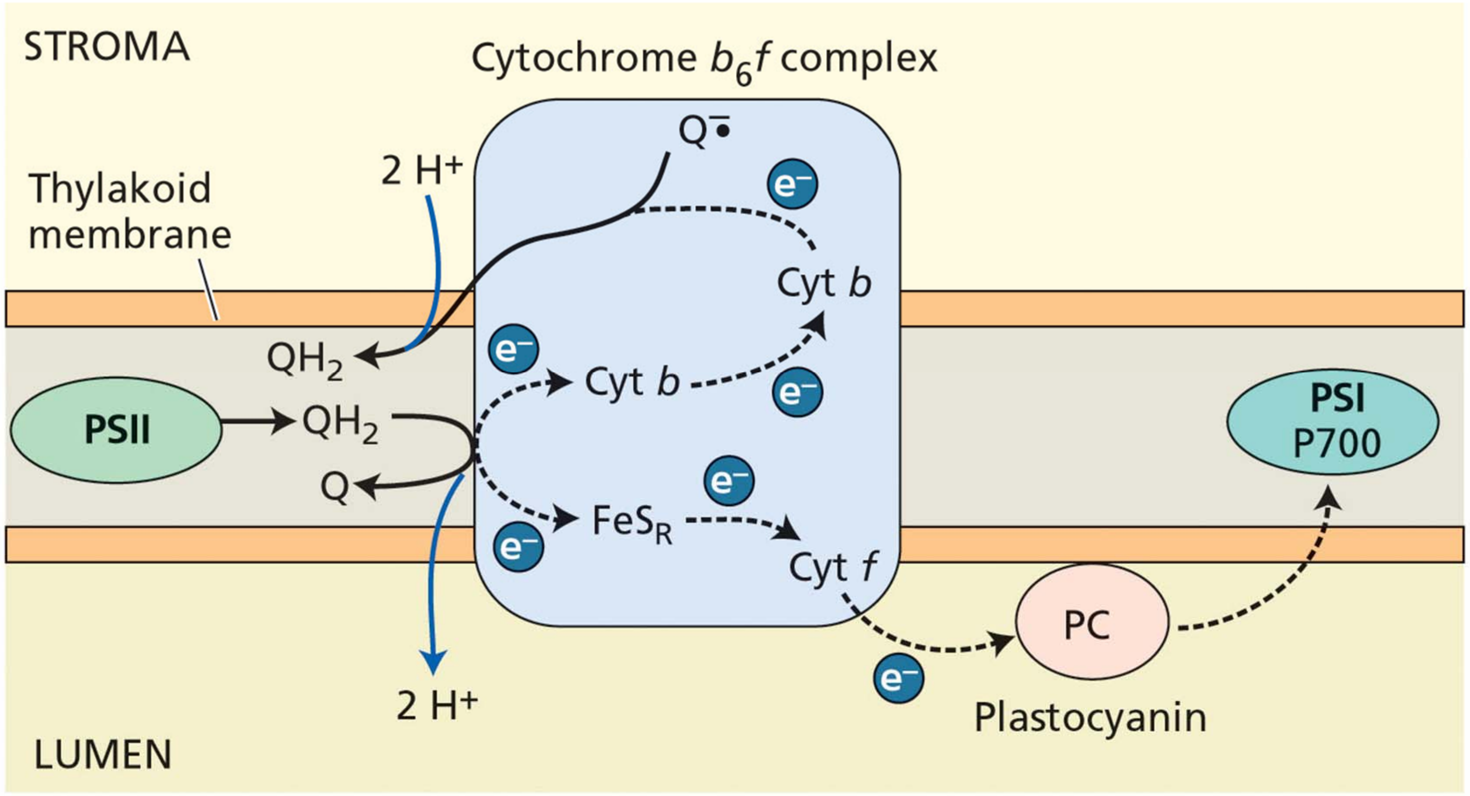
Q cyklus přenosu elektronů v cytb$_6$/f

(B) Second QH$_2$ oxidized



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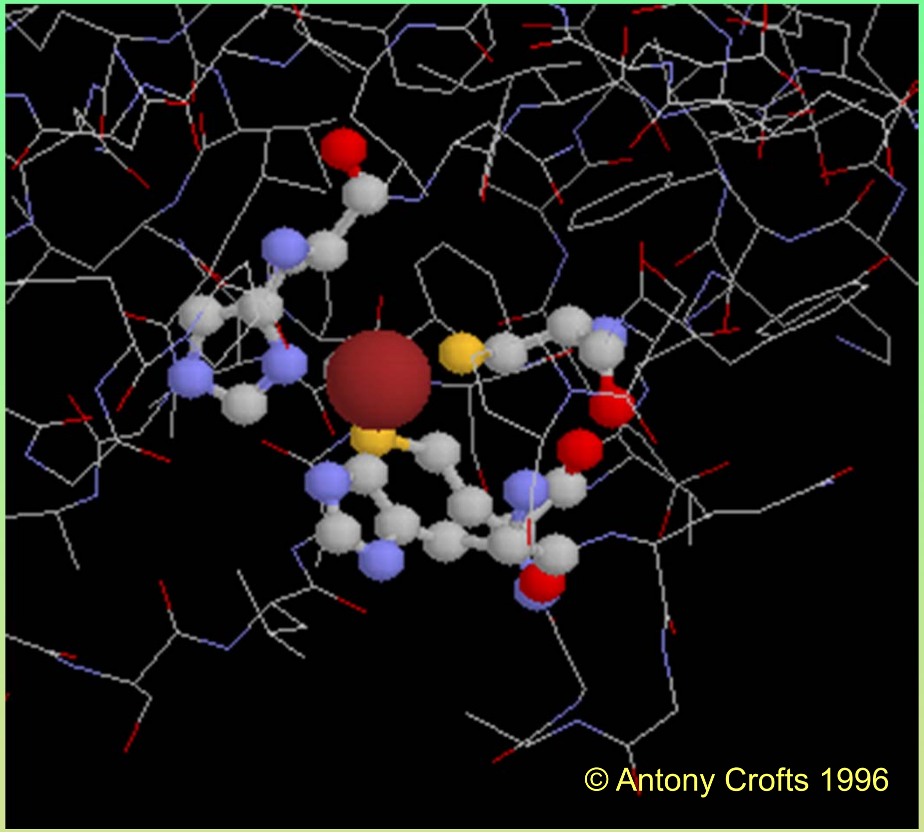
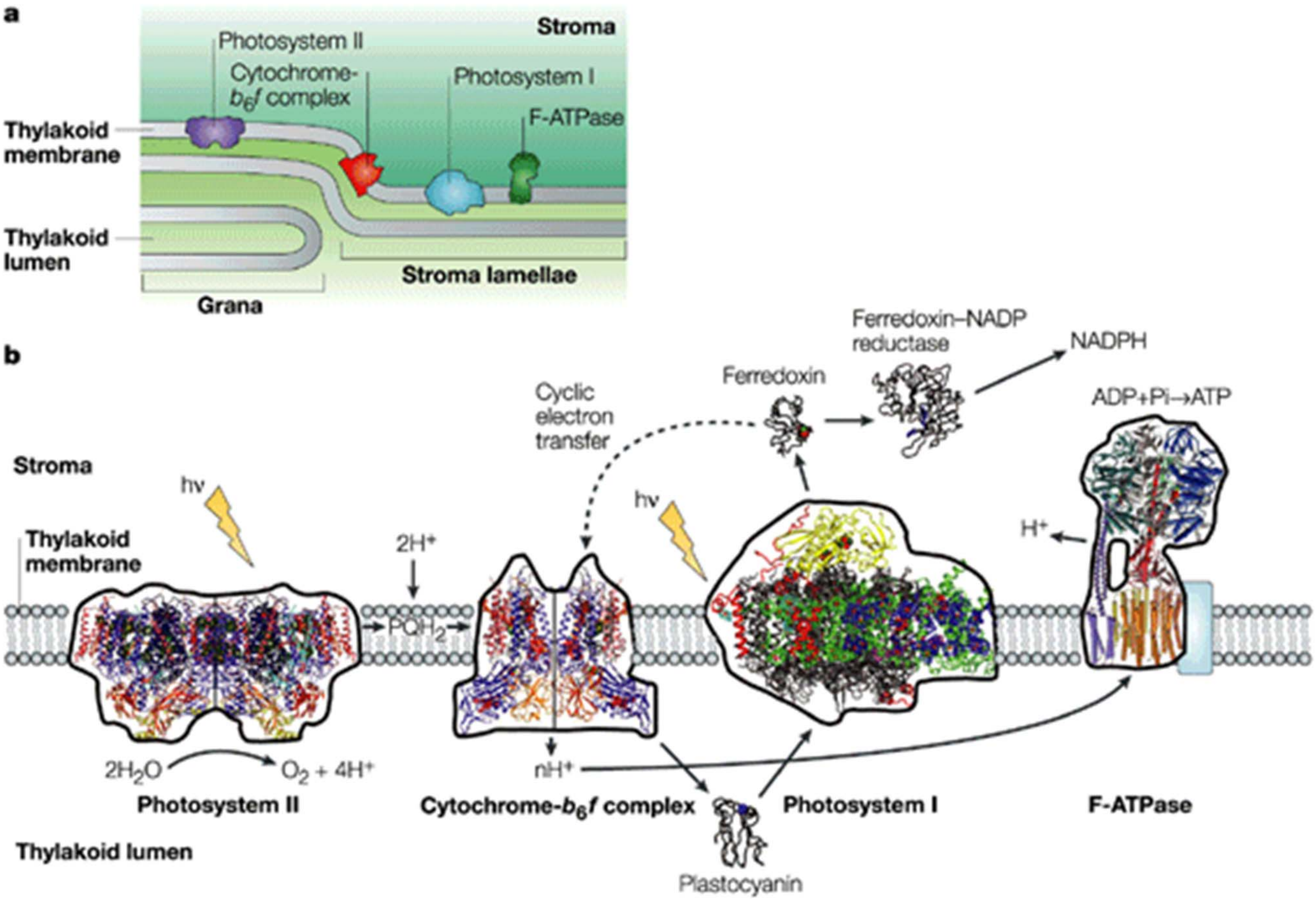
Plastocyanine


© Antony Crofts 1996

Cu center of plastocyanin

Cu^{2+} => Cu^{3+} + e^-

Midpoint redox potential: $E_{m,\ pH\ 7} = 360$ mV



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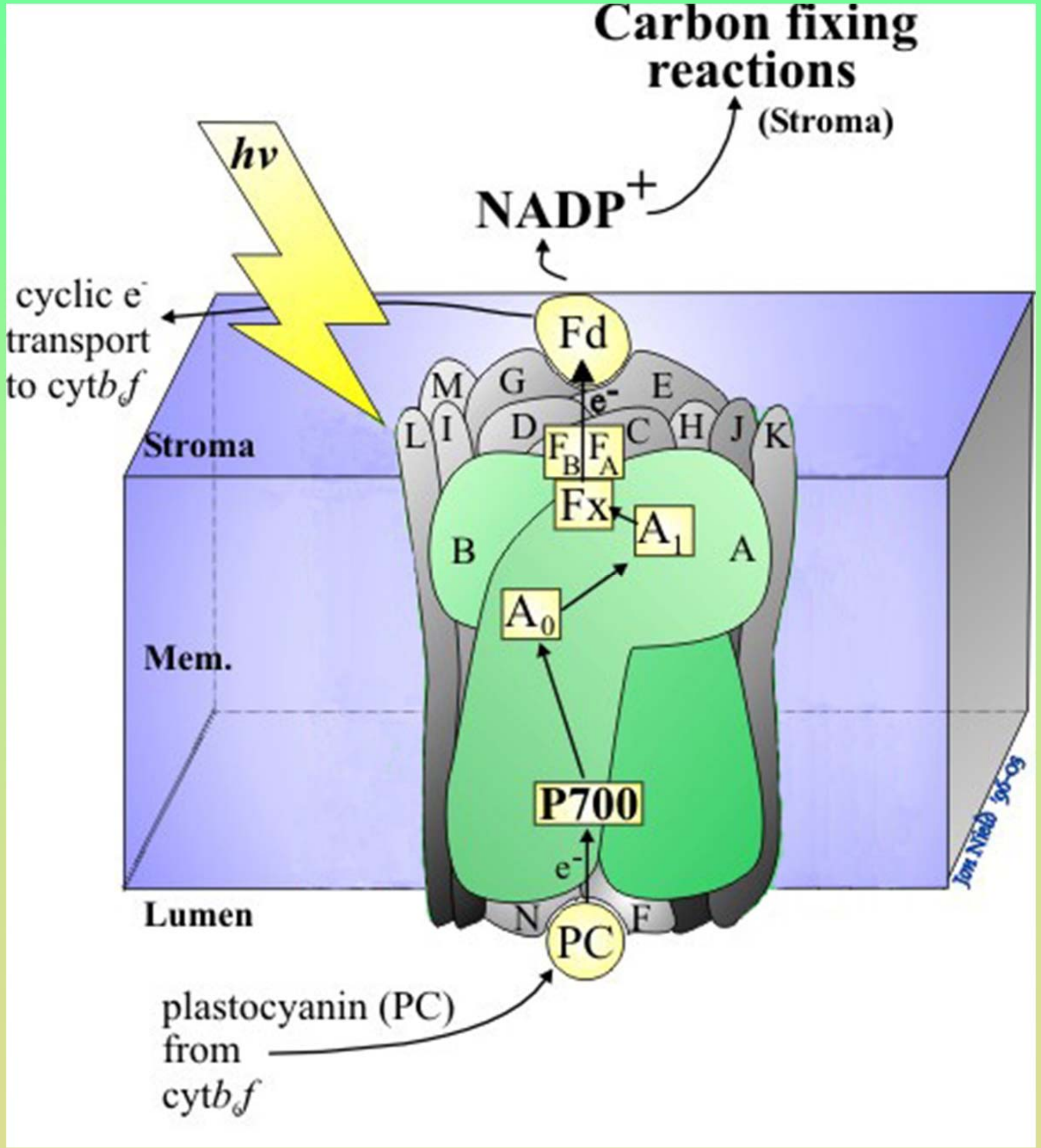
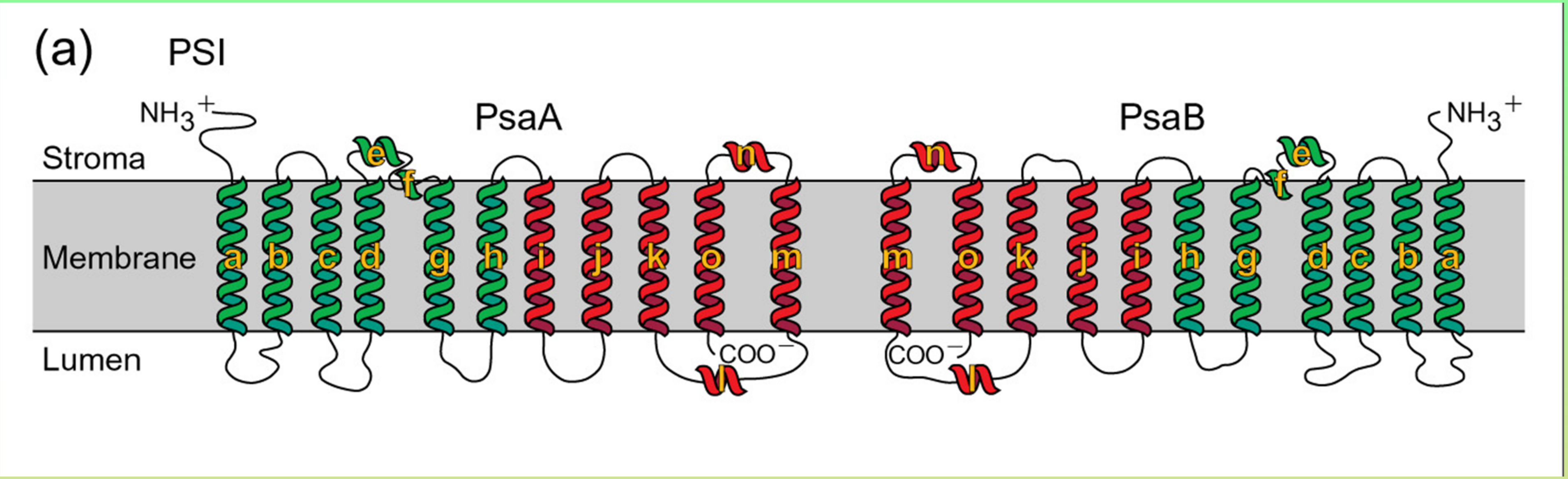
Fotosystém I

- z hlediska enzymové aktivity je to plastocyanin:ferredoxin oxidoreduktáza

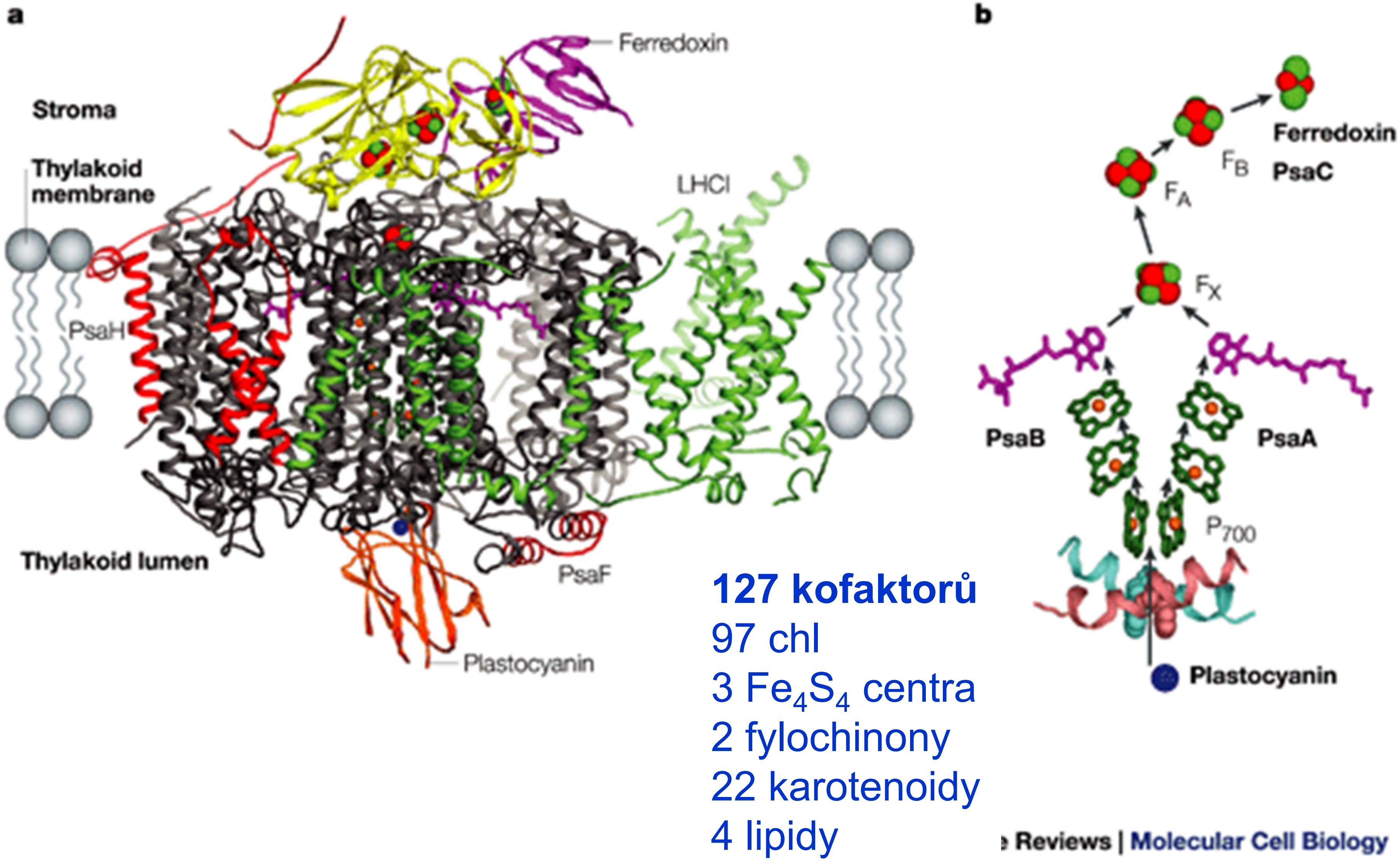


Velké membránové proteiny fotosystému I

- velké membránové podjednotky PSI označované jako PsaA a PsaB váží jak pigmenty reakčního centra, tak světlosběrné pigmenty

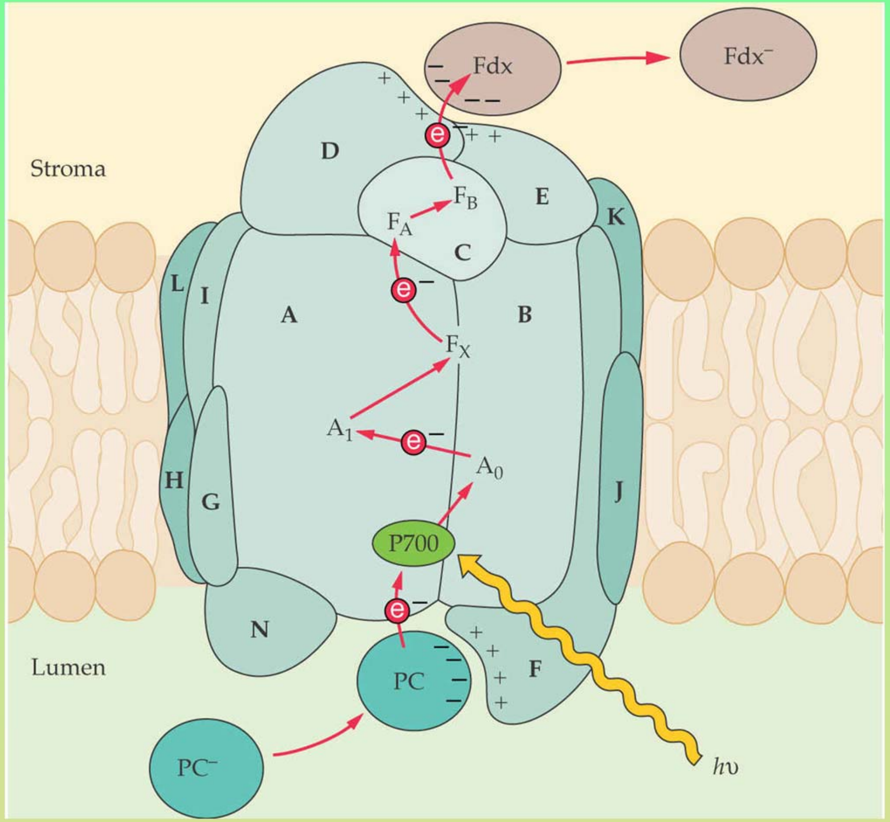


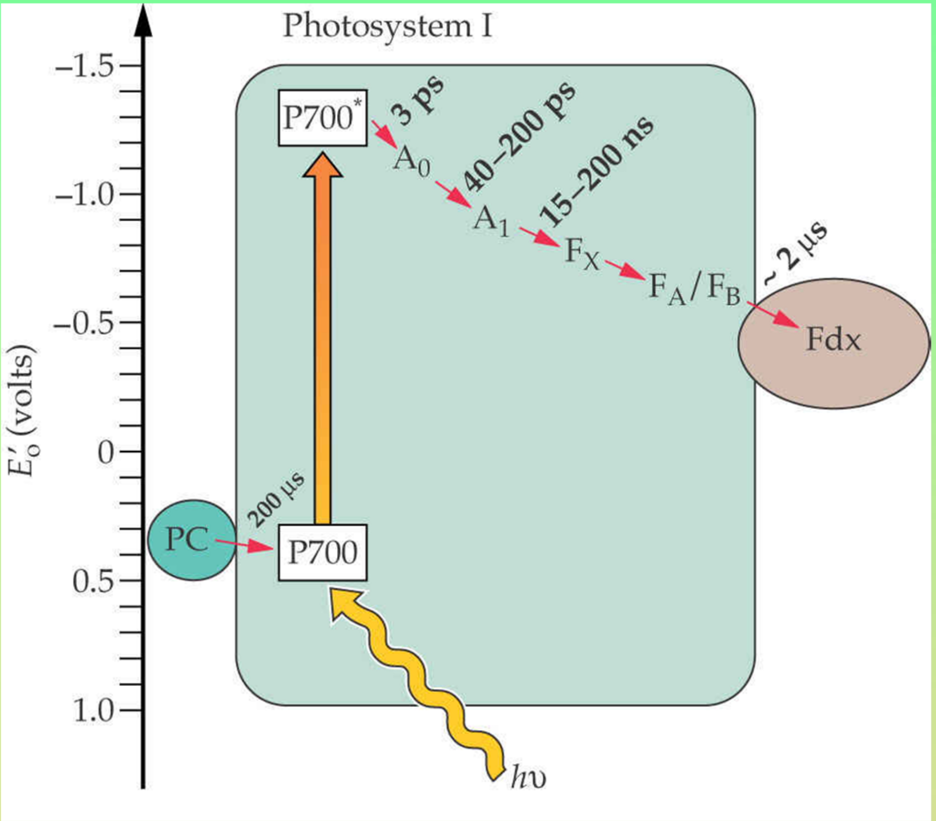
Fotosystém I



127 kofaktorů
97 chl
3 Fe_4S_4 centra
2 fylochinony
22 karotenoidy
4 lipidy

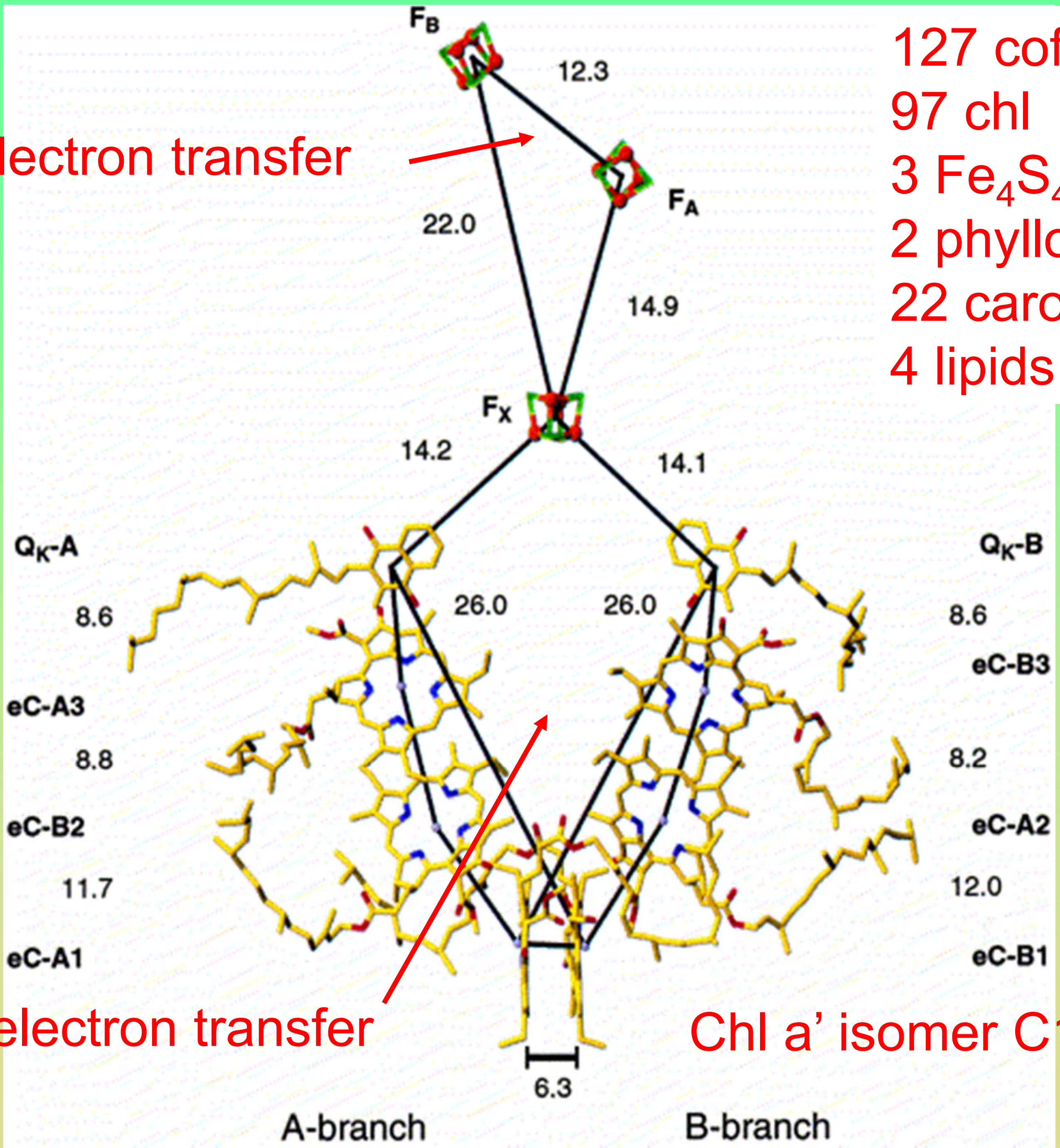
Fotosystém I





silný reduktant, slabý oxidant..

127 cofactors:
97 chl
3 Fe_4S_4 centers
2 phylloquinons
22 carotenoids
4 lipids

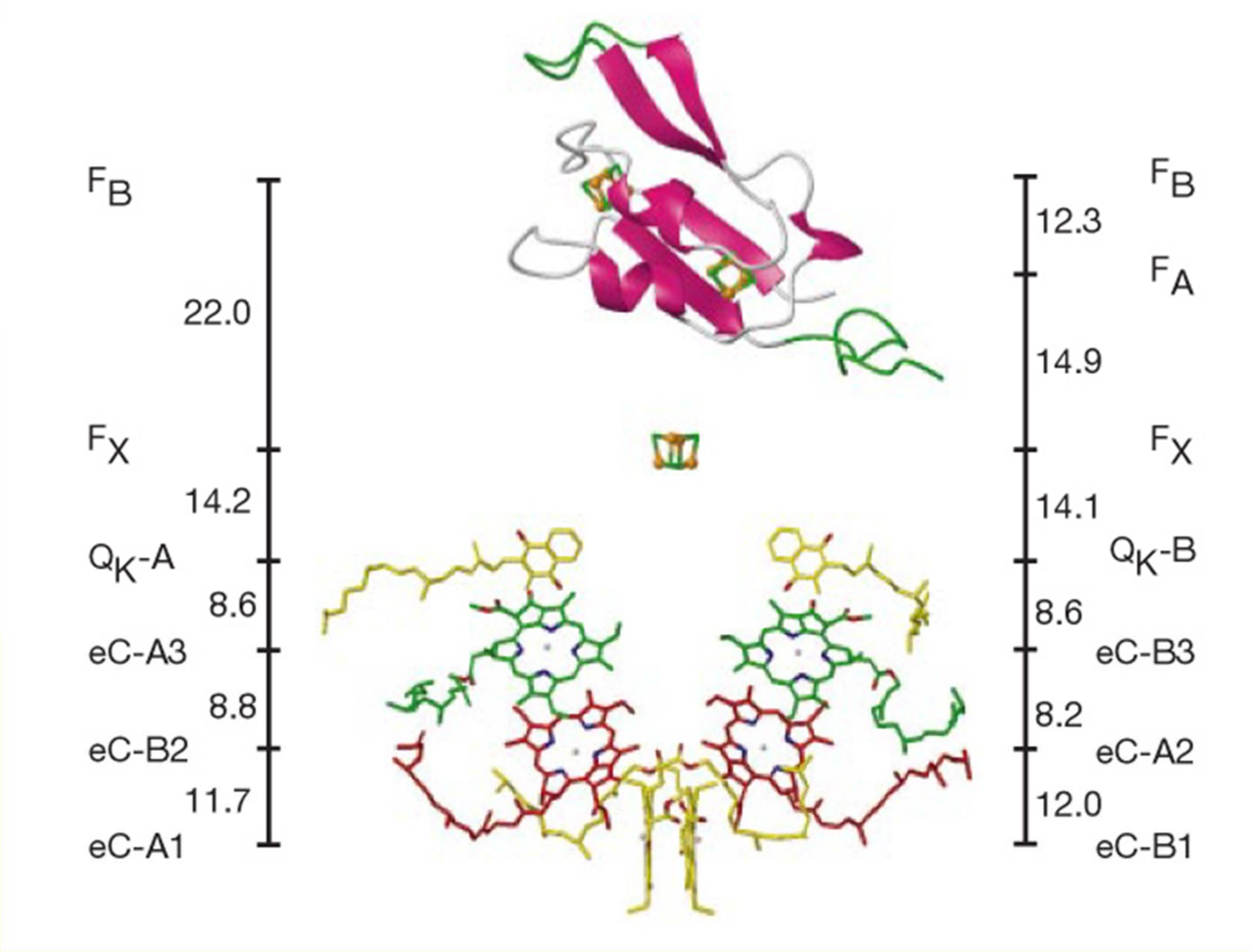


Uphill electron transfer

Parallel electron transfer

Chl a’ isomer C13

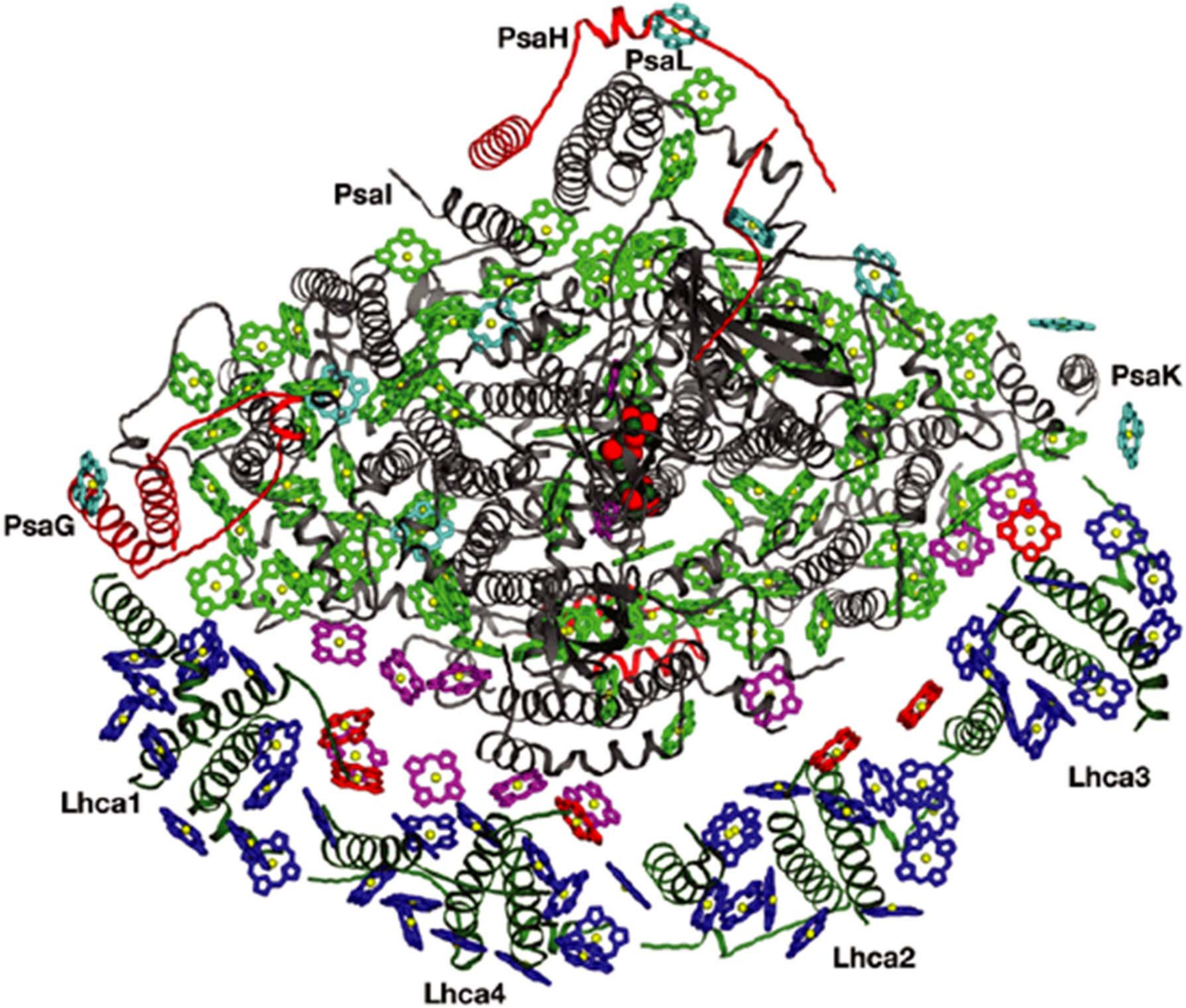
Elektron-transportní řetězec fotosystému I: obě větve funkční (Jordan et al., Nature 2001)



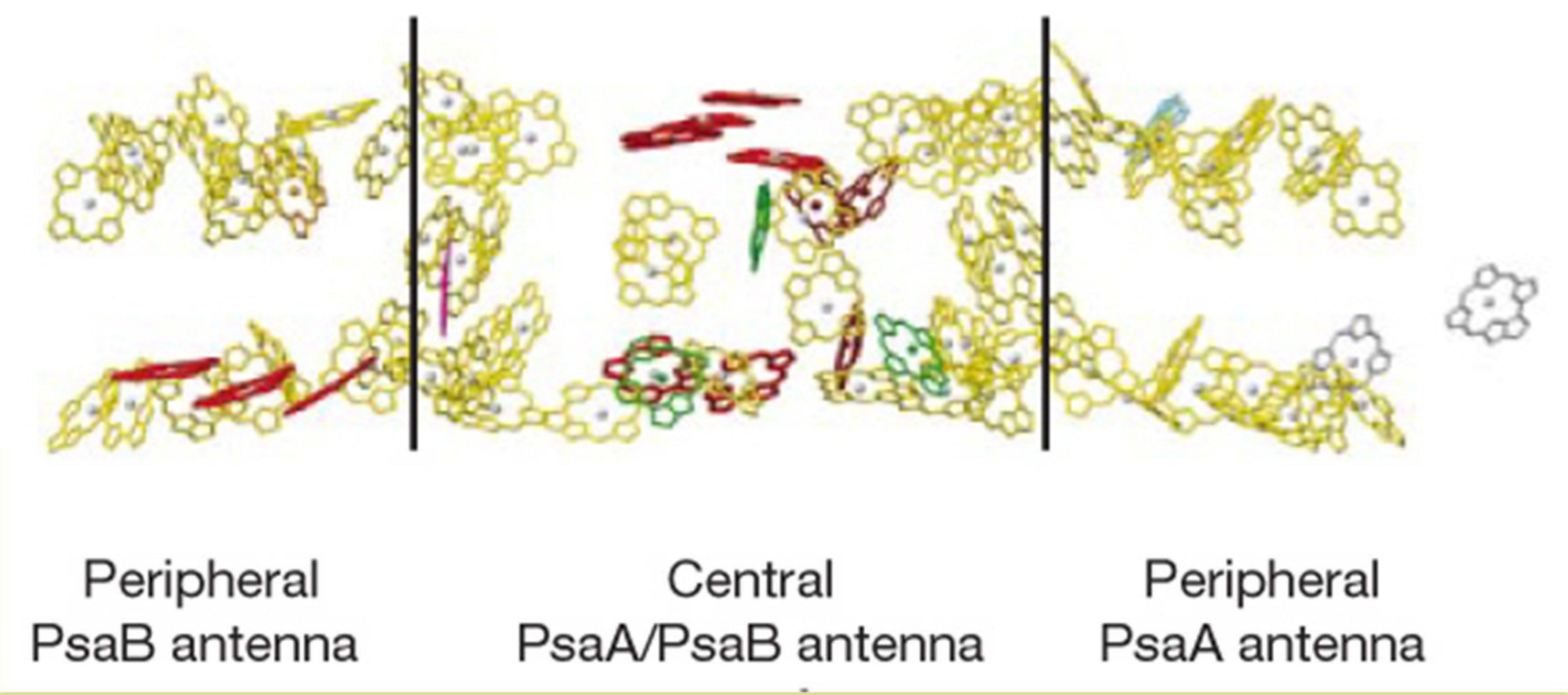
A_1 A_1

A_0 A_0

P700 P700

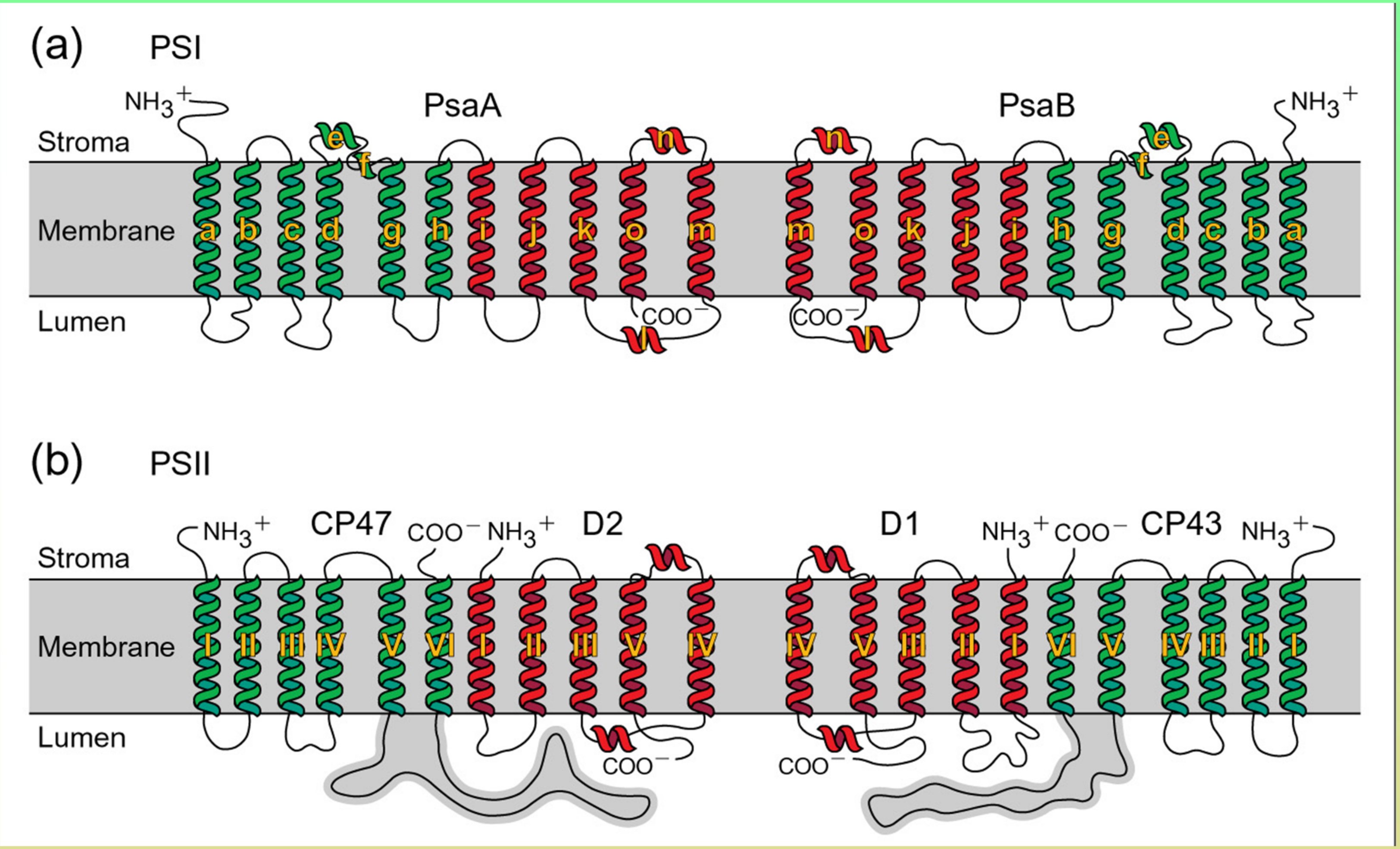

PsaH
PsaL
PsaI
PsaK
PsaG
Lhca3
Lhca1
Lhca2
Lhca4

Uspořádání molekul chlorofylu v PSI: na periferii dvě vrstvy pigmentů, v centrální části také velký počet anténních molekul (25) plus pigmenty RC P700 a A_0 (Jordan et al., Nature 2001)

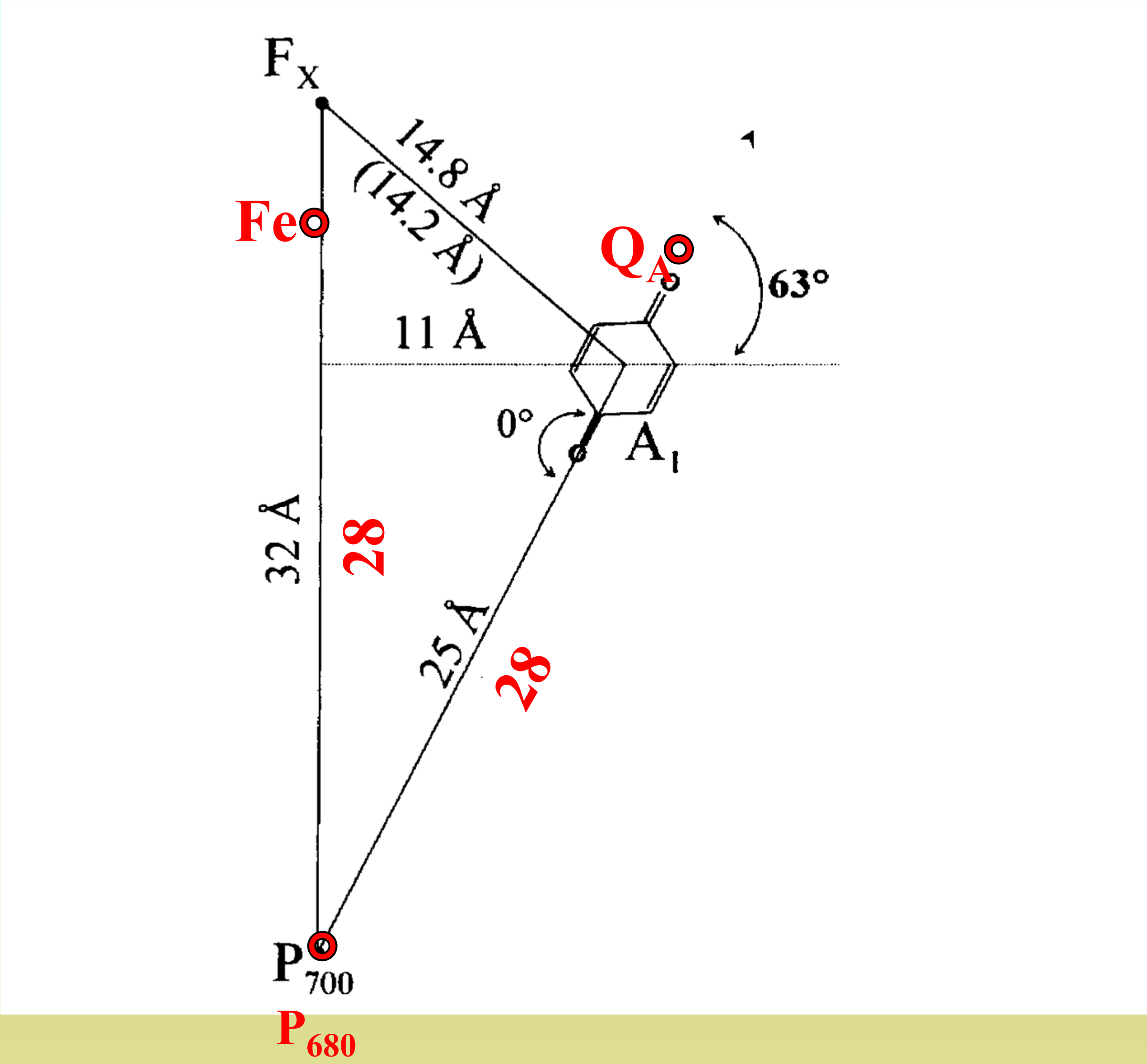


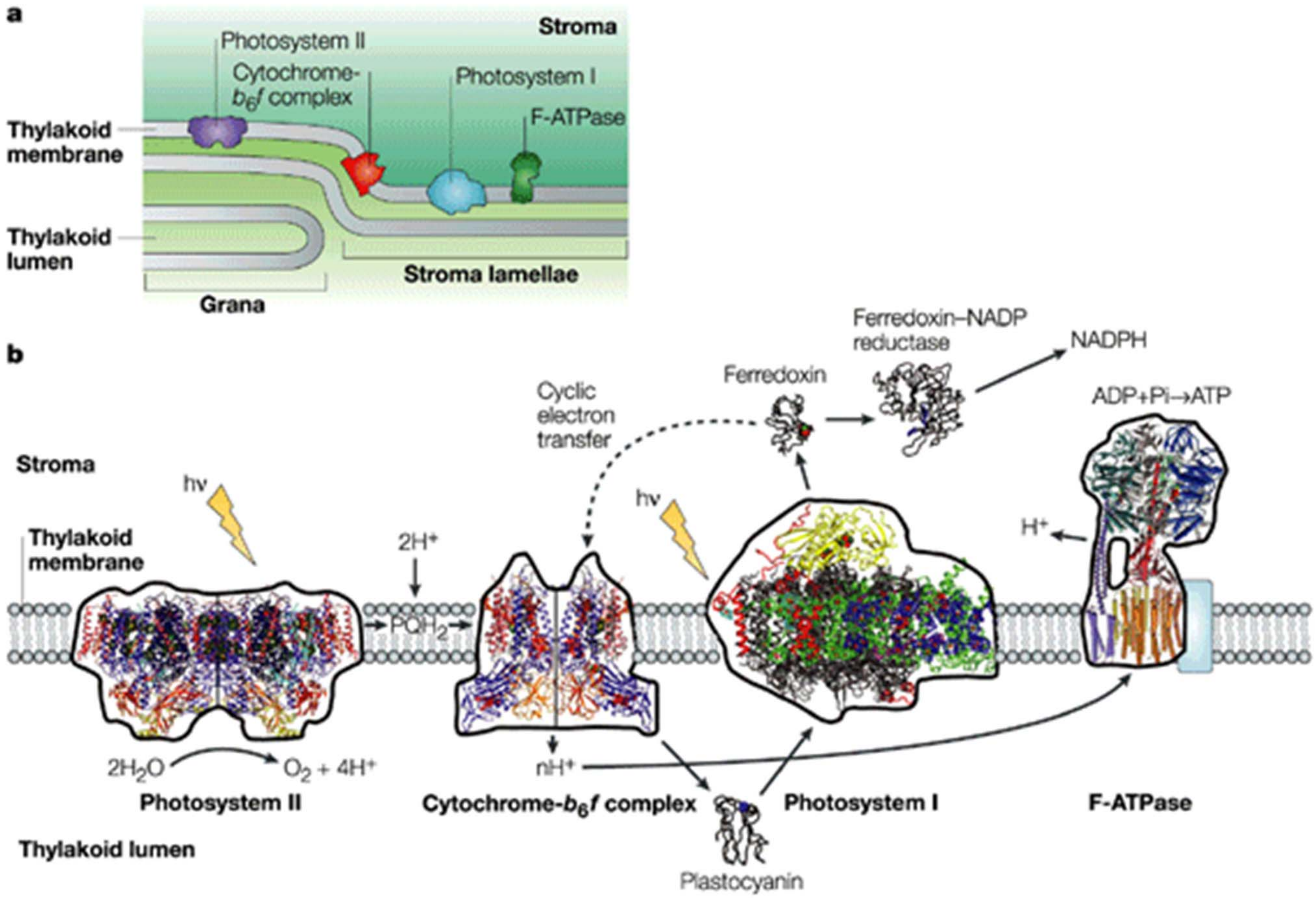
Proteiny fotosystému II

- velké membránové podjednotky PSII D1, D2, CP47 a CP43 lze získat hypotetickým rozštěpením obou velkých podjednotek fotosystému I PsaA a PsaB



Location of primary acceptors in RCI and RCII





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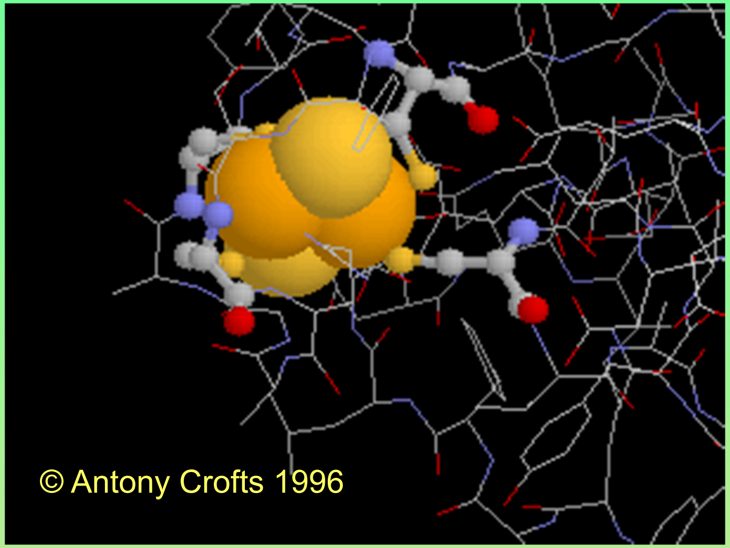
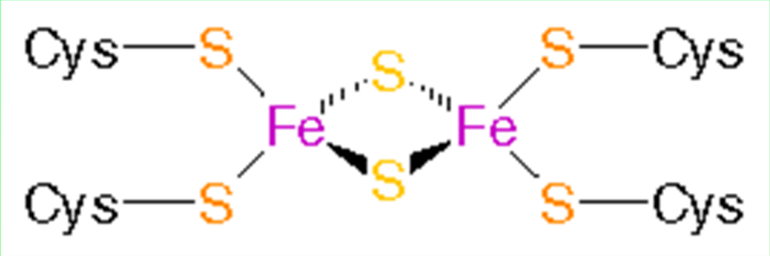
Ferredoxin


© Antony Crofts 1996



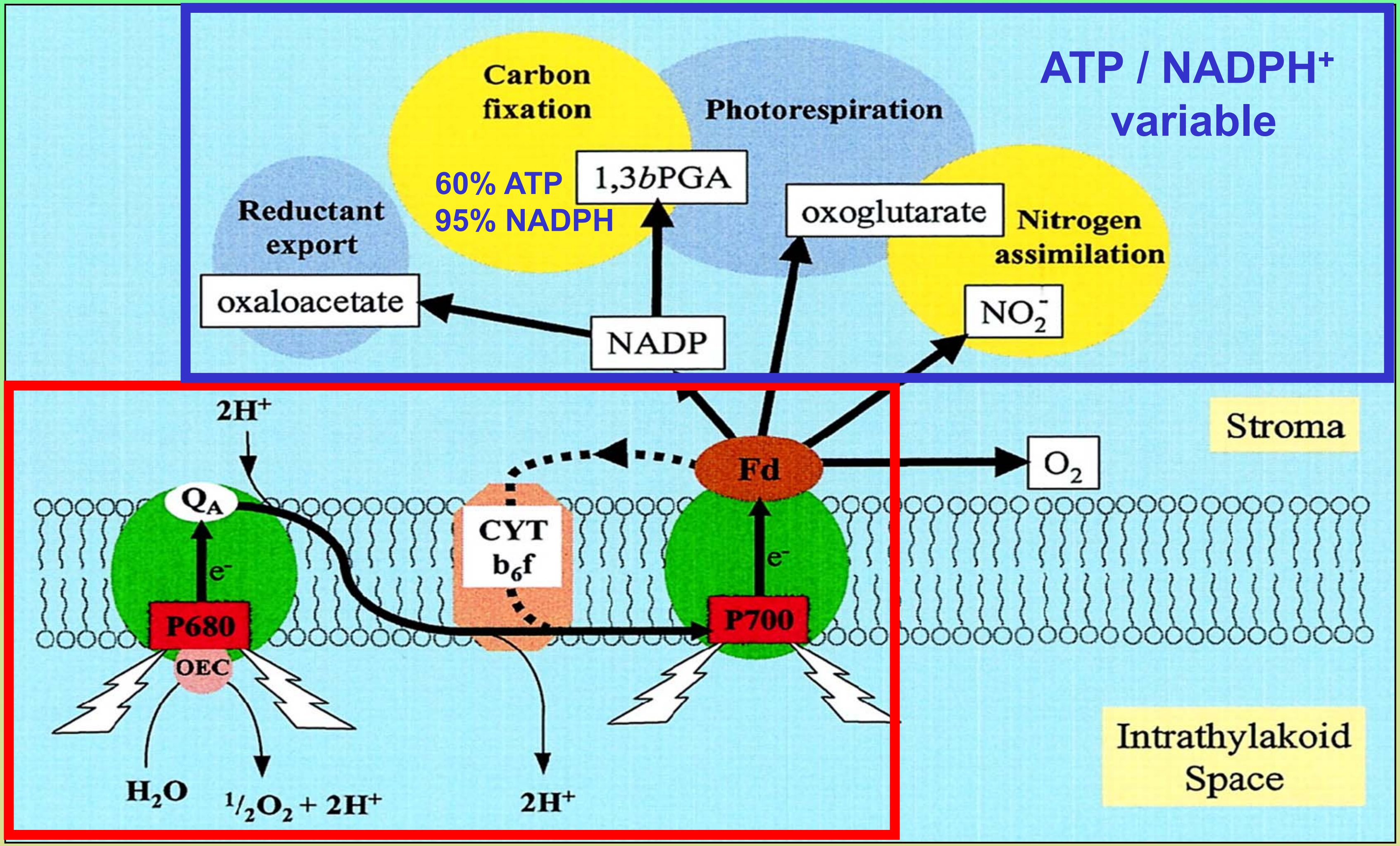
Plant like

2Fe.2S iron sulfur centers ferredoxin

$2Fe2S \Rightarrow 2Fe2S^{+} + e^{-}$

Midpoint redox potential: $E_{m,\ pH\ 7}$ = - 290 to - 400mV

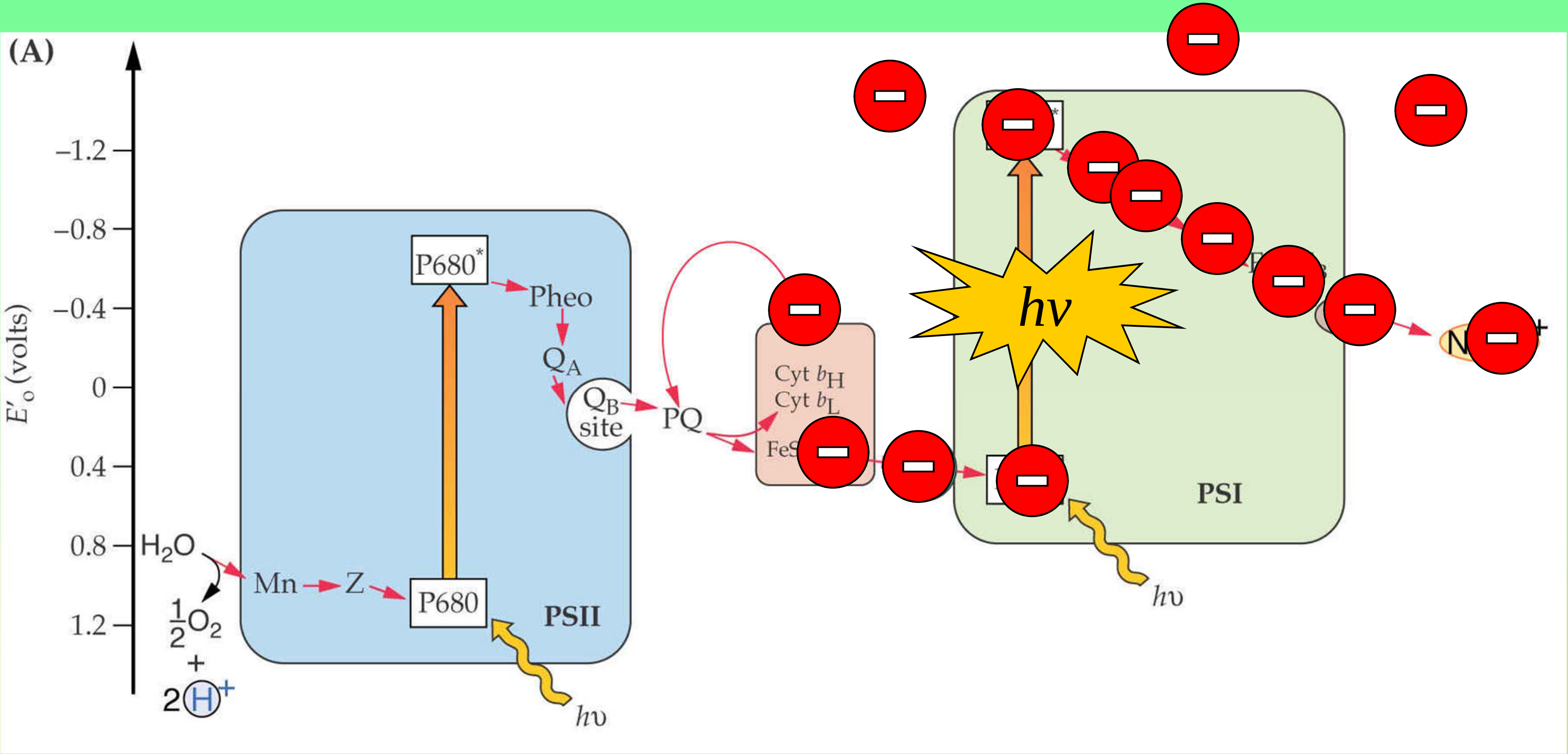
Multiple pathways for spending the photosynthetic currency



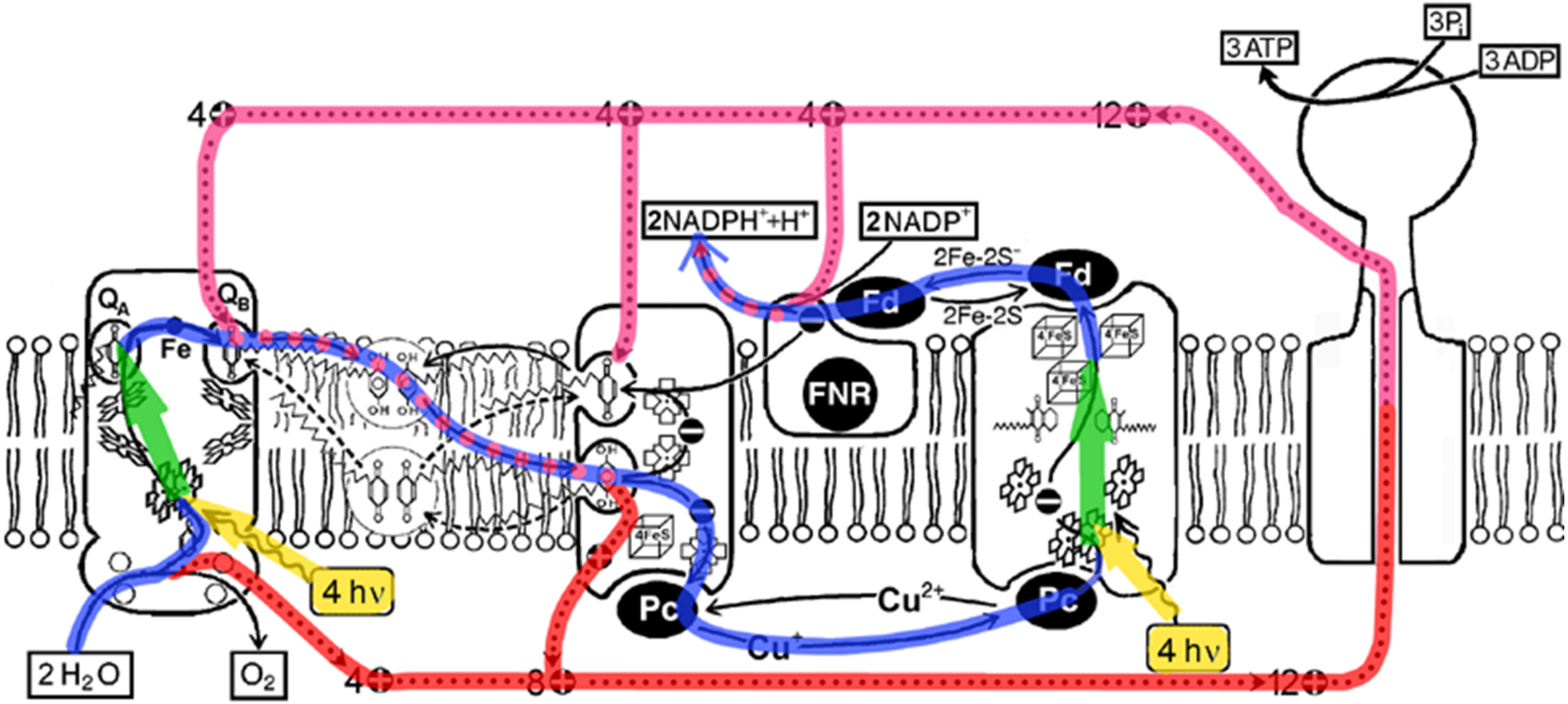
Noctor and Foyer 2000

ATP / NADPH$^+$ ~ 1.5

Cyclic electron transport



1 *hv* -> 0 e^-



$2H_2O + \mathbf{2NADP^+} + \mathbf{2H^+} \text{->} O_2 + \mathbf{2NADPH} + 4H^+$

$\mathbf{2NADPH} + 12H^+$

ETM (membrány přeměny energie)

Přeměny energie:

Energie fotonů $h\nu$

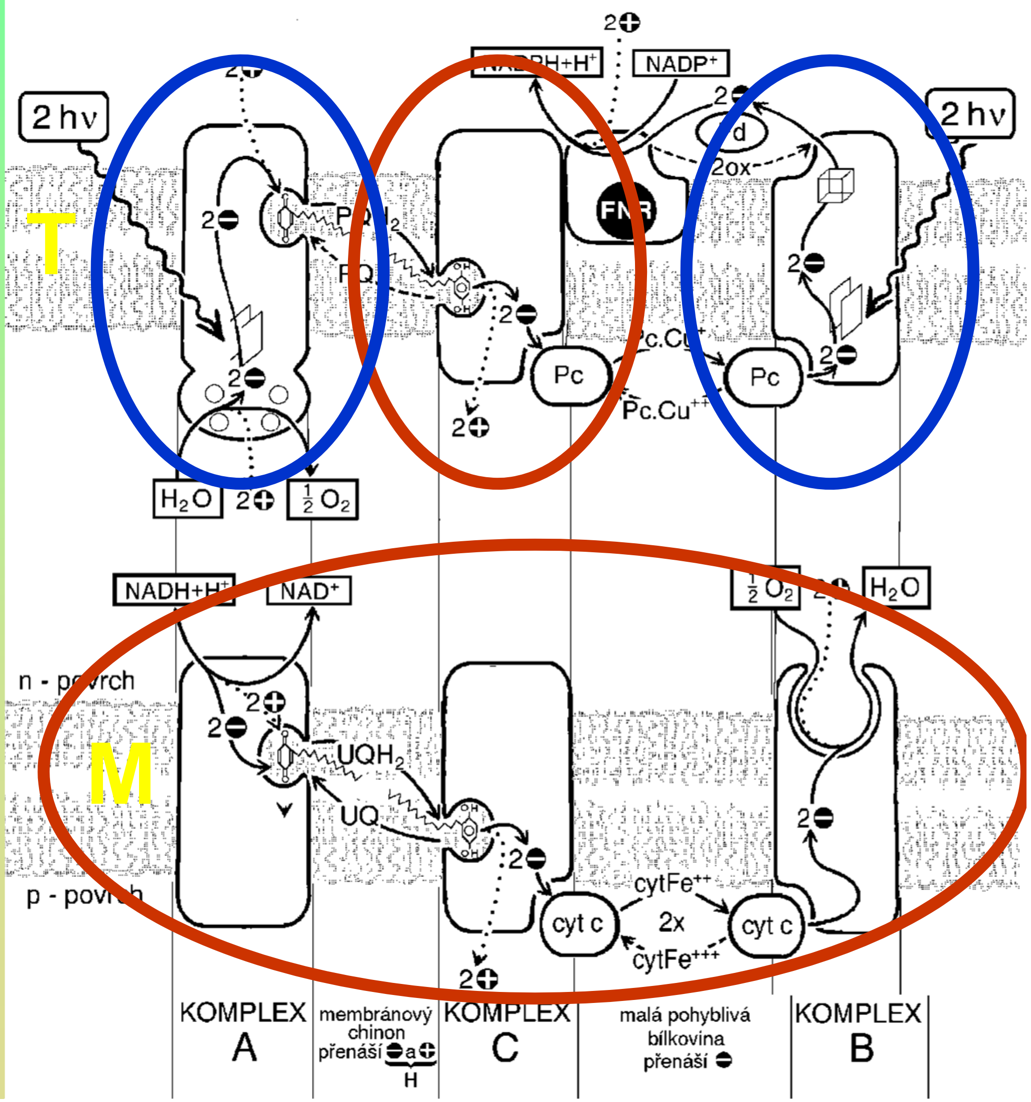
Excitační energie chlorofylu a

Energie elektronů na různých redoxních potenciálech:
membránové elektronové řetězce ΔE

Energie koncentrací iontů na membráně $\Delta\mu_{H^+}$

Energie anhydridové chemické vazby ATP ΔG_{ATP}

$h\nu \rightarrow \Delta E \rightarrow \Delta \mu_{H^+} \rightarrow \Delta G_{ATP}$



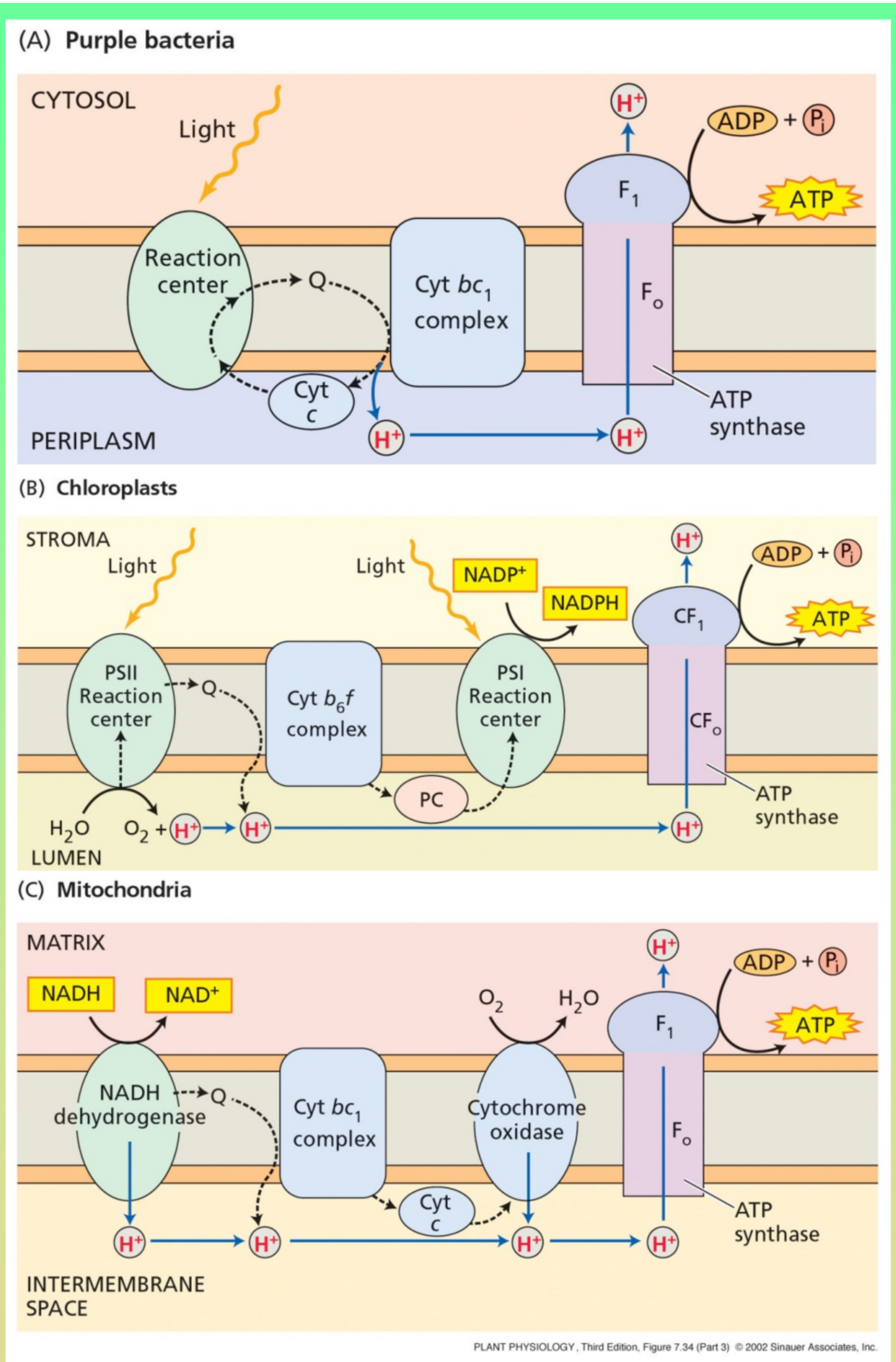
1. Generátory chemického potenciálu ***Δ μ*H+**

2. Generátory oxidačně redoxního potenciálu ***ΔE***

hν → ΔE

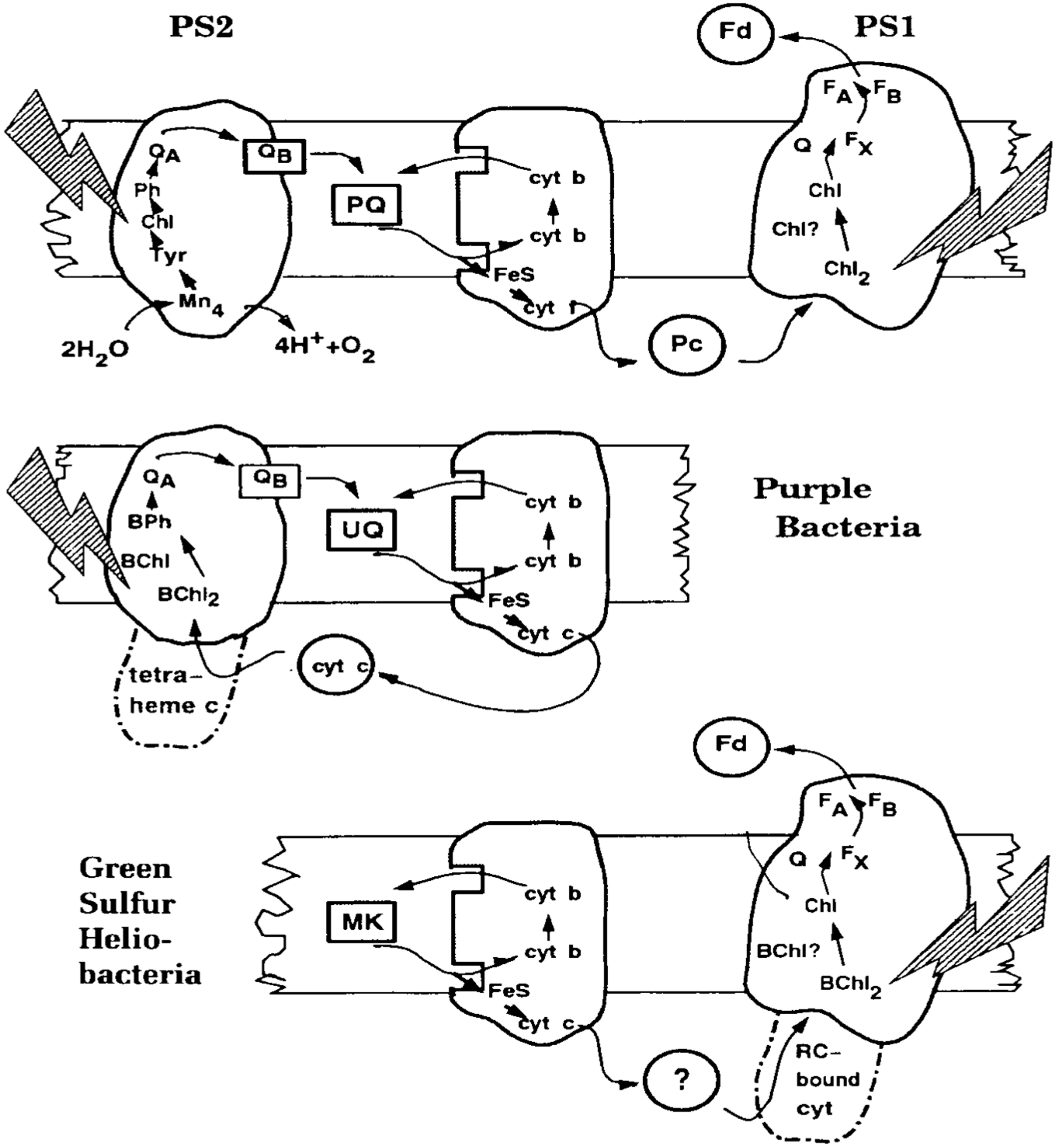
Podobnosti v uspořádání fotosyntetických a respiračních procesů

spřažení toku elektronů a protonů

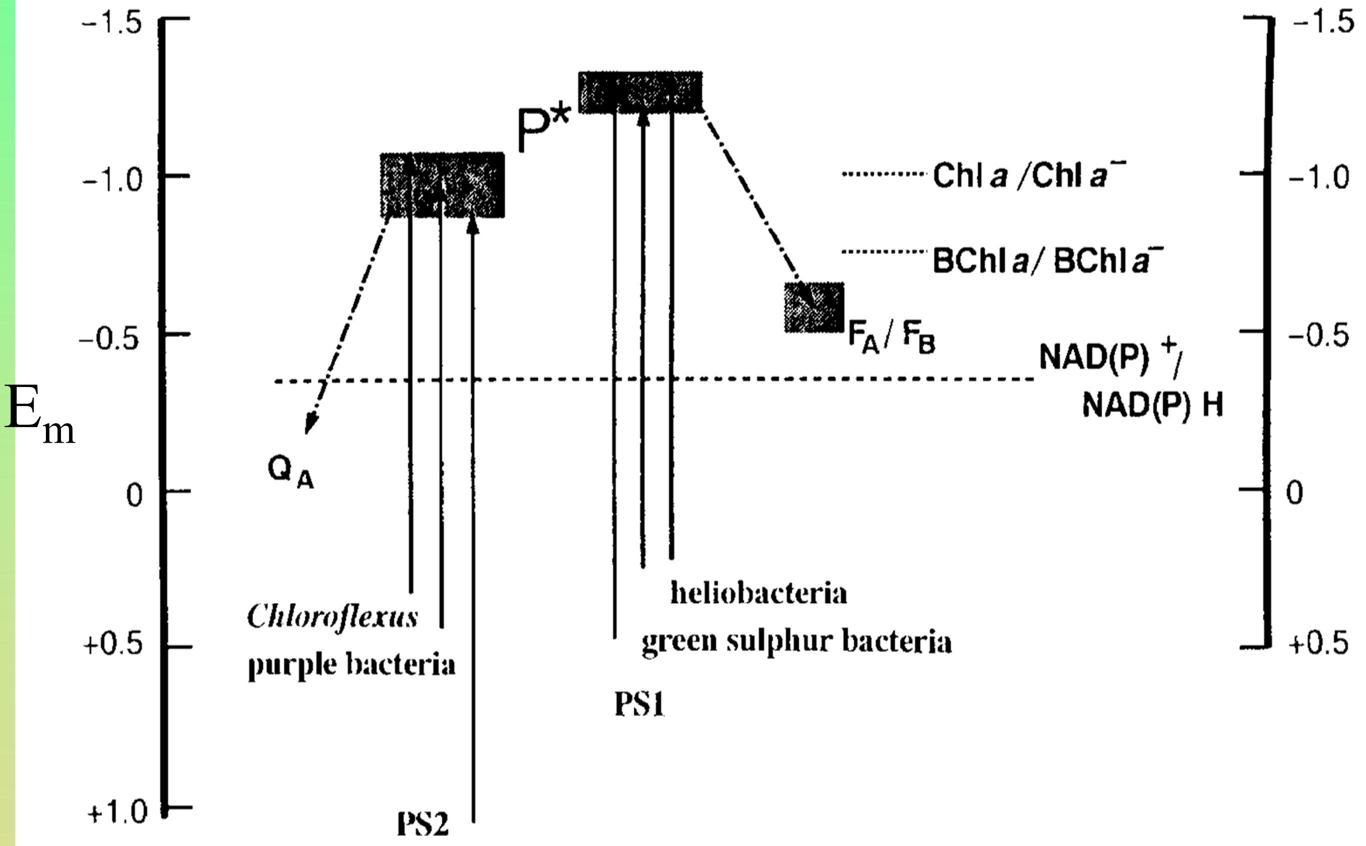


PLANT PHYSIOLOGY, Third Edition, Figure 7.34 (Part 3) © 2002 Sinauer Associates, Inc.

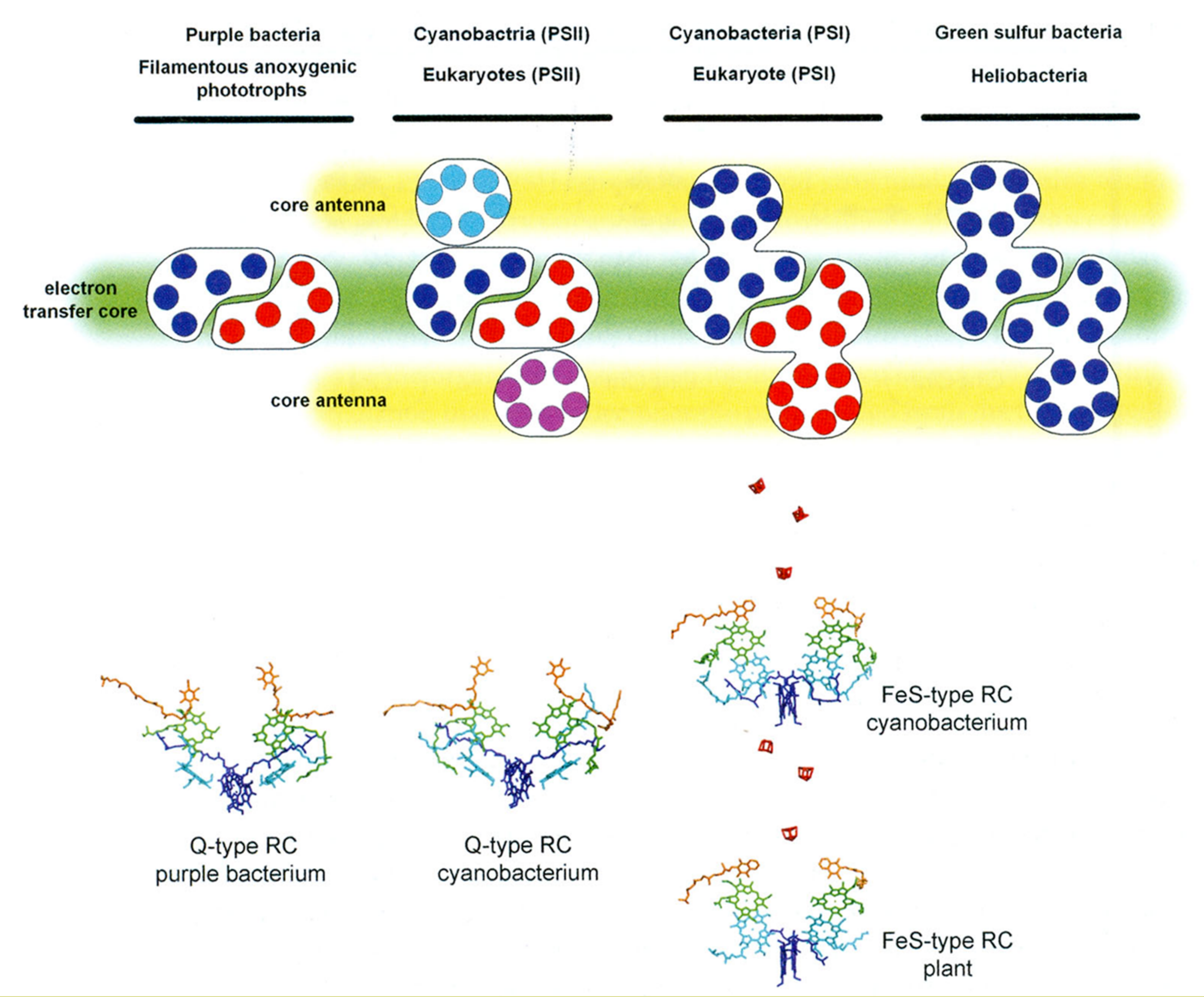
RC II **b/c/FeS** **RC I**

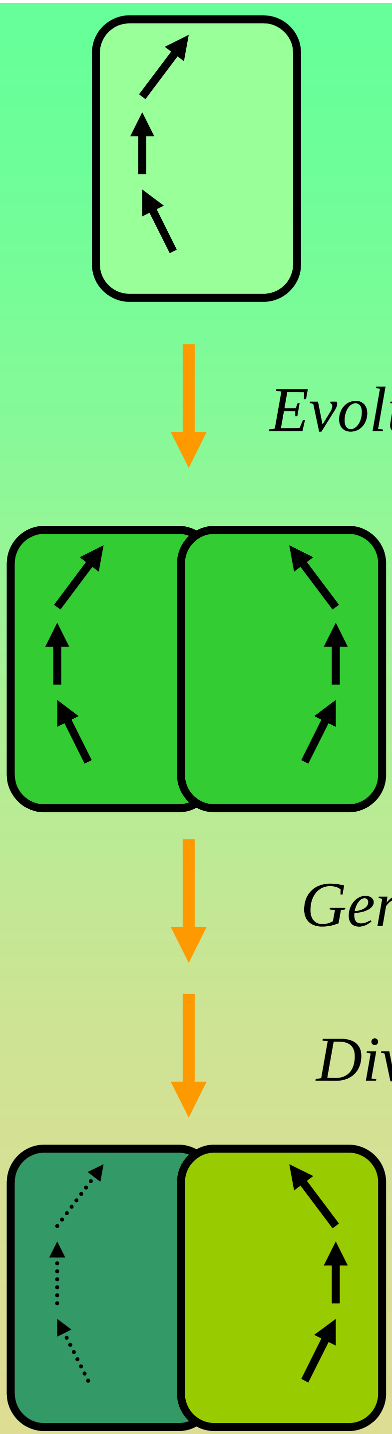


RC II **RC I**


-1.5
-1.0
-0.5
0
+0.5
+1.0
E_m
P*
Q_A
Chl *a* /Chl *a*⁻
BChl *a*/ BChl *a*⁻
F_A/ F_B
NAD(P) ⁺/
NAD(P) H
Chloroflexus
purple bacteria
PS2
heliobacteria
green sulphur bacteria
PS1
-1.5
-1.0
-0.5
0
+0.5

Vztah mezi hlavními podjednotkami RC různých fotosyntetických RC naznačuje společného prapředka





Monomeric Reaction Center

Evolutionary change

Homodimeric Reaction Center

Gene duplication

Divergence

Heterodimeric Reaction Center

RC II

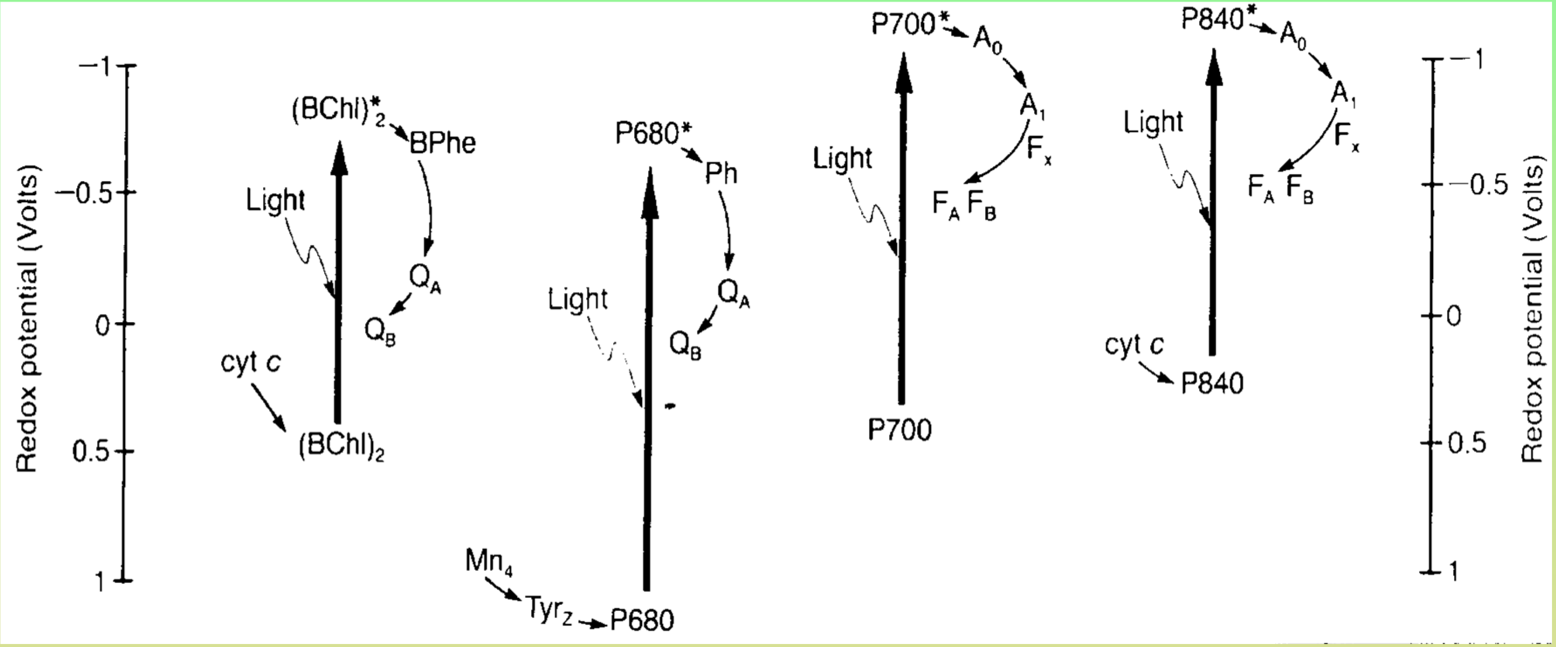
RC I

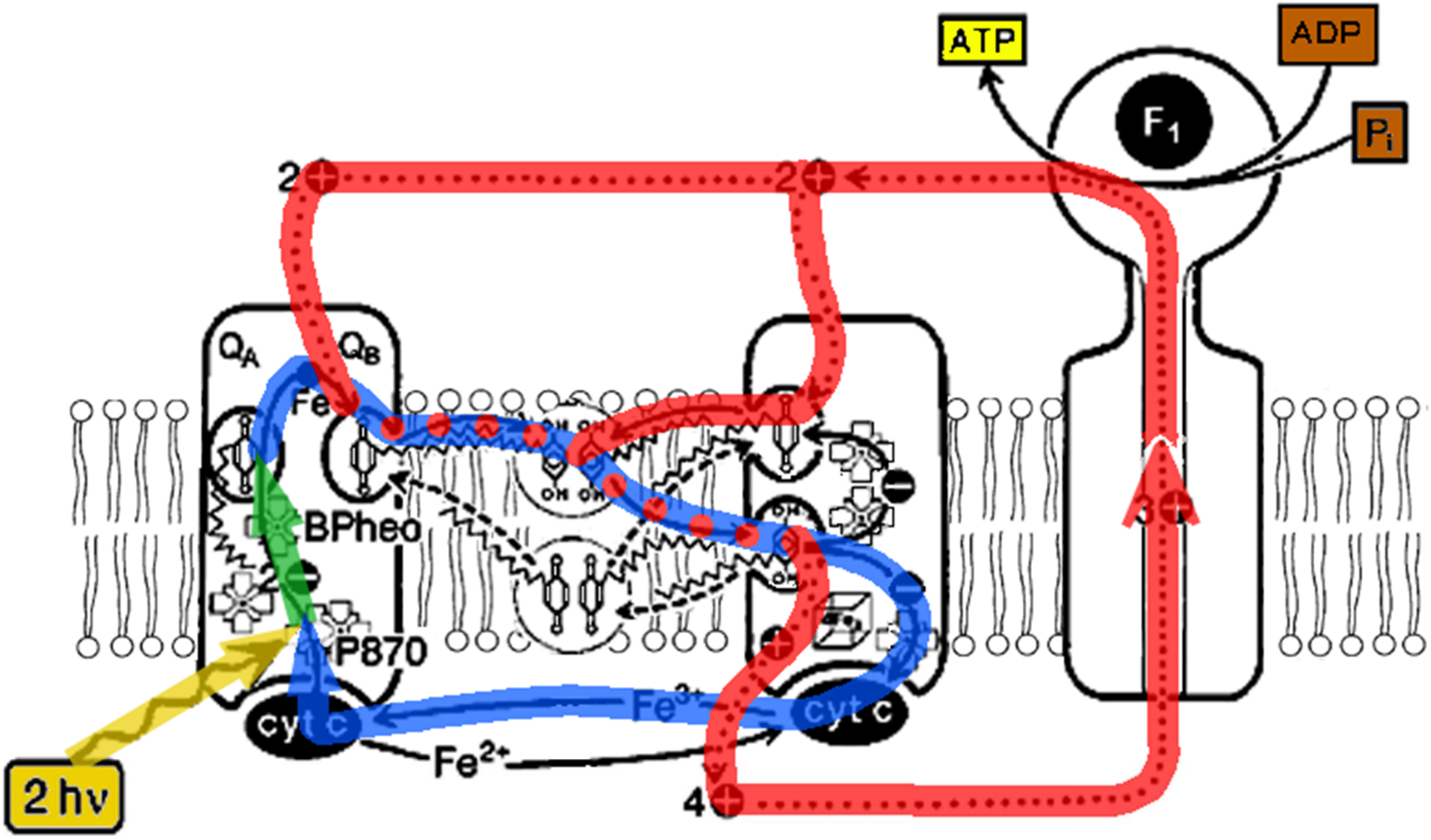
Purple bacteria

Photosystem I

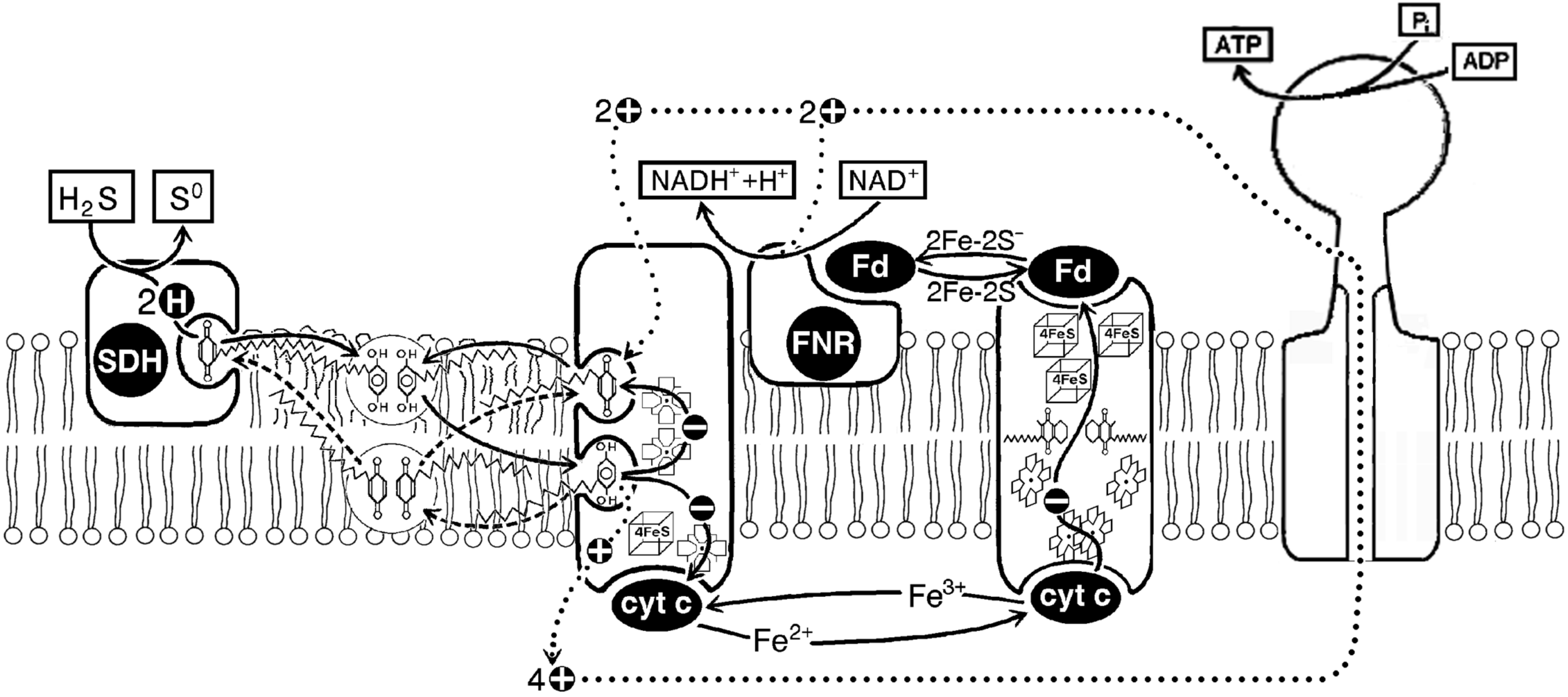
Photosystem II

Green sulfur bacteria





Purpurové bakterie



Zelené sirné bakterie

Konec