Měření rychlosti fotosyntézy oxymetricky a fluorometricky – srovnání metod

Photosynthetic reactions

- general equation:
  - » $nCO_2 + nH_2O + \text{light} \xrightarrow{\text{Chl } a} (CH_2O)_n + nO_2$
- light dependent reactions
  - light interception, l. energy transfer
  - excitation, charge separation
  - ETR
    - » linear
    - » cyclic
  - O_2 evolution
  - $4NADP^+ + 2H_2O + 4ADP + 4P_i \xrightarrow{\text{8 photons + 4}e^-} 4NADPH + 4ATP + O_2$
- light independent reactions
  - CBB – Calvin Benson Bassham Cycle
  - RuBisCO
  - CA

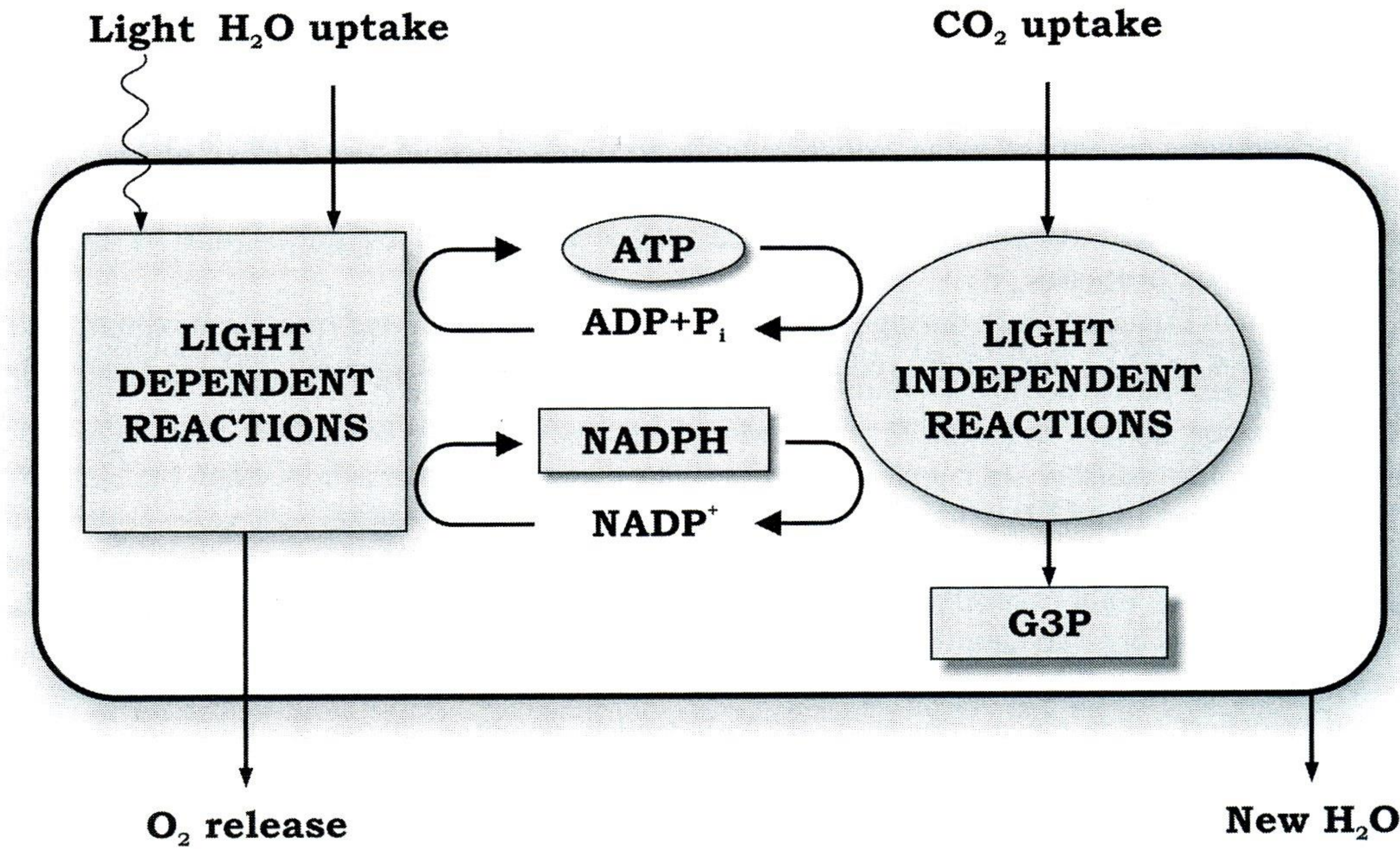
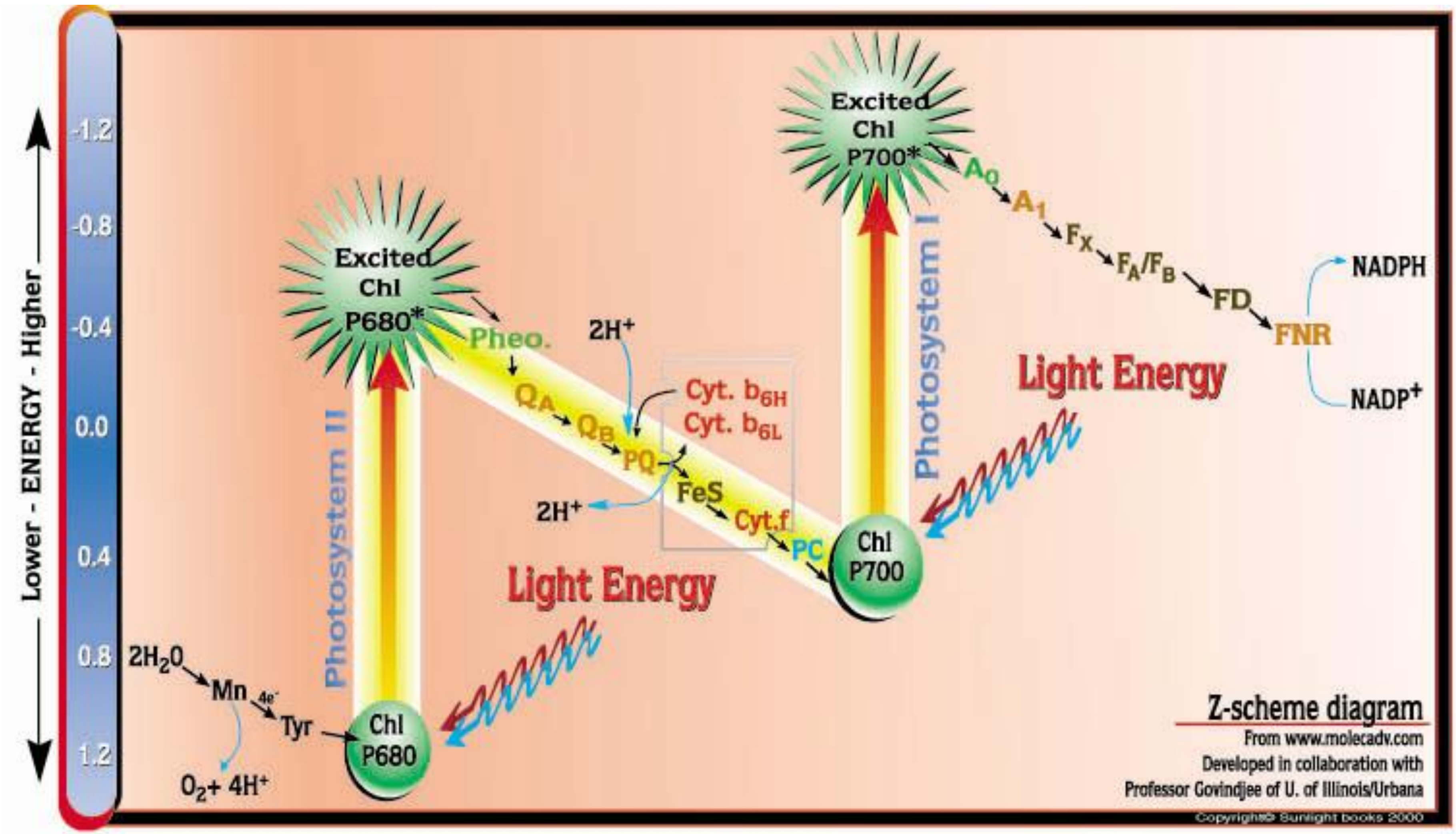


FIGURE 3.1 Schematic drawing of the photosynthetic machinery.



Lower - ENERGY - Higher
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Excited Chl P680*
Excited Chl P700*
Pheo.
QA
QB
PQ
2H+
2H+
Cyt. b6H
Cyt. b6L
FeS
Cyt.f
PC
Chl P700
Chl P680
A0
A1
FX
FA/FB
FD
FNR
NADPH
NADP+
Photosystem II
Photosystem I
Light Energy
Light Energy
2H2O
Mn
4e-
Tyr
O2+ 4H+
Z-scheme diagram
From www.molecadv.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana
Copyright© Sunlight books 2000

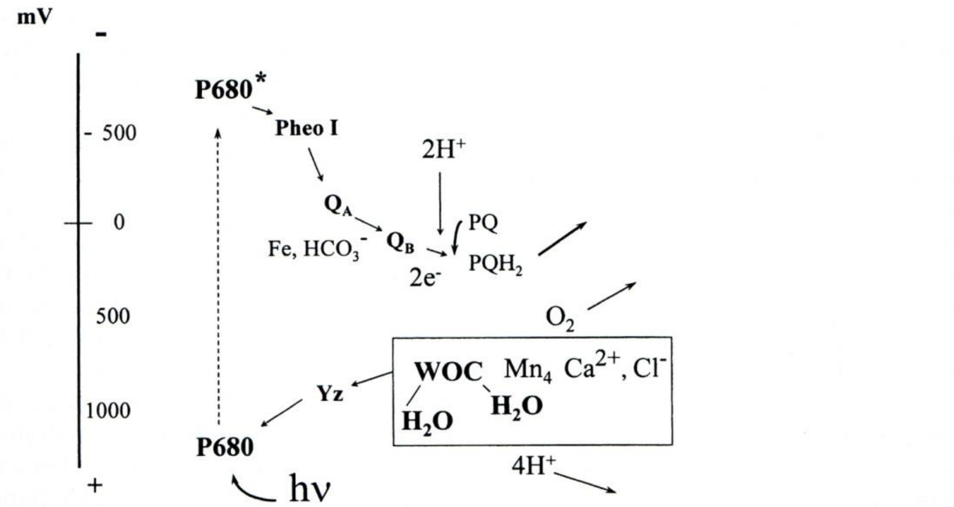


Fig. 1. Diagram showing the main cofactors of PS II, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Electron flow occurs from water to Q_B. Absorption of light energy ($h\nu$) or excitons by P680, producing P680*, leads to a photochemical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

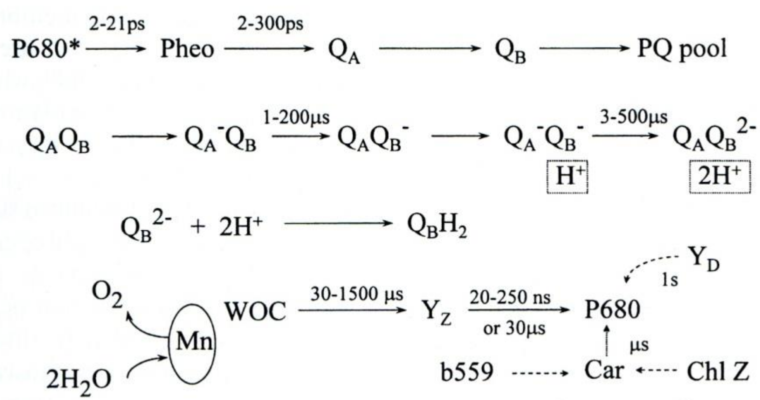


Fig. 2. Diagram showing the main kinetics of the main pathway of electrons through the PS II reaction center. Y_D (D2 Tyr161), Chlorophyll Z (Chl Z) and Cytochrome b_{559} (b_{559}) are alternate electron donors under certain conditions. See also Figure 1.

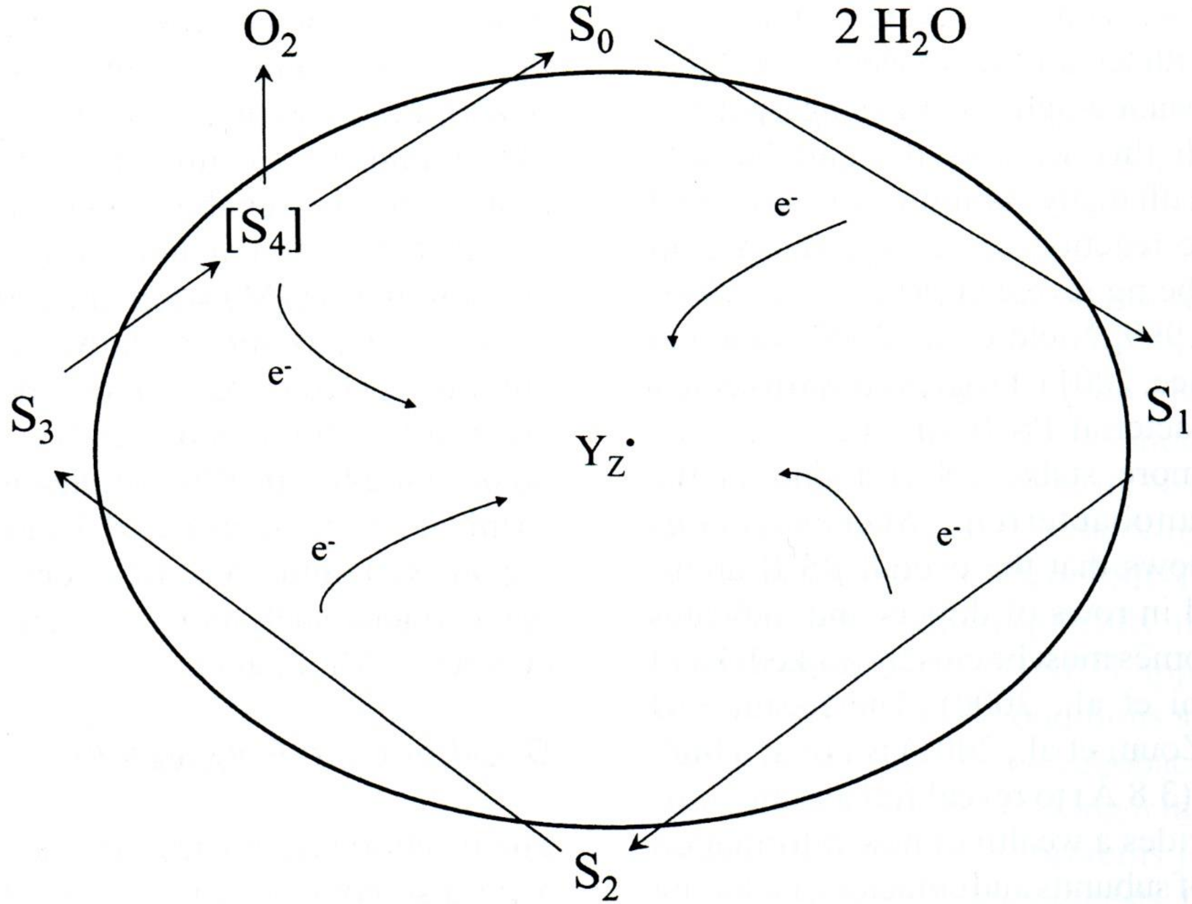


Fig. 3. The S-states of the water oxidizing complex (WOC). Electrons are removed sequentially by $P680^+$ via Y_Z. The S-state number indicates the number of oxidizing equivalents stored. On reaching S_4, oxygen is released and the cycle reset. The steps at which water may be bound, oxidized and protons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

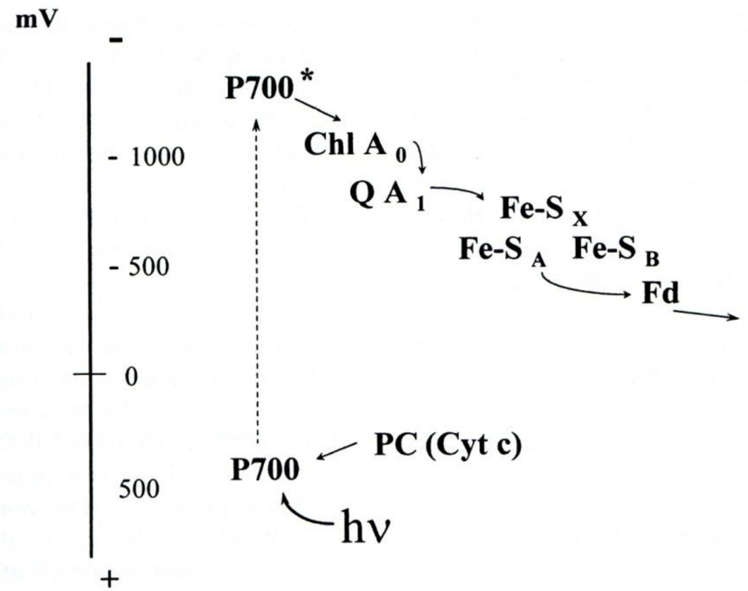


Fig. 4. Diagram showing the main cofactors of PS I, the pathway of electrons through the reaction center and the probable redox potential relationship between the cofactors. Absorption of light energy (hν) or excitons by P700 leads to a photochemical charge separation. Electron flow occurs from Plastocyanin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

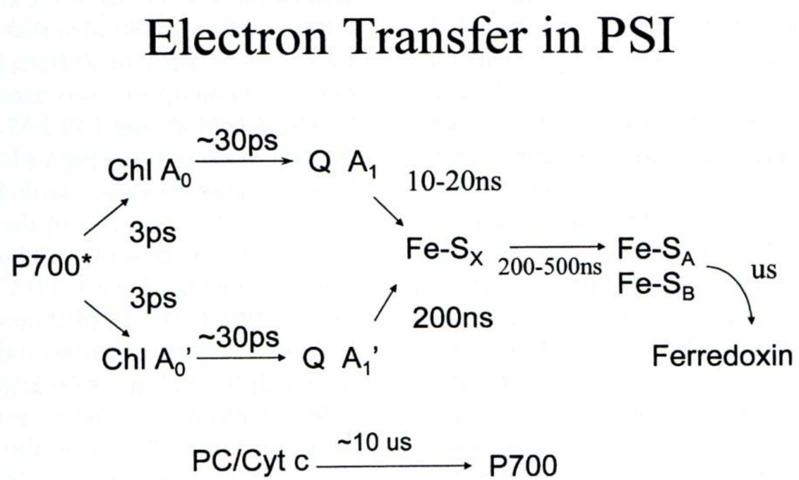
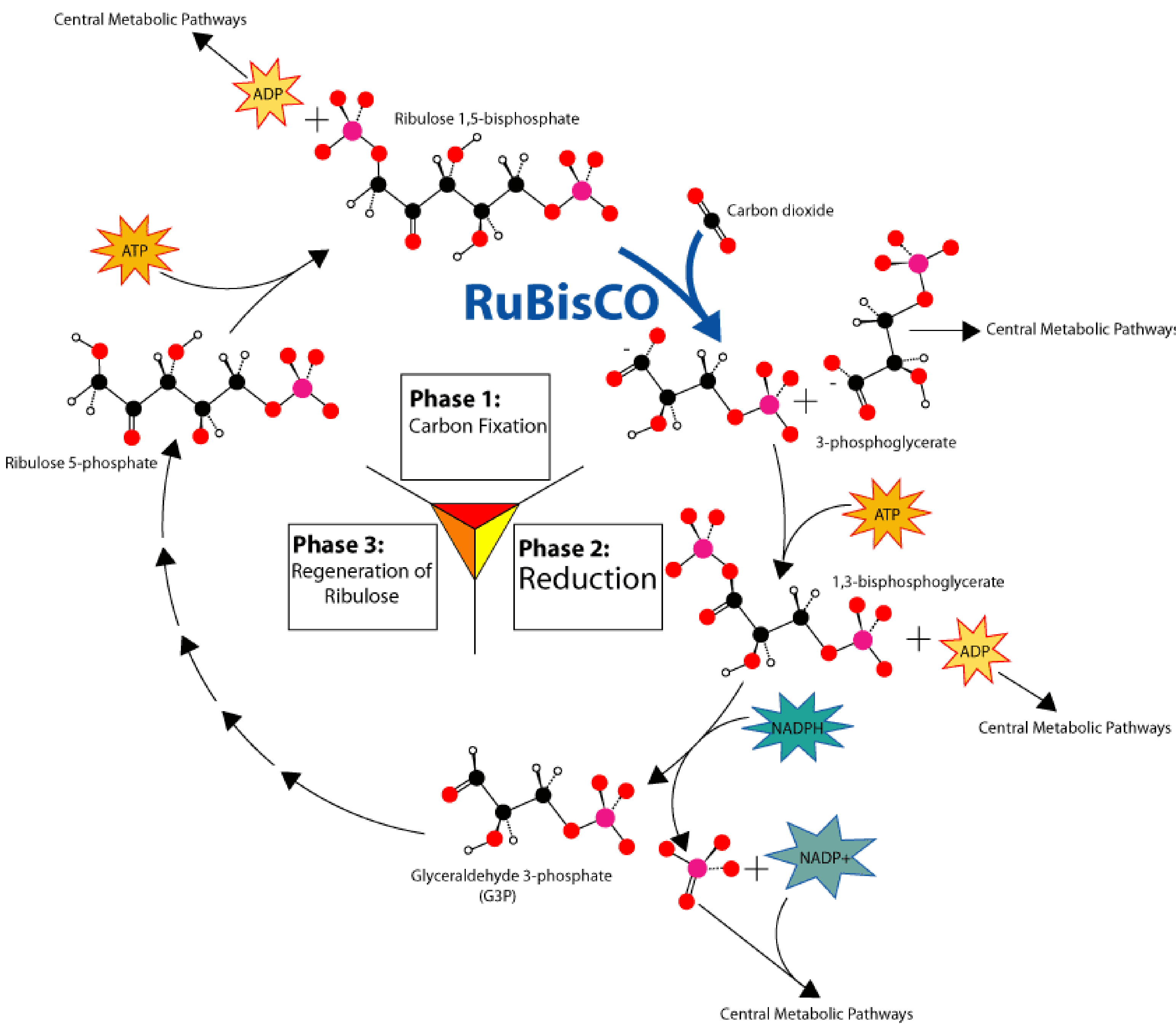


Fig. 5. Diagram showing the main kinetics of the main pathway of electrons through the PS I reaction center.



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

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

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

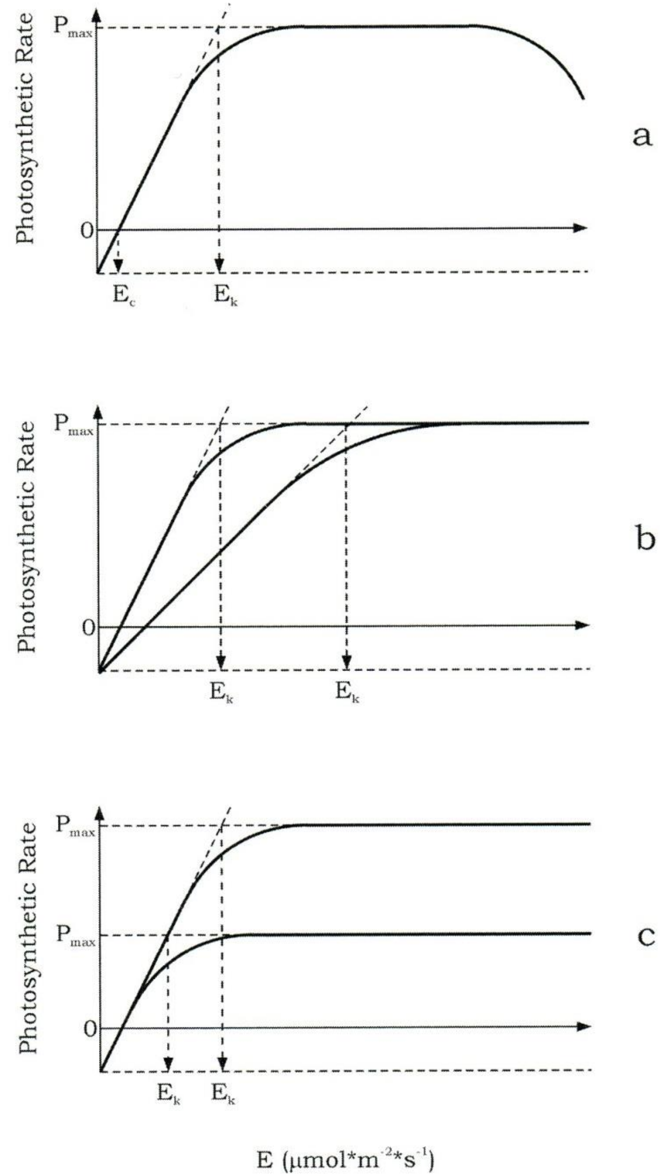
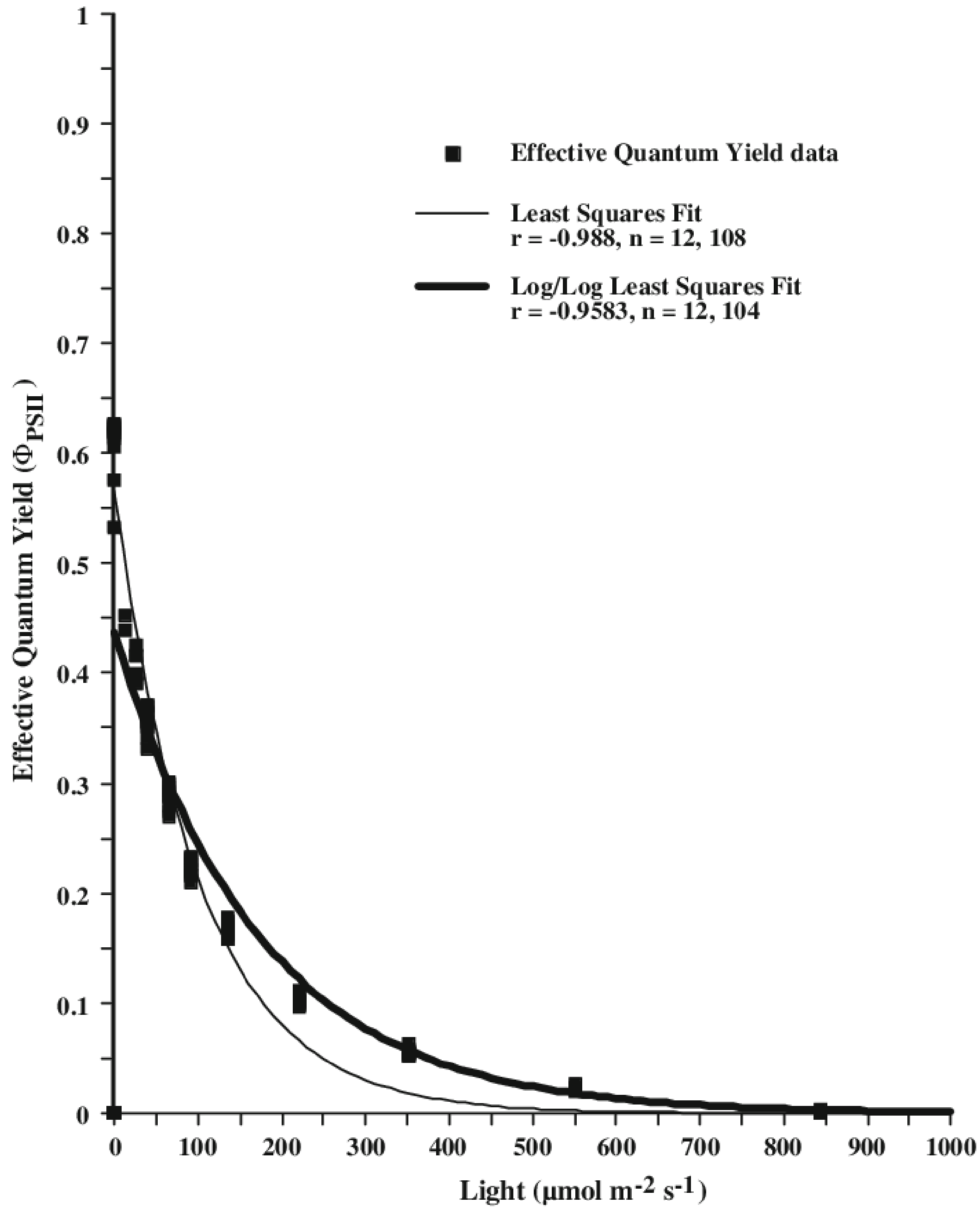


FIGURE 5.15 Photosynthesis–irradiance response curves: E_c, irradiance compensation point; E_k, saturating irradiance; and P_{max}: maximum photosynthetic rate. (a) typical plot; (b) comparison of two curves with different slopes: keeping constant the number of photosynthetic units, but increasing the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



Effective Quantum Yield data
Least Squares Fit
r = -0.988, n = 12, 108
Log/Log Least Squares Fit
r = -0.9583, n = 12, 104
Effective Quantum Yield (Φ_{PSII})
Light (μmol m^{-2} s^{-1})
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
800
900
1000

Oxygen measurement

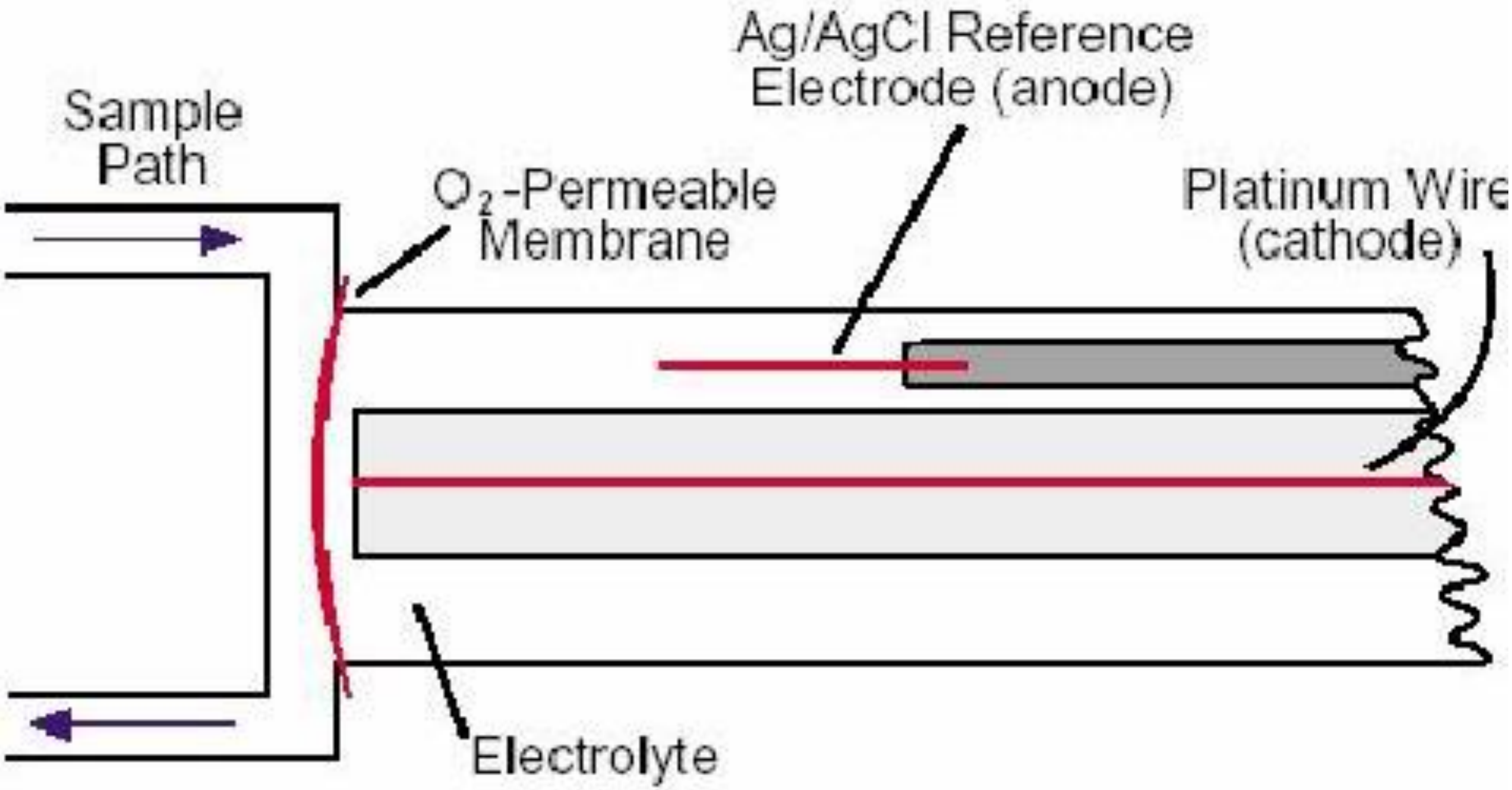
- Clarc- type electrode
  - Cathode reaction:

    $O^2 + 2\ H_2O + 4e^- \ \text{-->}\ 4\ OH^-$
  - Anode reaction:

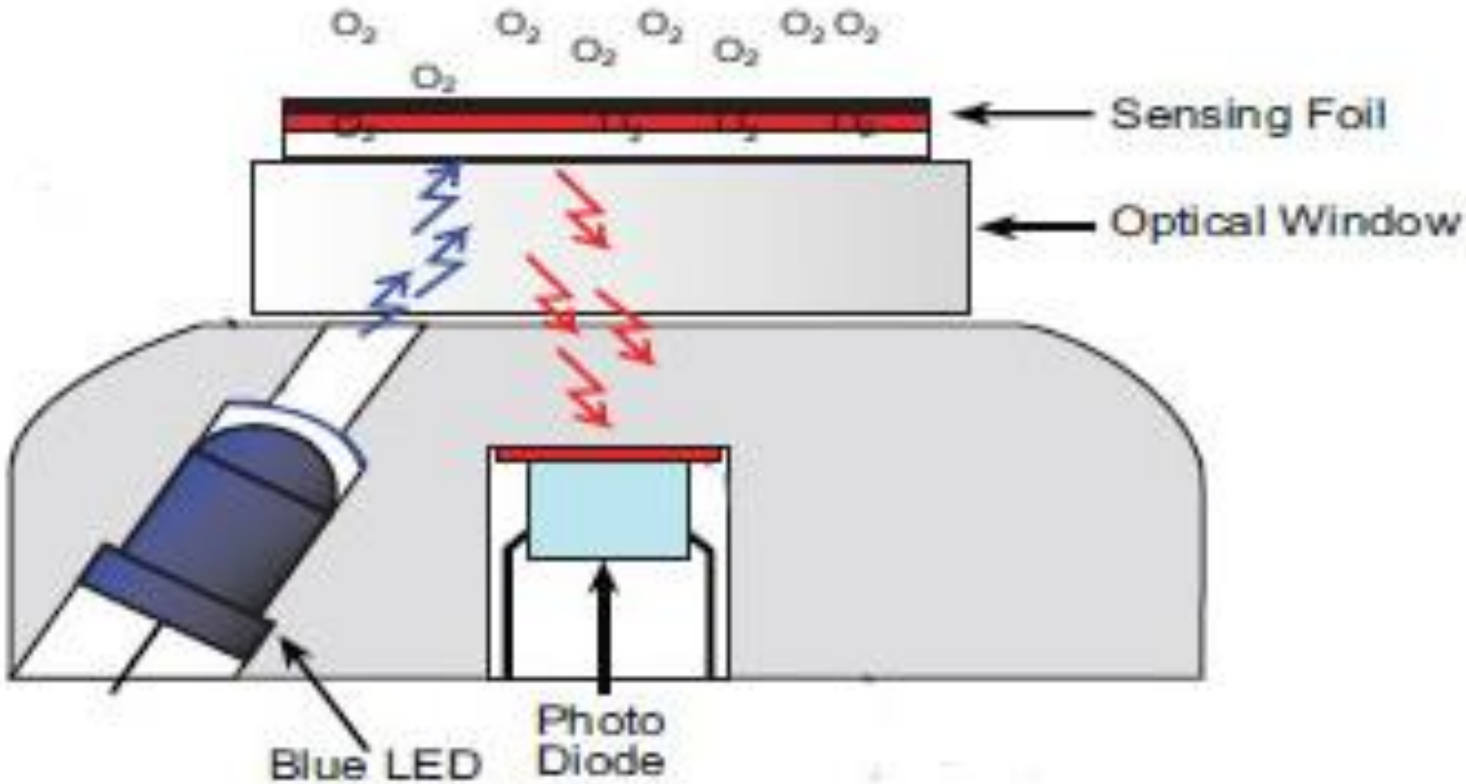
    $4\ Ag + 4\ Cl^- \rightarrow 4\ AgCl + 4e^-$

- Joliot – type electrode
- Fluorescence oxygen probe

Clark type oxygen electrode



Oxygen optode



Světelná křivka rychlosti vývinu kyslíku a kvantového výtěžku fotochemických reakcí v PS II

Metoda:

Souběžné měření rychlosti vývinu kyslíku (OER) a kvantového výtěžku (Φ_{II}) za postupně se zvyšujícího toku fotosynteticky aktivní radiace (PPFD).

OER

Měření koncentrace kyslíku probíhá pomocí kyslíkové elektrody ve stanovených intervalech.

Okamžitou rychlost OER stanovíme z rozdílů koncentrací v čase a vyjádříme v μmol O_2 za jednotku času (h) a množství chlorofylu *a* (mg)

$$OER = \frac{\delta\ c_{O_2}\ [\mu mol.l^{-1}]}{\delta\ t\ [h] * c_{chl\ \mathrm{a}}\ [mg.l^{-1}]}\ [\mu mol(O_2).mg(chl\ a)^{-1}.h^{-1}]$$

Φ_{II}

- kvantový výtěžek fotochemických procesů v PS II stanovíme fluorometricky metodou saturačních pulsů
- výsledky prezentujeme graficky vůči ozářenosti

Chlorofyly stanovíme v průběhu cvičení spektrofotometricky extrakci v DMSO dle Welburn (1994)

- Odebereme známé množství kultury (2ml), centrifugujeme při 5000rpm 5 min., odsajeme supernatant a extrahujeme s DMSO (1min vortex) hodinu při 70°C

- Centrifugujeme při 5000rpm 5min. supernatant měříme pomocí spektrofotometru (A=665, 649, 750), v případě potřeby naředíme pro OD=0,2-0,8

- Chlorofyl *a* stanovíme dle rovnice

$$C_{chl\ a}=12{,}19*A_{665} - 3{,}45*A_{649}$$

Závěr

- Naměřená data prezentujte formou grafů závislosti OER a Φ_{II} na PPFD (světelná křivka OER a Φ_{II}); zjištěné závislosti popište
- Ze světelné křivky OER zjištěte
  - Temnostní respiraci R_D
  - Kompenzační ozářenost I_c
  - Maximální rychlost evoluce kyslíku OERmax
  - Saturační ozářenost I_s
- Graficky prezentujte a popište vzájemný vztah OER na Φ_{II} za jednotlivých ozářeností