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..)

TABLE 3.1
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

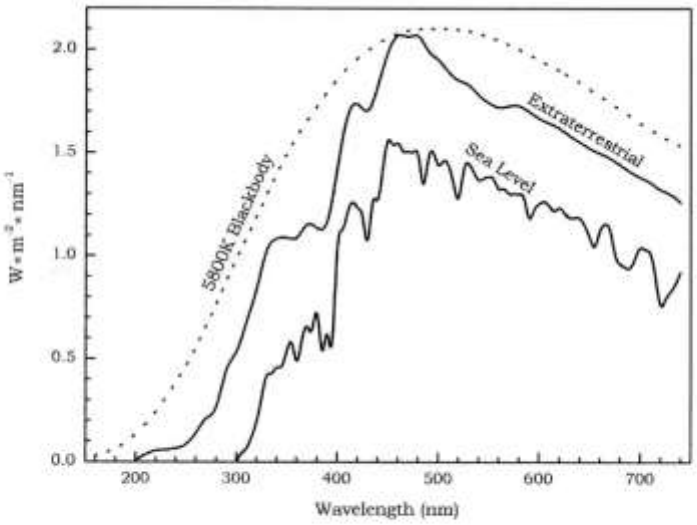


FIGURE 5.10 Spectral irradiance of the incoming sun radiation outside the atmosphere and at sea level compared with that of a perfect blackbody at 5800 K

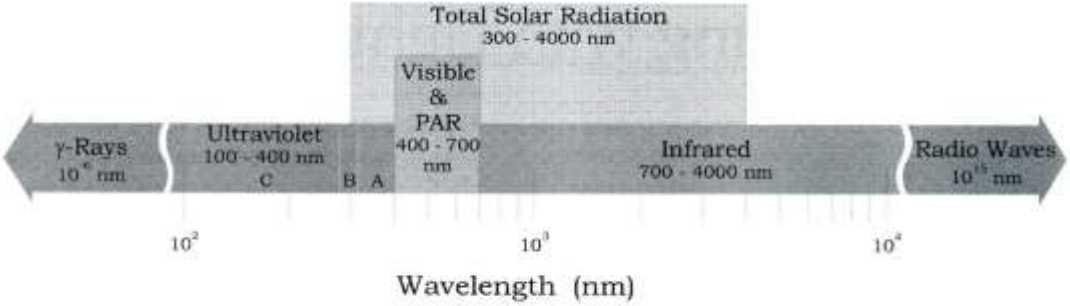


FIGURE 5.1 The electromagnetic spectrum from γ-rays (10^{-6}) to radio waves (10^{15}).

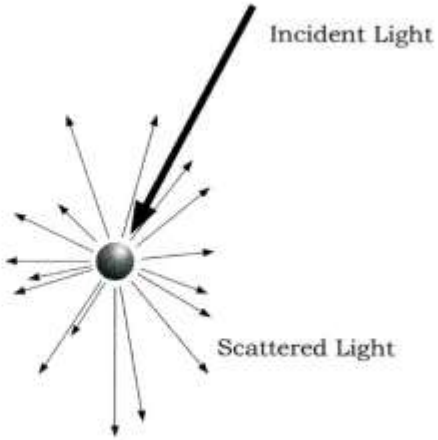


FIGURE 5.2 Light interaction with matter: the scattering process.

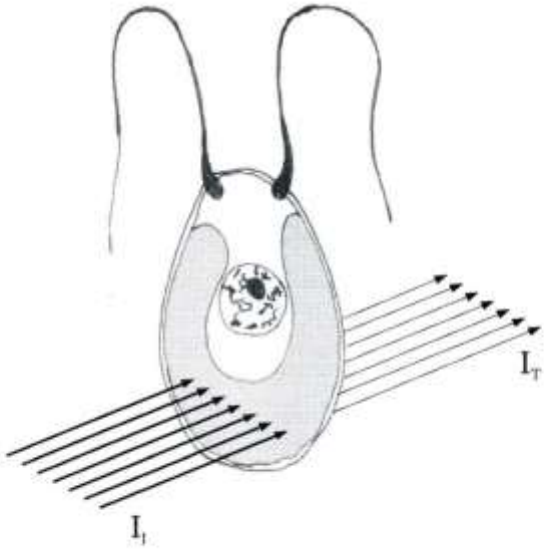


FIGURE 5.3 Light absorption by a unicellular alga: I_I, light incident on the cell and I_T, light transmitted by the cell.

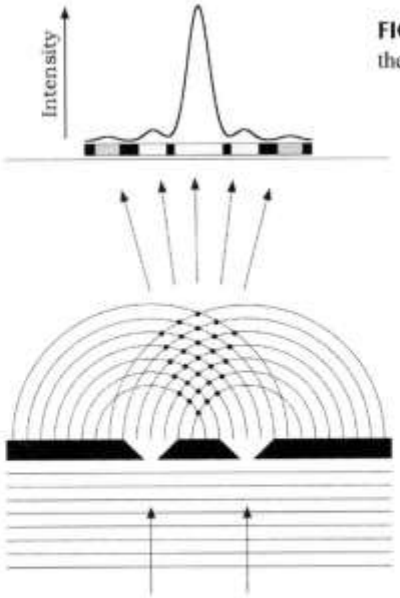
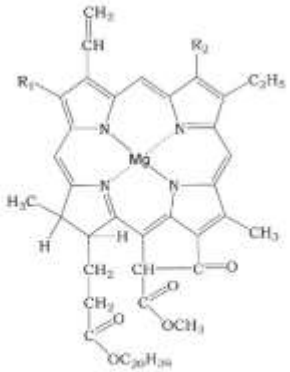


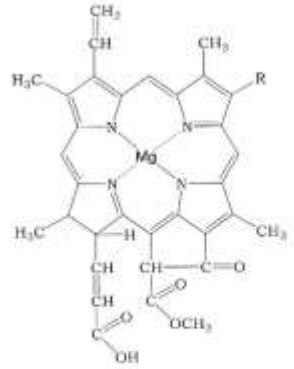
FIGURE 5.4 Interference of light passing through two narrow slits, each acting as a source of waves. The superimposition of waves produces a pattern of alternating bright and dark bands. When crest meets crest or trough meets trough, constructive interference occurs, which makes bright bands; when crest meets trough destructive interference occurs, which makes dark bands. The dots indicate the points of constructive interference. The light intensity distribution shows a maximum that corresponds to the highest number of dots.

Structure & function

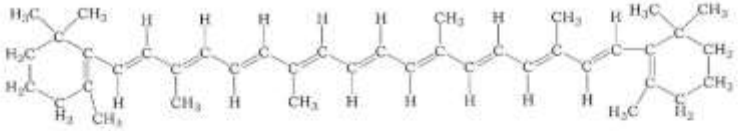
- thylakoid membrane structure
- pigments
- photosystems



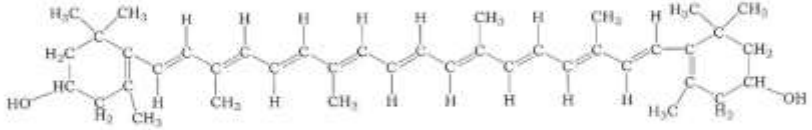
Chlorophyll a : $R_1 = CH_3$ $R_2 = CH_3$
Chlorophyll b : $R_1 = CH_3$ $R_2 = CHO$



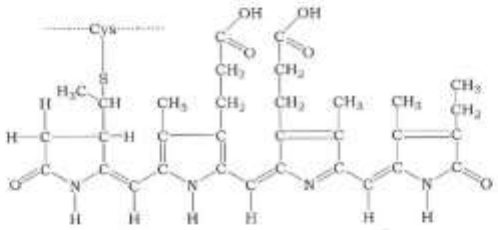
Chlorophyll c_1 : $R = C_2H_5$
Chlorophyll c_2 : $R = CH{=}CH_2$



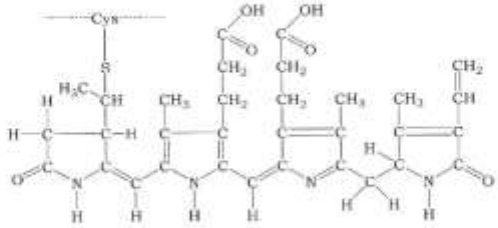
β-carotene



Lutein



Phycocyanobilin



Phycoerythrobilin

FIGURE 3.2 Structure of the main pigments of the thylakoid membrane.

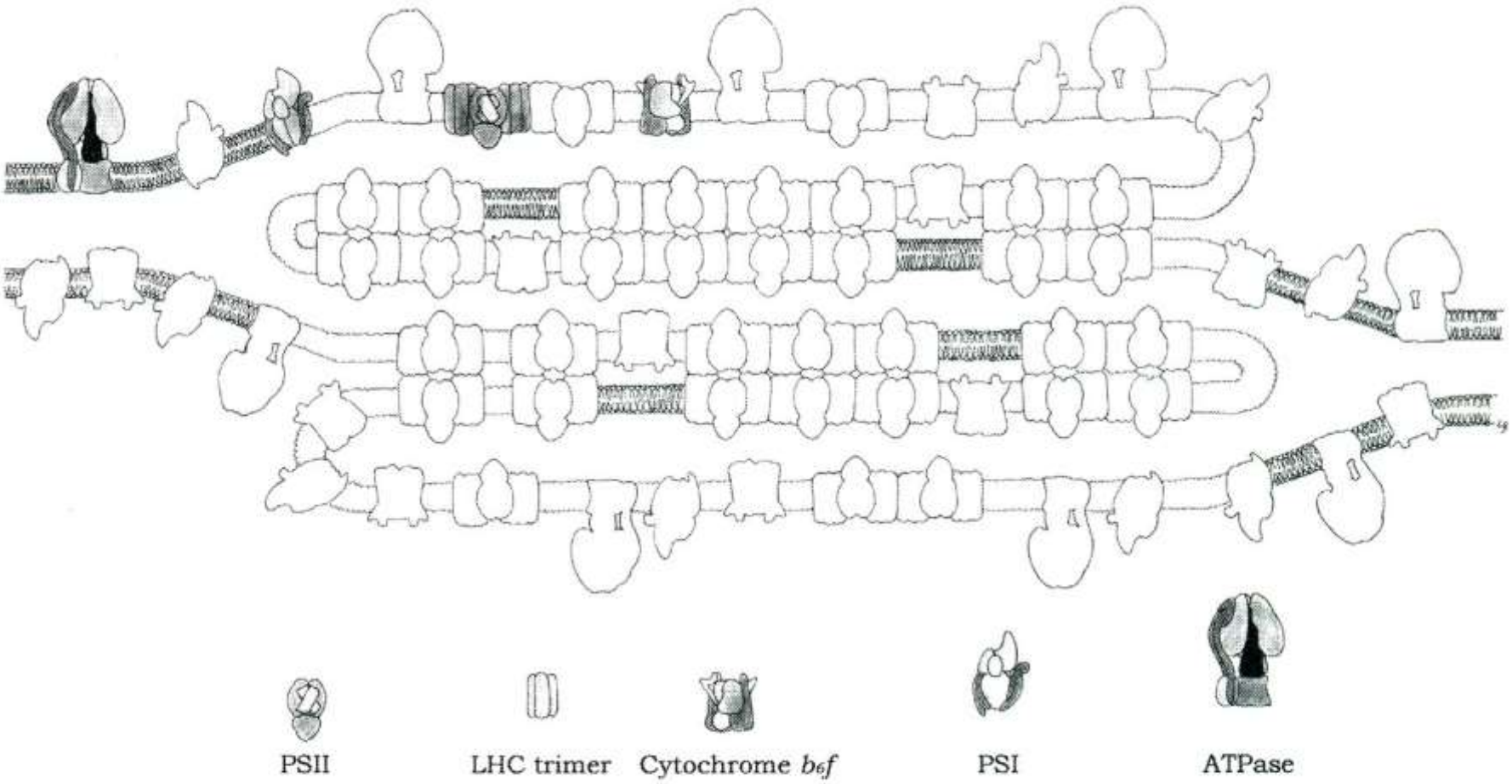


FIGURE 3.3 Model for the topology of chloroplast thylakoid membrane, and for the disposition within the chloroplast of the major intrinsic protein complexes, PSI, PSII, LHCII trimer, Cytochrome b_6f dimer and ATPase. (Redrawn after Allen and Forsberg, 2001.)

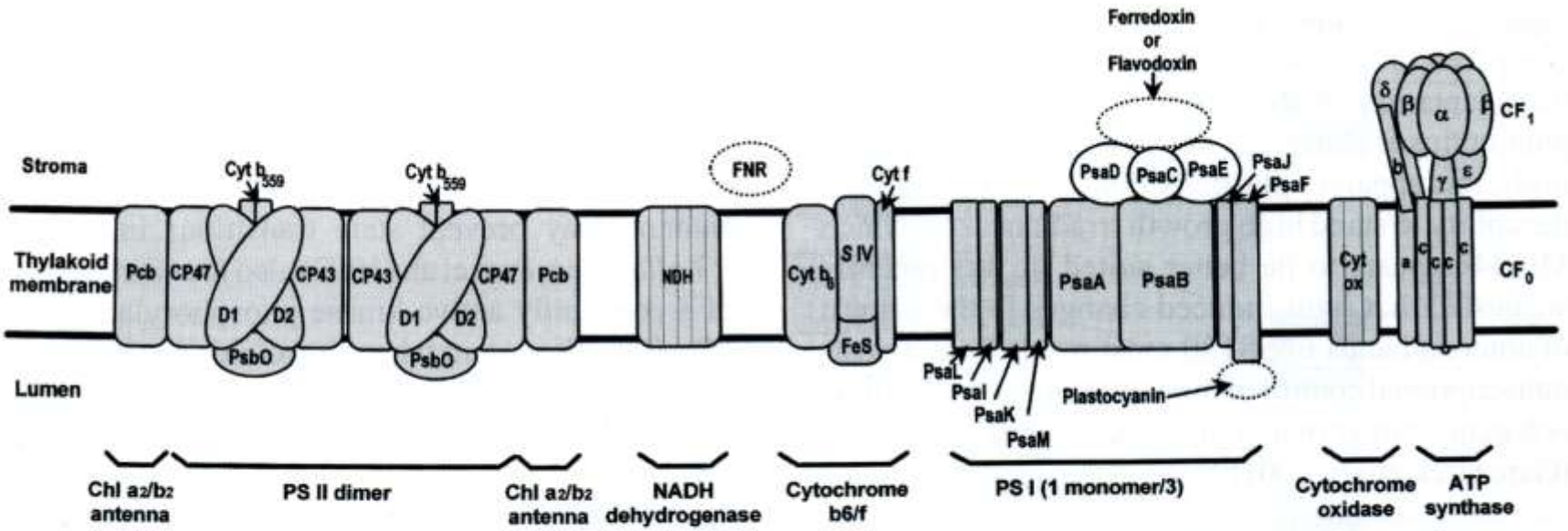


Fig. 5. Diagram showing the photosynthetic apparatus of *Prochlorococcus* sp. MED4. Genes encoding the proteins shown here have either been individually sequenced (see text) or are present in the MED4 genome and have been identified by homology with those of model organisms, such as *Synechocystis* PCC6803. Note that the MED4 strain possesses a single Pcb protein type which is thought to be associated with PS II (T. S. Bibby, F. Partensky, J. Barber (unpublished). A number of putative minor PS II proteins identified in *Synechocystis* (PsbH-M, PsbP, PsbY, PsbZ, Psb27 and Psb28; Kashino et al., 2002) have homologs in the MED4 genome, but are not shown for readability. PsaM, PsbU and PsbV are lacking from the MED4 genome. PS I is organized as trimers (see text), but only one monomer is shown here. The precise organization of the different subunits of NADH dehydrogenase (NDH; 11 subunits) and cytochrome *c* oxidase (Cyt Ox; 3 subunits) are not shown. Dotted forms indicate nonmembrane polypeptides which in Cyanobacteria are known to move and/or exchange with another protein type, depending on physiological conditions (for details, see Bryant, 1994). The localization of the ferredoxin-NADP+ oxidoreductase (FNR), which in Cyanobacteria exists under several isoforms, including one associated with the peripheral rods of PBS, is not yet known in green oxyphotobacteria.

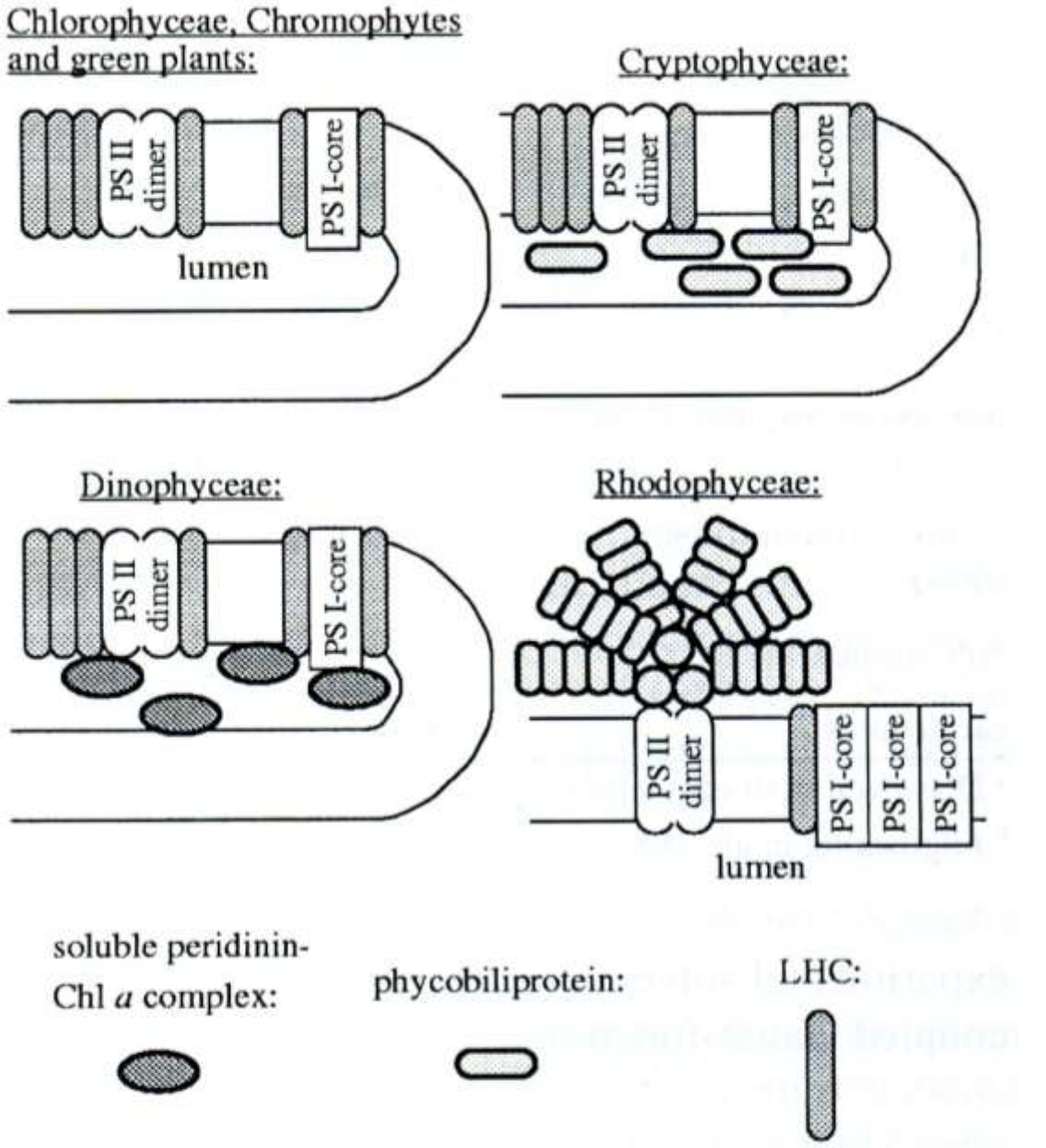


Fig. 3. Association of the different peripheral LHCs with the RCs of PS I and PS II in the thylakoid membranes of different groups of algae.

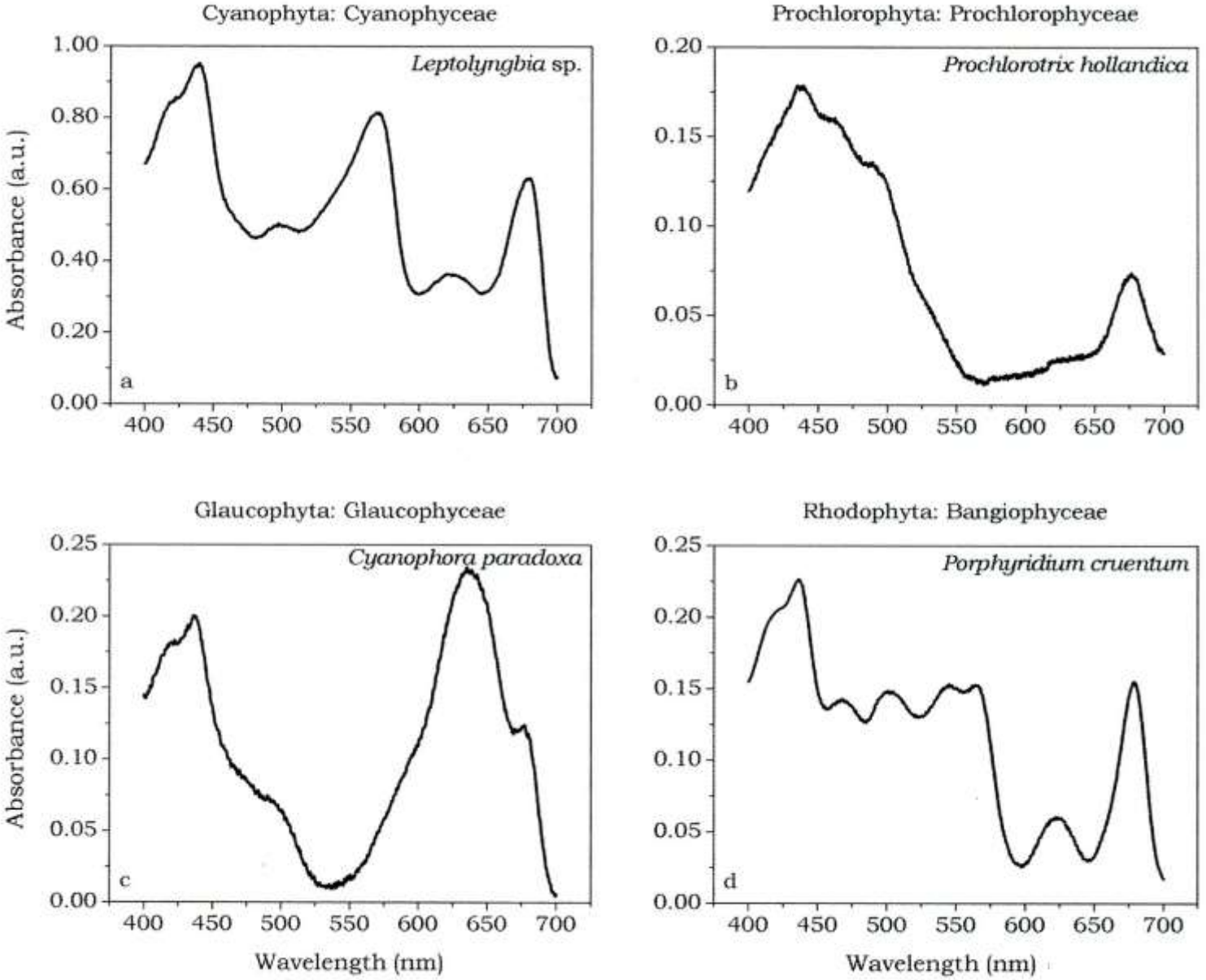


FIGURE 3.5 *In vivo* absorption spectra of photosynthetic compartments of Cyanophyta (a), Prochlorophyta (b), Glaucophyta (c), and Rhodophyta (d).

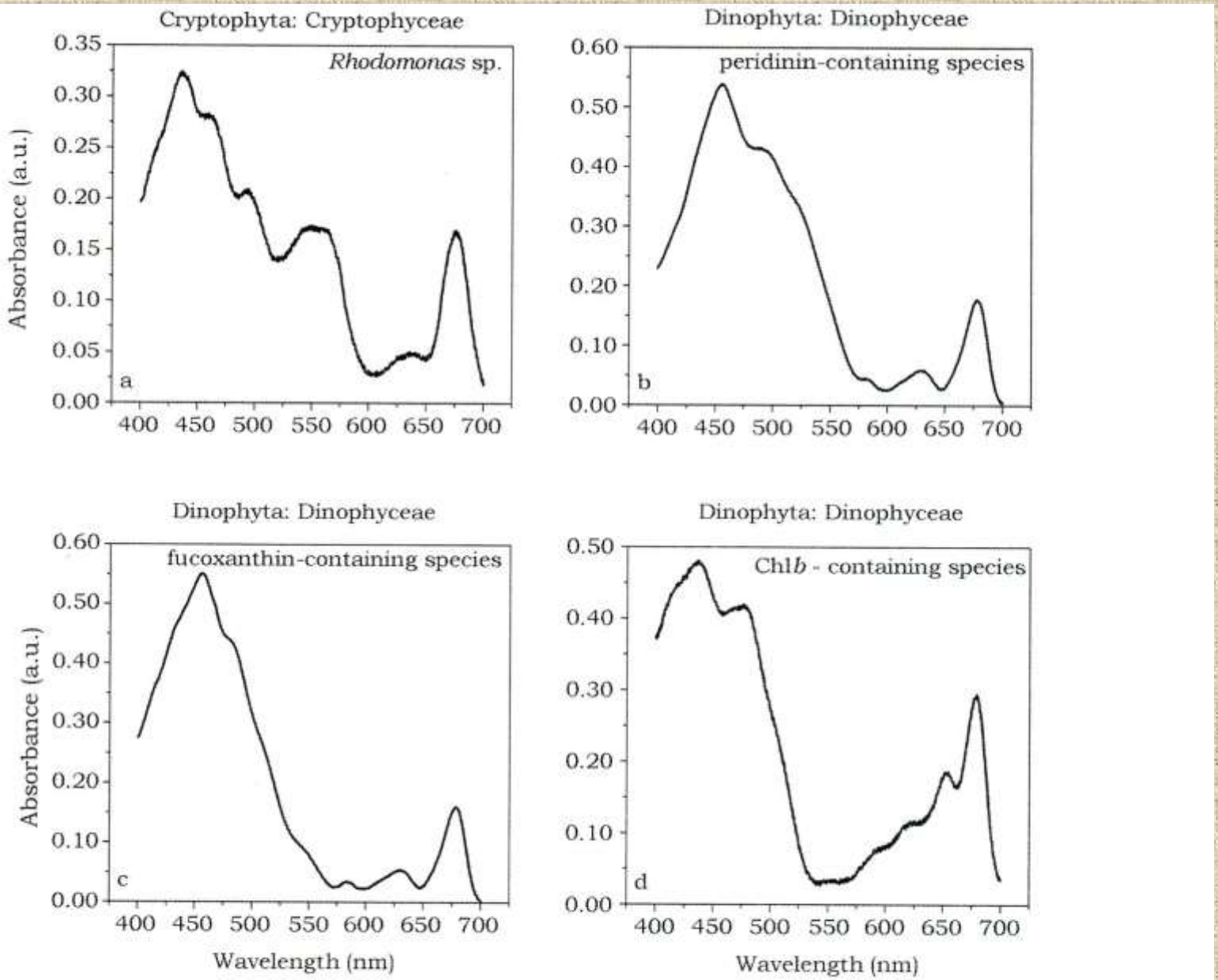


FIGURE 3.7 *In vivo* absorption spectra of photosynthetic compartments of Cryptophyta (a) and Dinophyta (b, c, and d).

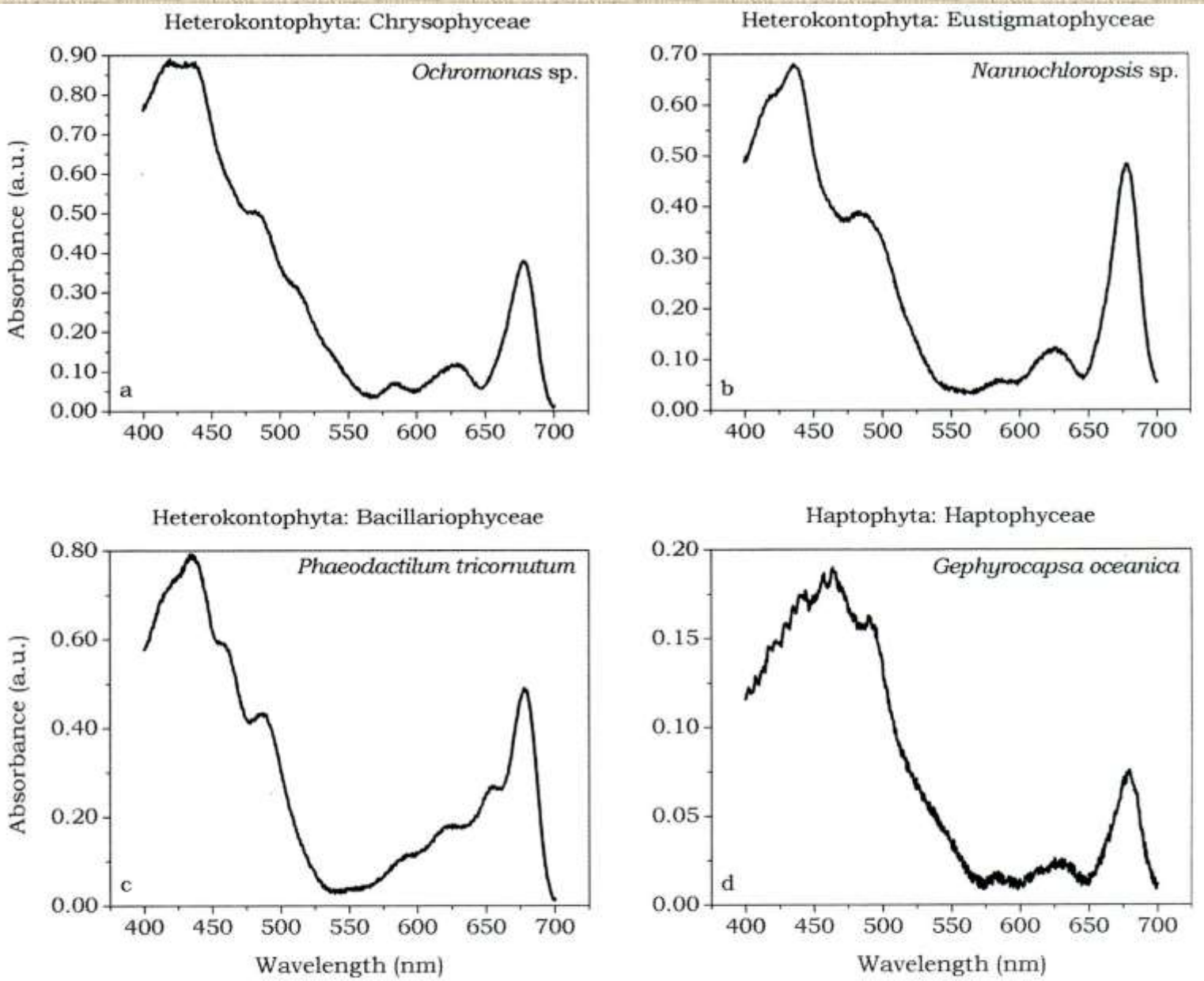


FIGURE 3.6 *In vivo* absorption spectra of photosynthetic compartments of Heterokontophyta (a, b, and c) and Haptophyta (d).

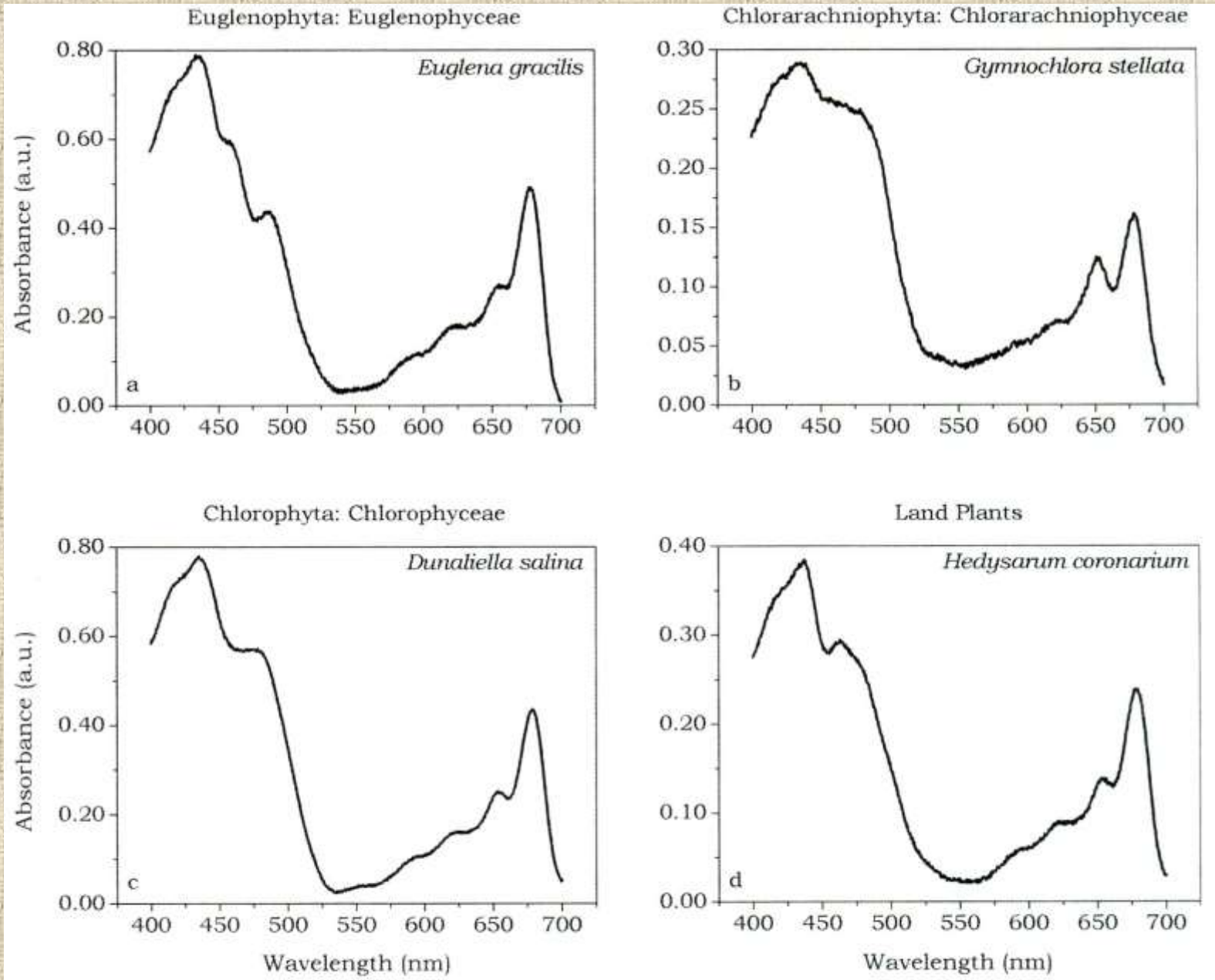


FIGURE 3.8 *In vivo* absorption spectra of photosynthetic compartments of Euglenophyta (a), Chlorarachnophyta (b), Chlorophyta (c), and Land Plants (d).

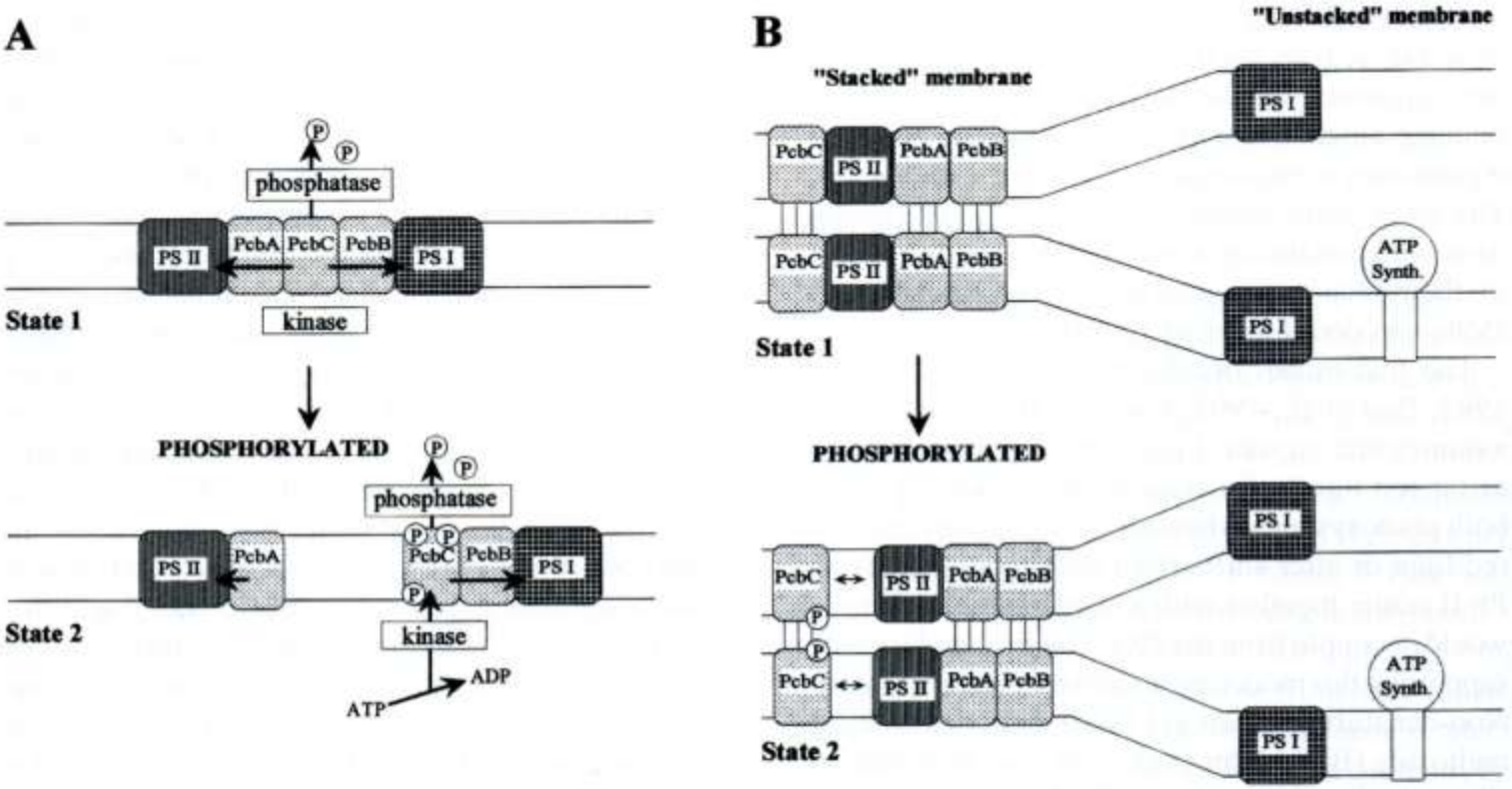


Fig. 6. Current models for the localization of the antenna proteins with regard to photosystems in thylakoid membranes of *Prochlorothrix hollandica* and effect of state 1-state 2 transitions. The original schemes have been redrawn in light of the recent identification of the three Pcb proteins and for 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.

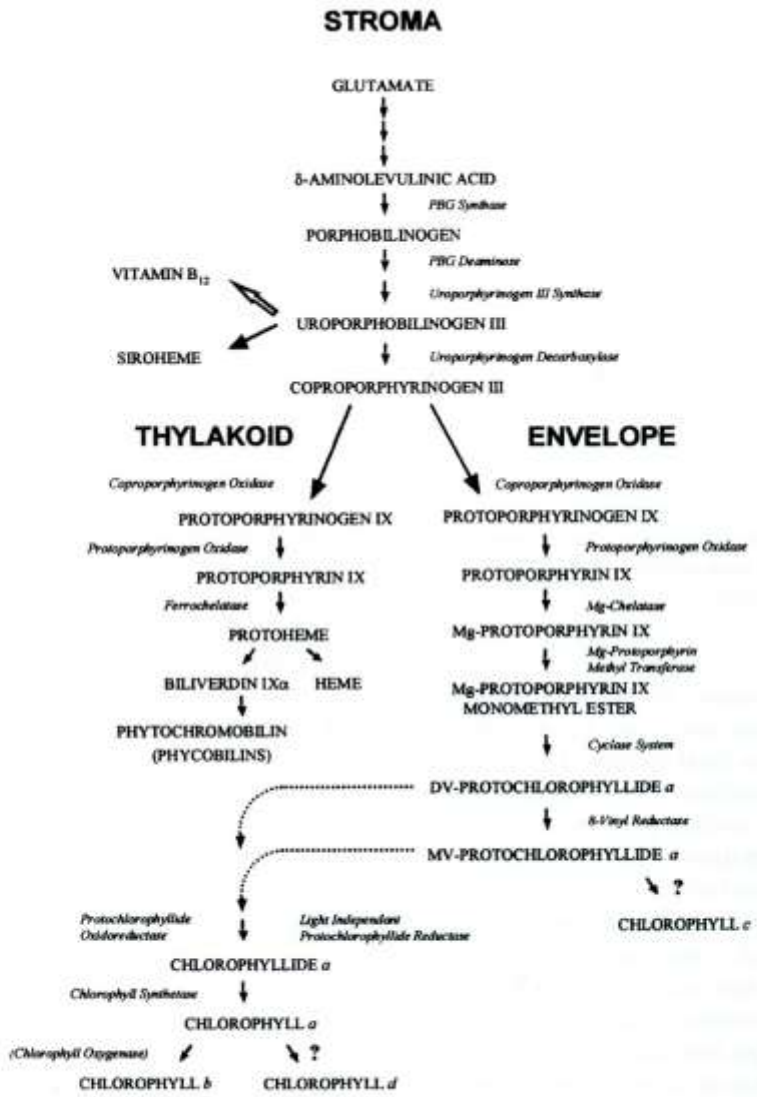


Fig 1. General outline of the tetrapyrrole biosynthesis pathway in photosynthetic organisms. Shown are the major intermediates and enzymes involved in tetrapyrrole formation in plants, algae, and photosynthetic bacteria and the proposed location of the enzymatic activities in plastids. The steps leading 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

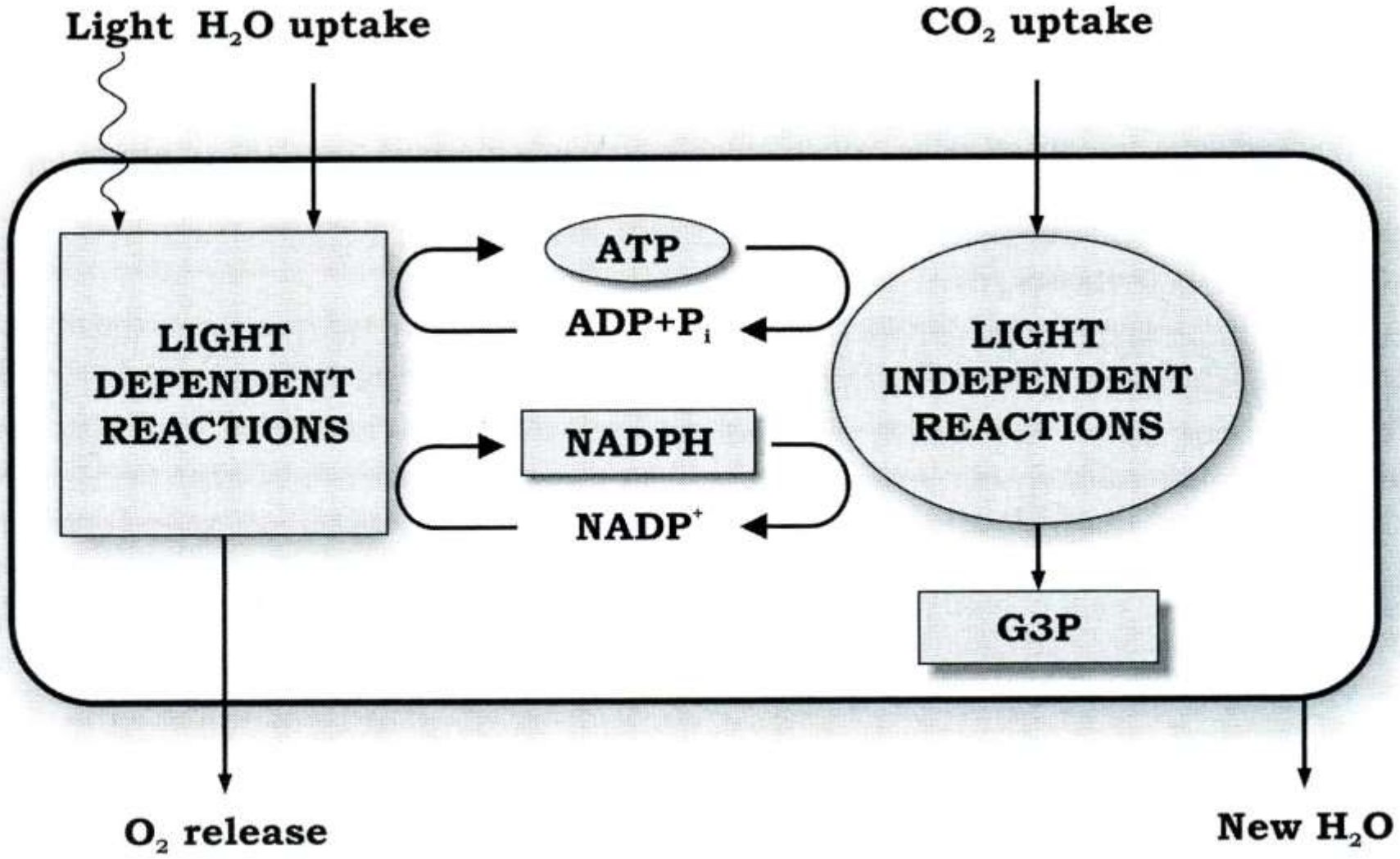
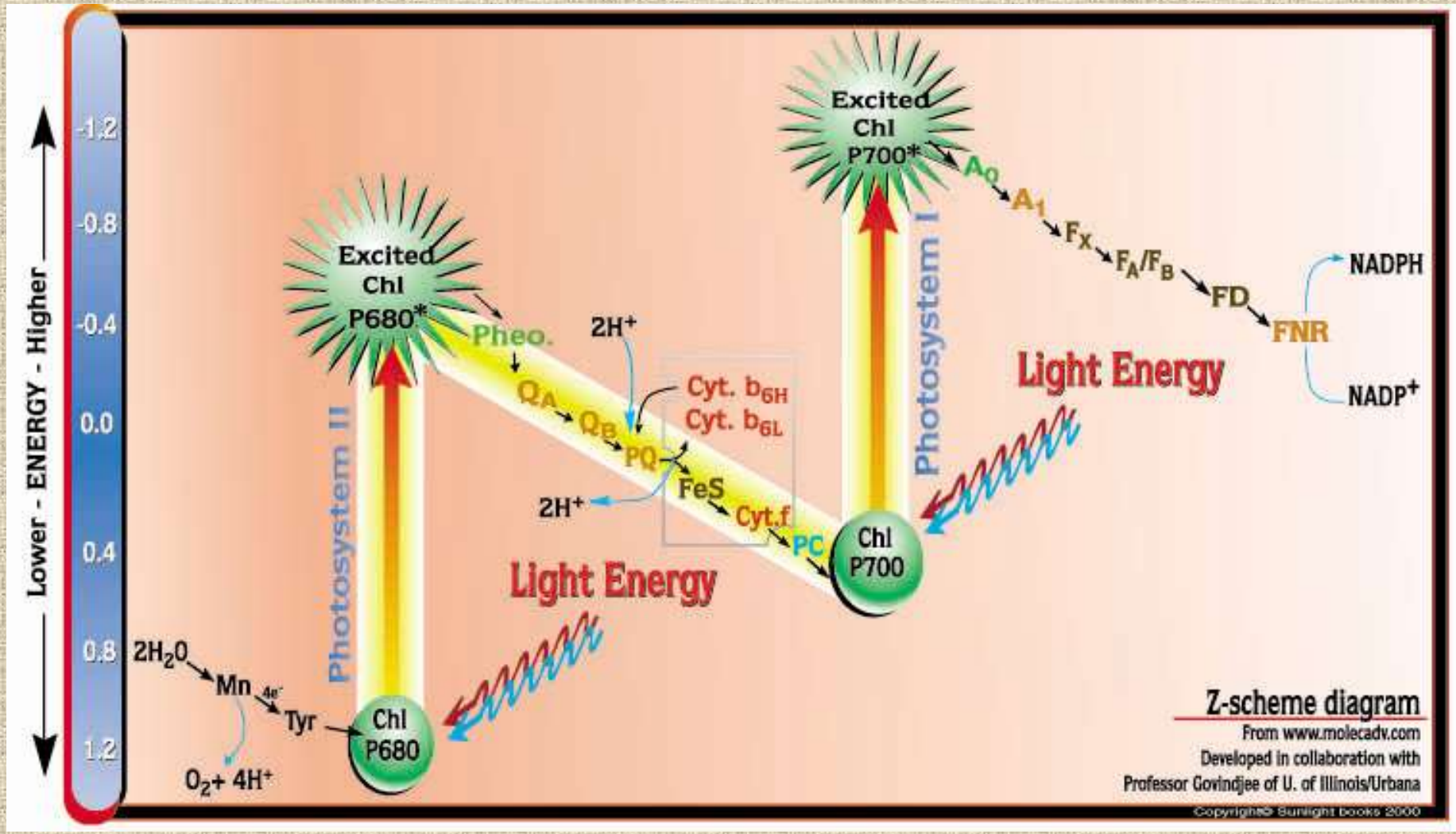


FIGURE 3.1 Schematic drawing of the photosynthetic machinery.


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
$2H_2O$
Mn
4e⁻
Tyr
O_2+ 4H+
A_0
A_1
F_X
F_A/F_B
FD
FNR
NADPH
$NADP^+$
Z-scheme diagram
From www.molecadv.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana
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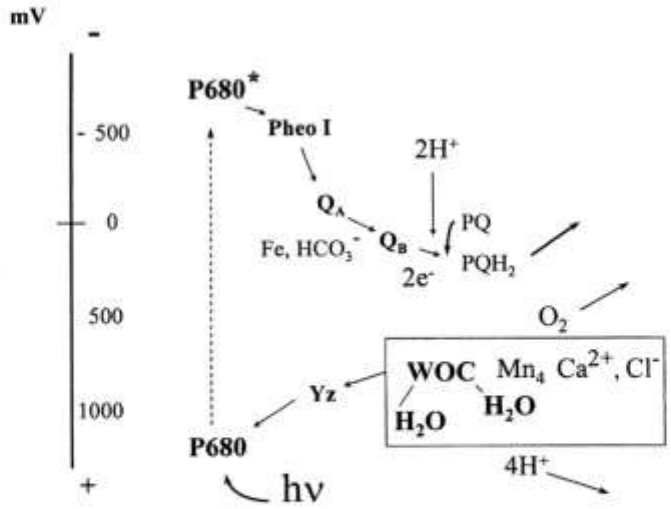


Fig. 1. Diagram showing the main cofactors of PS II, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Electron flow occurs from water to Q_B. Absorption of light energy ($h\nu$) or excitons by P680, producing P680*, leads to a photochemical 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

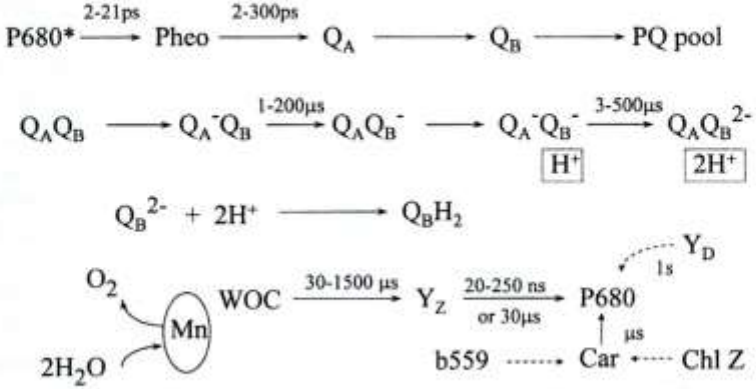


Fig. 2. Diagram showing the main kinetics of the main pathway of electrons through the PS II reaction center. Y_D (D2 Tyr161), Chlorophyll Z (Chl Z) and Cytochrome b_{559} (b_{559}) are alternate electron donors under certain conditions. See also Figure 1.

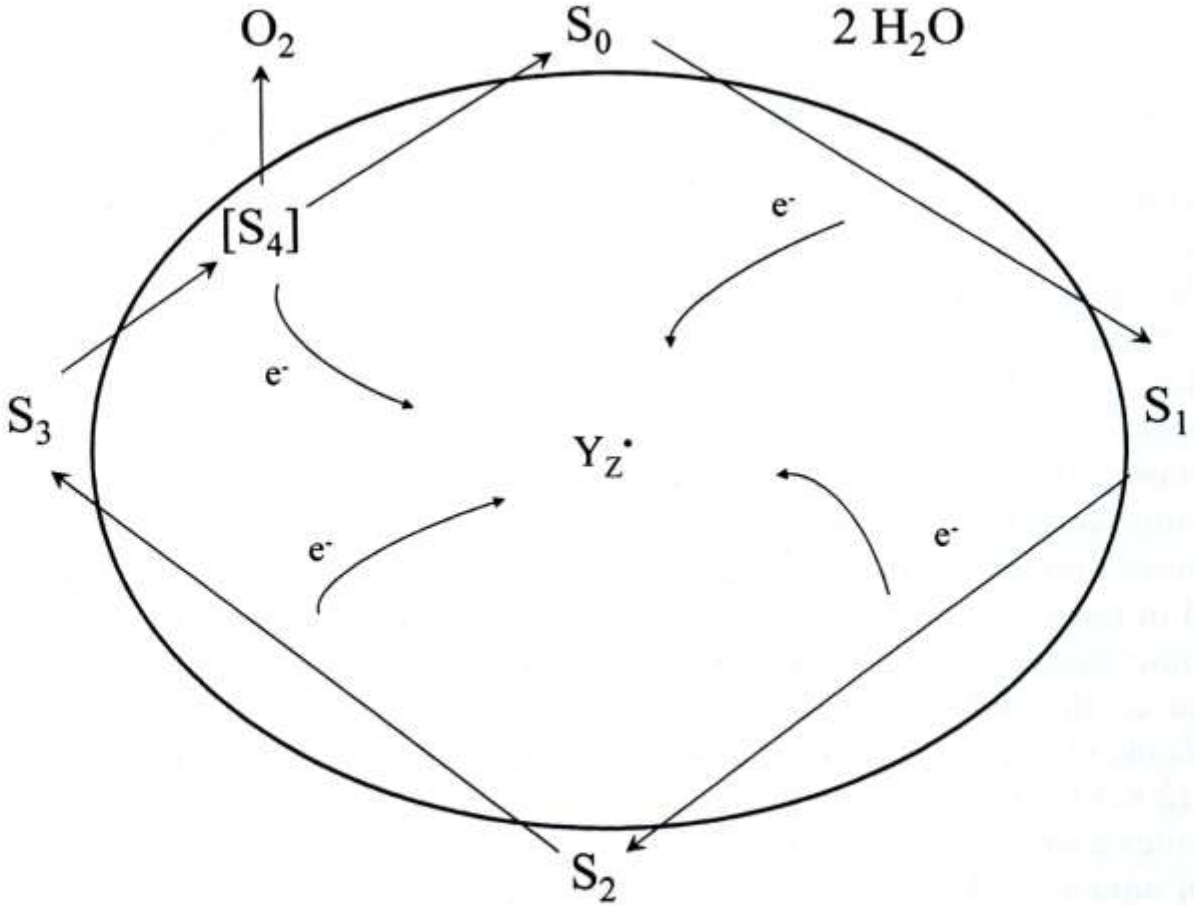


Fig. 3. The S-states of the water oxidizing complex (WOC). Electrons are removed sequentially by $P680^+$ via Y_Z. The S-state number indicates the number of oxidizing equivalents stored. On reaching S_4, oxygen is released and the cycle reset. The steps at which water may be bound, oxidized and protons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

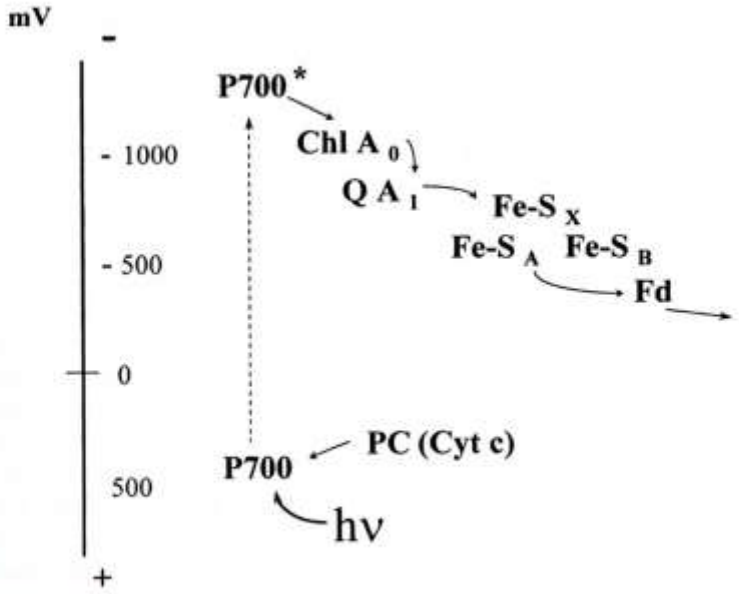


Fig. 4. Diagram showing the main cofactors of PS I, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Absorption of light energy ($h\nu$) or excitons by P700 leads to a photochemical charge separation. Electron flow occurs from Plastocyanin (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.

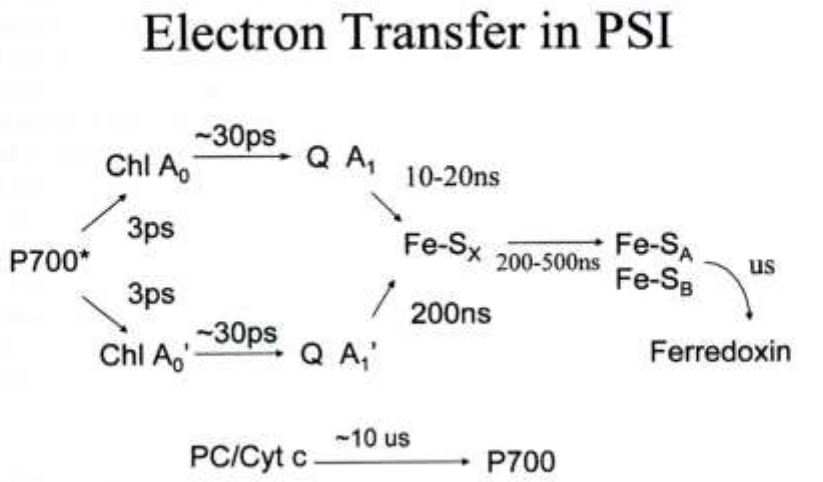
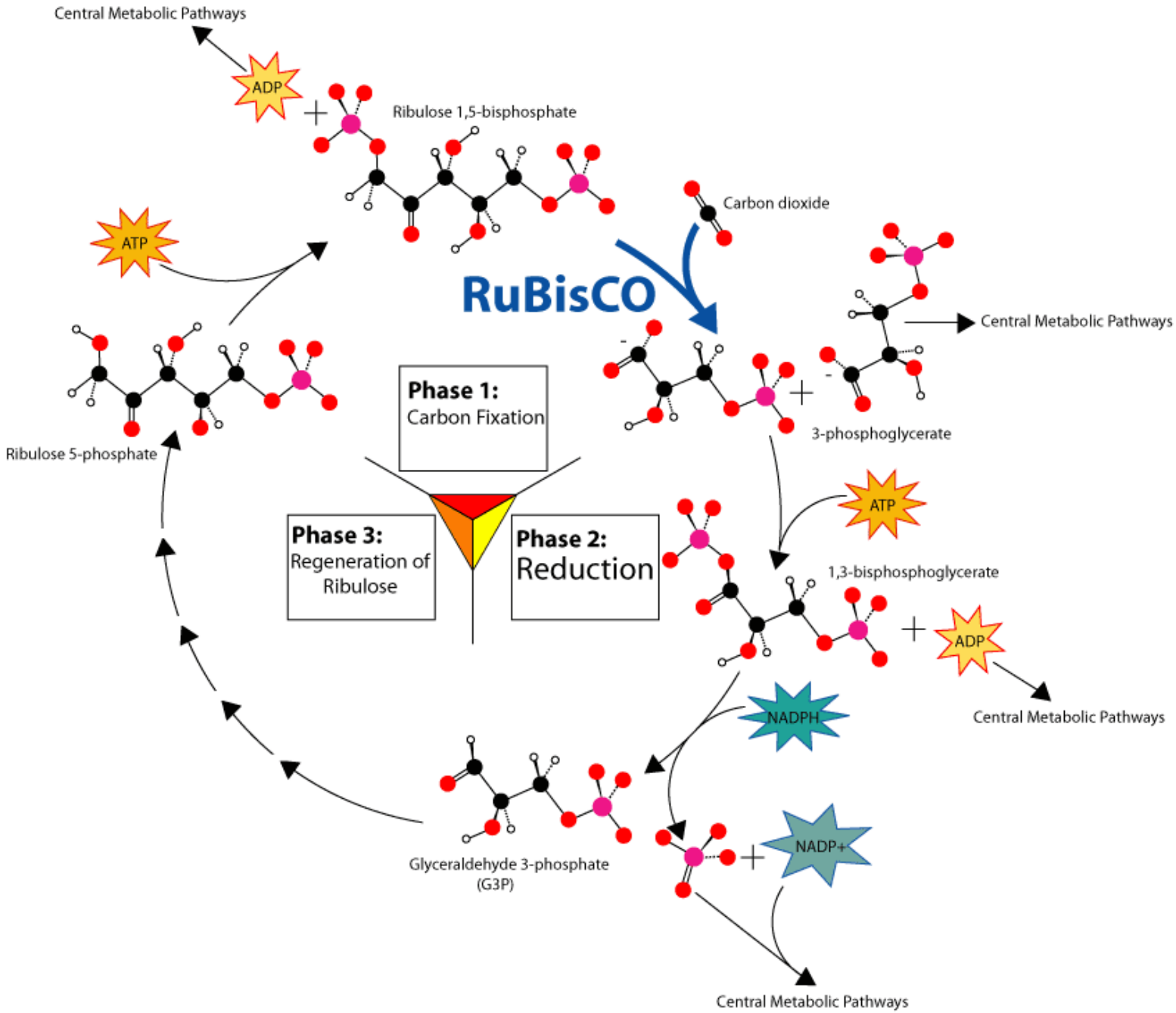


Fig. 5. Diagram showing the main kinetics of the main pathway of electrons through the PS I reaction center.


Central Metabolic Pathways
ADP
Ribulose 1,5-bisphosphate
ATP
Carbon dioxide
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

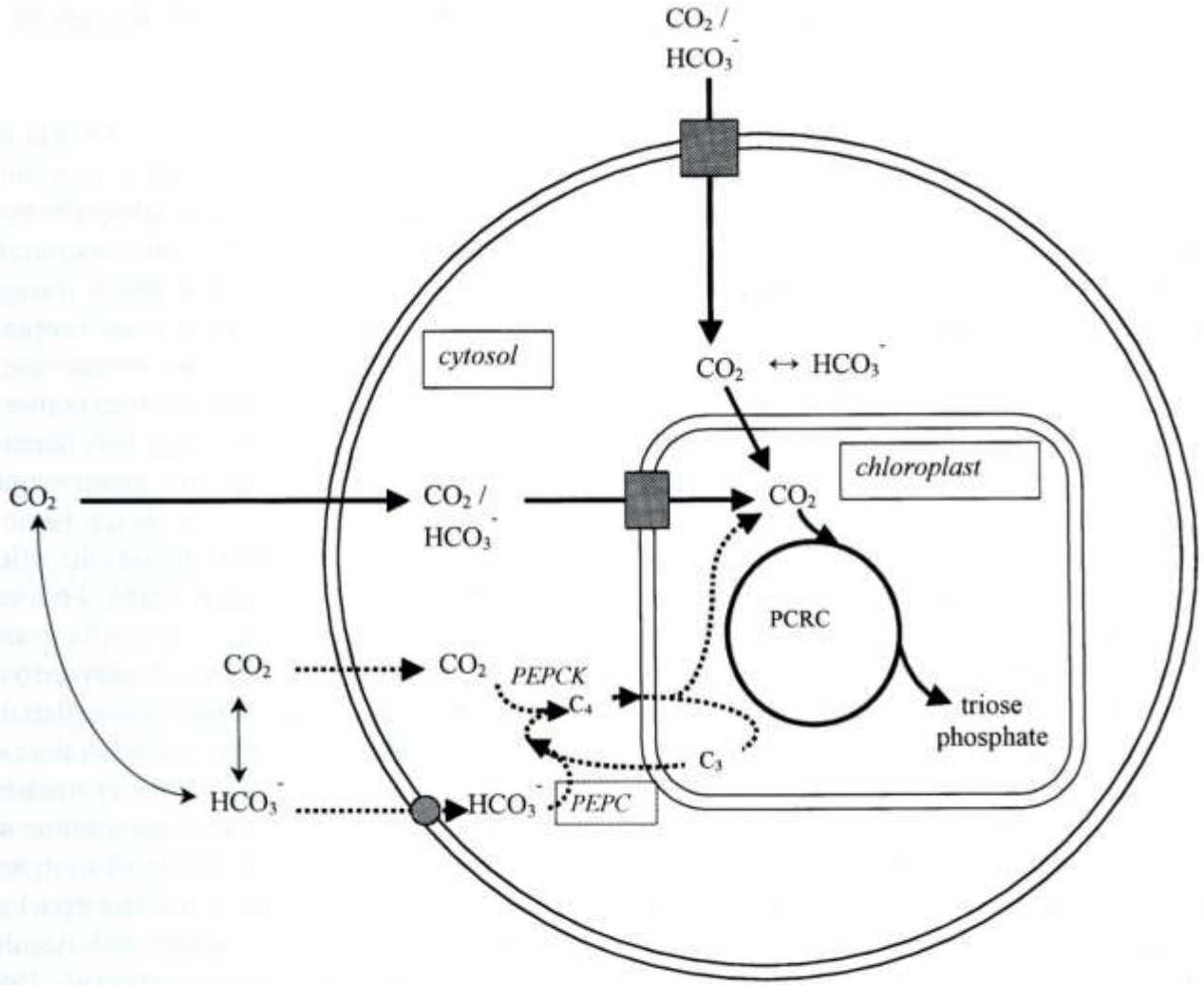


Fig. 1. A simplified scheme for transport of inorganic carbon into eukaryotic algal cells via active transport of CO_2 and/or bicarbonate. As explained in the text, CO_2 will cross membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of CO_2 or HCO_3^-. Active transport 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

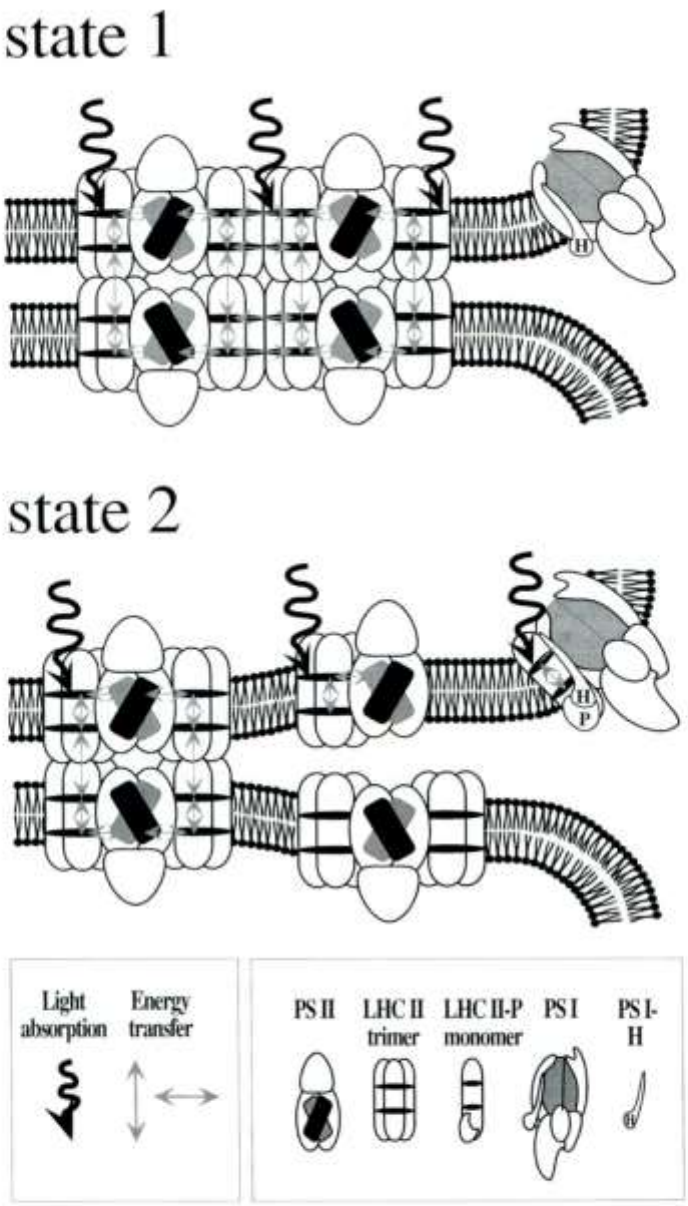
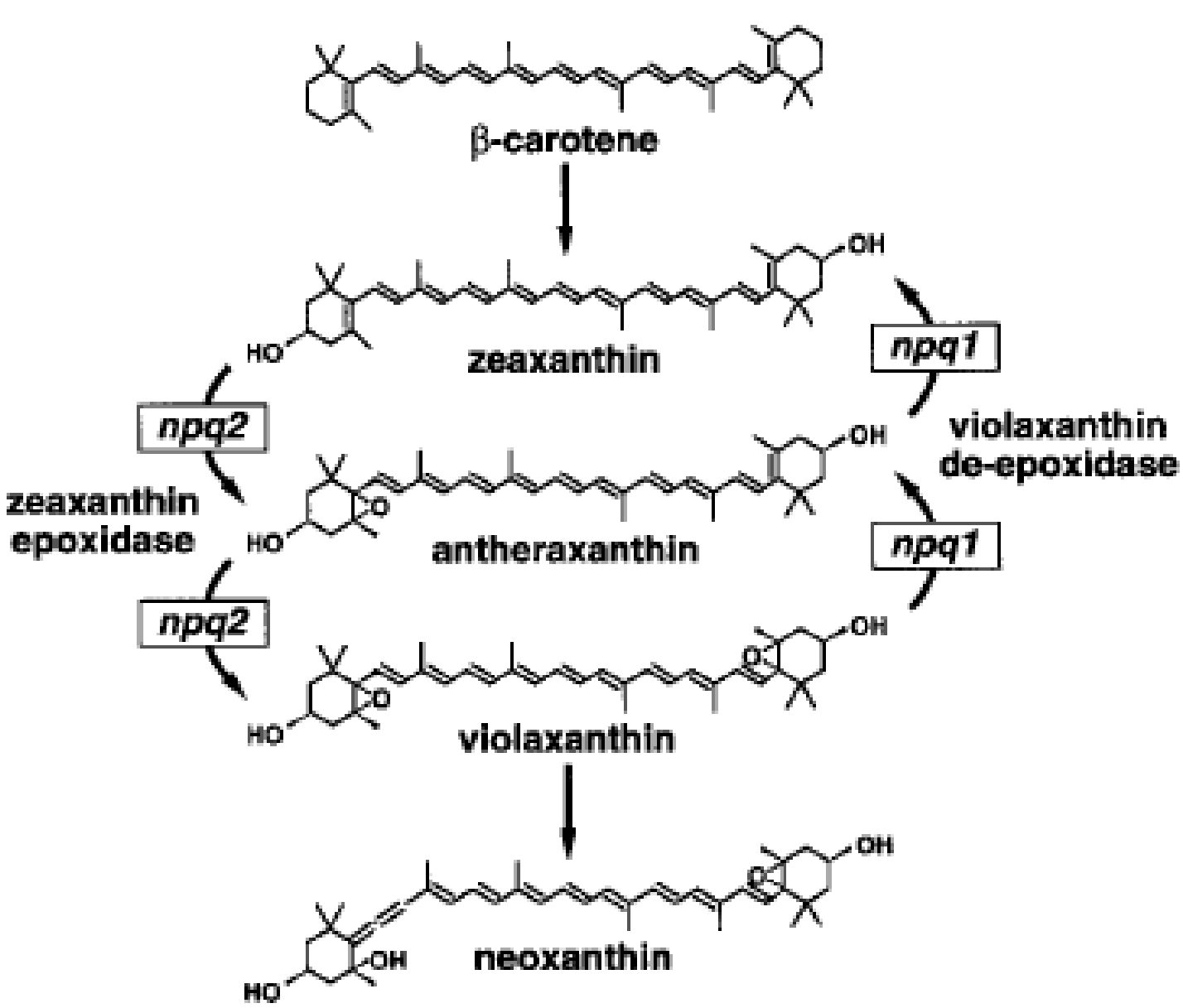


Fig 6. Artist's impression of the difference between State 1 and State 2 organization in appressed and non-appressed thylakoids in green plants. Redrawn with permission from Forsberg and Allen (2001a).


β-carotene
zeaxanthin
antheraxanthin
violaxanthin
neoxanthin
npq1
npq2
violaxanthin de-epoxidase
zeaxanthin epoxidase
HO
OH
O

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

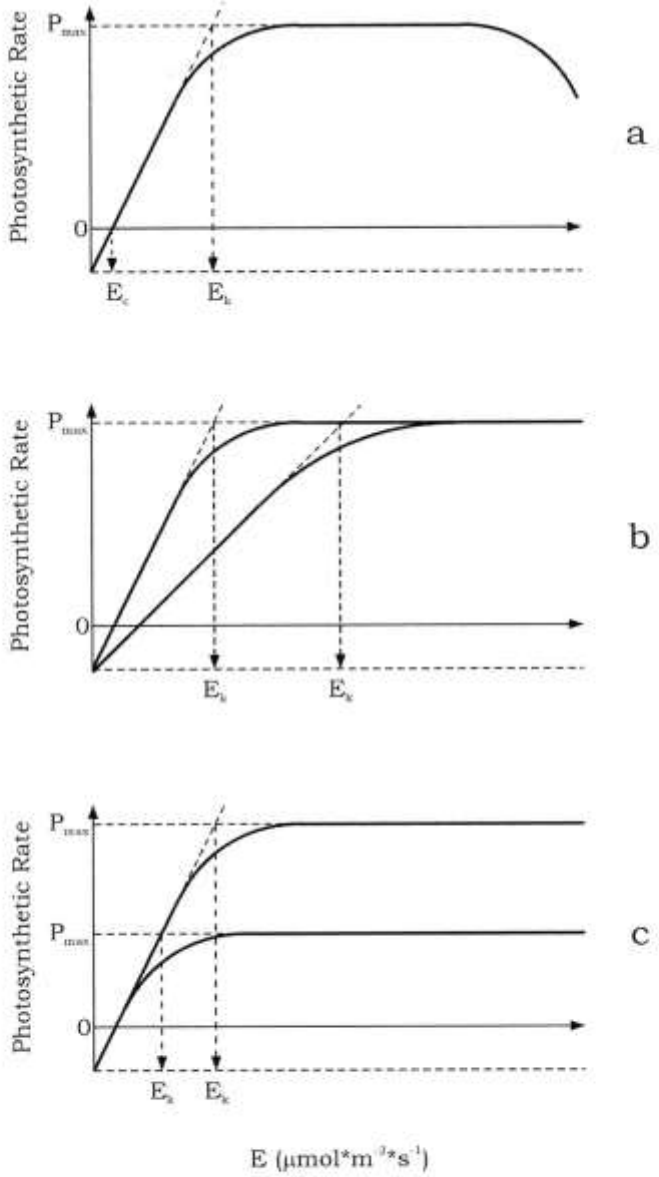


FIGURE 5.15 Photosynthesis–irradiance response curves: E_c, irradiance compensation point; E_k, saturating irradiance; and P_{max}: maximum photosynthetic rate. (a) typical plot; (b) comparison of two curves with different slopes: keeping constant the number of photosynthetic units, but increasing the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

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

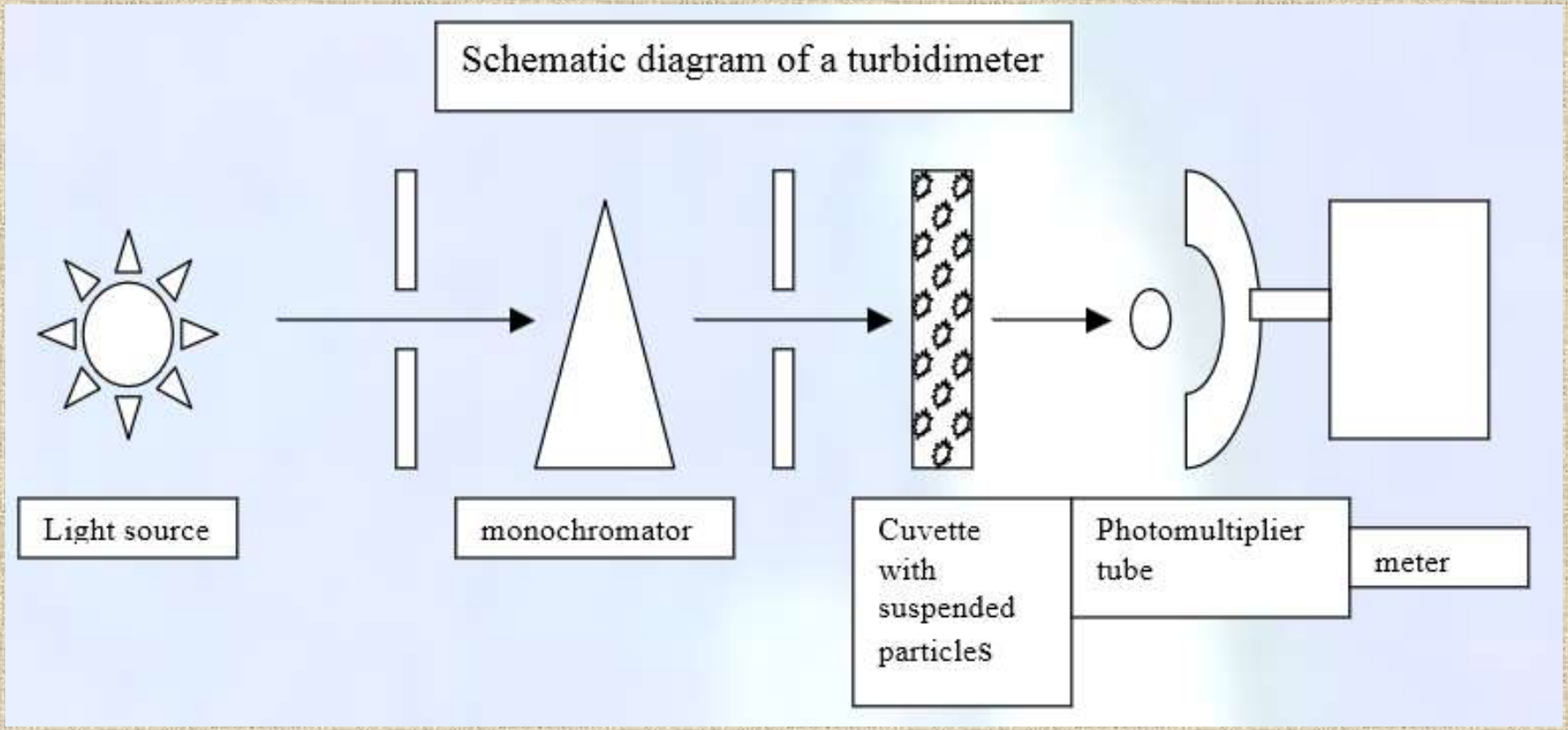
where: A = weight of filter + dried residue, mg
B = weight of filter, mg

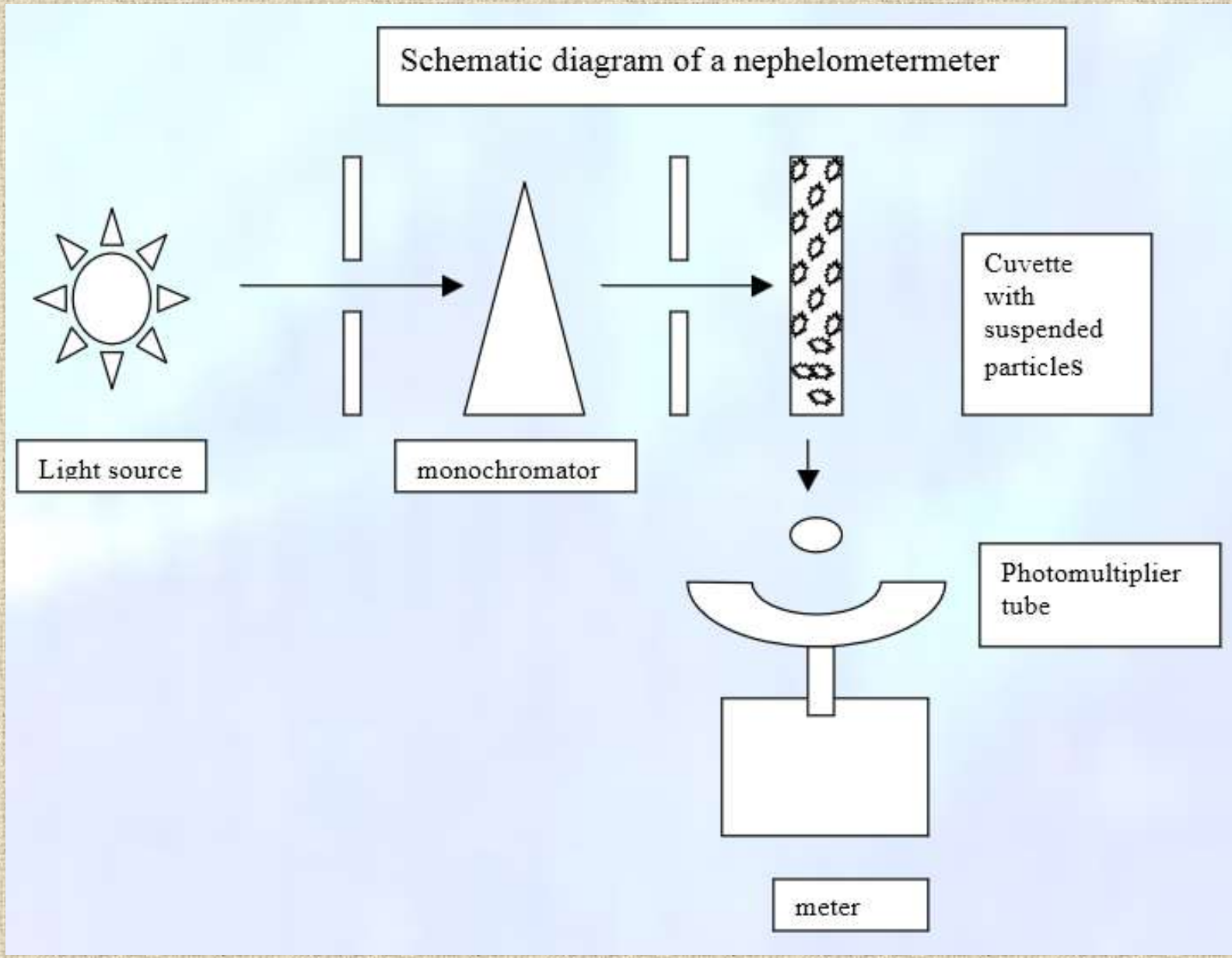
$$\text{AFDW, mg / L} = \frac{(C - D) \times 1000}{\text{sample volume, mL}}$$

- 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

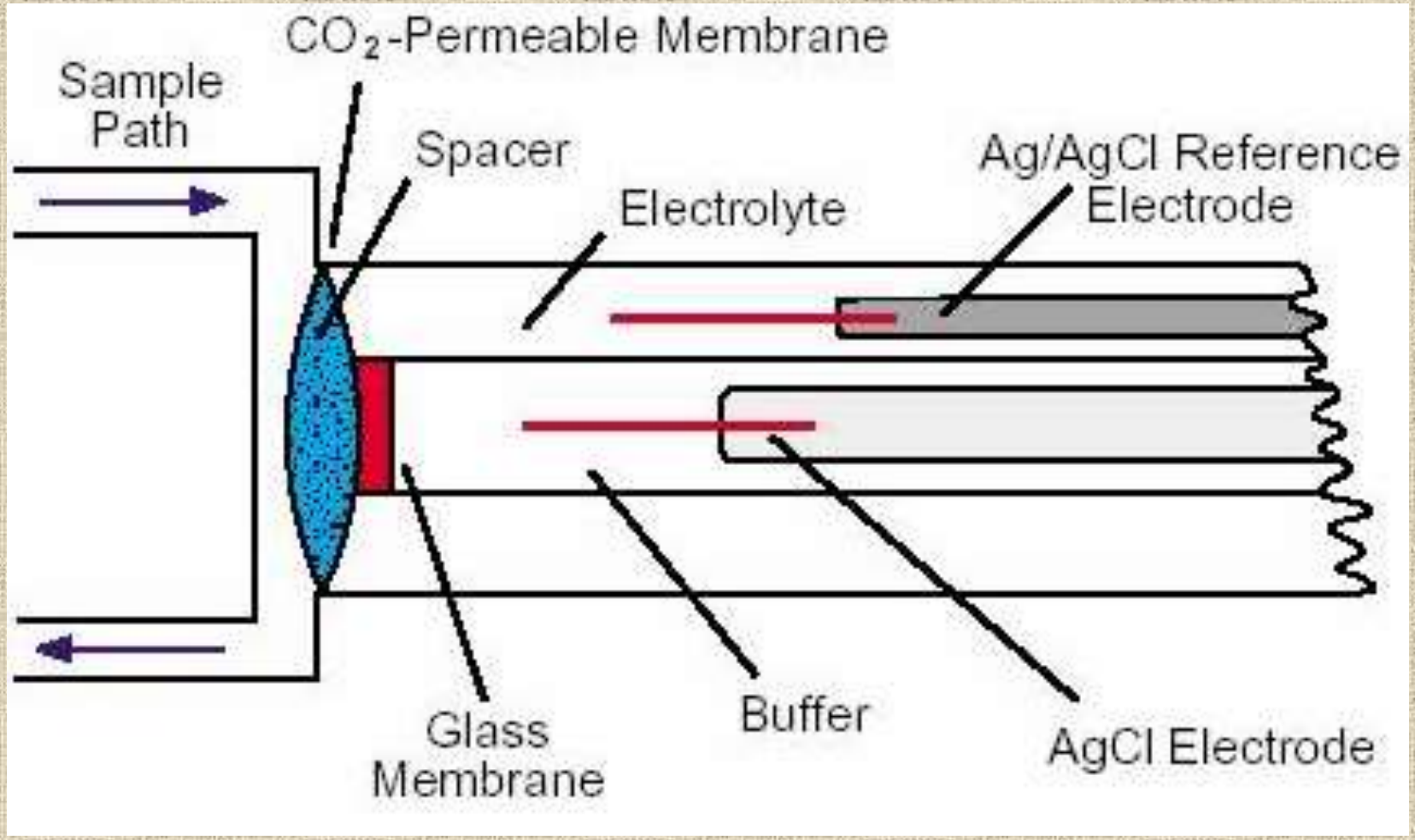
Turbidimetry & Nephelometry

- 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

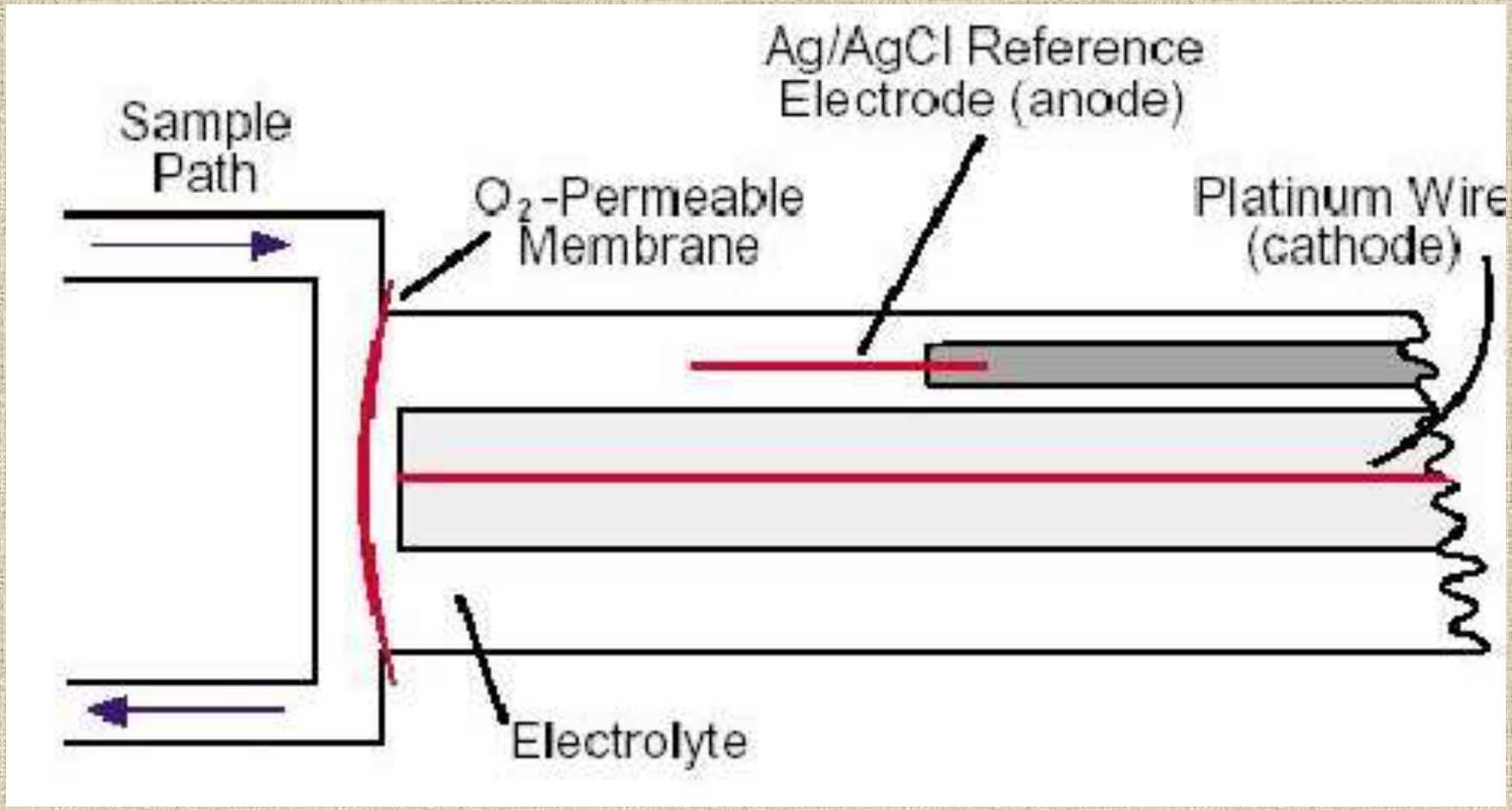
Gasometry

- 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

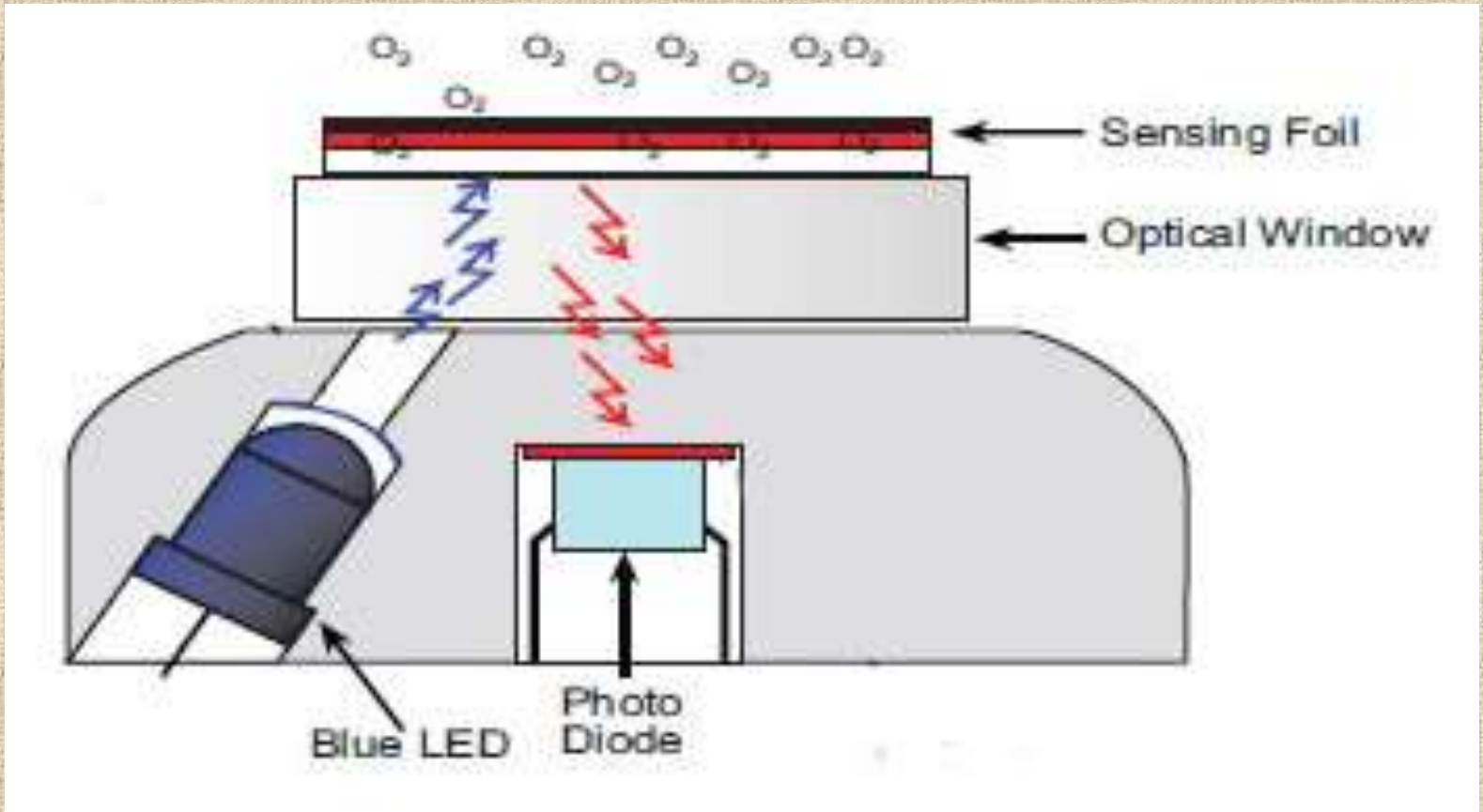
Carbon dioxide electrode



Clark type oxygen electrode



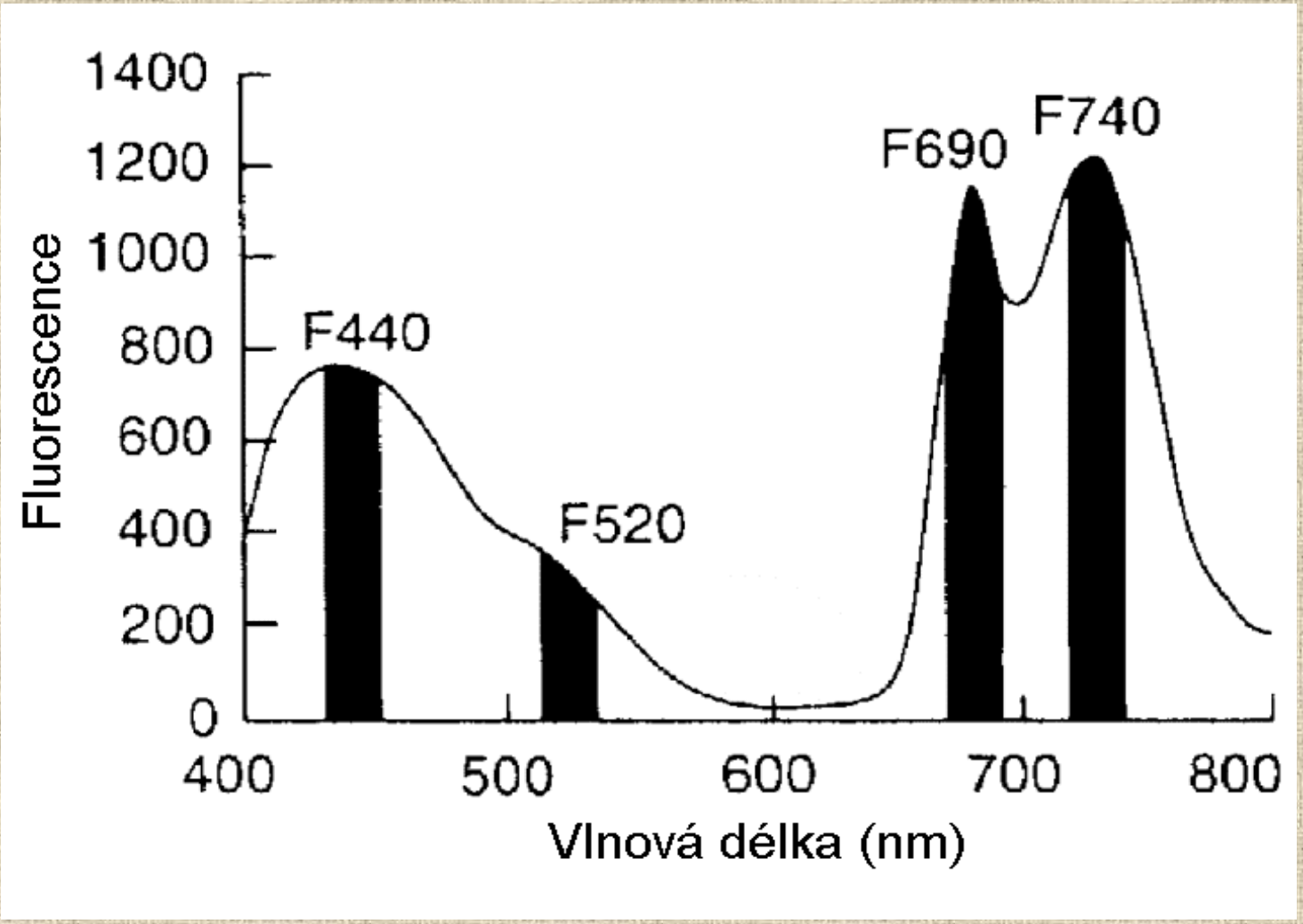
Oxygen optode



Chlorophyll fluorescence

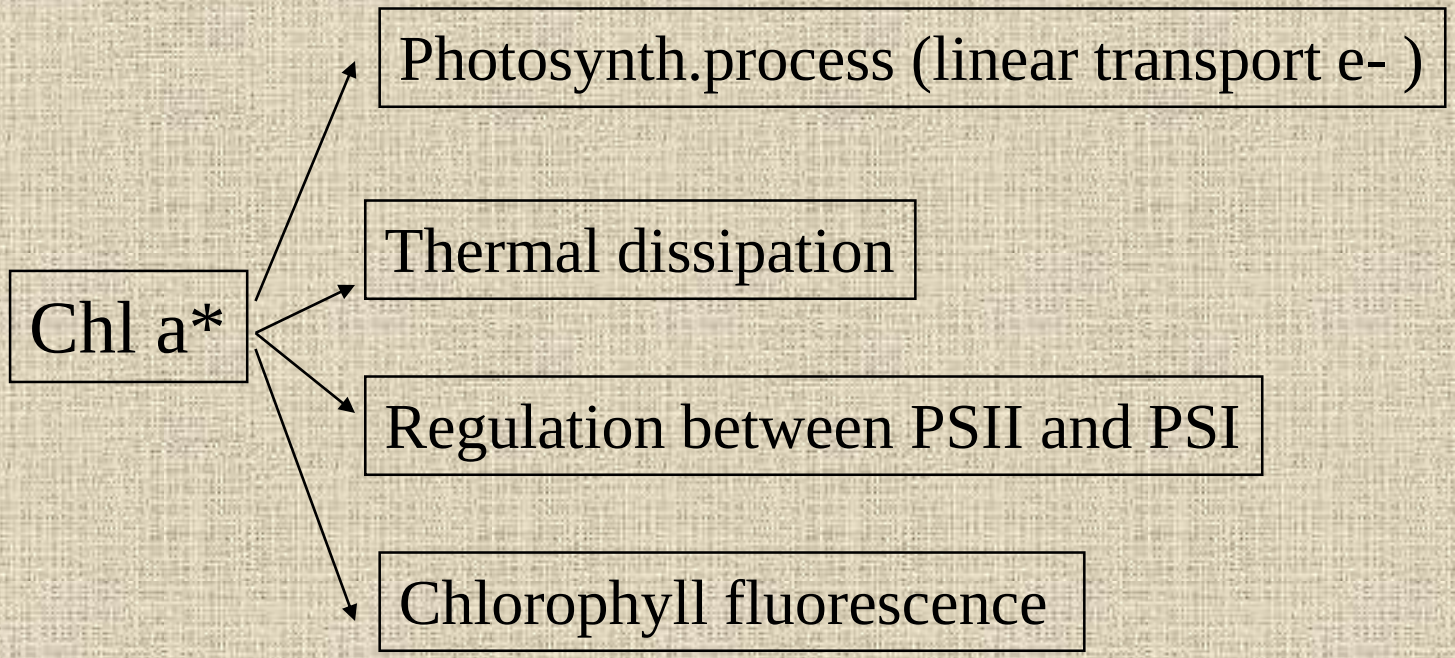
- What is chlorophyll fluorescence?
- Basic methods of Chl fluorescence measurements
- Signals, kinetics of Chl fuorescence
  - fast Chl fluorescence kinetics (parameters)
  - slow Chl fluorescence kinetics (parametrs)
  - saturation pulse method, quenching analysis
- Chl fluorescence imaging

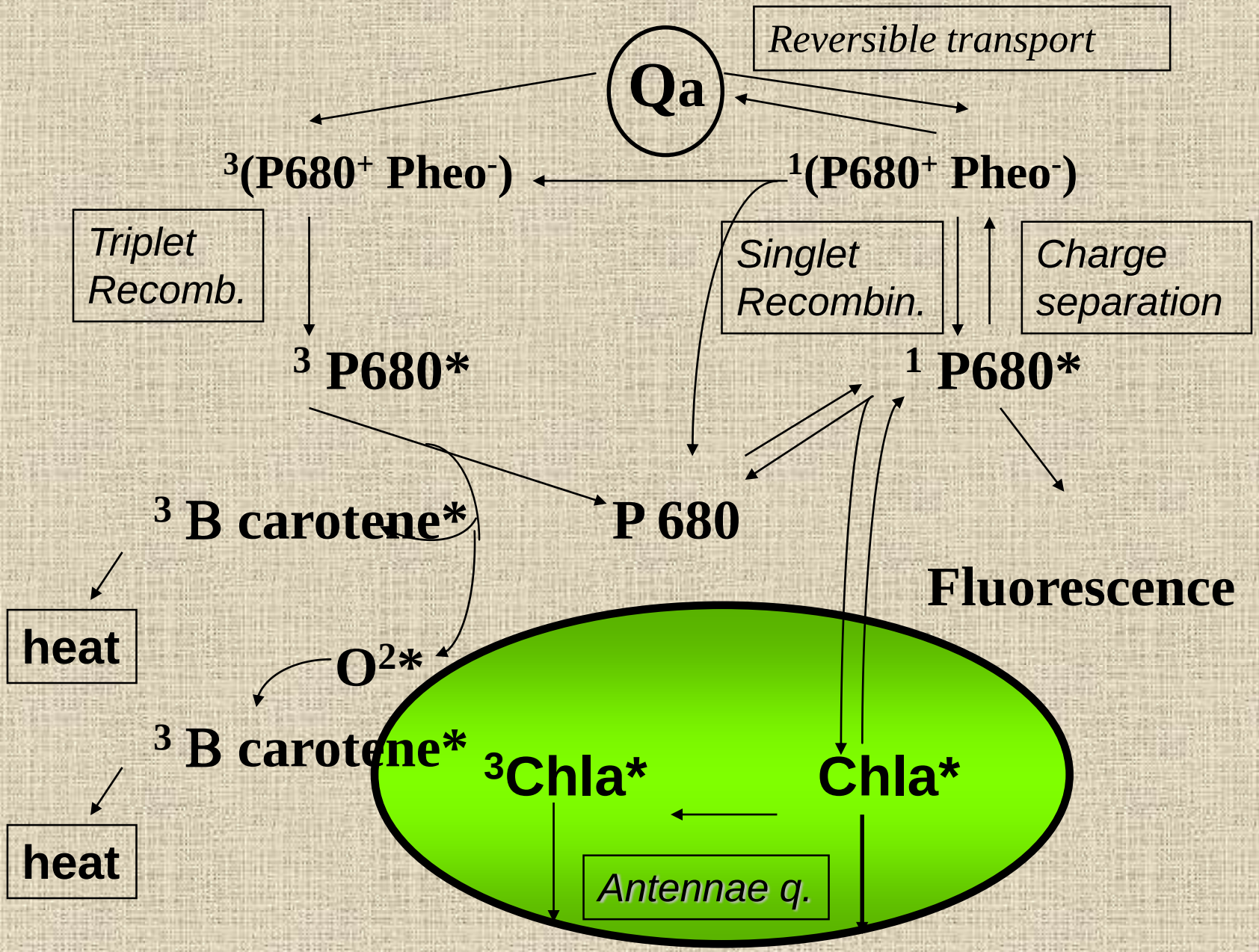
Fluorescence emission



Lichtenthaler et Miehe 1997

Basic de-excitation mechanisms of Chl *a*

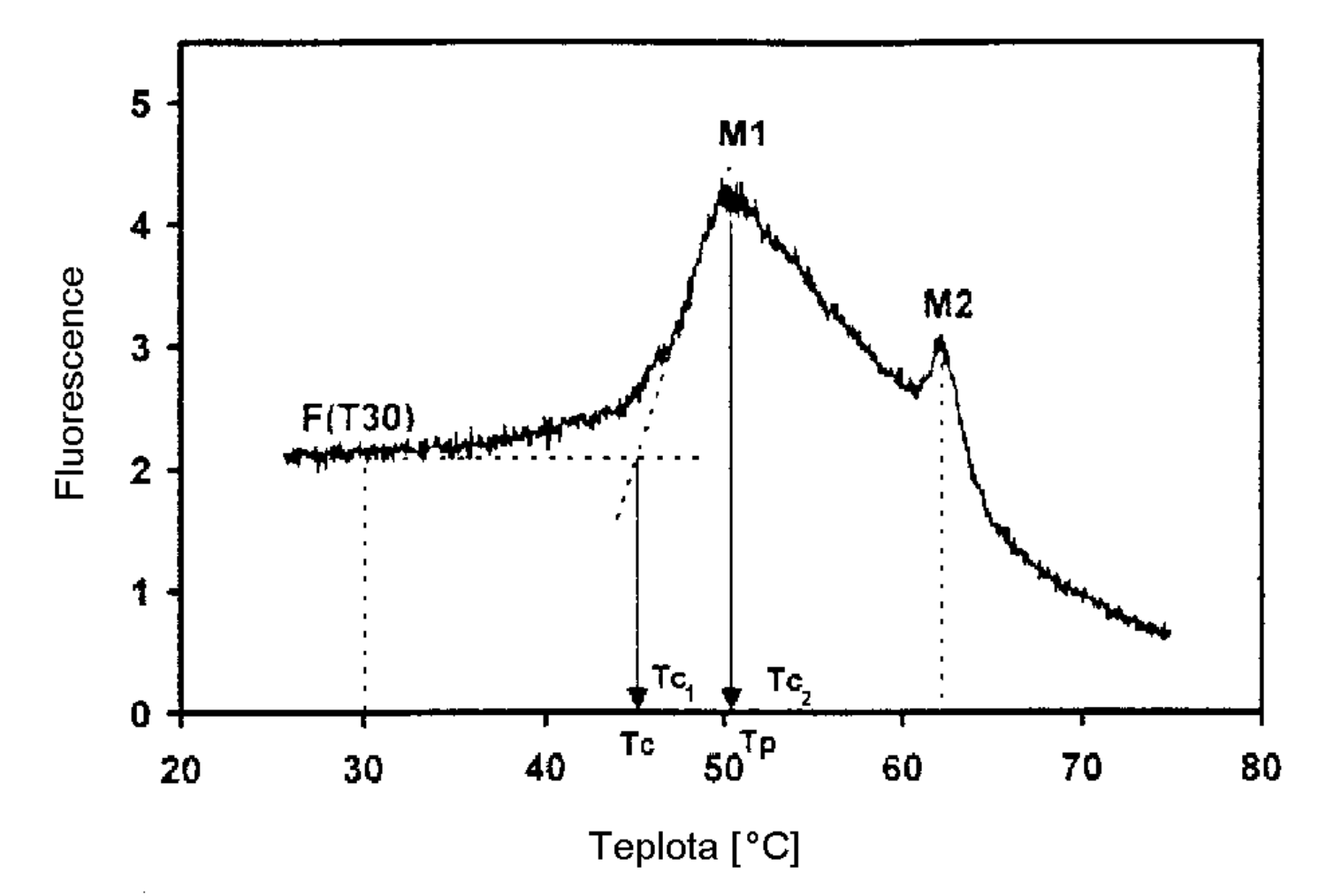



Reversible transport
Qa
$^3(P680^+ Pheo^-)$
$^1(P680^+ Pheo^-)$
Triplet Recomb.
Singlet Recombin.
Charge separation
^{3} P680*
^{1} P680*
^{3} B carotene*
P 680
Fluorescence
heat
O^{2*}
^{3} B carotene*
^{3}Chla*
Chla*
heat
Antennae q.

Chlorophyll fluorescence techniques

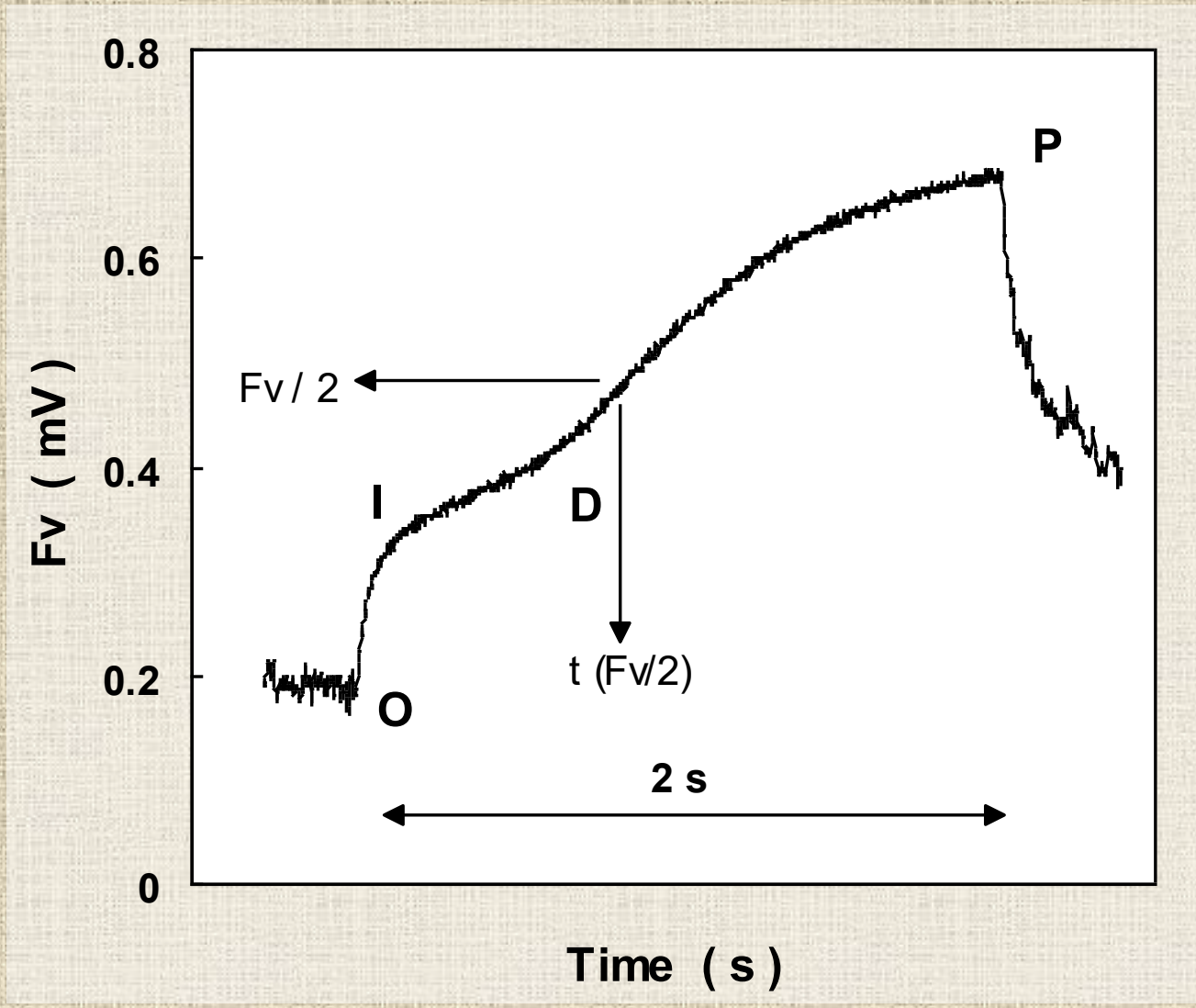
- Chl fluorescence signal
- Saturation pulse method
- Chl fluorescence kinetics (transients)
- Quenching analysis
- Rapid light/response curves
- Emission/absorption spectra
- Chl fluorescence imaging

Temperature response curve

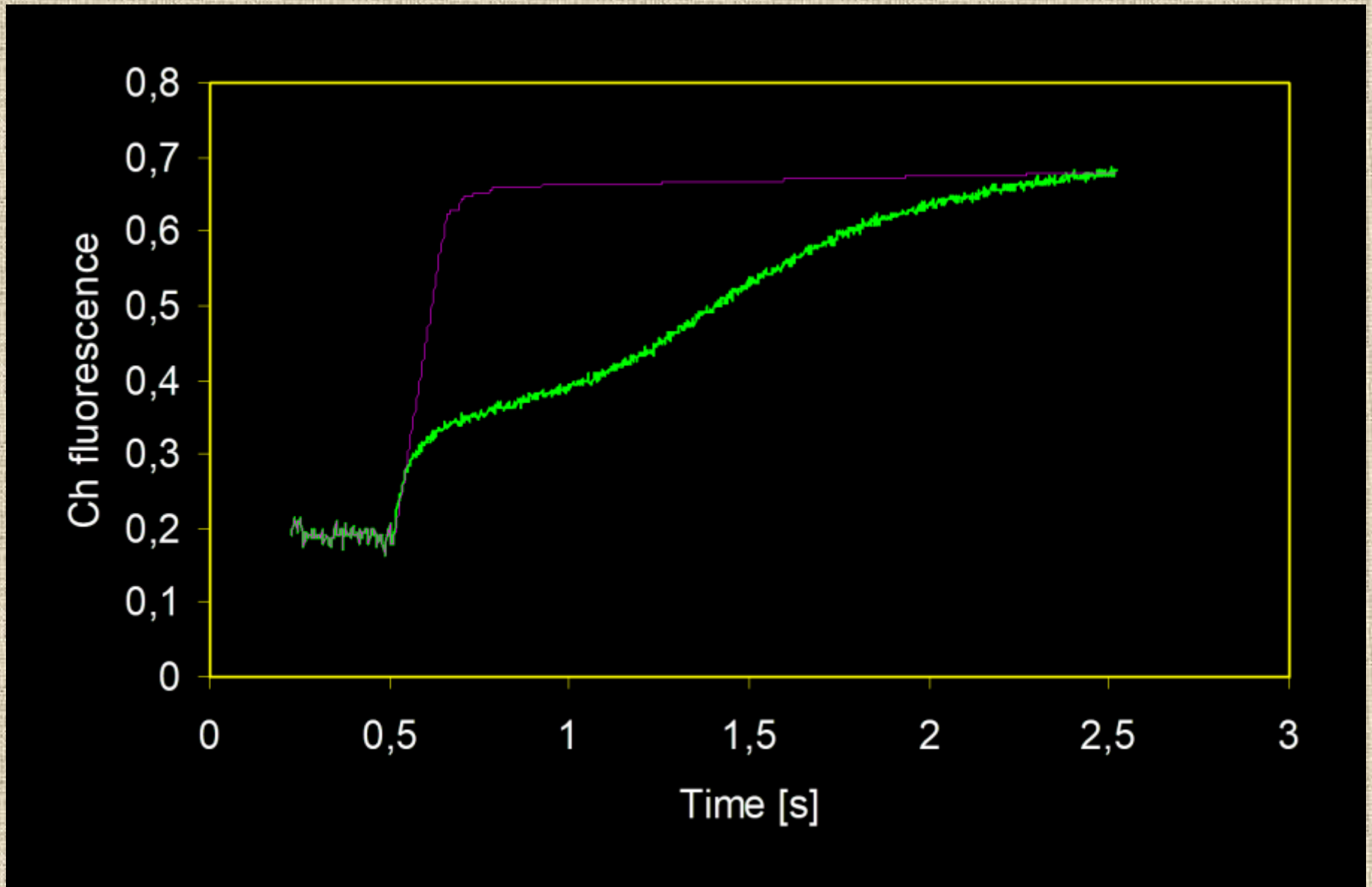


Kuropatwa 1994

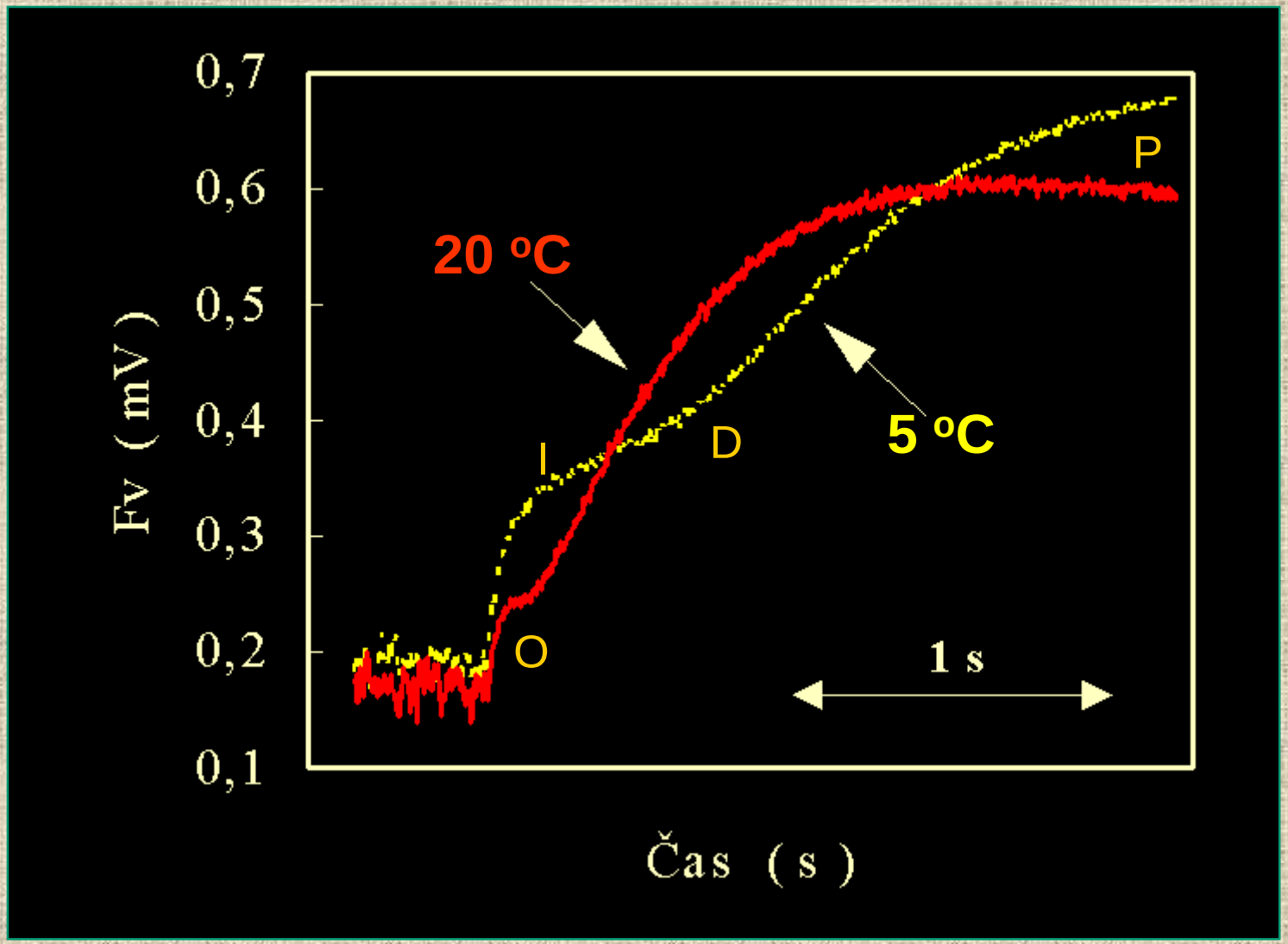
Fast Chl f. induction curve

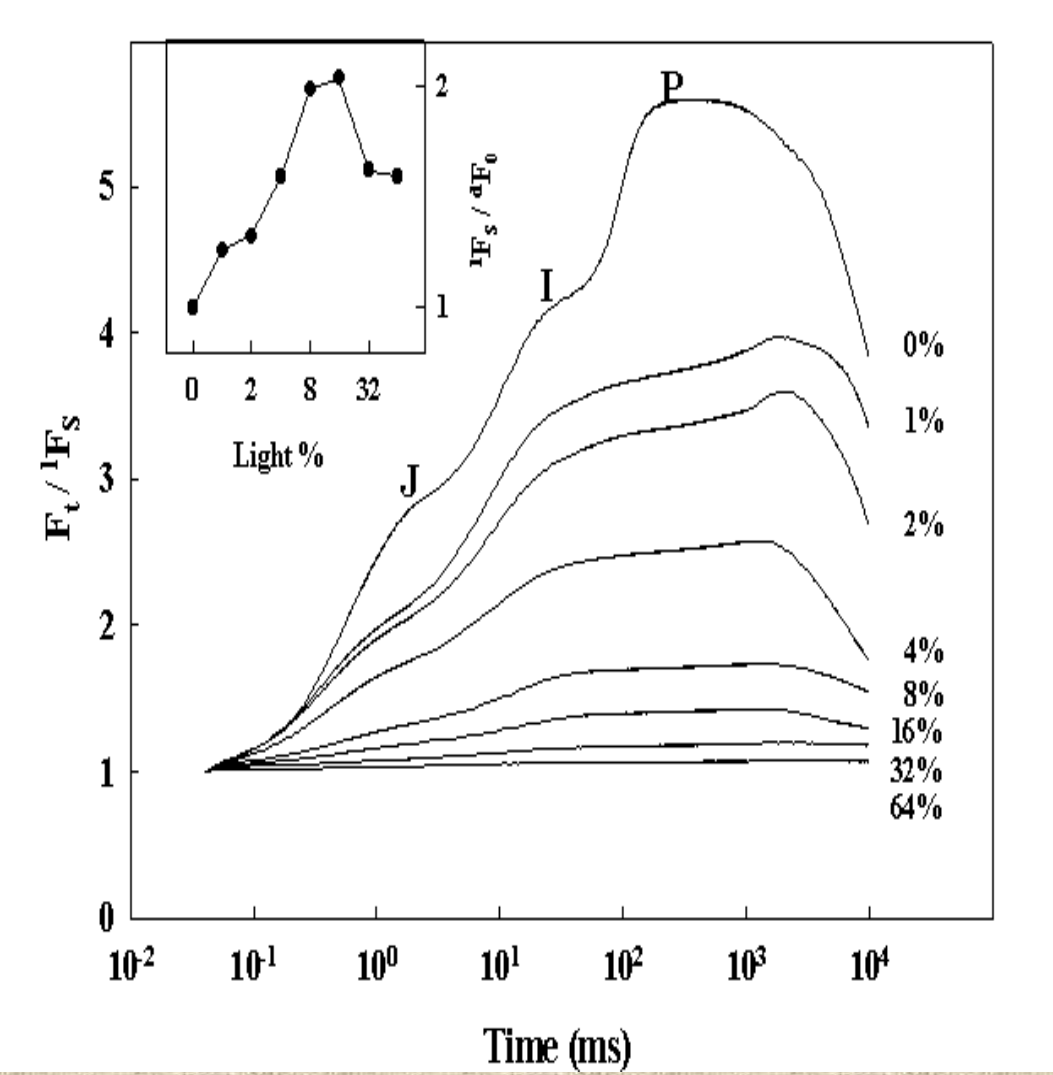


DCMU effect on fast kinetics



Effect of temperature on fast kinetics





OJIP taken after dark adaptation

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

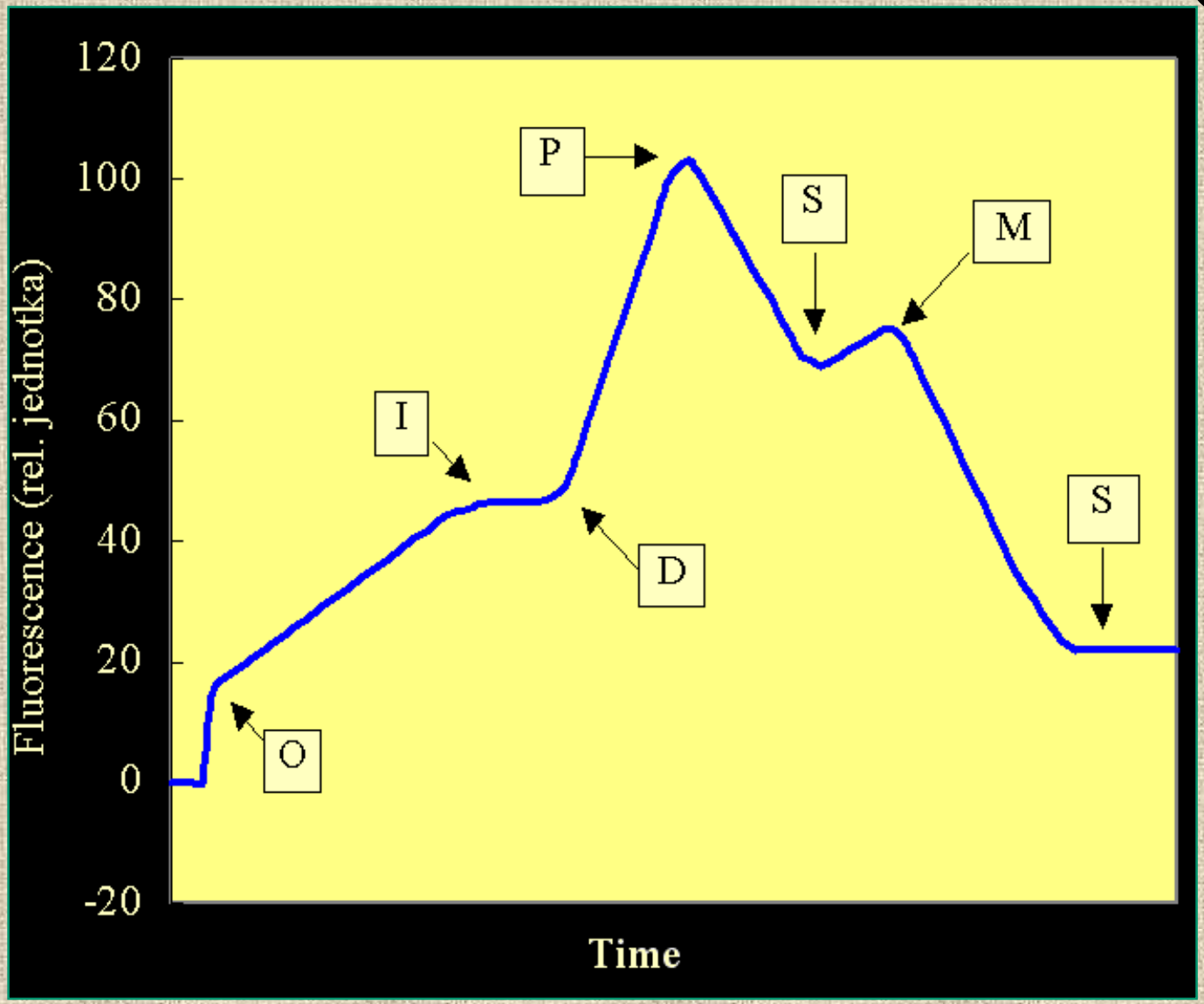
Fast Chl transient [R. Strasser]

Parameters derived from fast kinetics

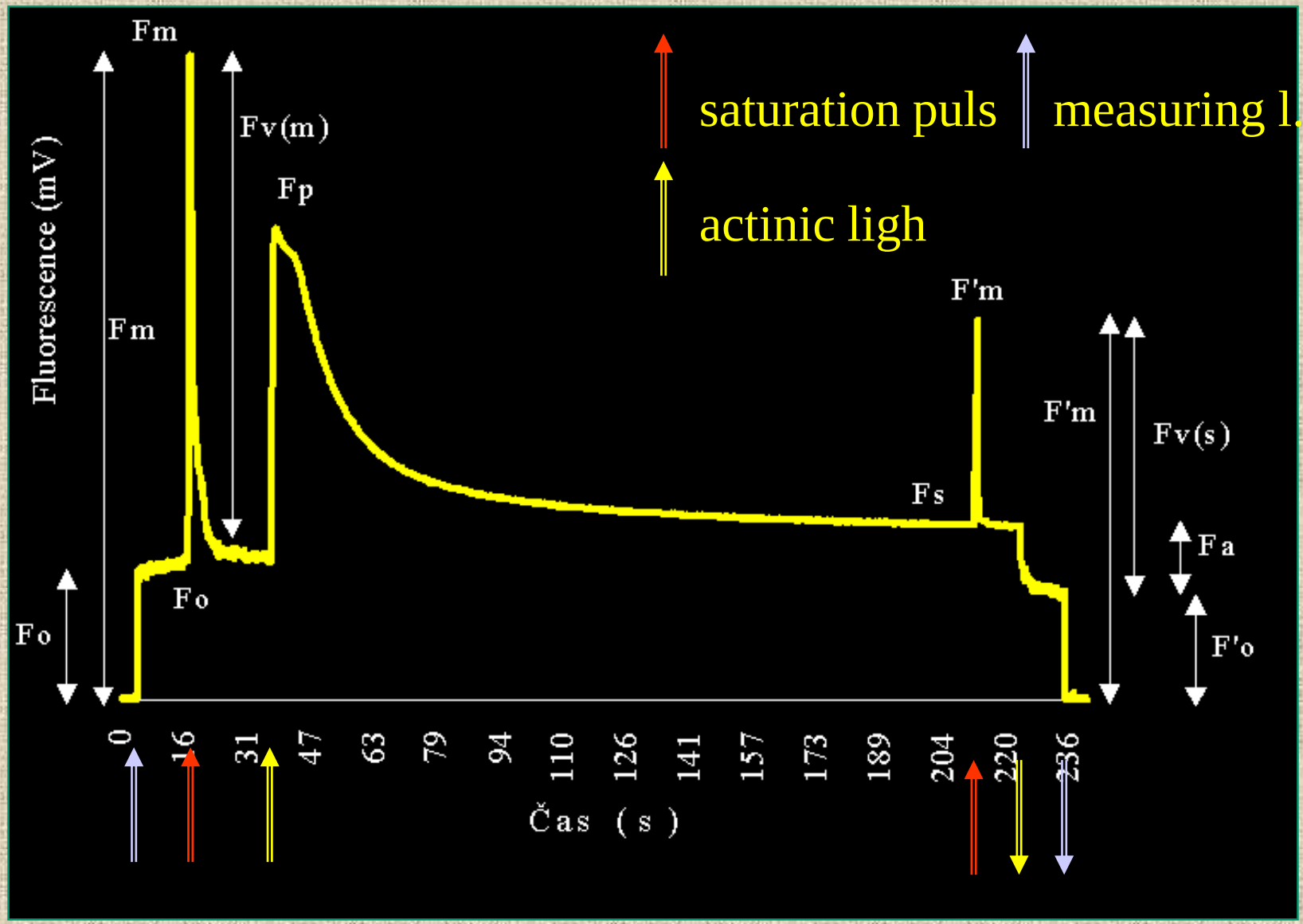
- Signals at O,I, D P points, O(K)JIP
- Ratios Fo/Fm Fv/Fm
- Half-times
- Rates of Chl fluorescence increase
- Area over rising part of curve
- Estimation of Active / inactive RC (Qb-reducing)

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