Physiology and Cultivation of Algae and Cyanobacteria

3.

Photosynthesis

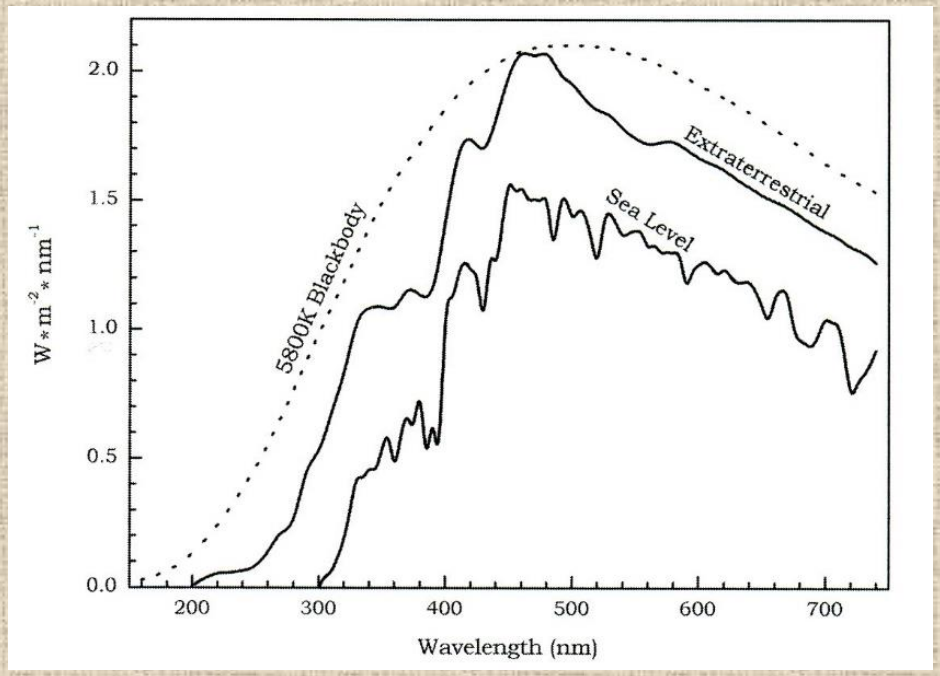
- light
- structure & function
  - membrane structure
  - pigments
  - photosystems
- photosynthesis – reactions
  - light dependent
  - light independent
- energy transfers

Light

- sunlight *vs.* PAR
- units; W m^{-2} s^{-1}; μmol m^{-2} s^{-1}
- spectrum
- absorption, transmissivity, reflection, scattering, interference
- environmental accessibility (spectrum, int..)

Sun Light Reflected by Sea Surface

Angle between Sun rays and zenith	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°
Percentage of reflected light	2	2	2.1	2.1	2.5	3.4	6	13.4	34.8	100

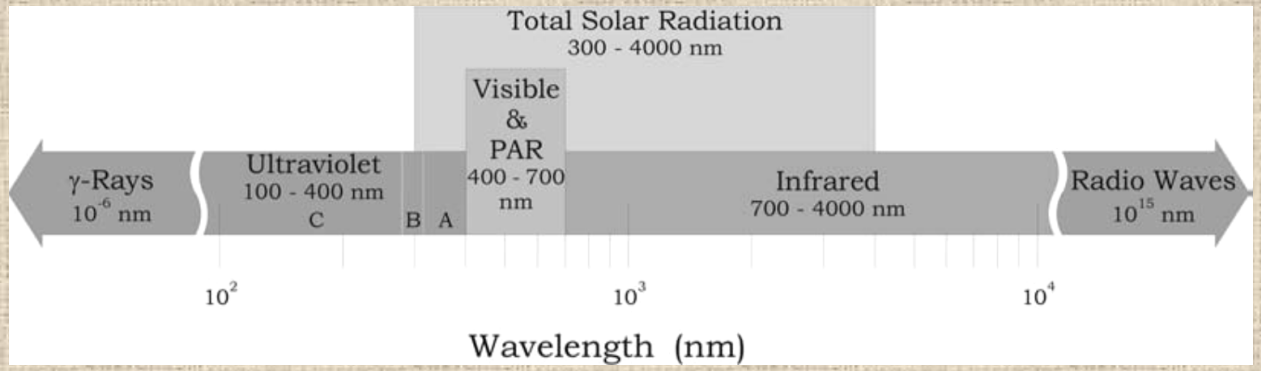


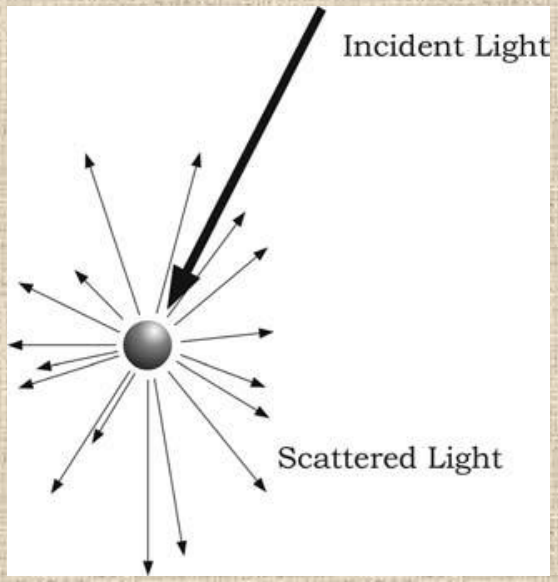
Relationship between Transmitted Light Percentage and Absorbance Value

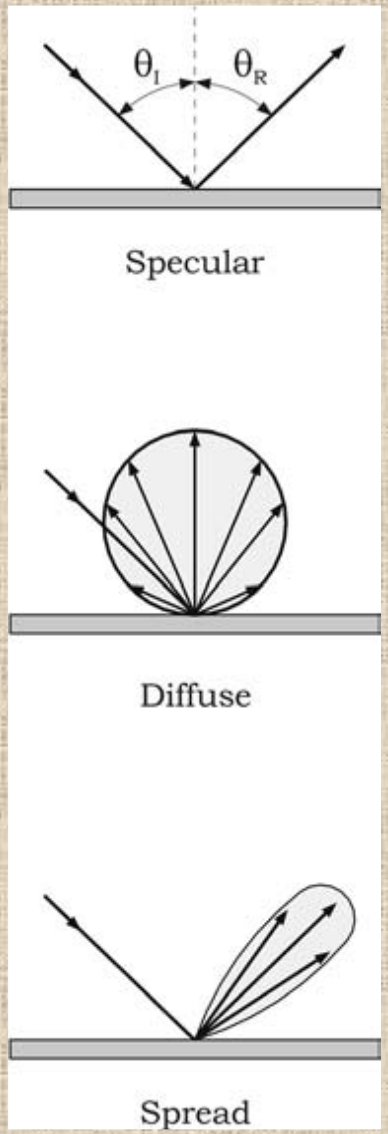
Transmittance	Absorbance
100	0.000
90	0.045
80	0.096
70	0.154
60	0.221
50	0.301
40	0.397
30	0.522
20	0.698
10	1.000
1	2.000
0.1	3.000

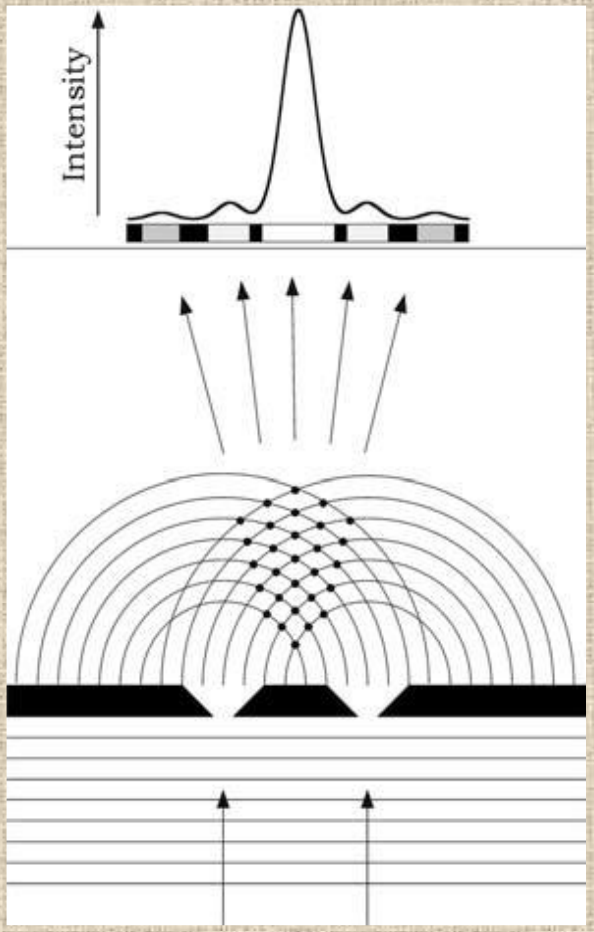
$$I_z = I_0 * e^{-kz}$$

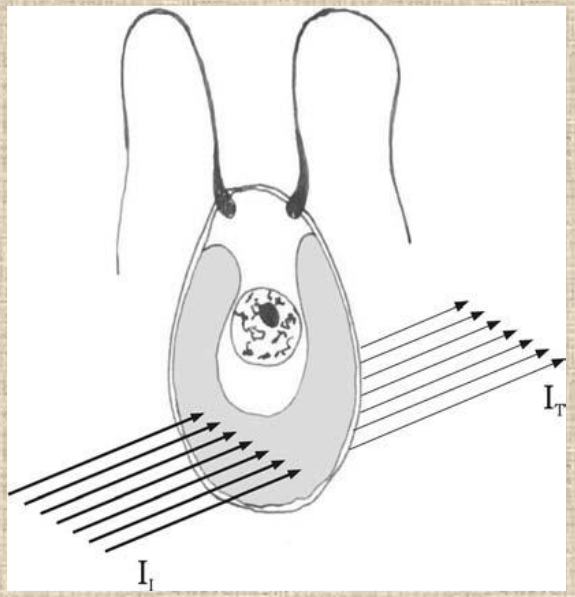
Beer's law
Light attenuation with depth (z)

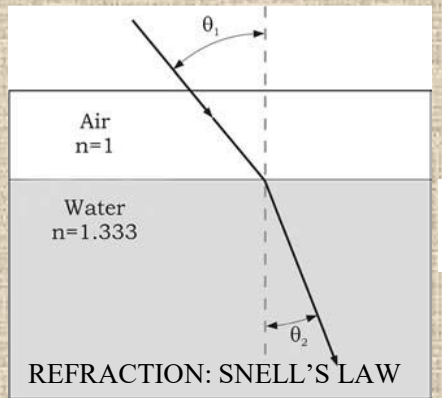












$$n = \frac{c_V}{c_S}$$

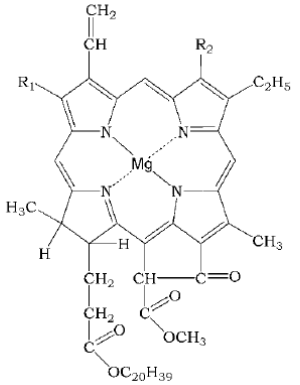
$$_1n_2 = \frac{\sin(\theta_1)}{\sin(\theta_2)}$$

REFRACTION: SNELL'S LAW

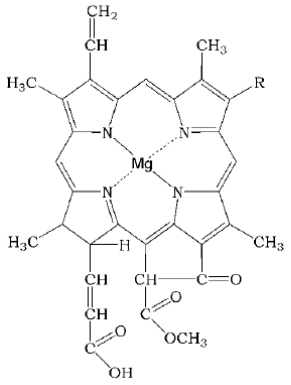
Structure & function

- thylakoid membrane structure
- pigments
- photosystems

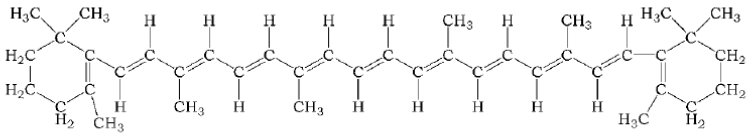
Structure of
the main pigments
of the thylakoid membrane.



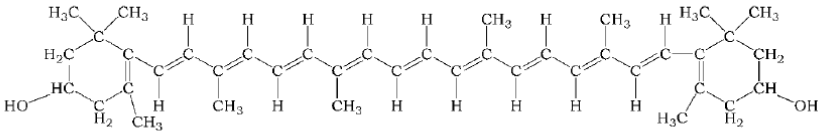
Chlorophyll *a* : $R_1 = CH_3$ $R_2 = CH_2$
Chlorophyll *b* : $R_1 = CH_3$ $R_2 = CHO$



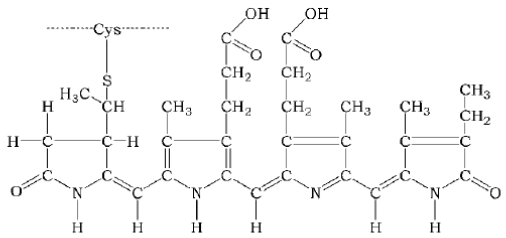
Chlorophyll c_1 : $R = C_2H_5$
Chlorophyll c_2 : $R = CH{=}CH_2$



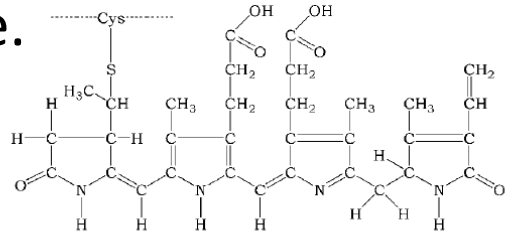
β–carotene



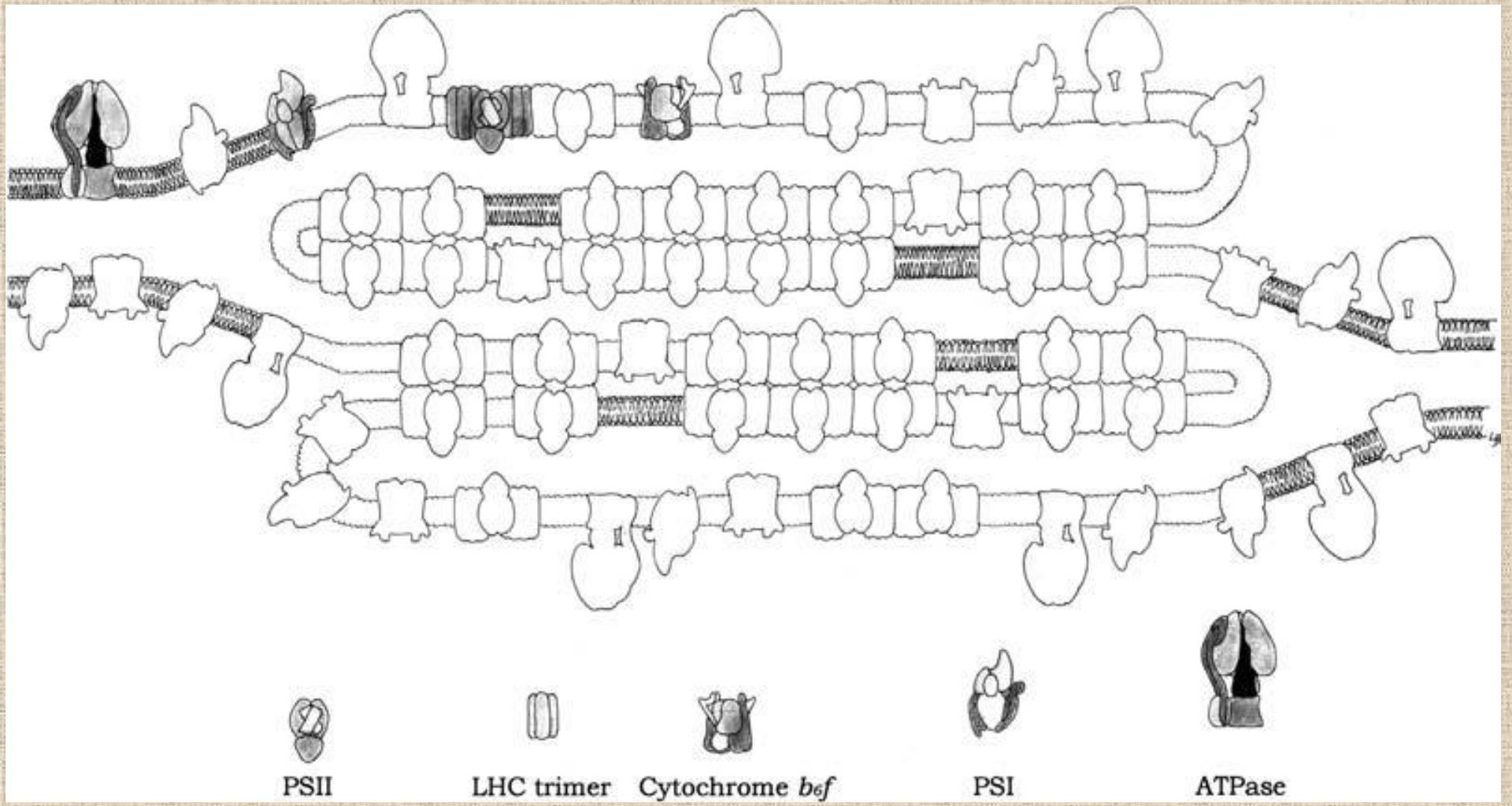
Lutein

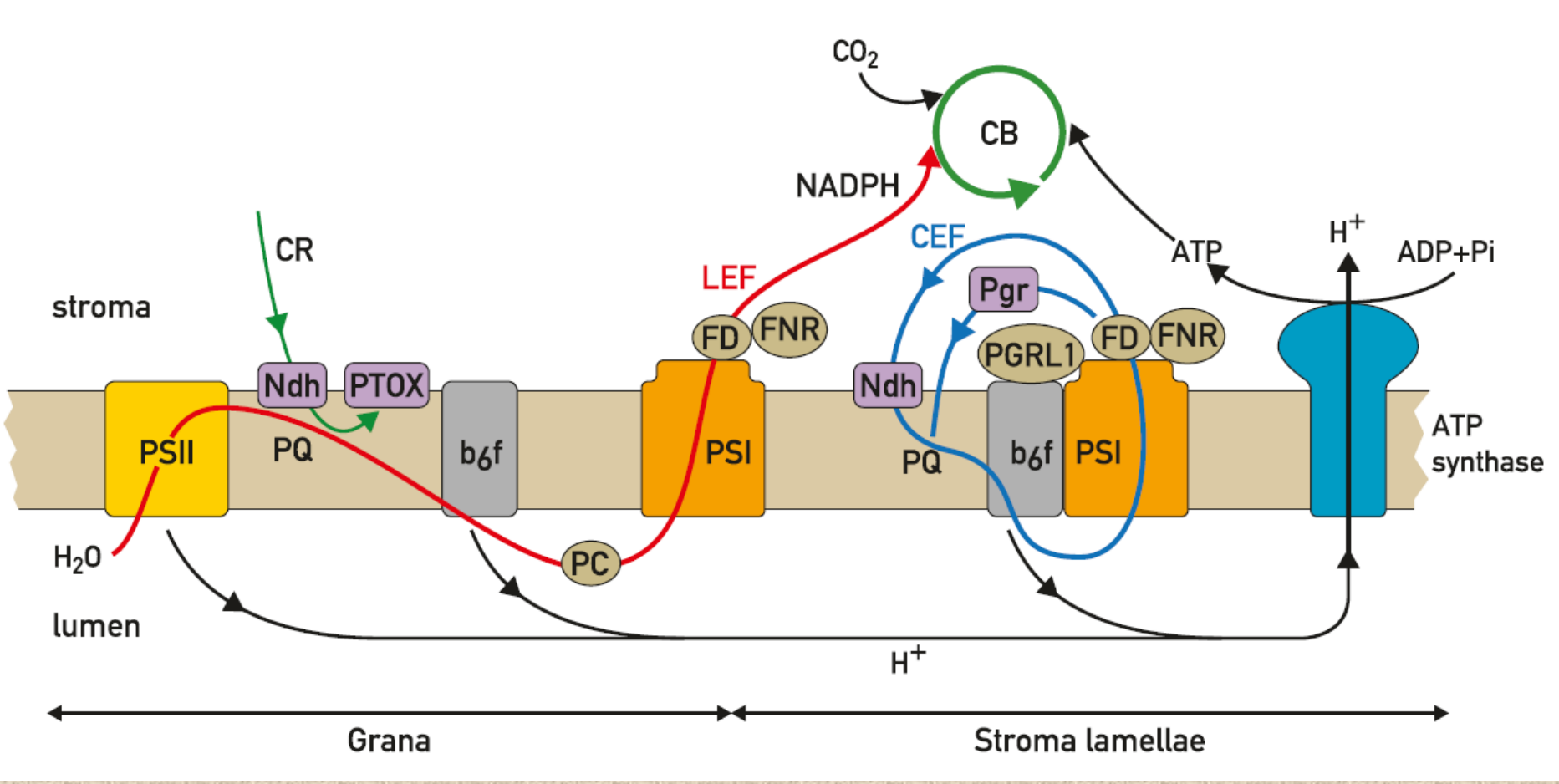


Phycocyanobilin



Phycoerythrobilin


PSII
LHC trimer
Cytochrome b_6f
PSI
ATPase


CO_2
CB
NADPH
CR
CEF
LEF
ATP
H^+
ADP+Pi
stroma
Pgr
FD
FNR
PGRL1
FD
FNR
Ndh
PTOX
Ndh
PSII
PQ
b6f
PSI
PQ
b6f
PSI
ATP
synthase
H_2O
PC
lumen
H^+
Grana
Stroma lamellae

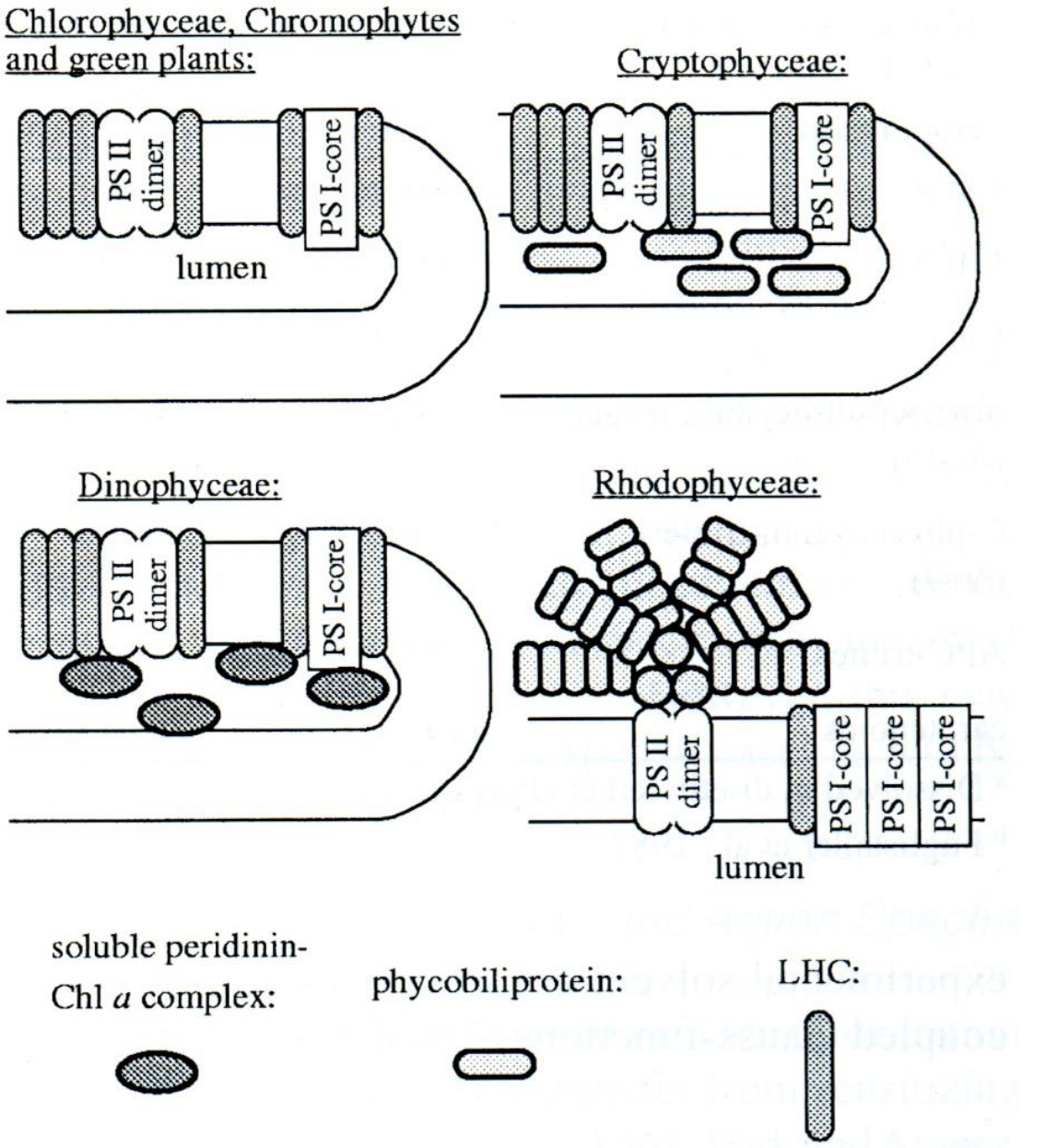
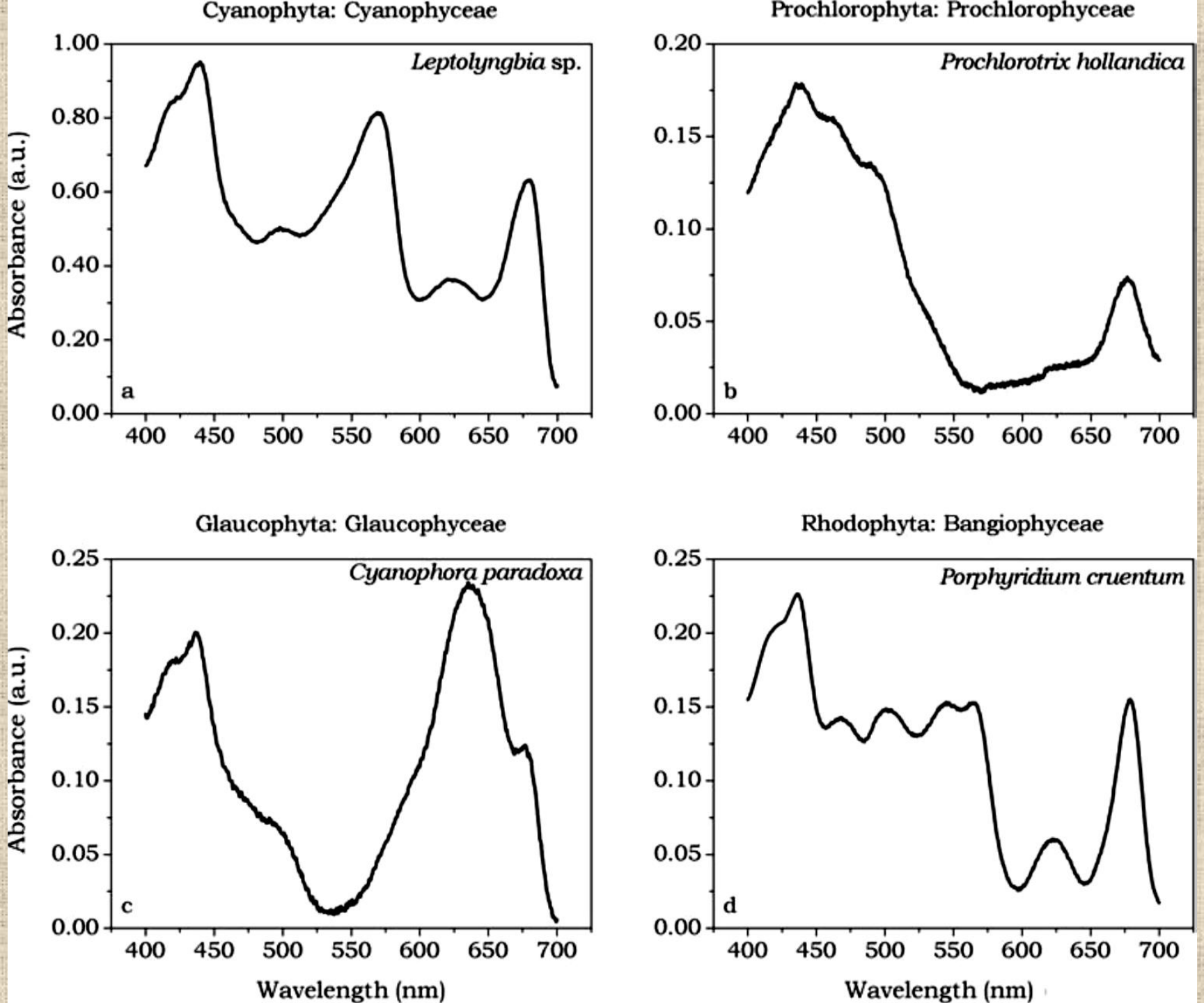
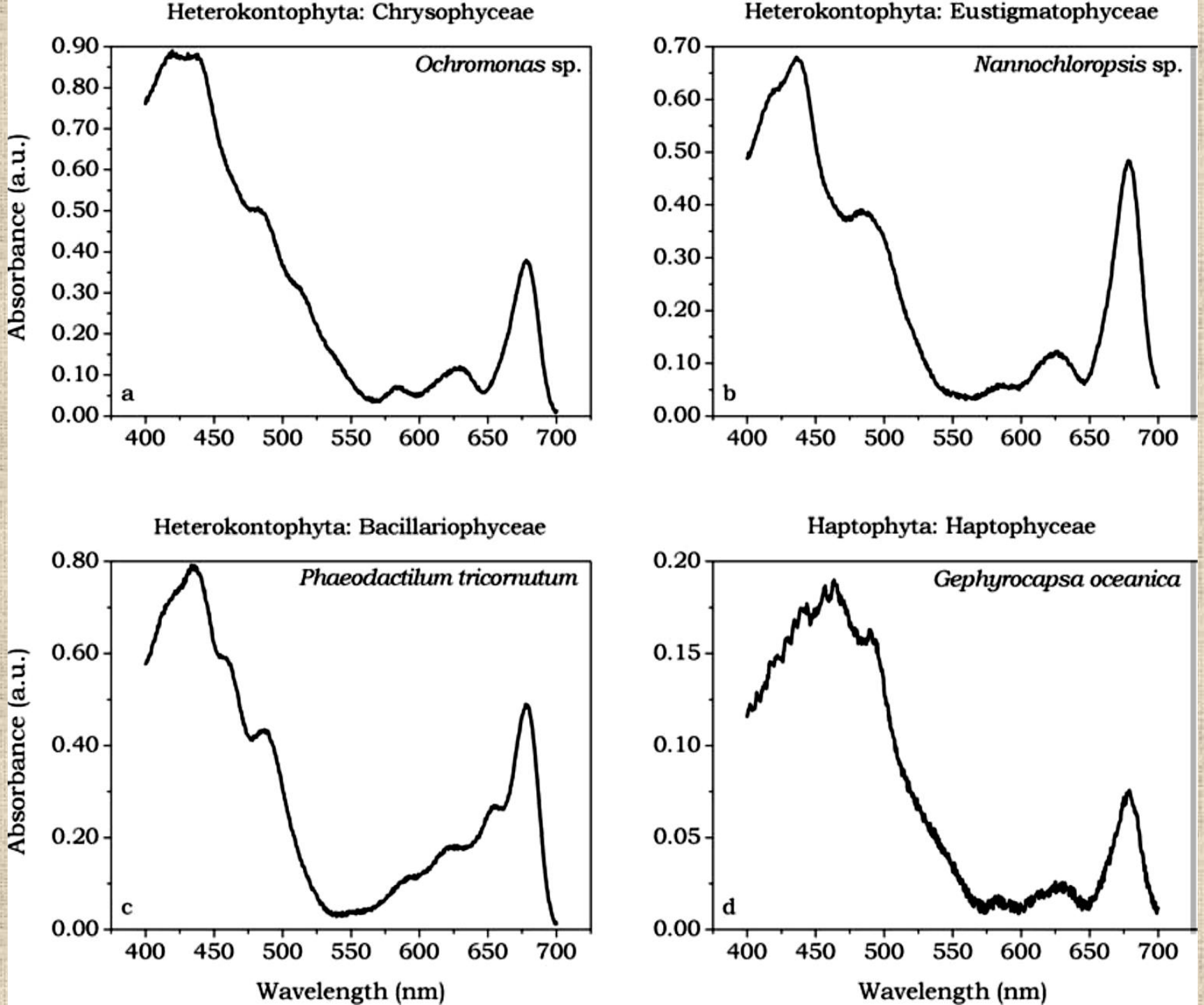
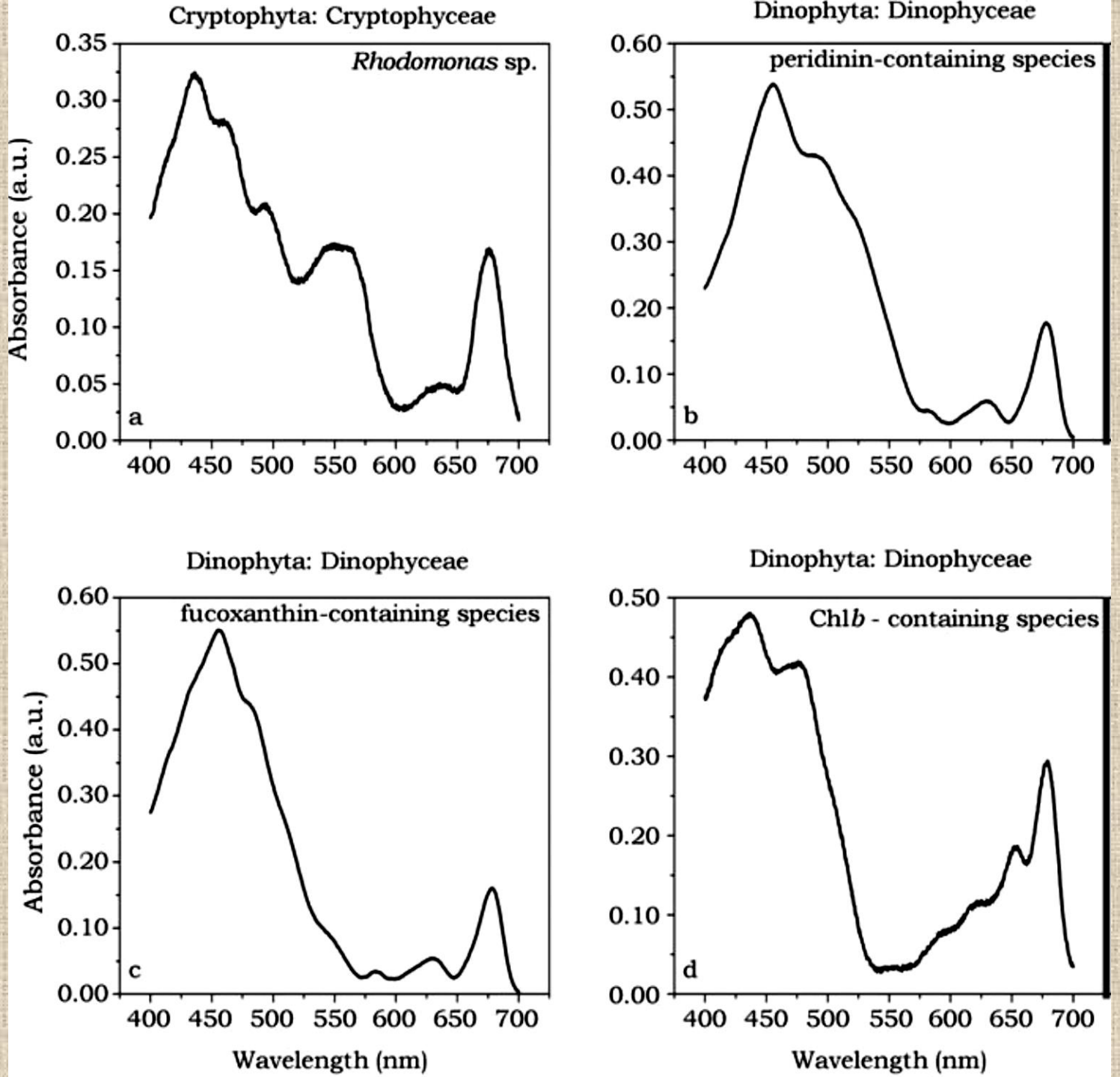
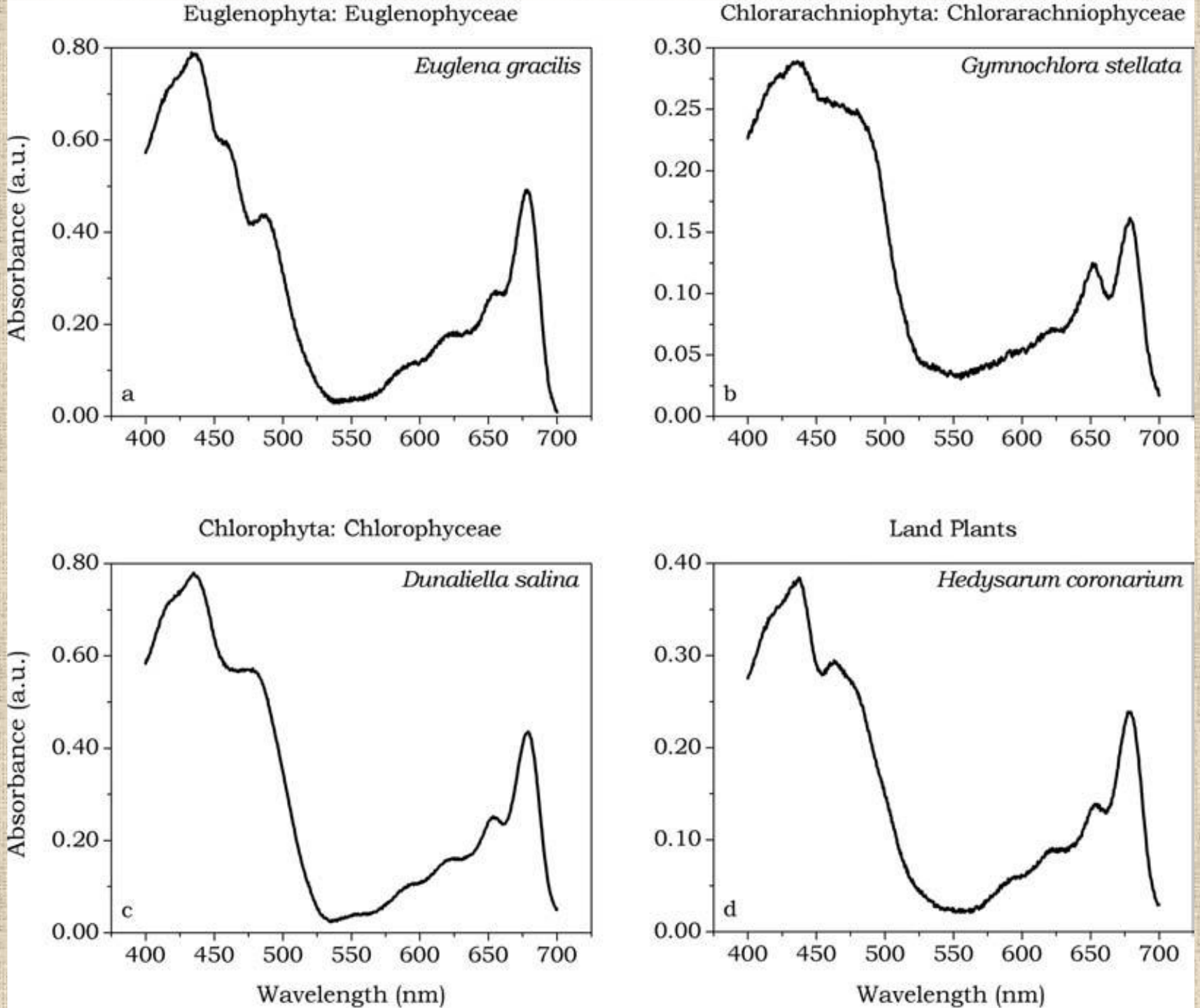


Fig. 3. Association of the different peripheral LHCs with the RCs of PS I and PS II in the thylakoid membranes of different groups of algae.


Cyanophyta: Cyanophyceae
Leptolyngbia sp.
a
Prochlorophyta: Prochlorophyceae
Prochlorotrix hollandica
b
Glaucophyta: Glaucophyceae
Cyanophora paradoxa
c
Rhodophyta: Bangiophyceae
Porphyridium cruentum
d
Absorbance (a.u.)
Wavelength (nm)
1.00
0.80
0.60
0.40
0.20
0.00
0.25
0.20
0.15
0.10
0.05
400
450
500
550
600
650
700


Heterokontophyta: Chrysophyceae
Ochromonas sp.
a
Heterokontophyta: Eustigmatophyceae
Nannochloropsis sp.
b
Heterokontophyta: Bacillariophyceae
Phaeodactilum tricornutum
c
Haptophyta: Haptophyceae
Gephyrocapsa oceanica
d
Absorbance (a.u.)
Wavelength (nm)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
0.05
0.15
400
450
500
550
600
650
700


Cryptophyta: Cryptophyceae
Rhodomonas sp.
a
Dinophyta: Dinophyceae
peridinin-containing species
b
Dinophyta: Dinophyceae
fucoxanthin-containing species
c
Dinophyta: Dinophyceae
Chl b - containing species
d
Absorbance (a.u.)
Wavelength (nm)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.50
0.60
400
450
500
550
600
650
700


Euglenophyta: Euglenophyceae
Euglena gracilis
a
Chlorarachniophyta: Chlorarachniophyceae
Gymnochlora stellata
b
Chlorophyta: Chlorophyceae
Dunaliella salina
c
Land Plants
Hedysarum coronarium
d
Absorbance (a.u.)
Wavelength (nm)
0.00
0.20
0.40
0.60
0.80
0.05
0.10
0.15
0.25
0.30
400
450
500
550
600
650
700

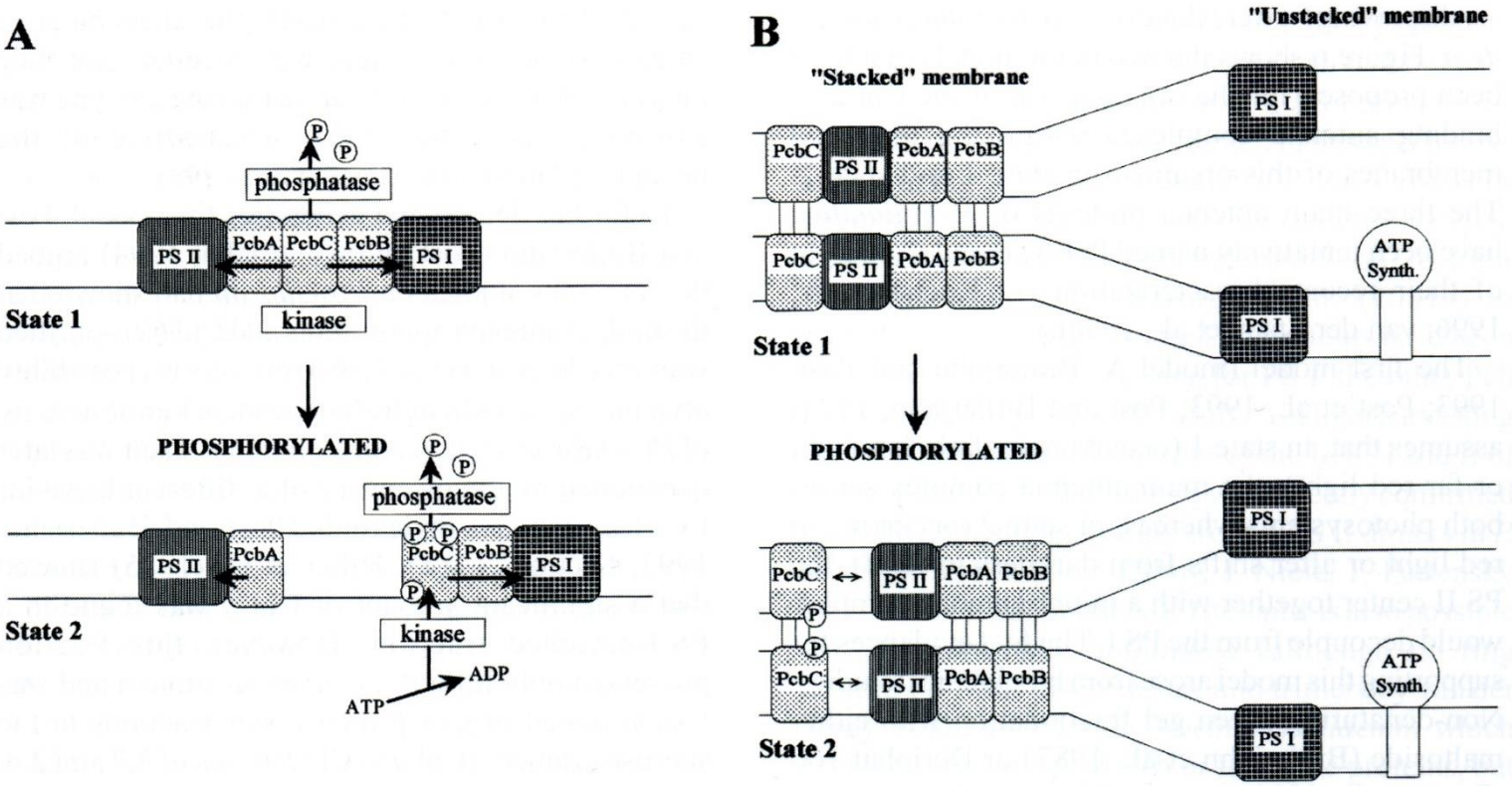


Fig. 6. Current models for the localization of the antenna proteins with regard to photosystems in thylakoid membranes of *Prochlorothrix hollandica* and effect of state 1-state 2 transitions. The original schemes have been redrawn in light of the recent identification of the three Pcb proteins and for a better homogenization. A: Model from Post et al. (1993). The Chl *a/b* antenna consists of a bulk antenna which is located on apoproteins of '30-kDa' (PcbA) and '35-kDa' (PcbC) and associated preferably with PS I. A minor Chl *a/b* antenna is carried by a '33-kDa' (PcbB) apoprotein and is found to co-purify with PS II. The '35-kDa' antenna protein forms the major target protein of light/redox controlled kinase activity. Upon phosphorylation, the bulk antenna excludes PS II centers and enters a tighter association with PS I. Under such conditions, the energy transfer to PS I is enhanced. This process reverses to a state of balanced energy transfer by the bulk antenna following dephosphorylation of the antenna in either darkness or far red illumination. B: Model from van der Staay and Staehelin (1994). As in chloroplasts, PS II and the Chl *a/b* antenna are located in 'stacked' parts of the thylakoid membrane, whereas PS I and the ATP synthase are restricted to the 'unstacked' membranes. The Chl *a/b* antenna associated with PS II comprises four polypeptides with apparent molecular masses of '32-kDa' (PcbA), '33.5-kDa', '35-kDa' (one or both of these probably correspond to PcbB) and '38-kDa' (PcbC), all of which are assembled into one antenna complex. The '38-kDa' antenna gets phosphorylated at high light. In accordance with the fluorescence data, an uncoupling of the phosphorylated antenna from PS II is assumed to occur, but it would not migrate into the 'unstacked' membranes.

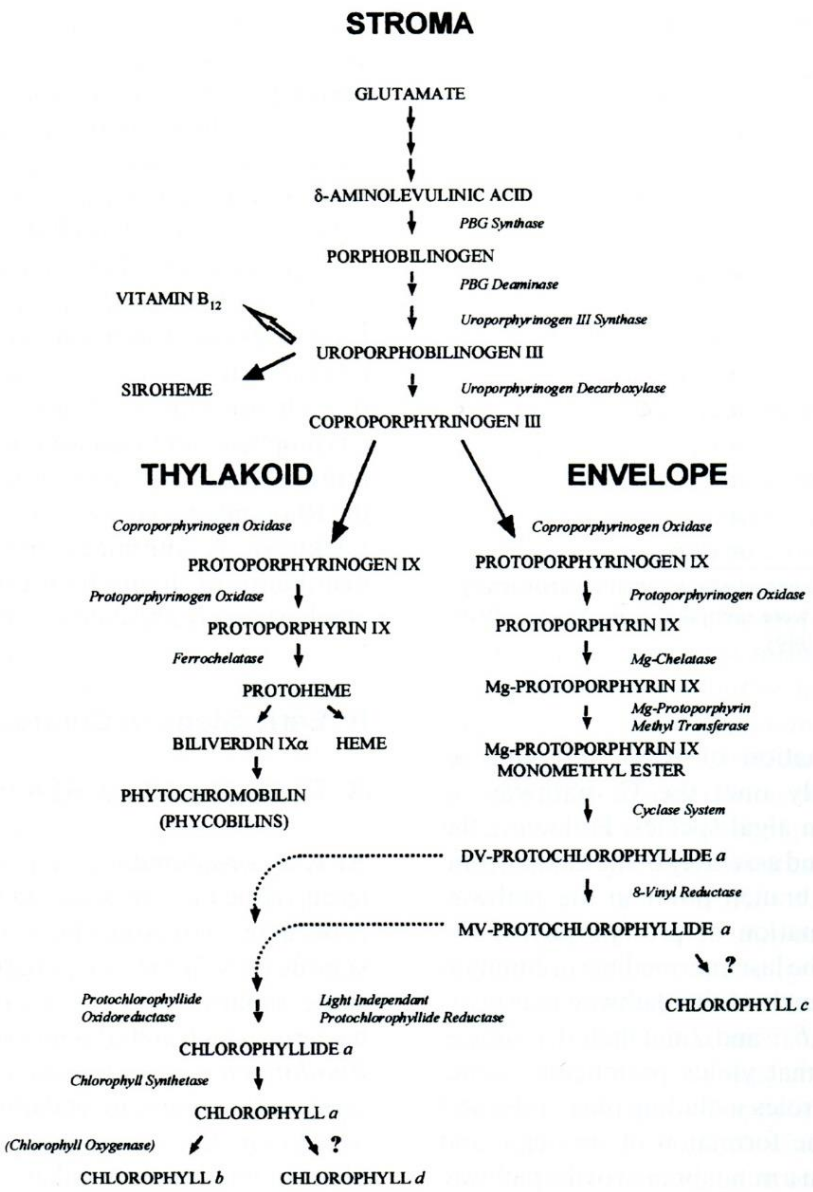
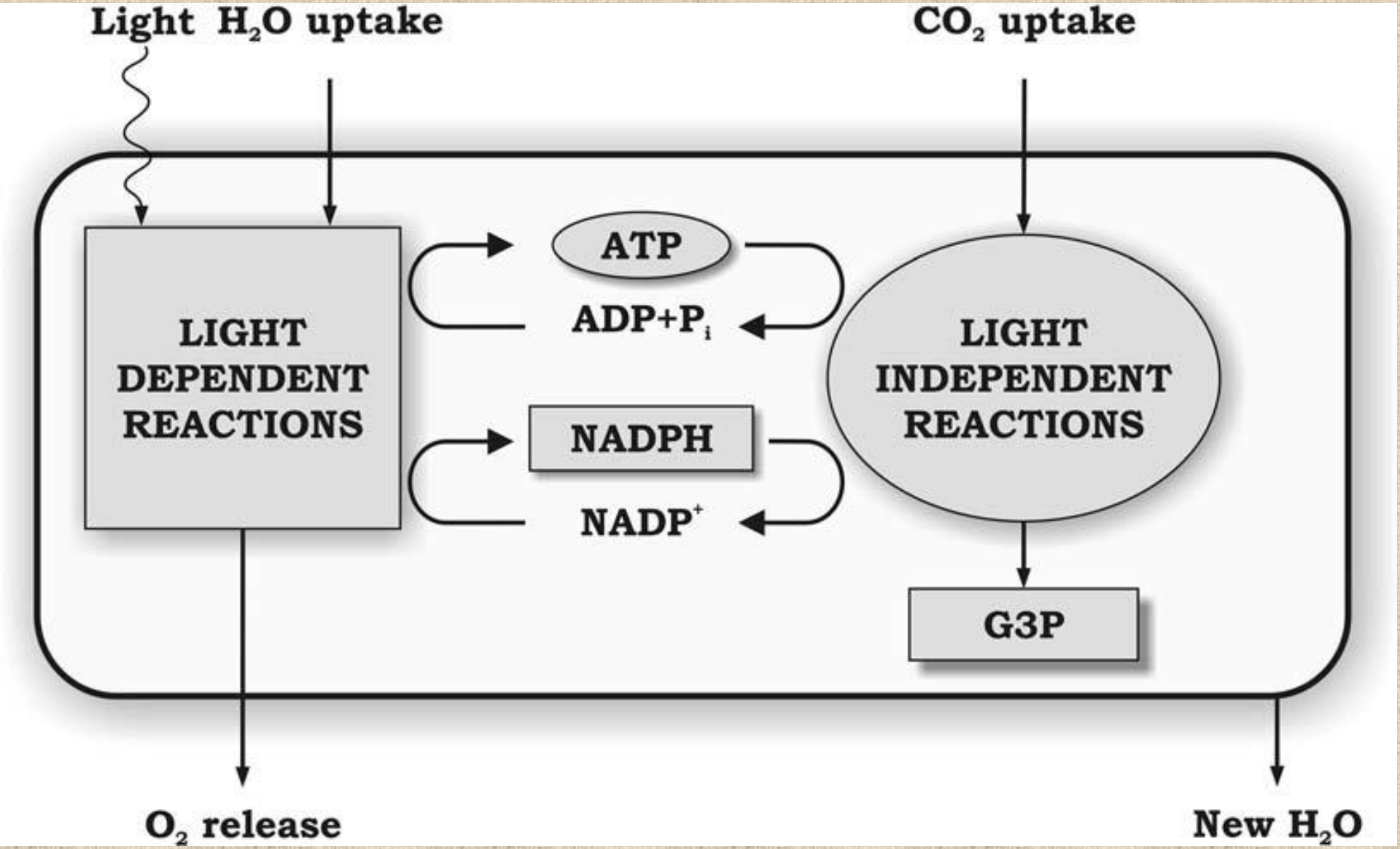
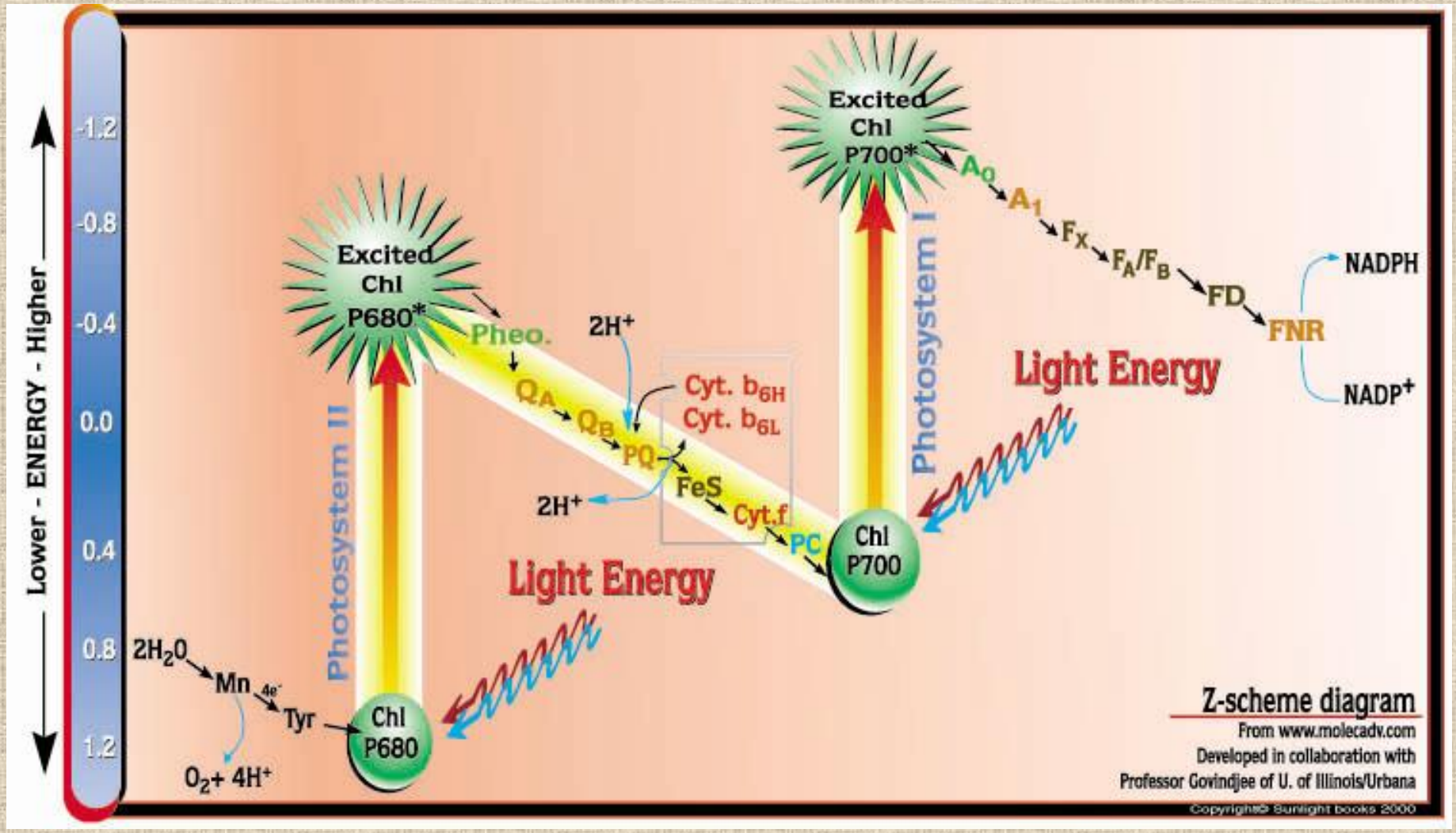


Fig 1. General outline of the tetrapyrrole biosynthesis pathway in photosynthetic organisms. Shown are the major intermediates and enzymes involved in tetrapyrrole formation in plants, algae, and photosynthetic bacteria and the proposed location of the enzymatic activities in plastids. The steps leading to phycobilin and vitamin B_{12} formation appear to be restricted to prokaryotic organisms. The dashed lines indicate the possible transport of these compounds from the plastid envelope to the thylakoids.

Photosynthetic reactions

- general equation:
  - » $n CO_2 + n H_2O + \text{light} \xrightarrow{\text{Chl } a} (CH_2O)_n + n O_2$
- light dependent reactions
  - light interception, l. energy transfer
  - excitation, charge separation
  - ETR
    - » linear
    - » cyclic
  - O_2 evolution
  - $4NADP^+ + 2H_2O + 4ADP + 4P_i \xrightarrow{8 \text{ photons} + 4e^-} 4NADPH + 4ATP + O_2$
- light independent reactions
  - CBB – Calvin Benson Bassham Cycle
  - RuBisCO
  - CA


Light
H_2O uptake
CO_2 uptake
LIGHT DEPENDENT REACTIONS
ATP
$ADP+P_i$
NADPH
$NADP^+$
LIGHT INDEPENDENT REACTIONS
G3P
O_2 release
New H_2O


Lower - ENERGY - Higher
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Excited Chl P680*
Excited Chl P700*
Photosystem II
Photosystem I
Pheo.
Q_A
Q_B
PQ
$2H^+$
Cyt. b_{6H}
Cyt. b_{6L}
FeS
Cyt.f
PC
Chl P700
Chl P680
Light Energy
Light Energy
A_0
A_1
F_X
F_A/F_B
FD
FNR
NADPH
$NADP^+$
$2H_2O$
Mn
4e⁻
Tyr
$O_2 + 4H^+$
Z-scheme diagram
From www.molecadv.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana
Copyright© Sunlight books 2000

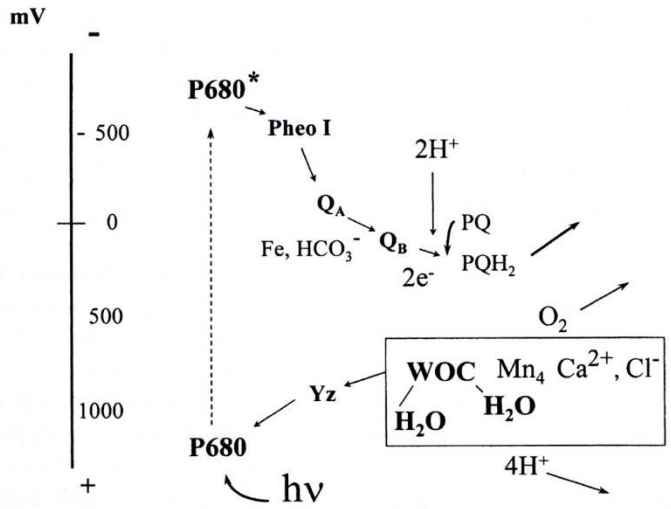


Fig. 1. Diagram showing the main cofactors of PS II, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Electron flow occurs from water to Q_B. Absorption of light energy ($h\nu$) or excitons by P680, producing P680*, leads to a photochemical charge separation between P680 and Pheo I. Water donates electrons to $P680^+$ via Y_Z (D1 Tyr161). The water oxidizing complex (WOC) involves Mn, plus possibly Ca^{++} and Cl^- cofactors, oxygen evolution requiring four turnovers of the reaction center. A non-heme iron (Fe) is located between Q_A and Q_B and bicarbonate (HCO_3^-) also binds in this region. Q_B picks up two electrons and two protons, transferring these to the membrane plastoquinone pool (PQ). Key: P680, the primary chlorophyll electron donor; P680*, excited state of P680; Pheo, pheophytin electron acceptor I; Q_A and Q_B, primary and secondary plastoquinones.

Electron Transfer in PSII

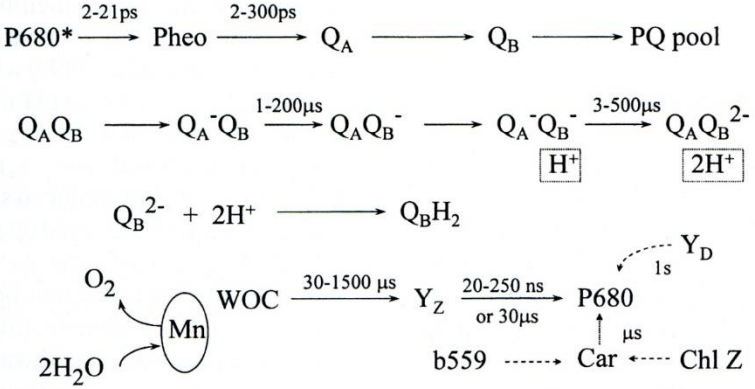


Fig. 2. Diagram showing the main kinetics of the main pathway of electrons through the PS II reaction center. Y_D (D2 Tyr161), Chlorophyll Z (Chl Z) and Cytochrome b_{559} (b_{559}) are alternate electron donors under certain conditions. See also Figure 1.

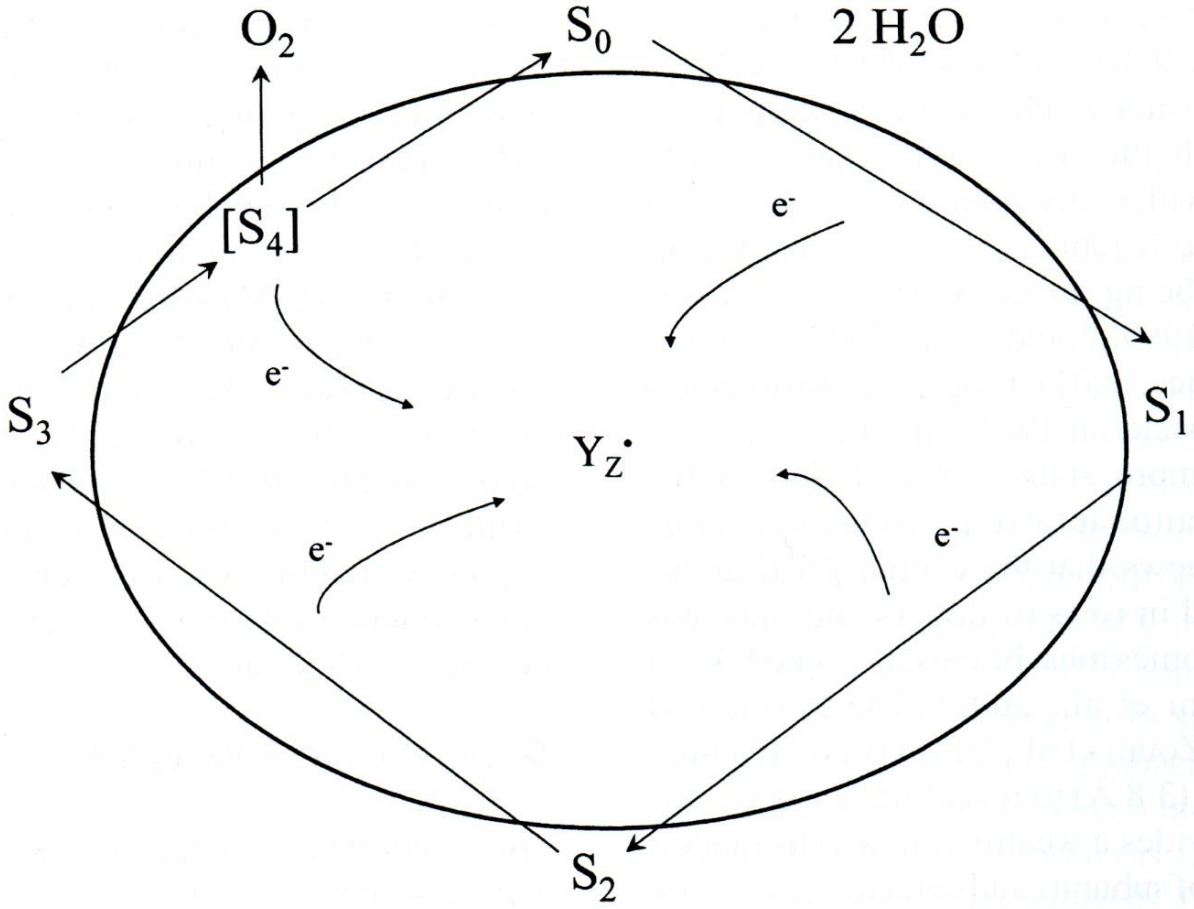


Fig. 3. The S-states of the water oxidizing complex (WOC). Electrons are removed sequentially by $P680^+$ via Y_Z. The S-state number indicates the number of oxidizing equivalents stored. On reaching S_4, oxygen is released and the cycle reset. The steps at which water may be bound, oxidized and protons released are discussed in the text.

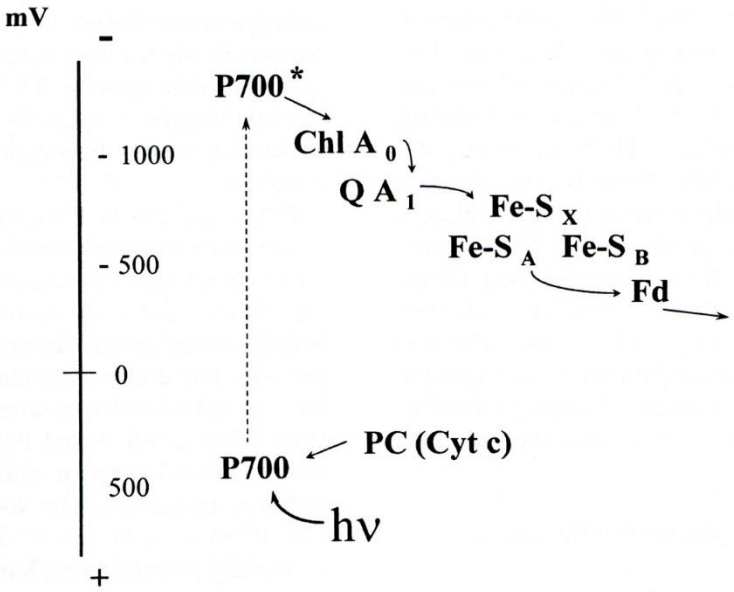


Fig. 4. Diagram showing the main cofactors of PS I, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Absorption of light energy (hν) or excitons by P700 leads to a photochemical charge separation. Electron flow occurs from Plastocyanin (PC) to Ferredoxin (Fd). Electron transfer from P700 occurs across the membrane to reduce the iron-sulfur centers. PC donates an electron to $P700^{+}$. Key: P700, primary electron donor; P700*, excited state of P700; A_0, Chl electron acceptor; A_1, phylloquinone (Q); Fe-S_X, Fe-S_A and Fe-S_B, are iron-sulfur centers; Cyt *c*, cytochrome *c* is an alternative electron donor.

Electron Transfer in PSI

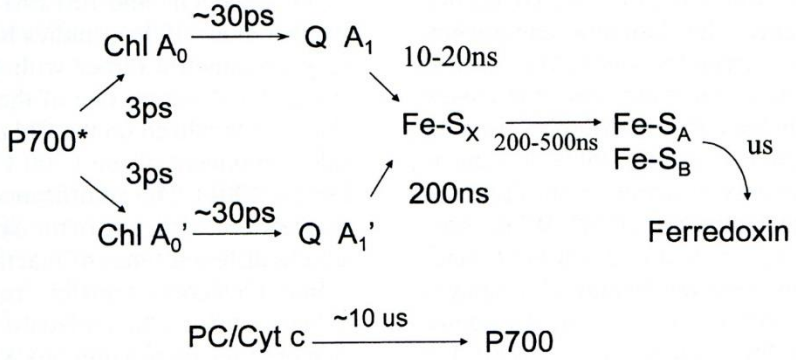
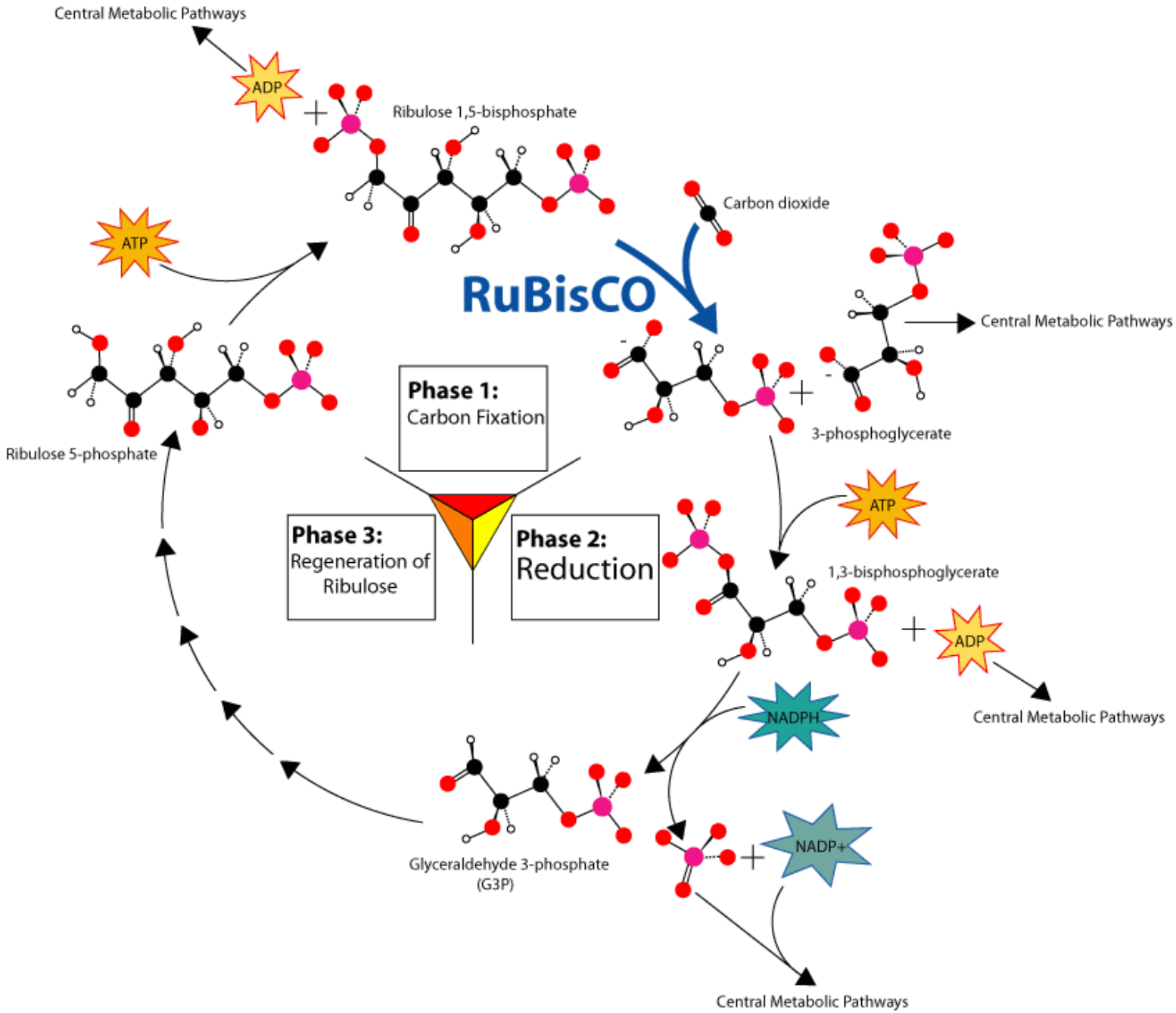


Fig. 5. Diagram showing the main kinetics of the main pathway of electrons through the PS I reaction center.


Central Metabolic Pathways
ADP
Ribulose 1,5-bisphosphate
Carbon dioxide
ATP
RuBisCO
Central Metabolic Pathways
Ribulose 5-phosphate
Phase 1:
Carbon Fixation
3-phosphoglycerate
ATP
Phase 3:
Regeneration of
Ribulose
Phase 2:
Reduction
1,3-bisphosphoglycerate
ADP
Central Metabolic Pathways
NADPH
NADP+
Glyceraldehyde 3-phosphate
(G3P)
Central Metabolic Pathways

Role of Carbon Concentrating Mechanism

- Carbonic Anhydrase
  - periplasmic space, carboxysomes (b-g algae), pyrenoid (algae)
  - CA *vs.* RuBisCO
- mechanisms of HCO_3^- uptake
  - active transp. via CMP (enough ATP)
  - symport Na^+ HCO_3^- - via icpB complex
  - Na^+/H^+ antiport help
  - difusion
- CO_2 uptake - difusion

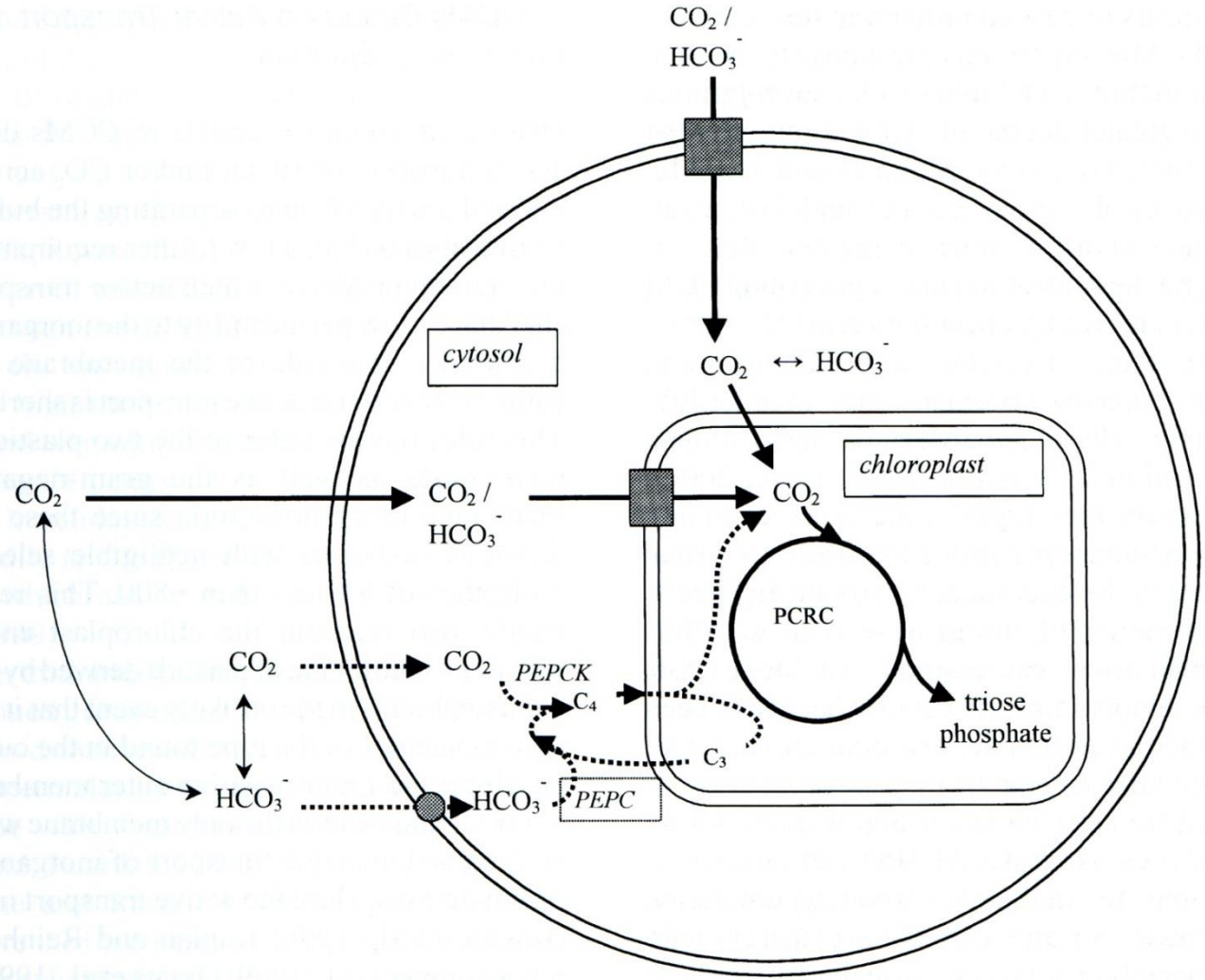


Fig. 1. A simplified scheme for transport of inorganic carbon into eukaryotic algal cells via active transport of CO_2 and/or bicarbonate. As explained in the text, CO_2 will cross membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of CO_2 or HCO_3^-. Active transport can occur at the plasmalemma or at the chloroplast envelope or at both membranes. Carbonic anhydrases in the periplasmic space, cytosol and chloroplast maintain equilibrium between CO_2 and HCO_3^-. Also shown (dotted line) is a putative role for C_4-like metabolism in CO_2 concentration (see text for details). PCRC – photosynthetic carbon reduction cycle; PEPC – phosphoenolpyruvate cartboxylase; PEPCK – phosphoenolpyruvate carboxykinase. Redrawn after Sültemeyer (1998).

Energy transfers & regulations

- excitation energy (excess) dissipation pathways
  - photosynthesis
  - state stransitions
  - heat production (Xanthophyll cycle)
  - fluorescence
- photoaclimation
  - light dependent motions; state transitions; non-photochemical processes
- photoinhibition
  - photomodification
  - photodamage

- photorespiration & chlororespiration
    - RuBisCo – generation of glycolate
    - enigma; PQ pool reducion

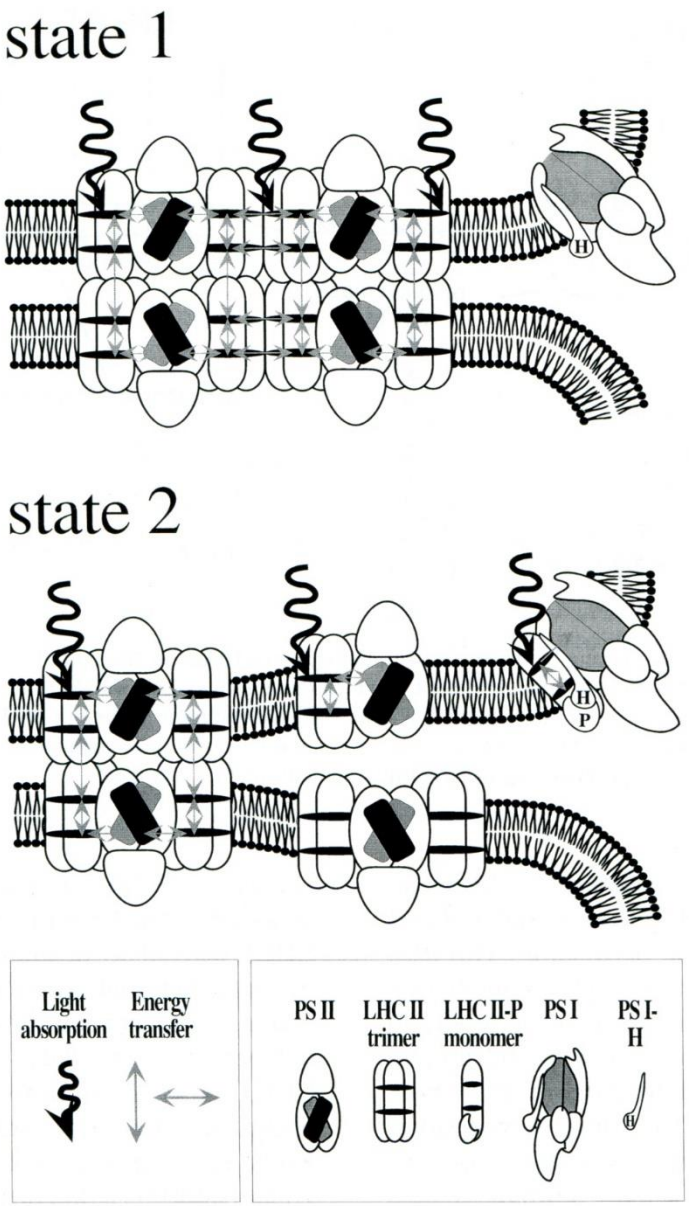
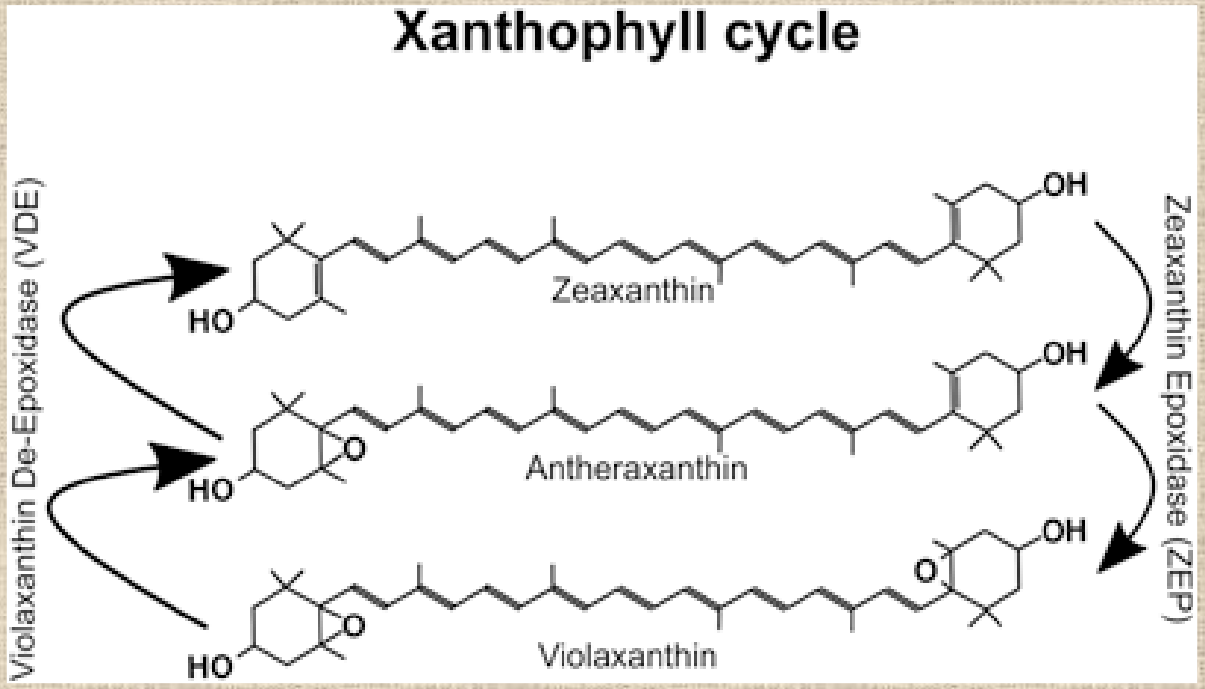
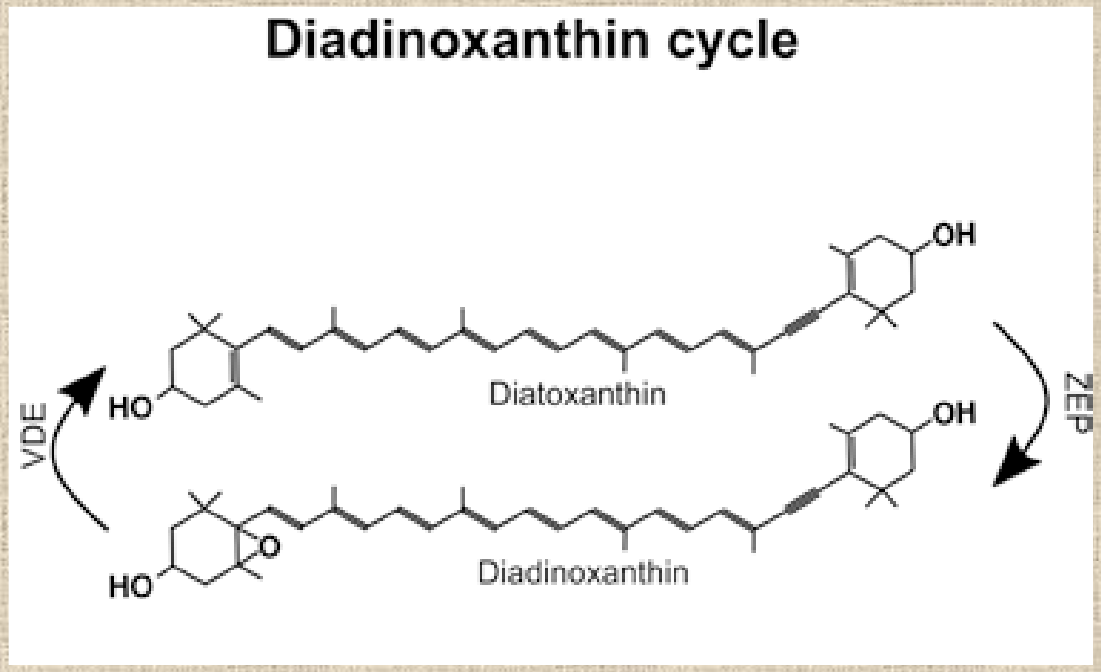
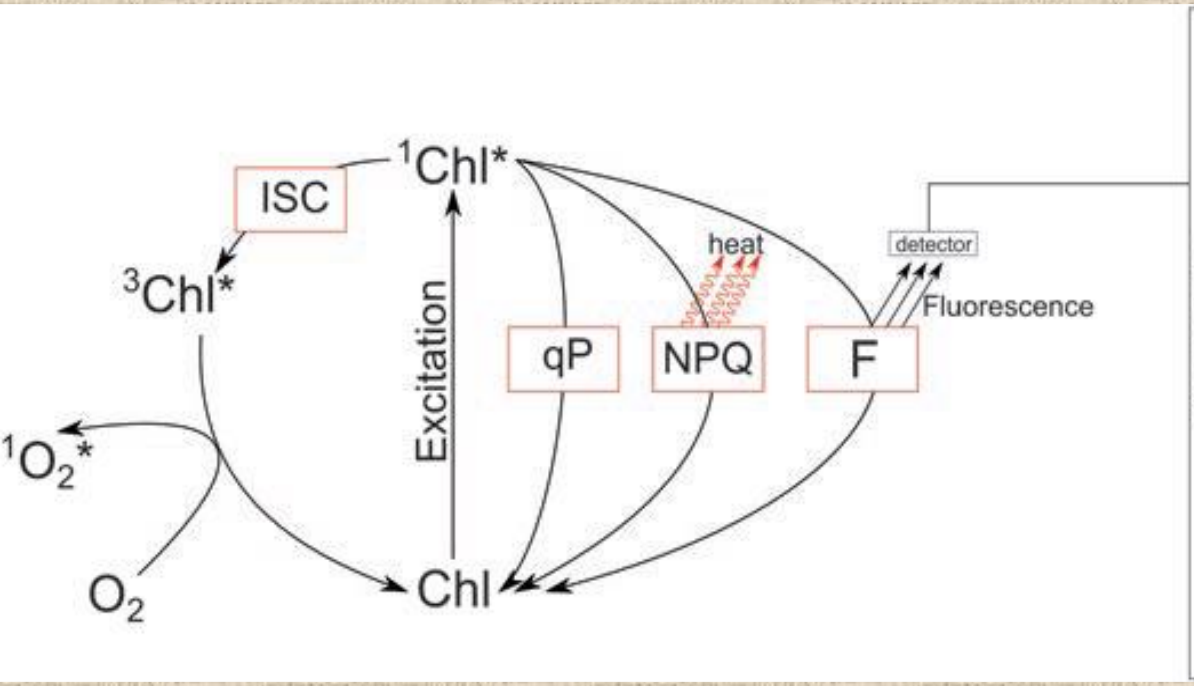
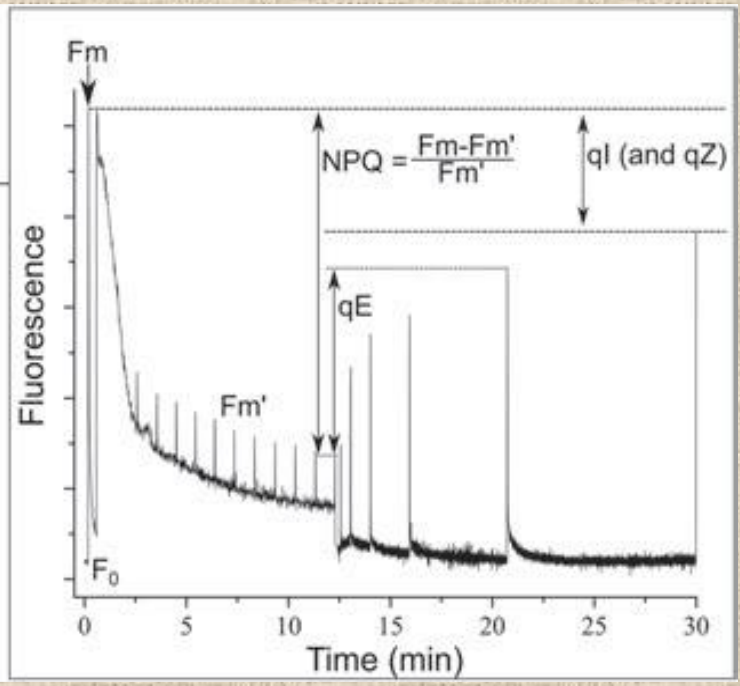


Fig 6. Artist's impression of the difference between State 1 and State 2 organization in appressed and non-appressed thylakoids in green plants. Redrawn with permission from Forsberg and Allen (2001a).


Xanthophyll cycle
Violaxanthin De-Epoxidase (VDE)
Zeaxanthin Epoxidase (ZEP)
Zeaxanthin
Antheraxanthin
Violaxanthin
HO
OH
O


Diadinoxanthin cycle
VDE
ZEP
Diatoxanthin
Diadinoxanthin
HO
OH
O


ISC
^{1}Chl*
^{3}Chl*
heat
detector
Fluorescence
qP
NPQ
F
Excitation
$^1O_2$*
O_2
Chl


Fm
NPQ = Fm-Fm' / Fm'
qI (and qZ)
qE
Fm'
F_0
Fluorescence
0
5
10
15
20
25
30
Time (min)

Photosynthesis – Irradiance response curve (P *vs.* E curve)

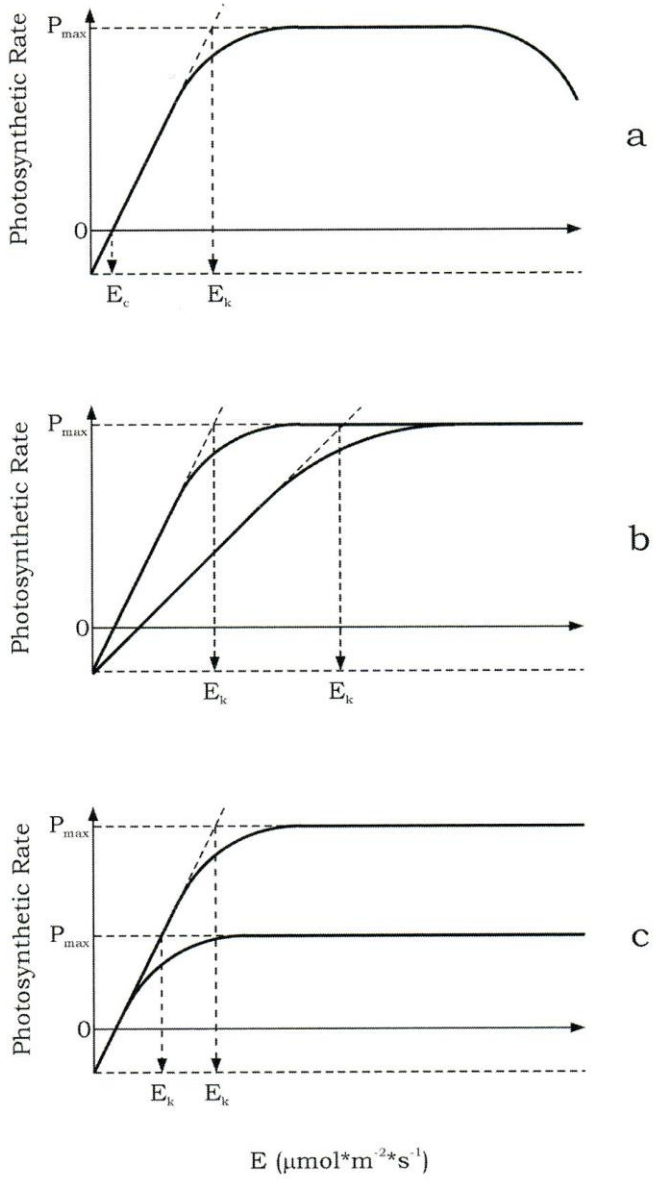


FIGURE 5.15 Photosynthesis–irradiance response curves: E_c, irradiance compensation point; E_k, saturating irradiance; and P_{max}: maximum photosynthetic rate. (a) typical plot; (b) comparison of two curves with different slopes: keeping constant the number of photosynthetic units, but increasing the functional absorption cross-section, the slope increases; and (c) comparison of two curves with different maximum photosynthetic rate: increasing the number of photosynthetic units, P_{max} increases.

Measurement of photosynthetic production

Methods:

- gravimetric / voluntometric
- turbidimetric / nephelometric
- fluorometric
- gasometric
  - IRGA; O_2 measurement
- Light response curve
  - Pn = GP - R

Gravimetry / Voluntometry

- Dry weight, fresh weight
- PCV (packed cell volume)
- AFDW (Ash free dry weight)

$$\text{Dry weight mg / L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where: A = weight of filter + dried residue, mg
B = weight of filter, mg

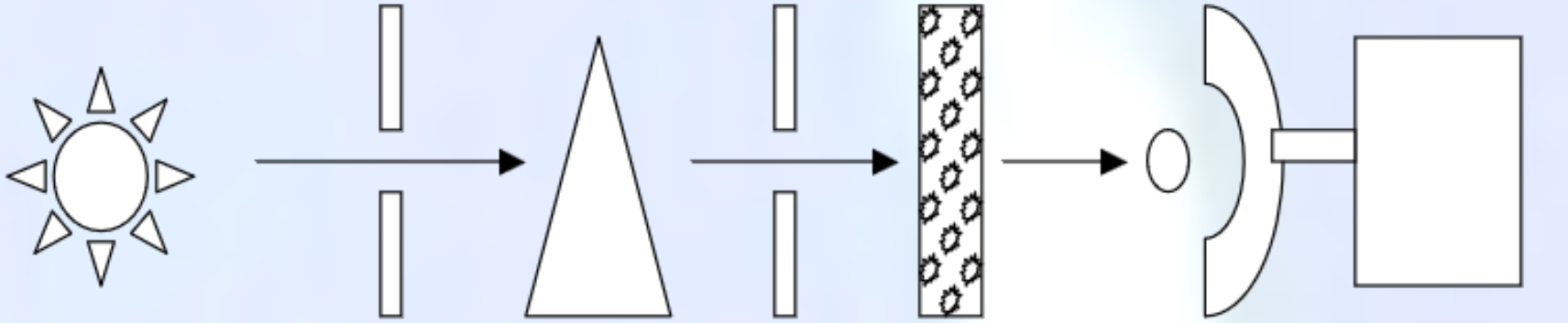
$$\text{AFDW, mg / L} = \frac{(C - D) \times 1000}{\text{sample volume, mL}}$$

- Content of primary compound
  - chl a, Total chl, proteins

Turbidimetry / Nephelometry

- Turbidimetry
  - amount of absorbed light *vs.* number & size of particles (cells)
  - spectrophotometers (750, 735, 680nm,..)
- Nephelometry
  - amount of scattered light (90°, 70°, 37°) *vs.* number & size of particles (cells)
  - amount of scattered light is far greater than the transmitted light >> offers higher sensitivity than turbidimetry

Schematic diagram of a turbidimeter



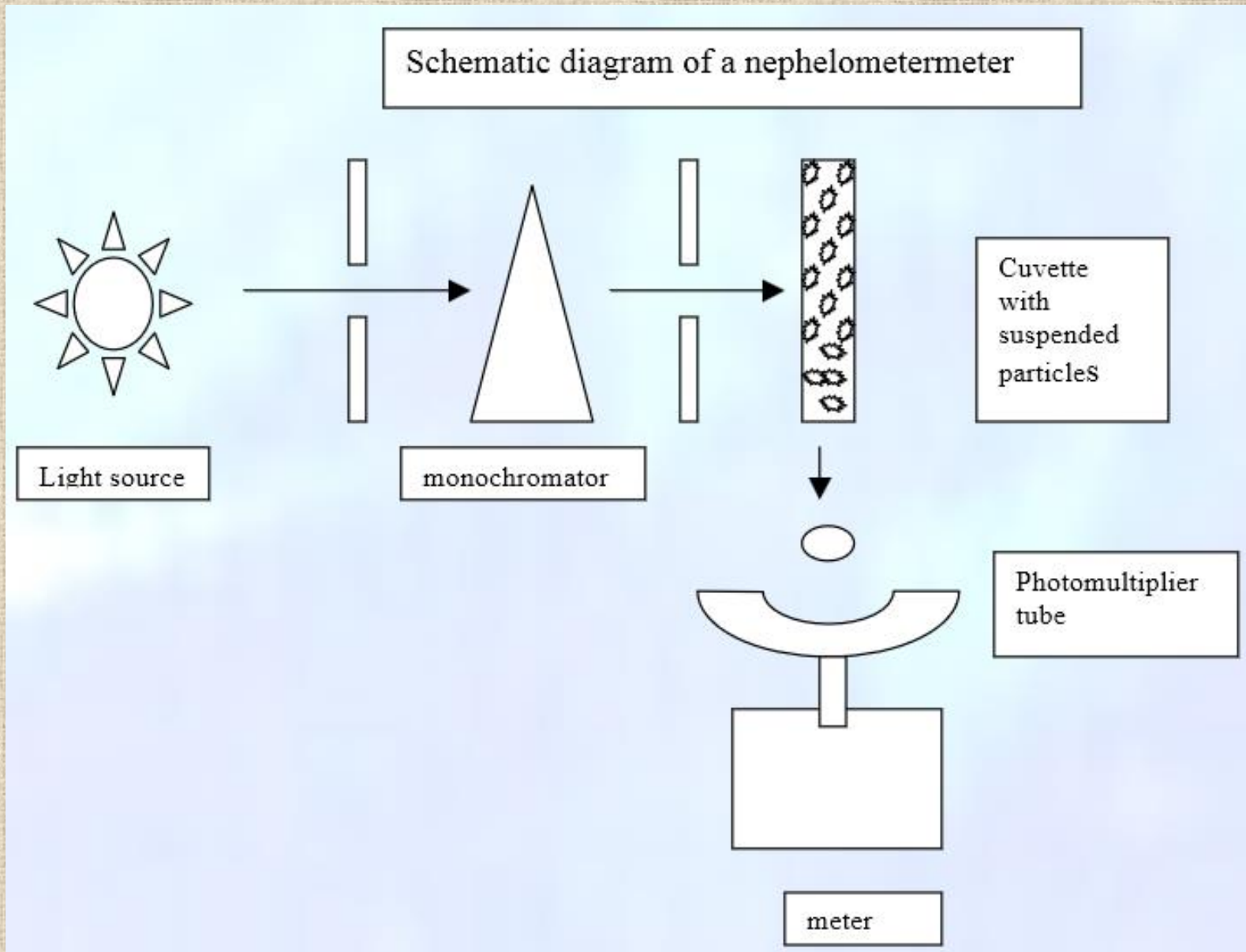
Light source

monochromator

Cuvette with suspended particleS

Photomultiplier tube

meter


Schematic diagram of a nephelometermeter
Cuvette with suspended particleS
Light source
monochromator
Photomultiplier tube
meter

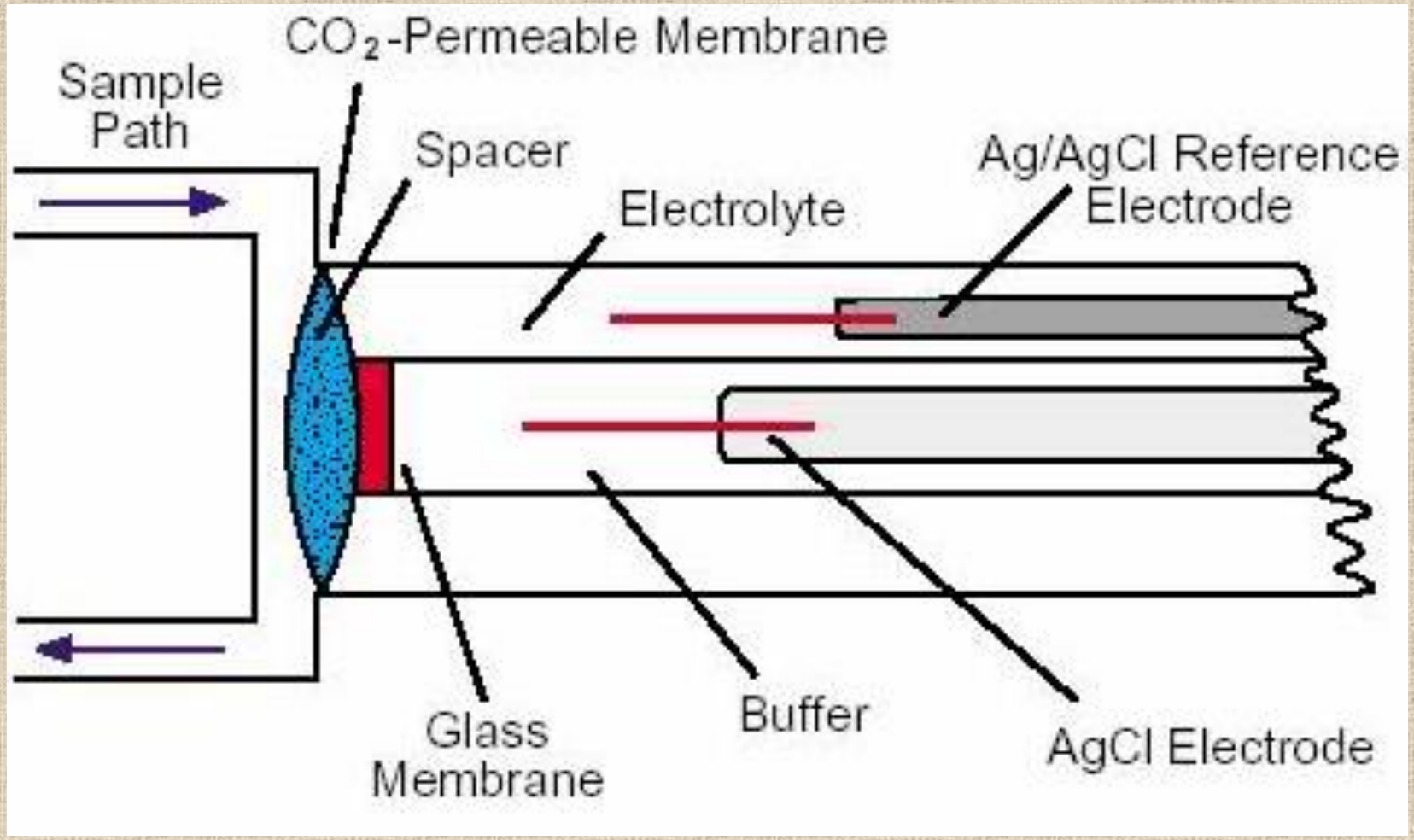
Turbidimetry & Nephelometry

- blank
- selection of wavelenght (750, 735, 680nm,..)
- standard curves must be plotted
  - similar cell size
- precipitation and settlement may occur
  - mix the sample well prior to measurement
  - keep the timing
- Kinetic reaction
  - provides additional information

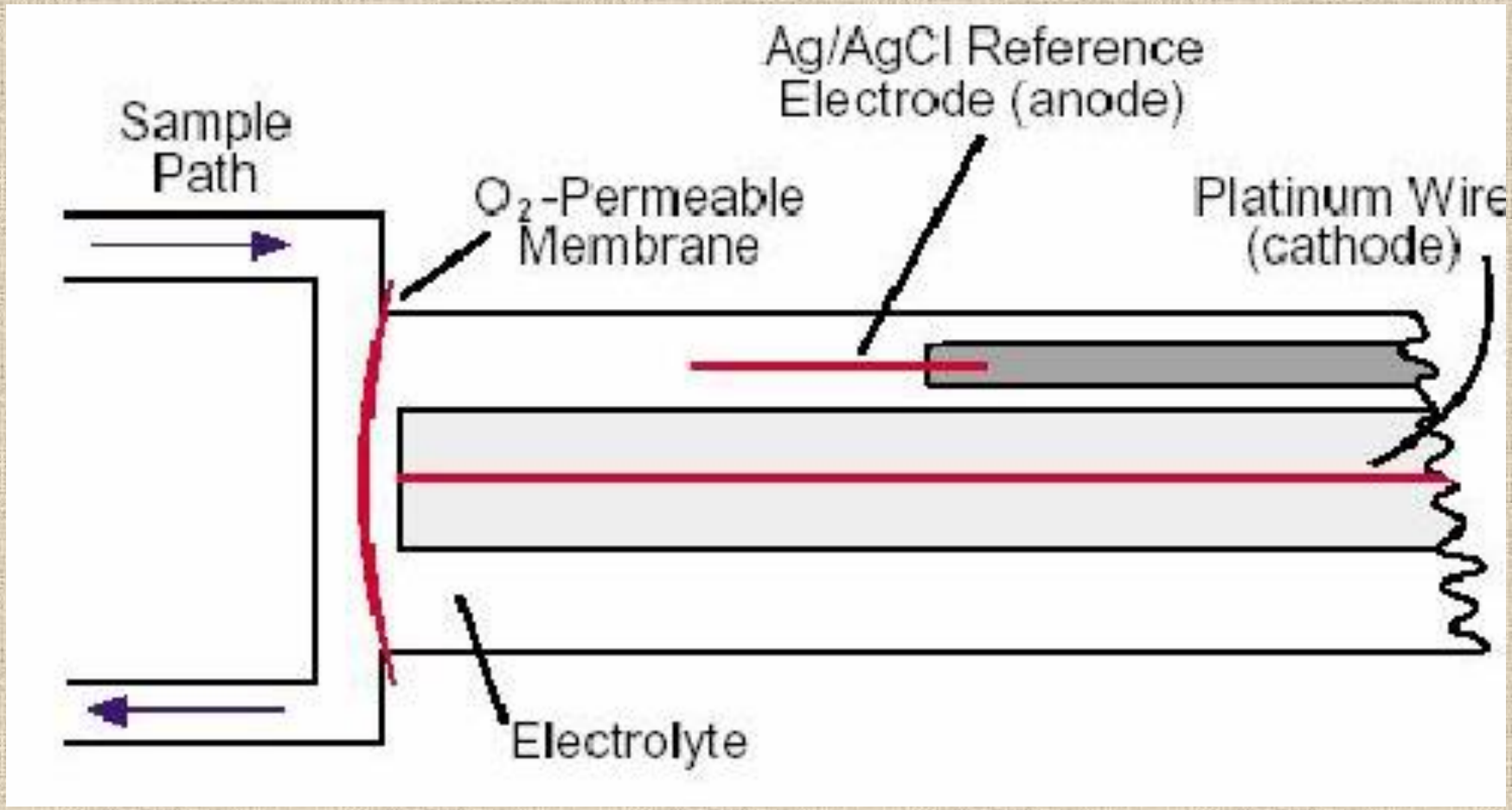
Gasometry

- Manometry, Formation of titratable compounds (NO>NO2)
- measure CO_2, O_2
- IRGA cuvette
  - aerophytic algae, soil crusts
  - water vapor
- CO2 electrode, O2 electrode, optode
  - Fast & sensitive, dimension vary
  - Cathode vs. background consumption !!
    - » Membrane type – Clark
    - » Bare type – ZrO2
  - Optode – luminescence quenching

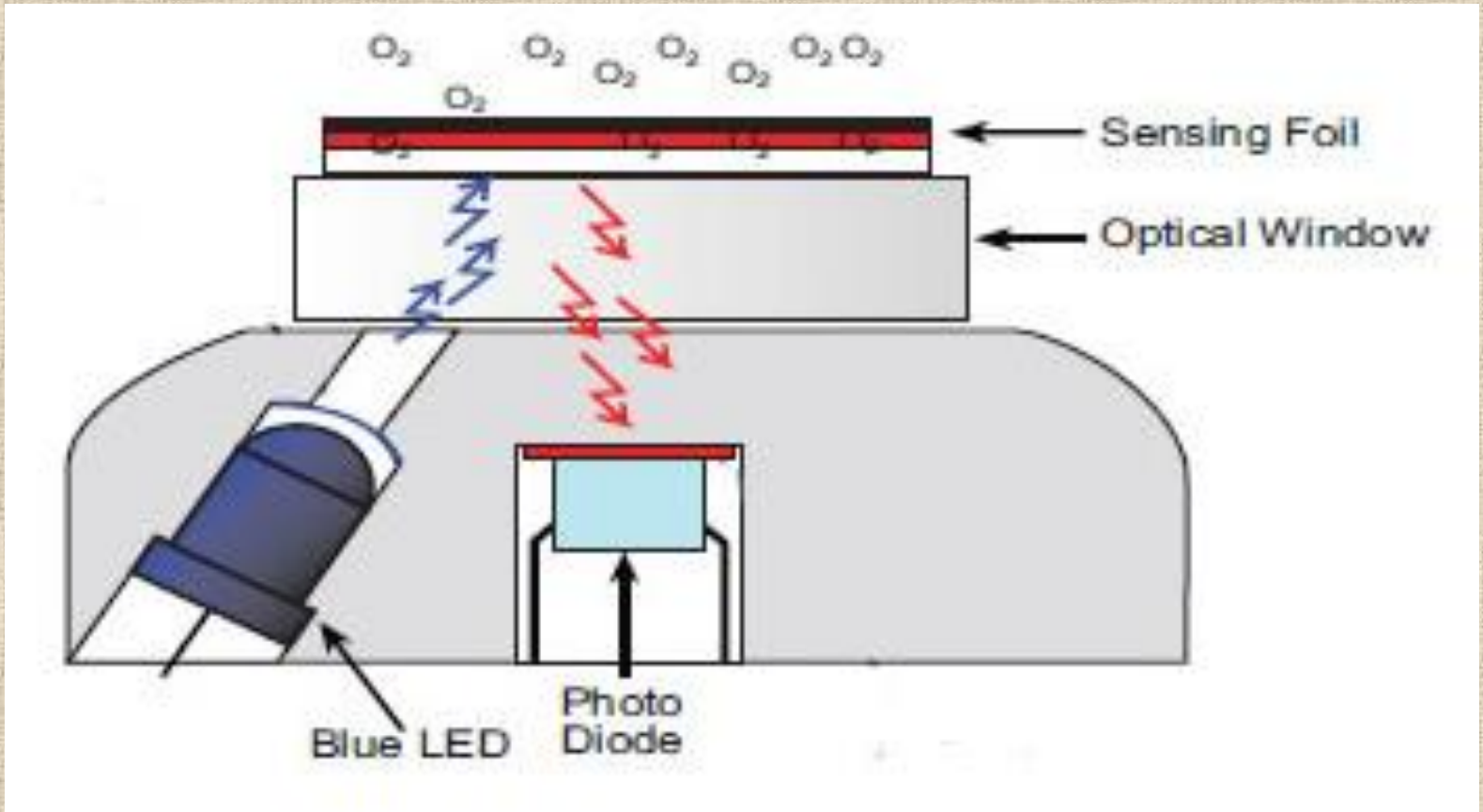
Carbon dioxide electrode



Clark type oxygen electrode



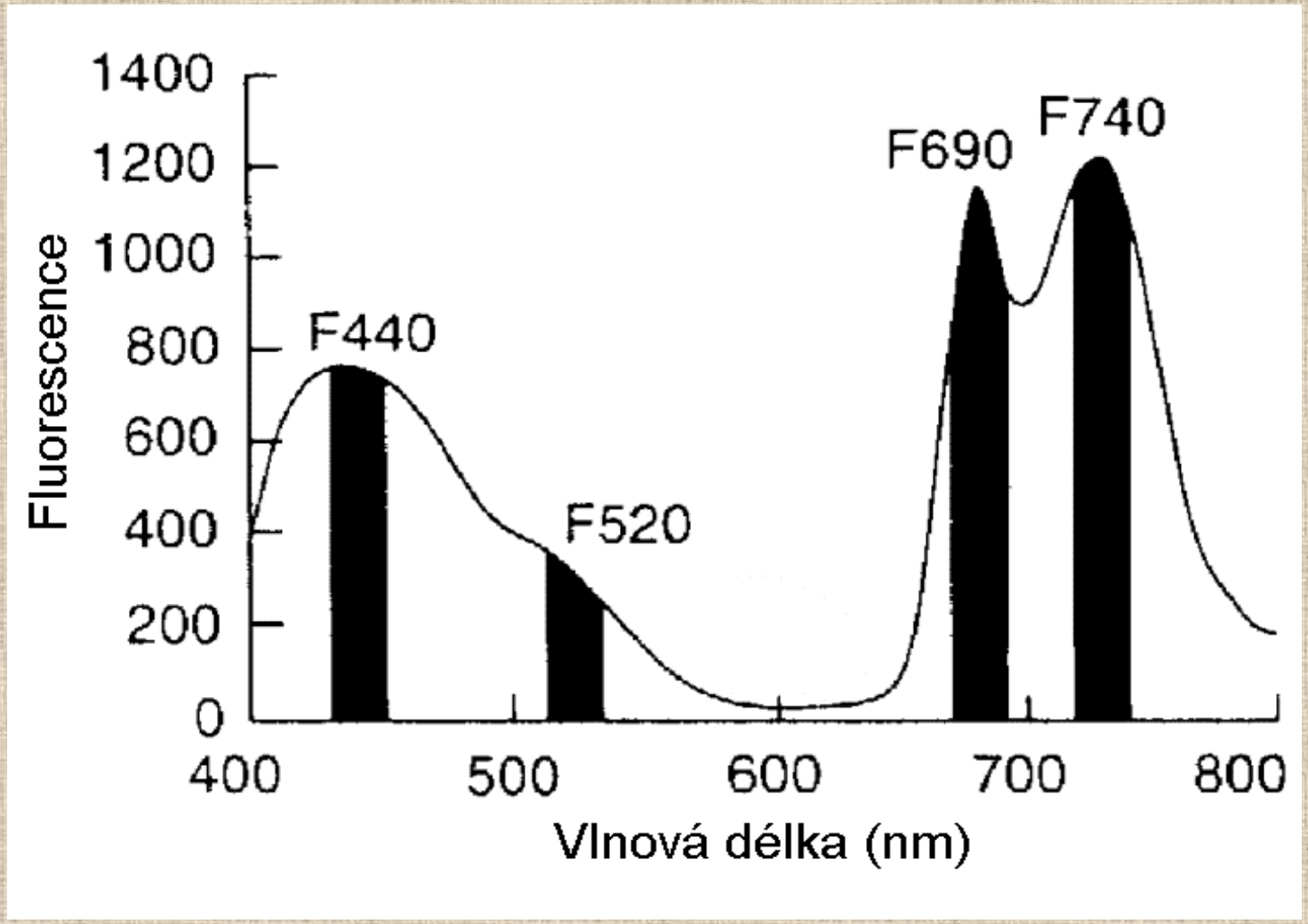
Oxygen optode



Chlorophyll fluorescence

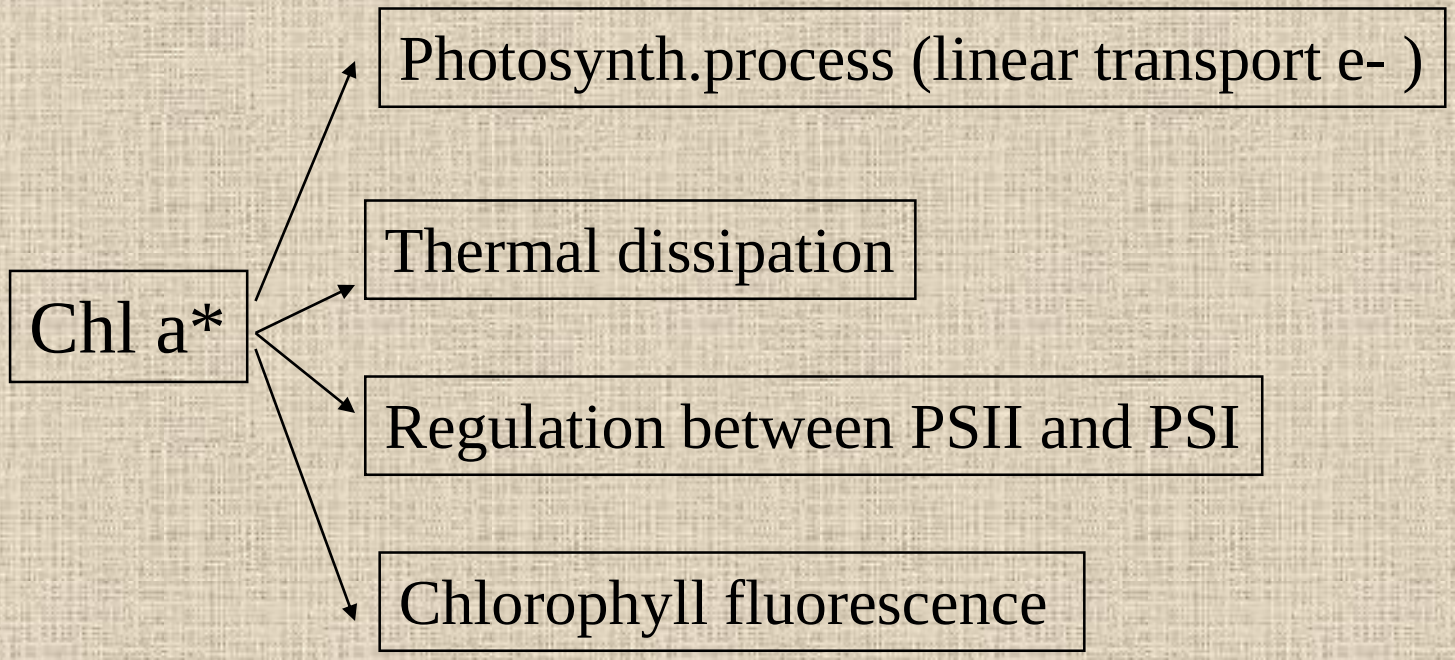
- What is chlorophyll fluorescence?
- Basic methods of Chl fluorescence measurements
- Signals, kinetics of Chl fuorescence
  - fast Chl fluorescence kinetics (parameters)
  - slow Chl fluorescence kinetics (parametrs)
  - saturation pulse method, quenching analysis
- Chl fluorescence imaging

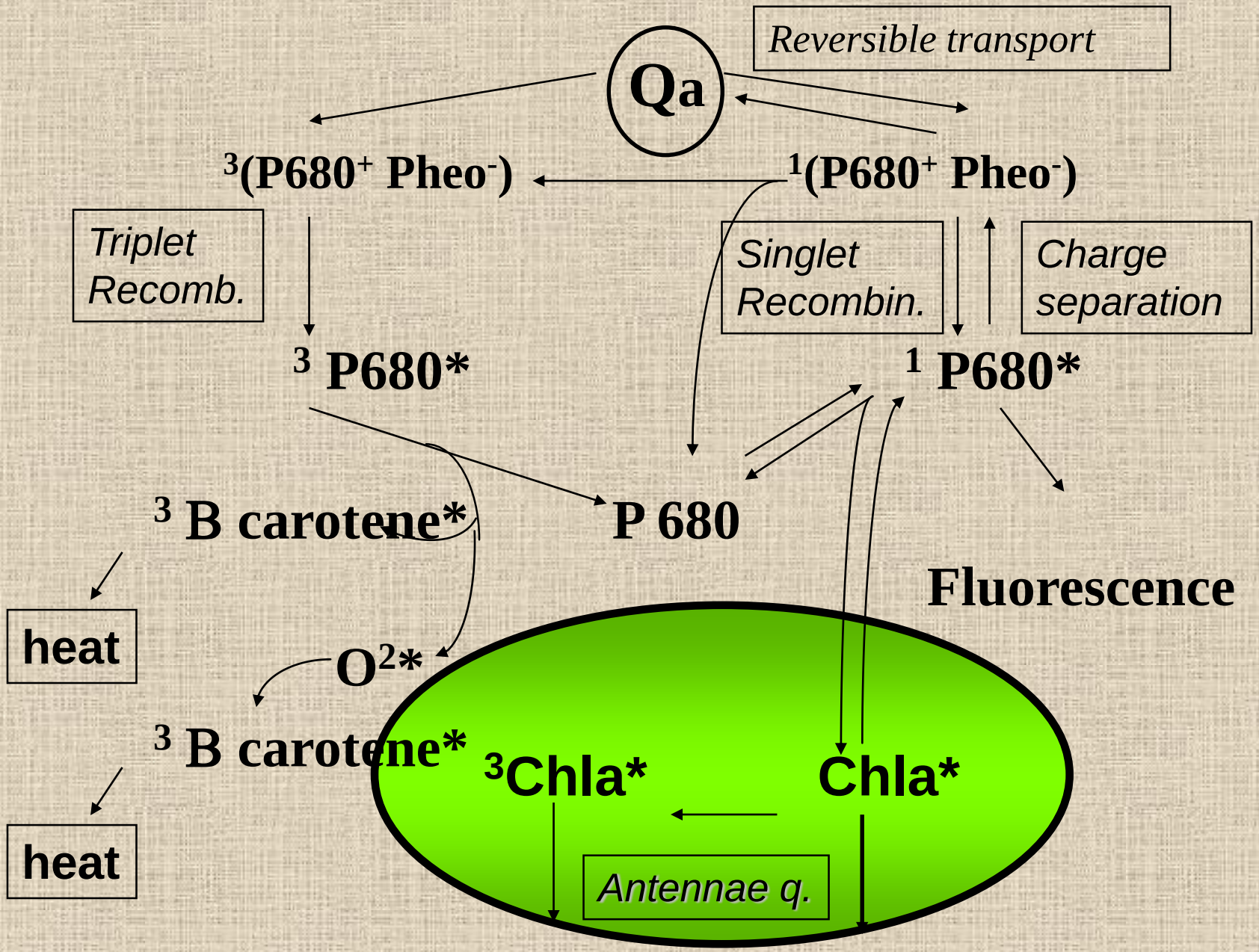
Fluorescence emission



Lichtenthaler et Miehe 1997

Basic de-excitation mechanisms of Chl *a*

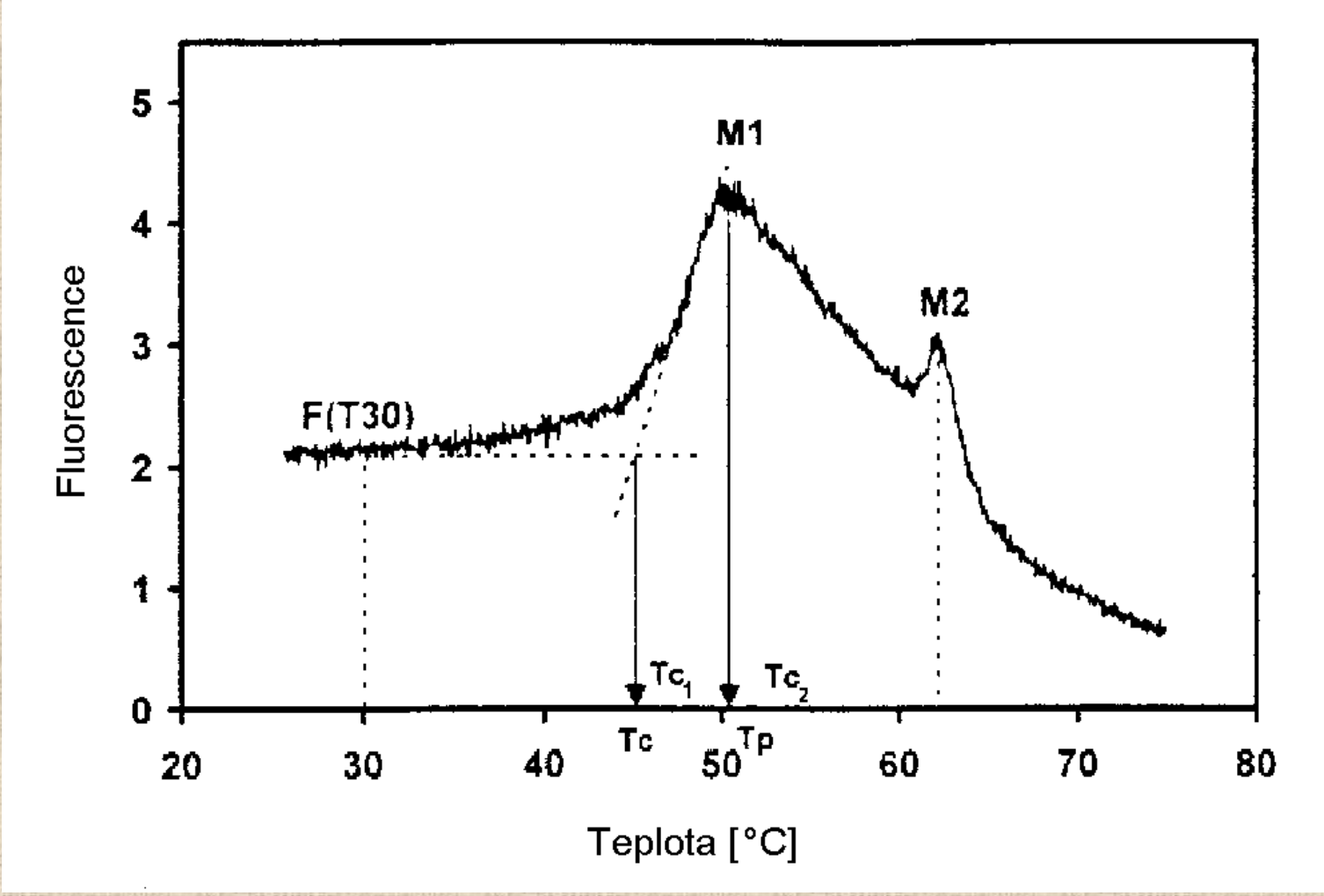



Reversible transport
Qa
$^3(P680^+ Pheo^-)$
$^1(P680^+ Pheo^-)$
Triplet Recomb.
Singlet Recombin.
Charge separation
^{3} P680*
^{1} P680*
^{3} B carotene*
P 680
Fluorescence
heat
O^{2*}
^{3} B carotene*
^{3}Chla*
Chla*
heat
Antennae q.

Chlorophyll fluorescence techniques

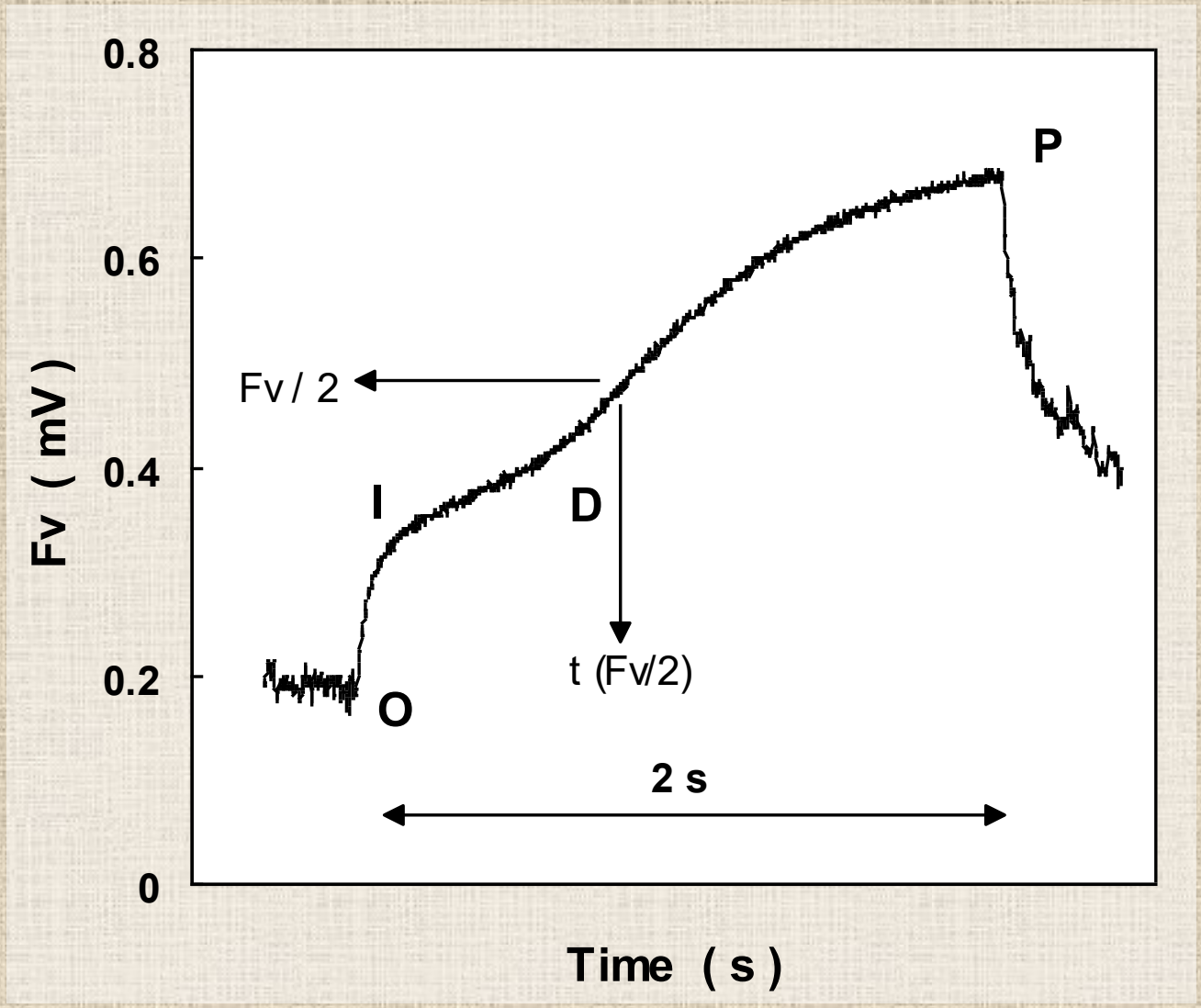
- Chl fluorescence signal
- Saturation pulse method
- Chl fluorescence kinetics (transients)
- Quenching analysis
- Rapid light/response curves
- Emission/absorption spectra
- Chl fluorescence imaging

Temperature response curve

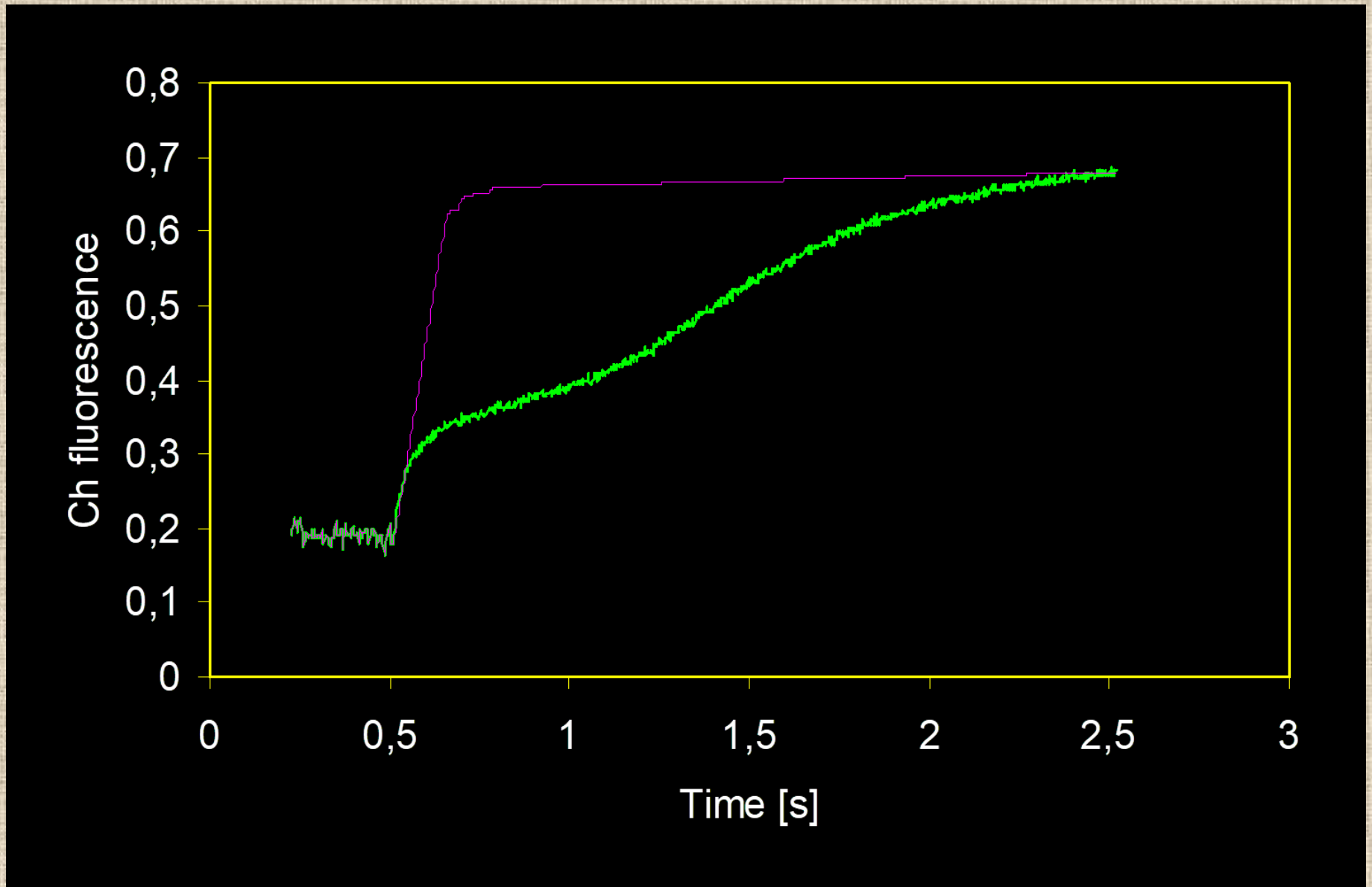


Kuropatwa 1994

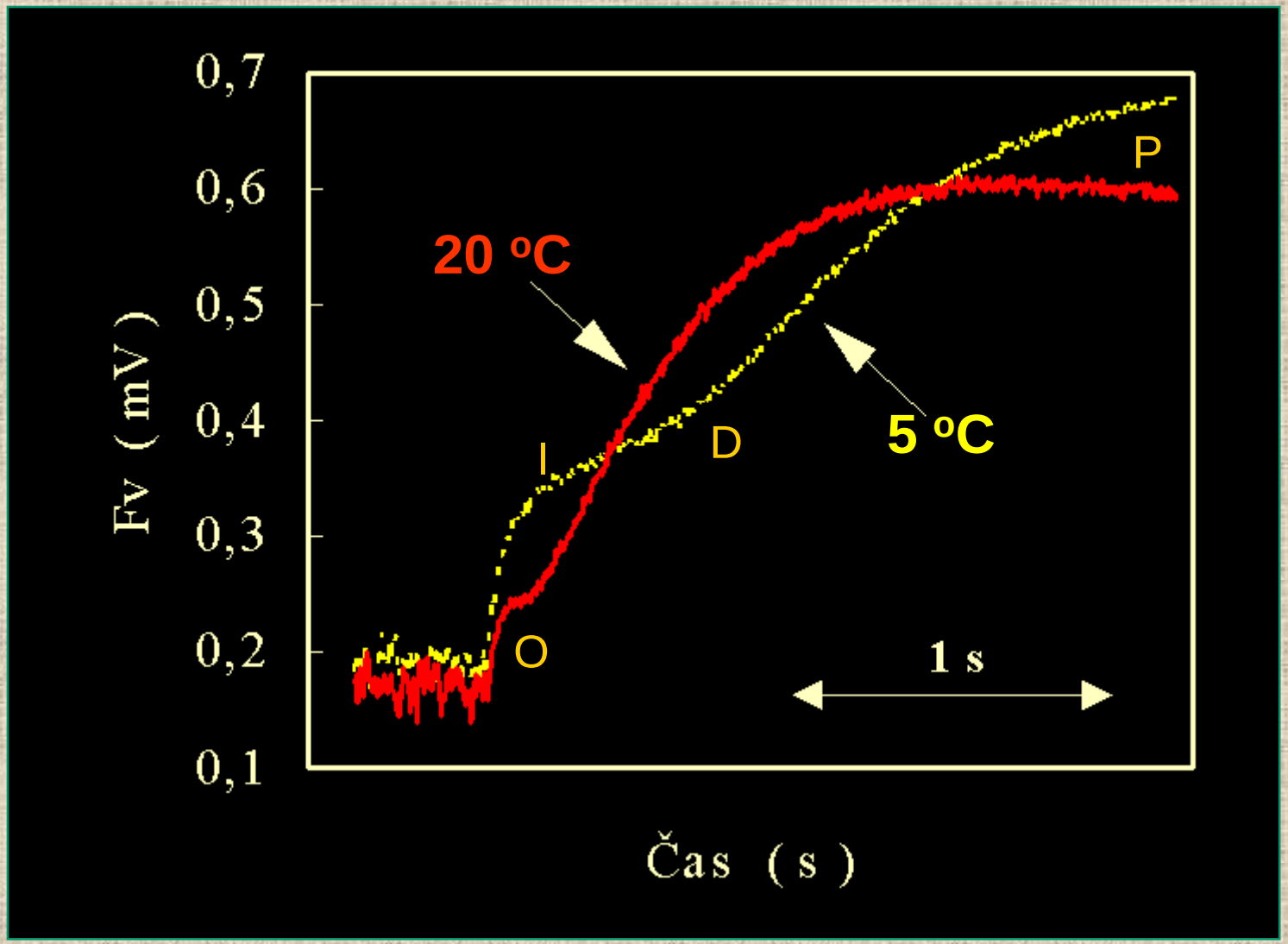
Fast Chl f. induction curve

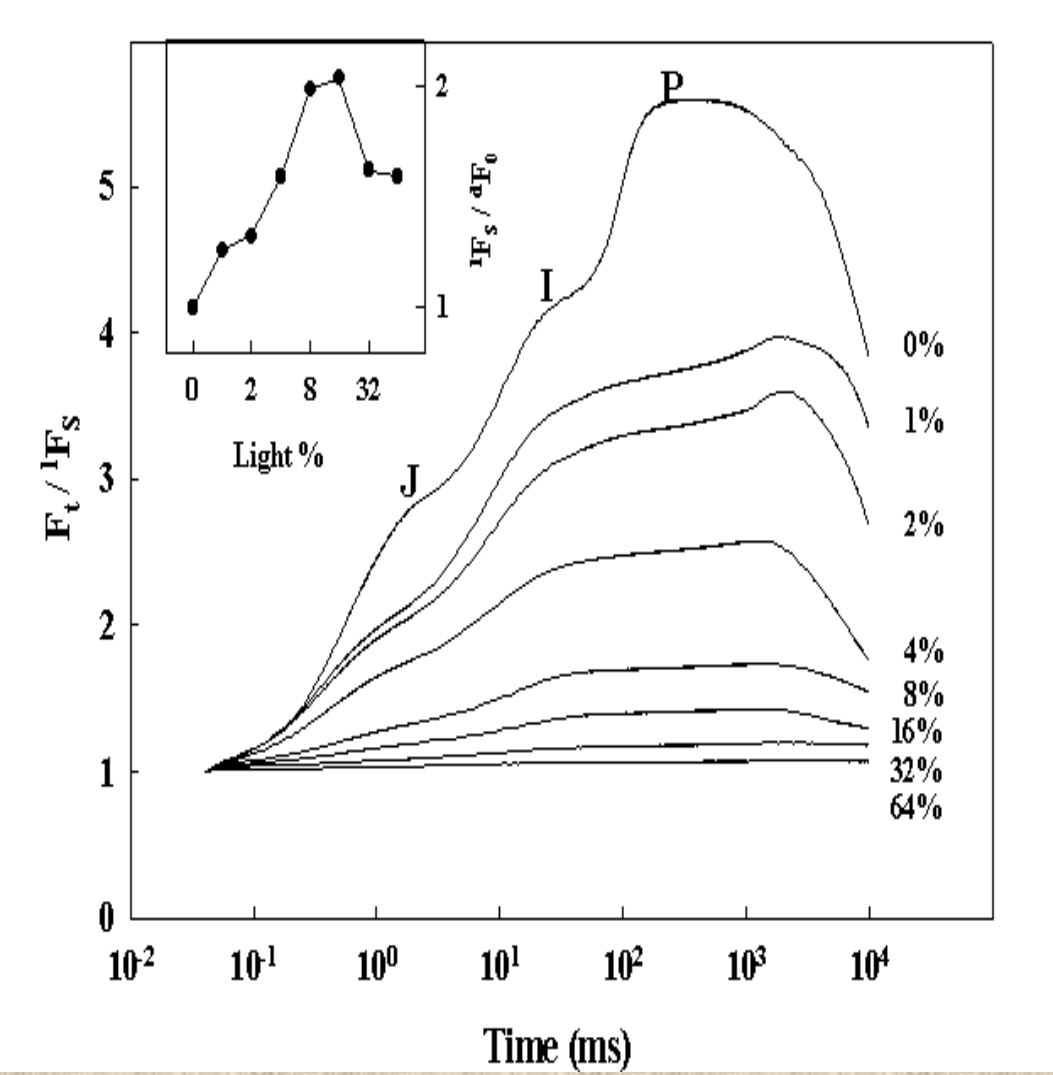


DCMU effect on fast kinetics



Effect of temperature on fast kinetics





OJIP taken after dark adaptation

OJIP taken after % light pretreatment (1-64 %)

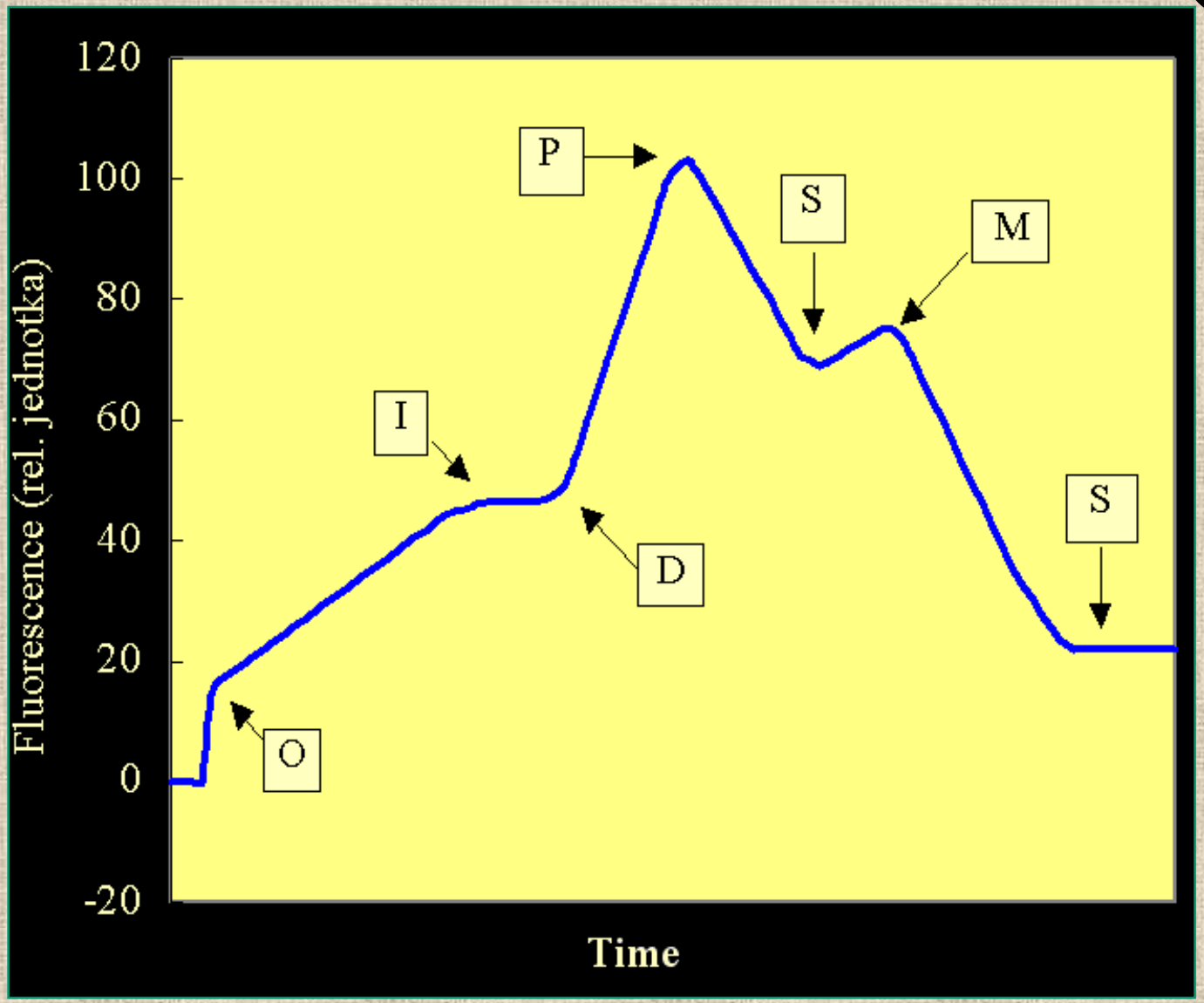
Fast Chl transient [R. Strasser]

Parameters derived from fast kinetics

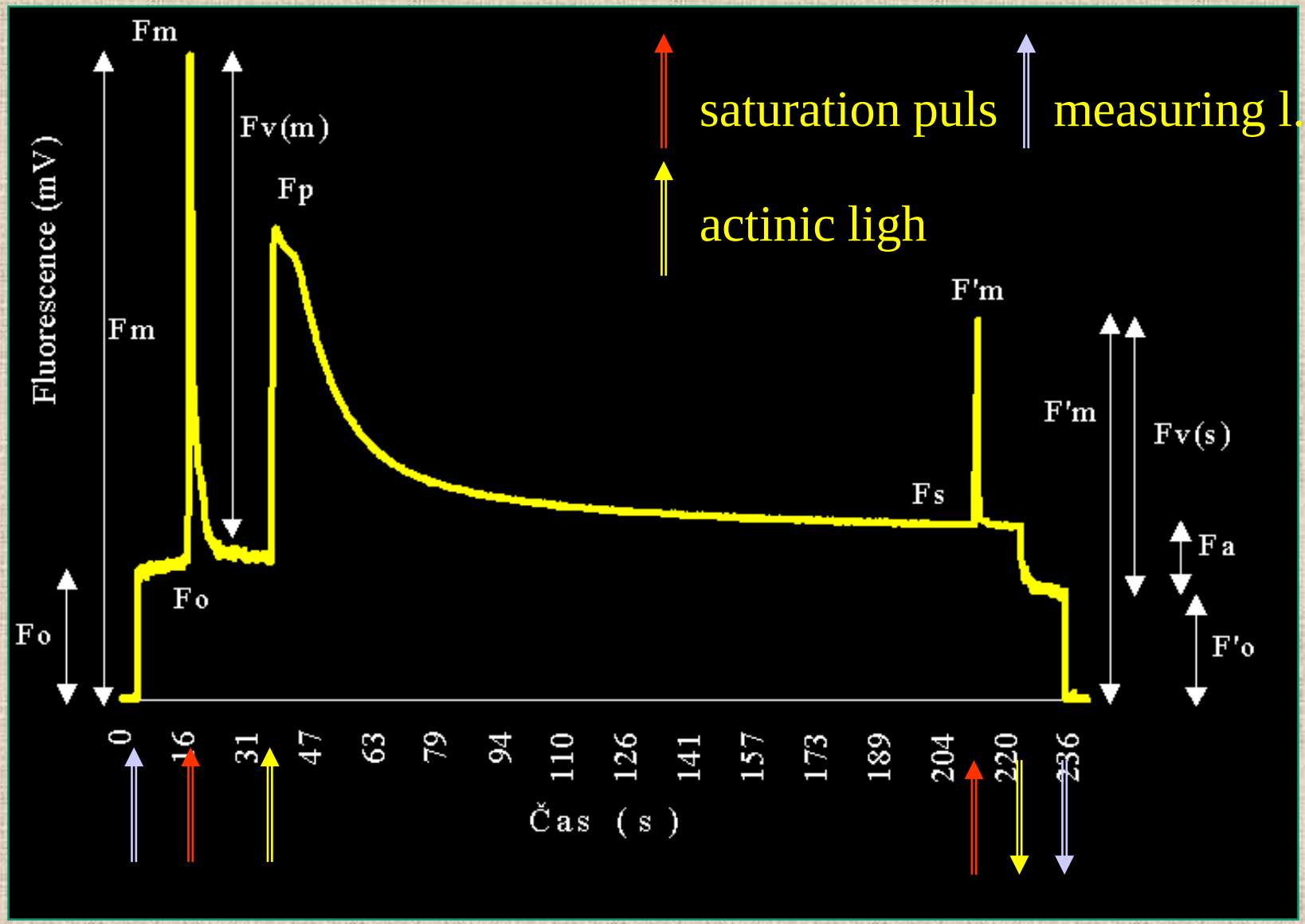
- Signals at O,I, D P points, O(K)JIP
- Ratios Fo/Fm Fv/Fm
- Half-times
- Rates of Chl fluorescence increase
- Area over rising part of curve
- Estimation of Active / inactive RC (Qb-reducing)

Parameters are related to structure and function of photosystem II

Slow Chl fluorescence kinetics (log)



Slow kinetics of Chl fluorescence +SP



Parameters derived from slow kinetics

- Fv / Fm *("potential" photochemistry)*
- Yield PS II (Φ_{II}) *("actual" photochemistry)*
- Rdf *("vitality")*

- Photochemical, non-photochemical quenching
  - qP, qN, NPQ, qPrel, qNrel, qFo
  - Components of qN: qE, qT, qI

Parameters are related to whole photochemistry of photosynthesis

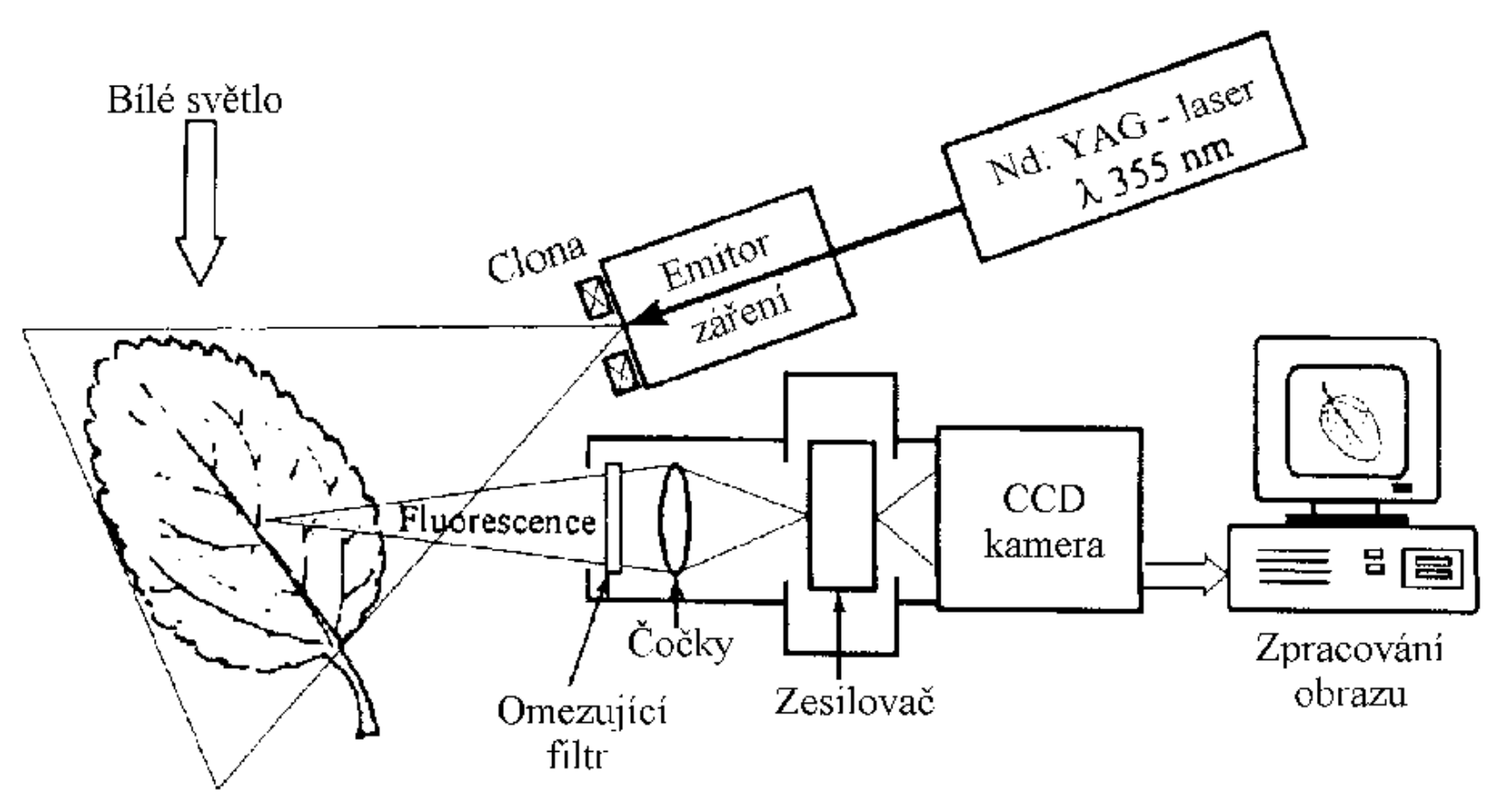
Rapid light reponse curves

- Actinic light (AL) is step-increased after 30 sec.
- At each AL level, saturation pulse is applied
- For each AL level, Yield of PS II is calculated

- Pn = (Yield * PAR * abs * 0.5) / c c = 8-14
- Light reponse curves of Pn

Chlorophyll fluorescence imaging

- Variable Chl fluorescence
  - Amount of Chl, mechanical load
  - Growing/active zones on crusts

- Chl fluorescence parameters
  - Stress (HL, heavy metals…*etc)*
  - Water status

Schematic diagraph of CCD camera