Chlorophyll-*a* fluorescence – what can we learn?

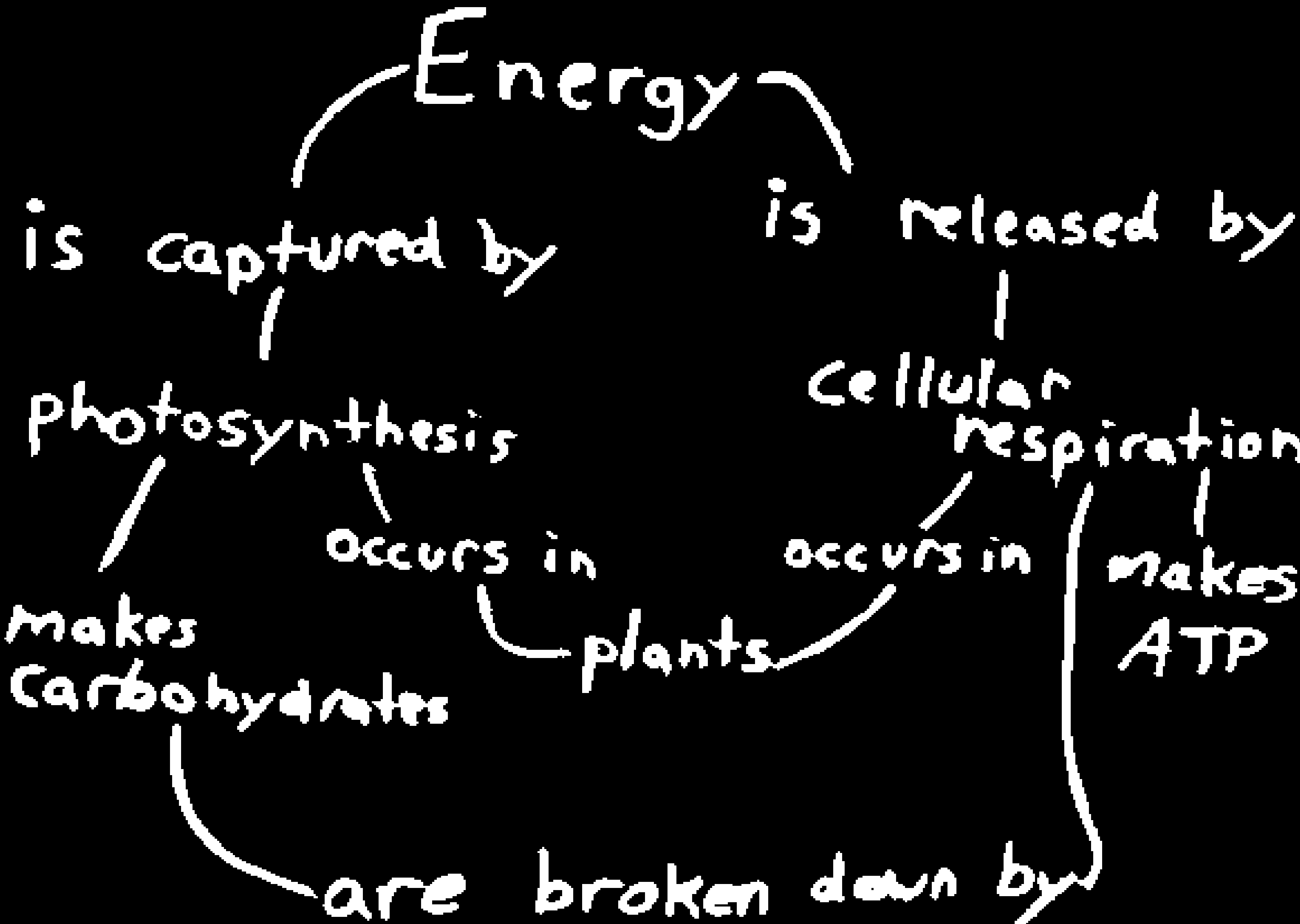
Dr. Hazem M. Kalaji

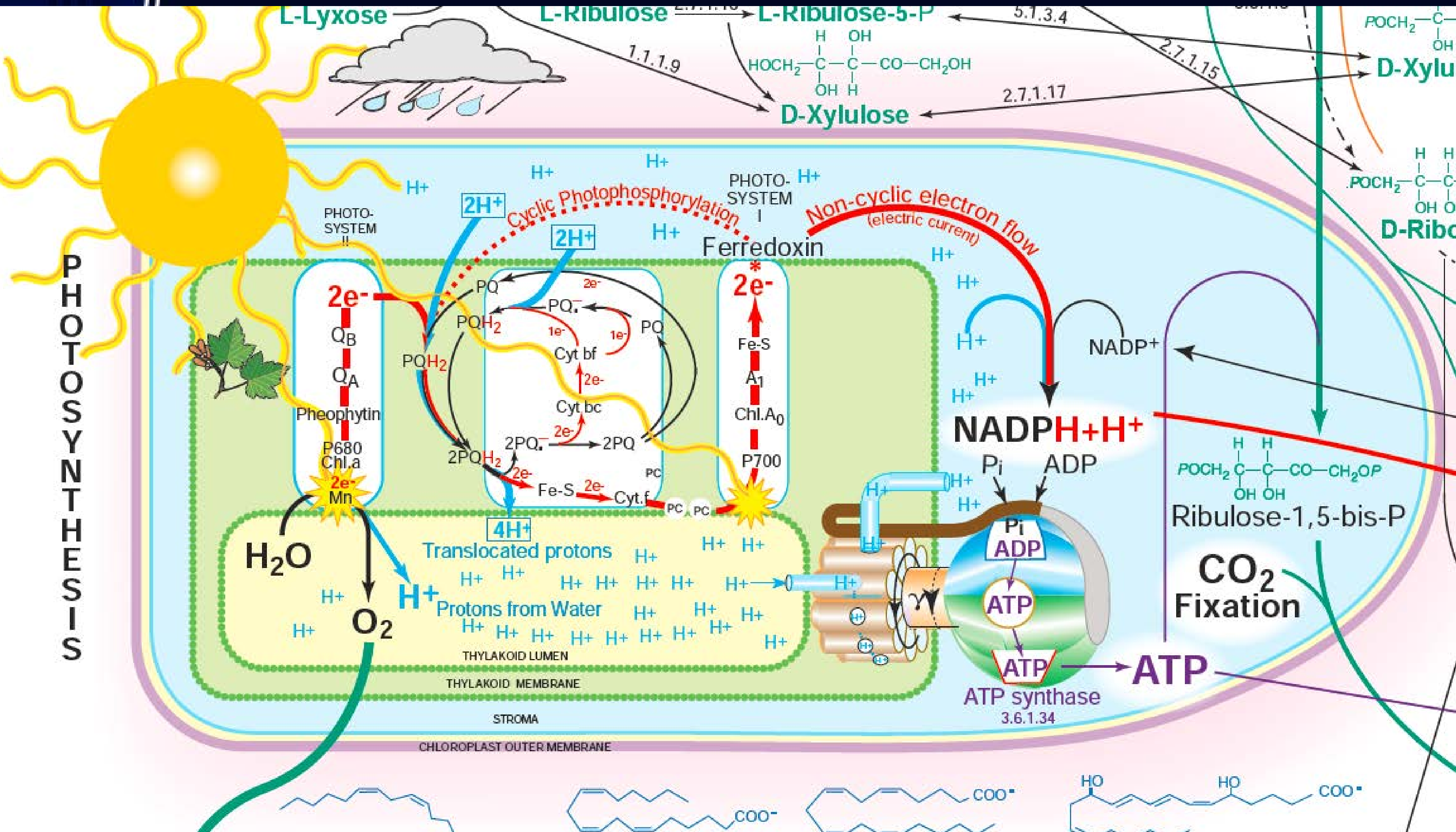
Natural Phenomena

- Bioindicator
- Biomarker
- Stress fingerprint
- WHY ?
- Sensitive
- Reliable
- Non-invasive
- Fast and low cost
- Applicable in all living organisms with chlorophyll


RUE DU MIDI
ZUIDSTRAAT
RUE
CHLOROPHYLLE
CHLOROPHYL
STRAAT

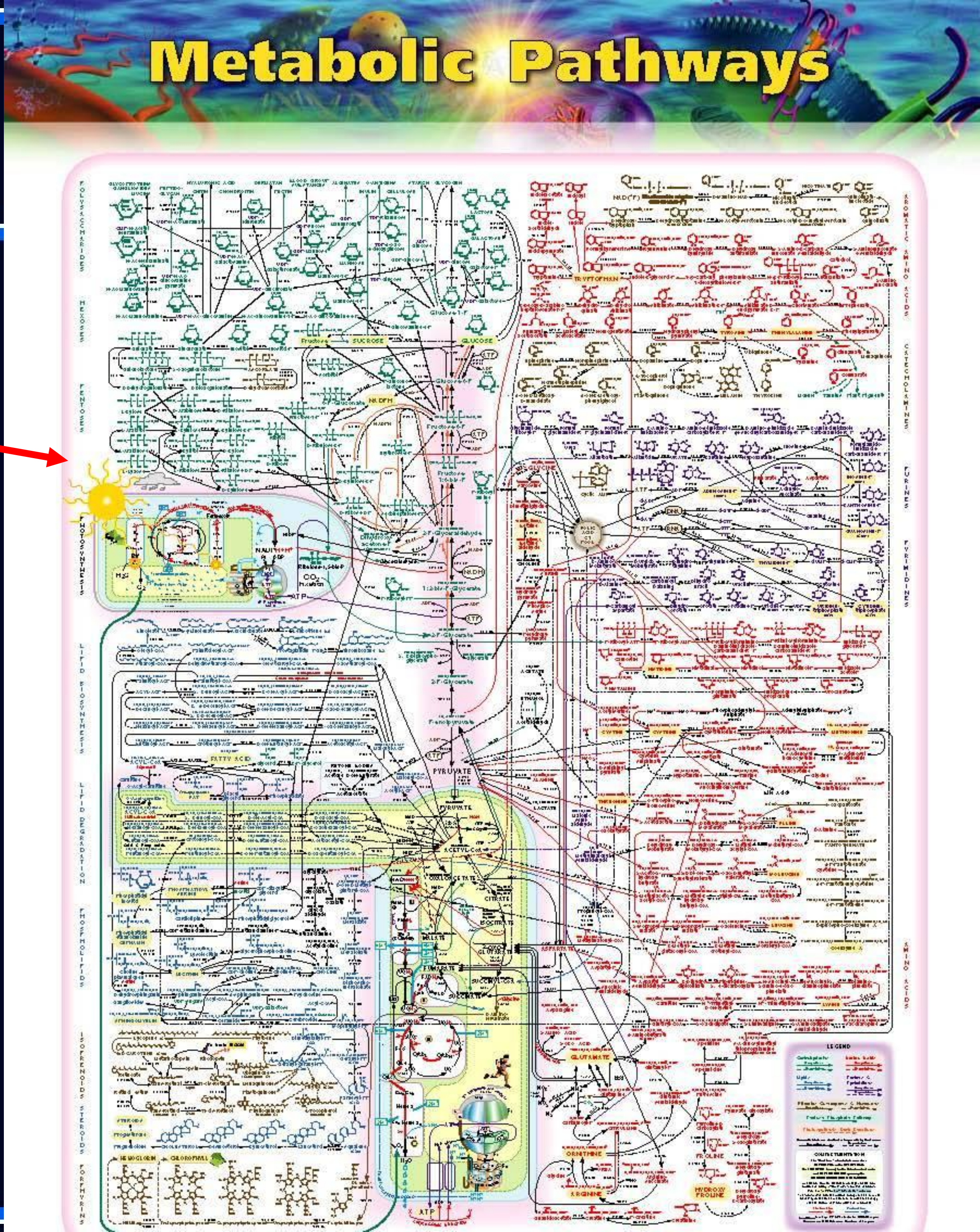

KOCHAM
CIE


Energy
is captured by
is released by
photosynthesis
cellular respiration
makes
carbohydrates
occurs in
plants
occurs in
makes
ATP
are broken down by

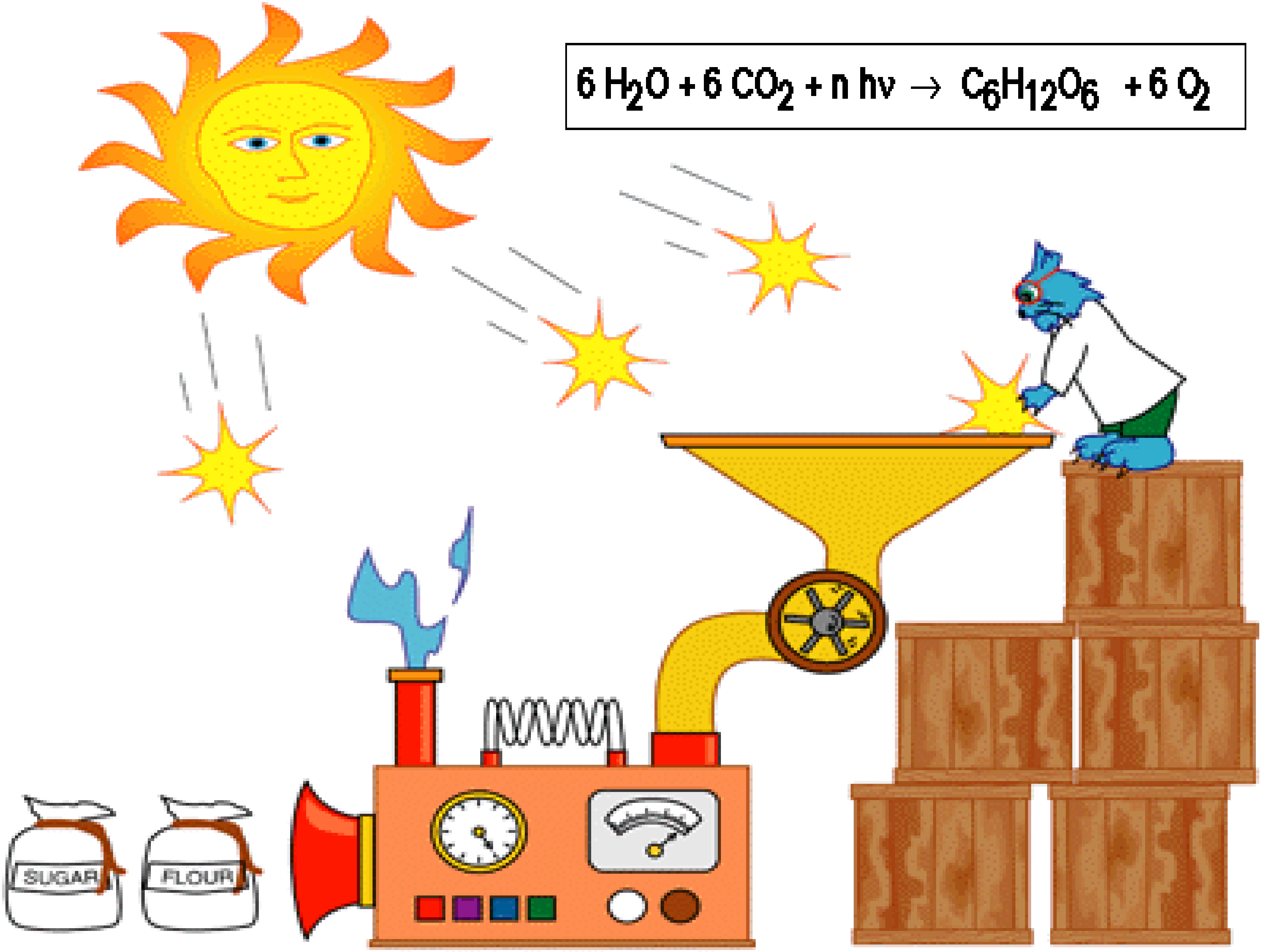


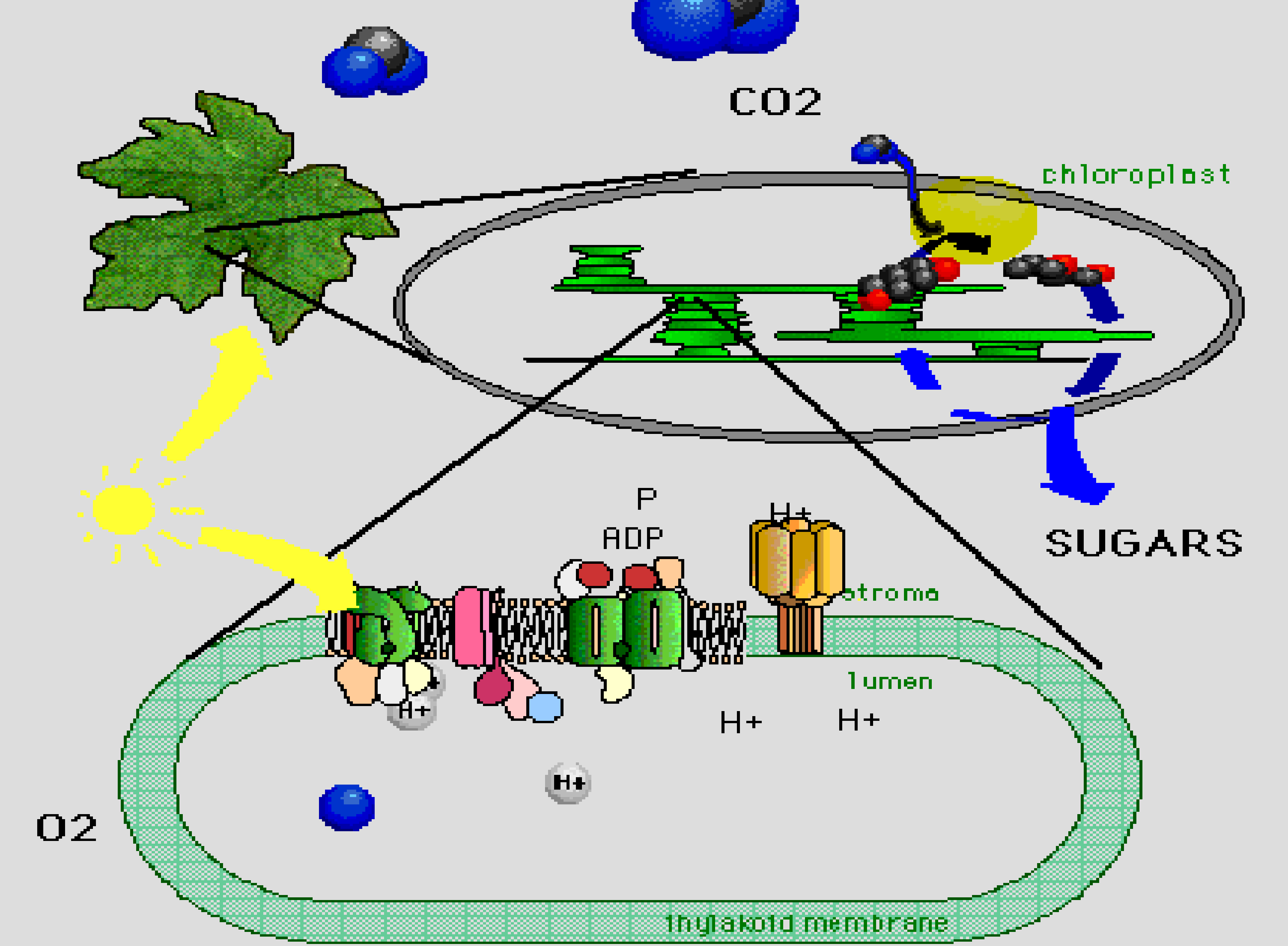
PHOTOSYNTHESIS
L-Lyxose
L-Ribulose
L-Ribulose-5-P
5.1.3.4
1.1.1.9
2.7.1.15
2.7.1.17
D-Xylulose
D-Xylu
D-Ribo
PHOTO-SYSTEM II
PHOTO-SYSTEM I
Cyclic Photophosphorylation
Non-cyclic electron flow
(electric current)
Ferredoxin
2H+
2e-
QB
QA
Pheophytin
P680
Chl.a
Mn
PQ
PQH2
2PQH2
2PQ
Cyt bf
Cyt bc
Fe-S
Cyt.f
PC
4H+
Fe-S
A1
Chl.A0
P700
H2O
O2
H+
Translocated protons
Protons from Water
THYLAKOID LUMEN
THYLAKOID MEMBRANE
STROMA
CHLOROPLAST OUTER MEMBRANE
NADP+
NADPH+H+
Pi
ADP
ATP
ATP synthase
3.6.1.34
Ribulose-1,5-bis-P
CO2 Fixation
COO-
HO

Photosynthesis

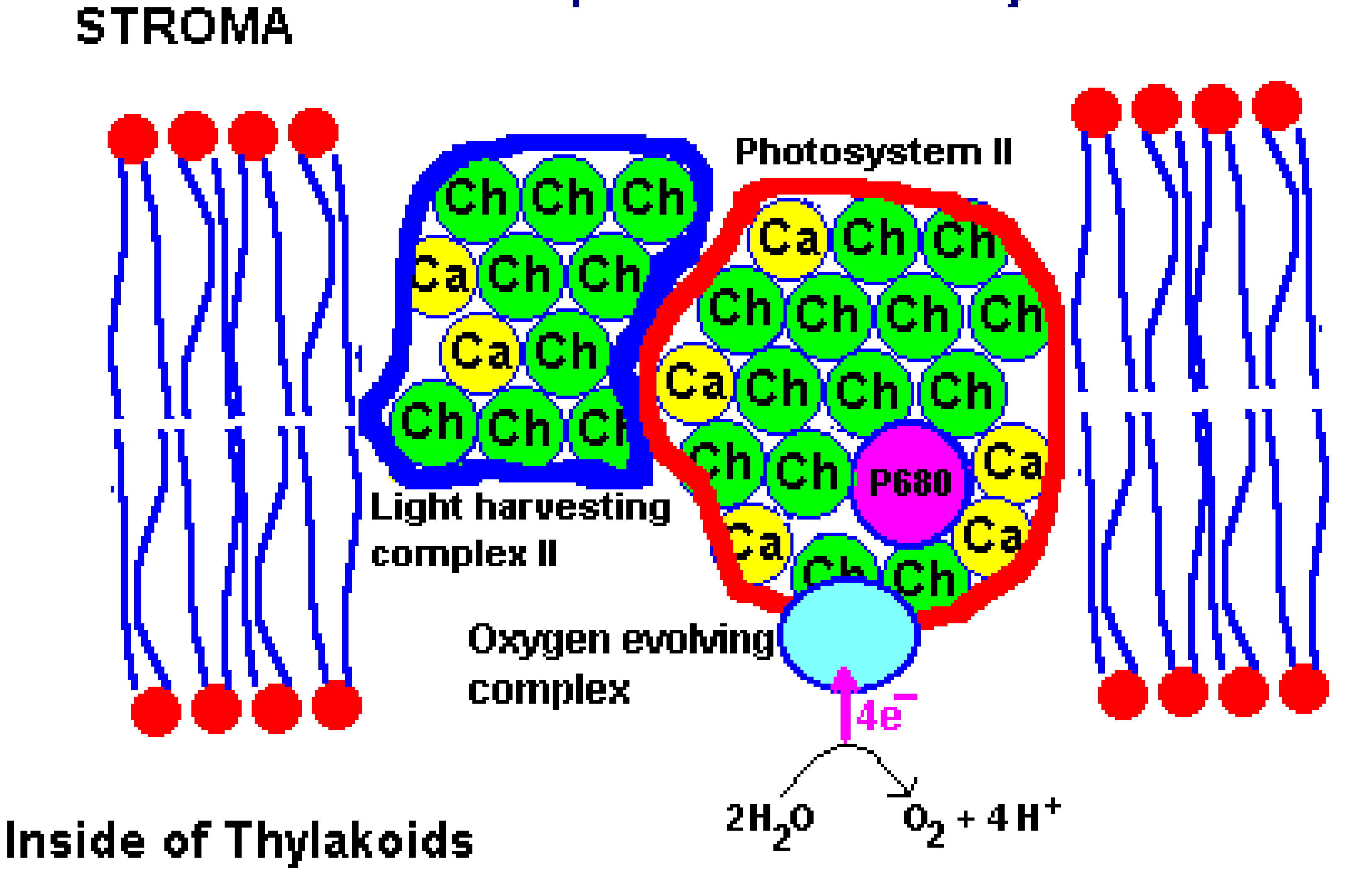


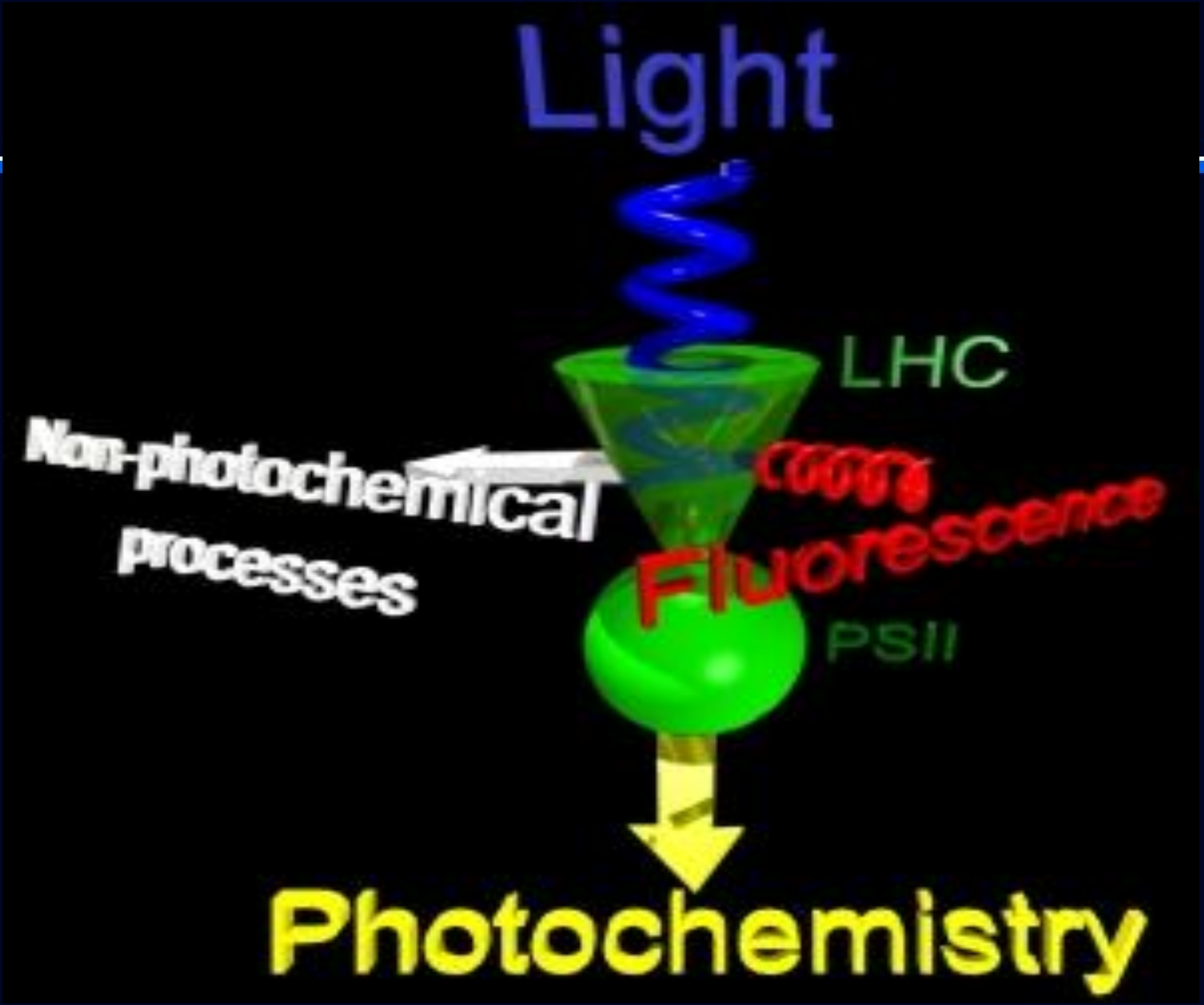
$$6\,H_2O + 6\,CO_2 + n\,h\nu \rightarrow C_6H_{12}O_6 + 6\,O_2$$

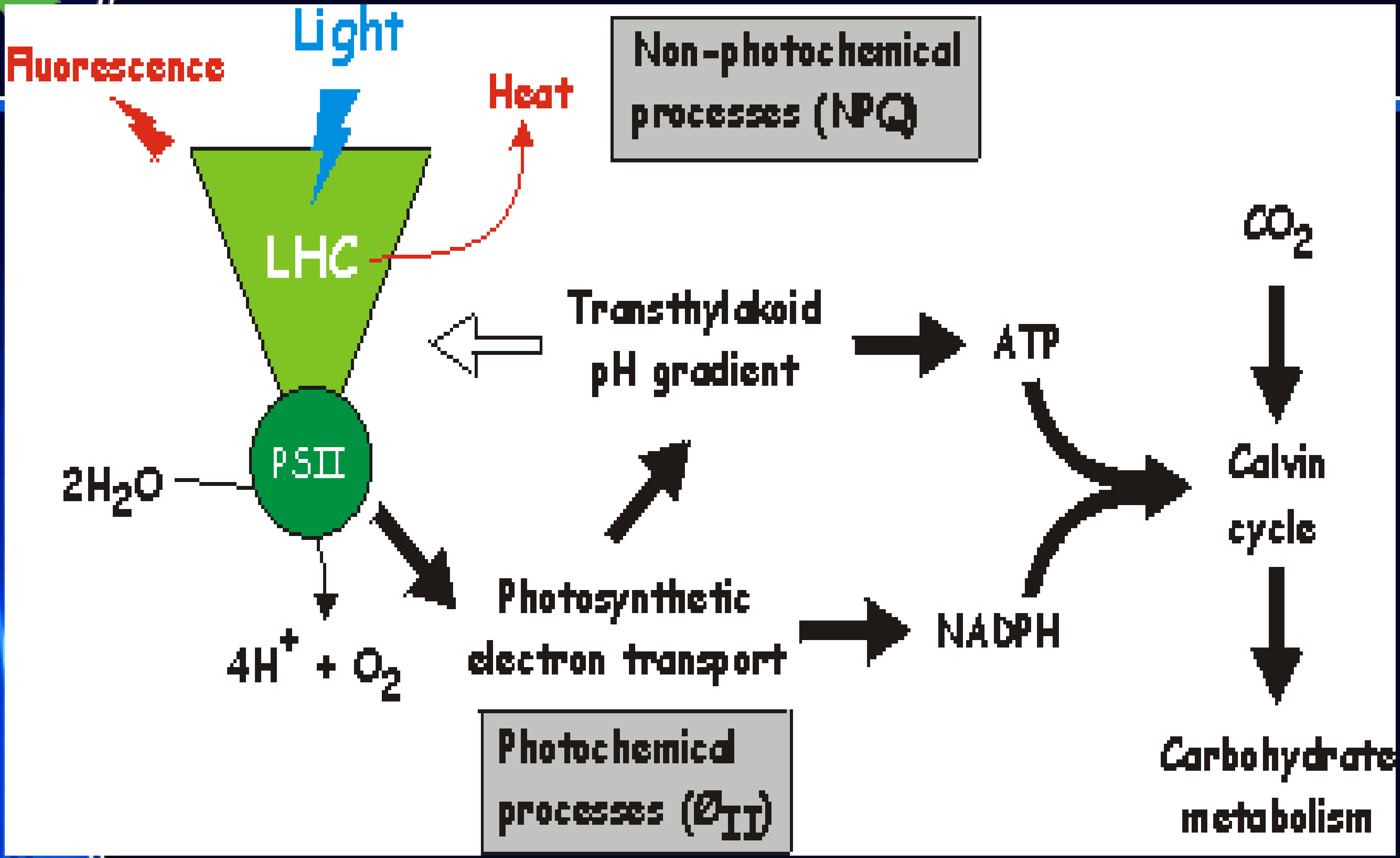



CO2
chloroplast
P
ADP
H+
SUGARS
stroma
lumen
H+
H+
H+
H+
O2
thylakoid membrane

Components of Photosystem II

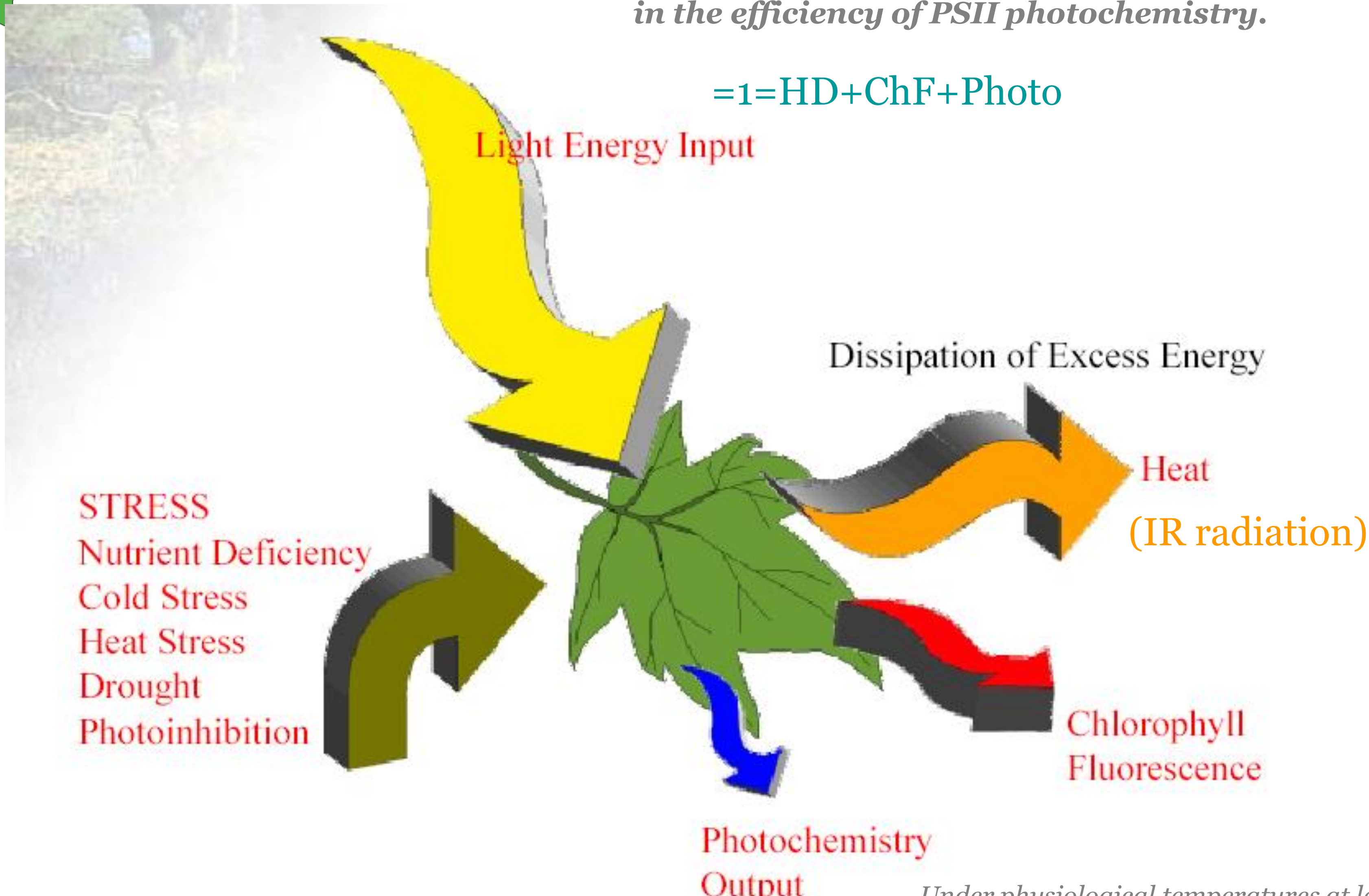



Light
LHC
Non-photochemical
processes
Fluorescence
PSII
Photochemistry



Fluorescence
Light
Heat
Non-photochemical processes (NPQ)
LHC
PSII
$2H_2O$
$4H^+ + O_2$
Transthylakoid pH gradient
ATP
CO_2
Calvin cycle
Photosynthetic electron transport
NADPH
Photochemical processes ($\varnothing_{II}$)
Carbohydrate metabolism

Fluorescence is re-emitted red / far red light (mostly from PSII). Changes in efficiency of fluorescence emission inversely relate to changes in the efficiency of PSII photochemistry.

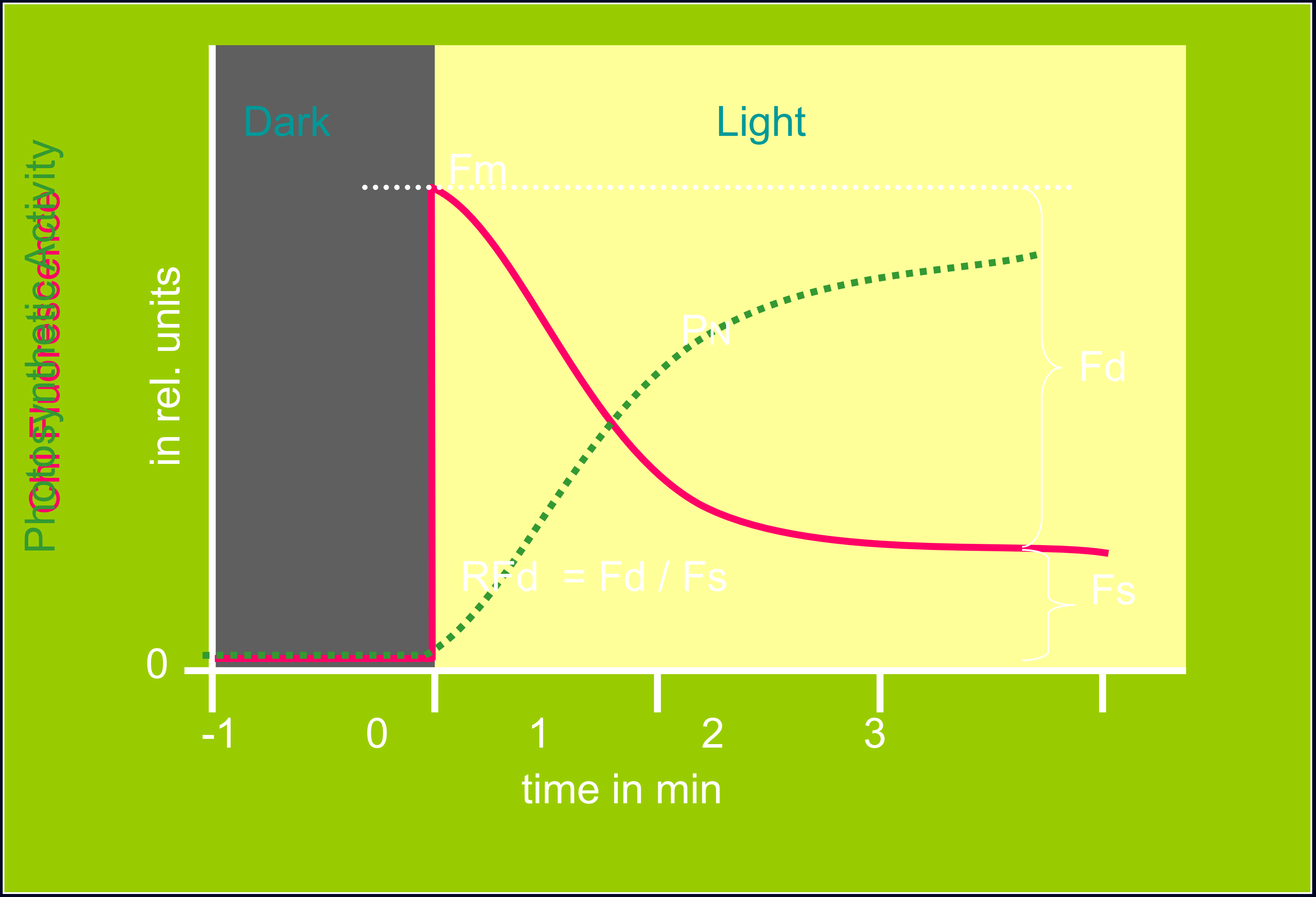


*Under physiological temperatures at least **95%** of the fluorescence emission is derived from chlorophyll associated with photosystem II.*

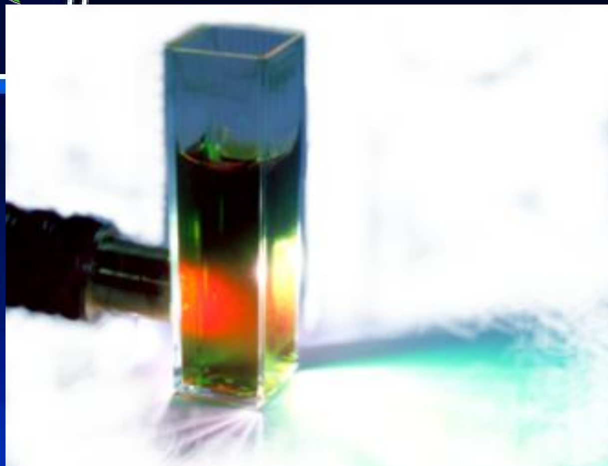
http://bioenergy.asu.edu/photosyn/education/experiments/protein_exp/rcreq4.htm

Fluorescence and Photosynthesis

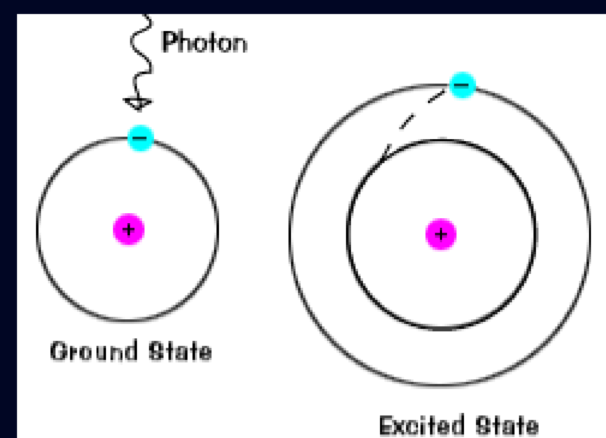
(Kautsky-Effect)

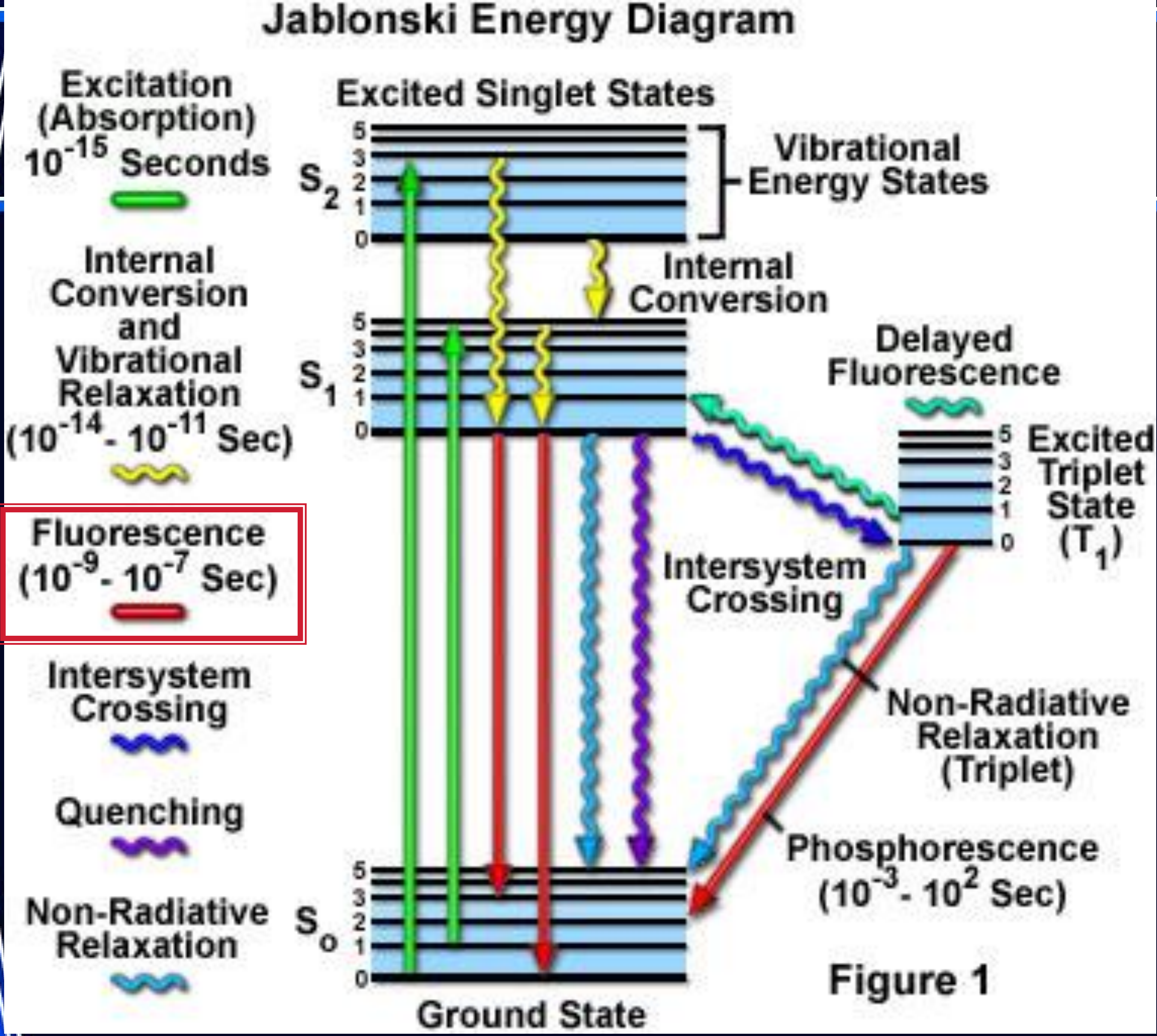


What is fluorescence ?



When light strikes chlorophyll molecules, absorbed quantum raises an electron from a ground state to an excited state. Upon chlorophyll de-excitation to a ground state, a small portion of the excitation energy is dissipated as red fluorescence (690 nm).





Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Internal Conversion and Vibrational Relaxation (10^{-14} - 10^{-11} Sec)
Fluorescence (10^{-9} - 10^{-7} Sec)
Intersystem Crossing
Quenching
Non-Radiative Relaxation
Excited Singlet States
S_2
S_1
S_0
Vibrational Energy States
Internal Conversion
Delayed Fluorescence
Excited Triplet State (T_1)
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Phosphorescence (10^{-3} - 10^{2} Sec)
Ground State
Figure 1

Chlorophyll fluorescence is a **re-emission of light energy** absorbed by chlorophyll molecules.

UNITS:

relative (r.u.)

arbitrary (a.u.)

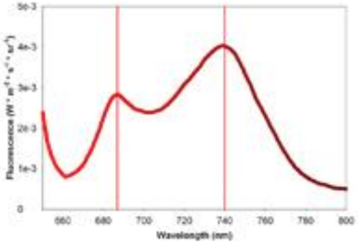
mv

II. Methodological Approach

Spectroradiometer (ASD FieldSpec Pro)

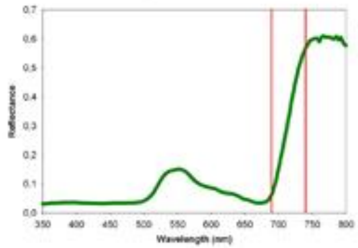
- Chlorophyll Fluorescence Emission Spectrum
  - Red to Far-red Ratio (RF/FRF)

Fig.1:
ChlF - spectrum with 2 maxima at 690 nm and 740 nm.
(*N. tabacum*)



- Leaf Reflectance Spectrum
  - Vegetation Indices e.g. Chlorophyll content

Fig.2:
Typical reflectance spectrum with green peak around 550 nm.
(*A. thaliana*)



Nicotiana tabacum var 'Samsun'

- Advantages:
  - broad leaves
  - fast response to stress factors
  - many different mutants available

Cold Light Source (Schott 2500 LCD)

- Short Pass Filter cut-off wavelengths over 650 nm for better ChlF determination

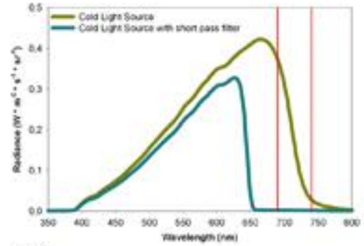
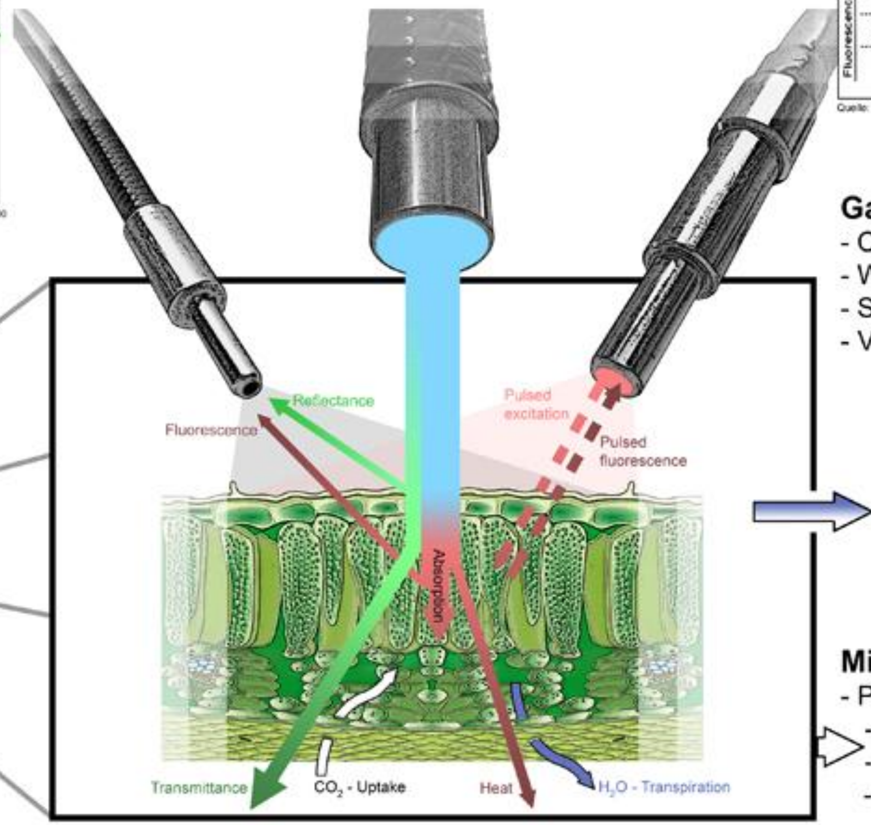
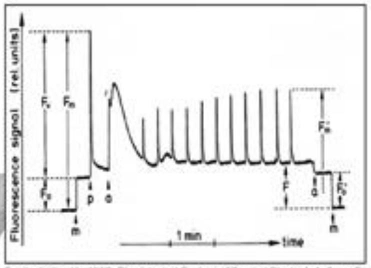


Fig.3:
Light emission spectra of the cold light source.
Yellow line (unfiltered), Cyan line (with filter)



PAM Fluorometer (Walz, Mini-PAM)

- Dark adapted leaf
  - Potential quantum efficiency (Fv/Fm)
- Light adapted leaf
  - Actual quantum efficiency (ΔF/Fm')
  - Electron Transport Rate (ETR)
  - Non-Photochemical Quenching (NPQ)



Quelle: Lüttge U. (1997) *Physiological Ecology of Tropical Plants*. 1. Auflage, Box 3-6

Fig.4:
Overview of common used parameters in PAM fluorometry.

Gas Exchange Analyzer (CMS-400)

- Carbondioxid flux (JCO_2)
- Water vapor flux (JH_2O)
- Stomatal conductance (g_s)
- Vapor pressure deficit (VPD)

Mini-Cuvette with climate modul

- Precise regulation and measurement of
  - Air temperature
  - Leaf temperature
  - Relative humidity

André Moersch, http://www2.fz-juelich.de/icg/icg-3/Mitarbeiter/Moersch

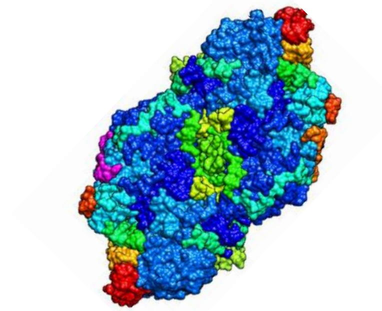
100%

Photosynthesis

Fluorescence
+
Heat

Pulse = 60-80/minute

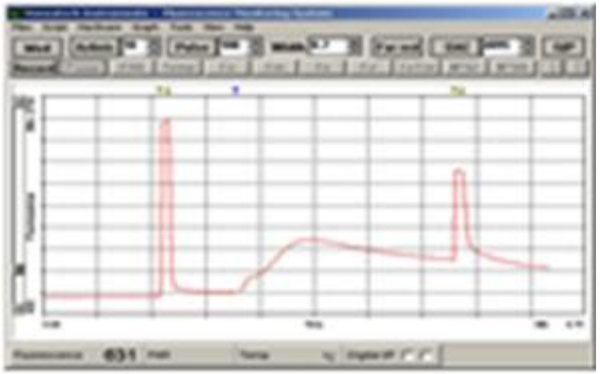
Maximal quantum yield
Fv/Fm = 0.83-0.85 r.u.



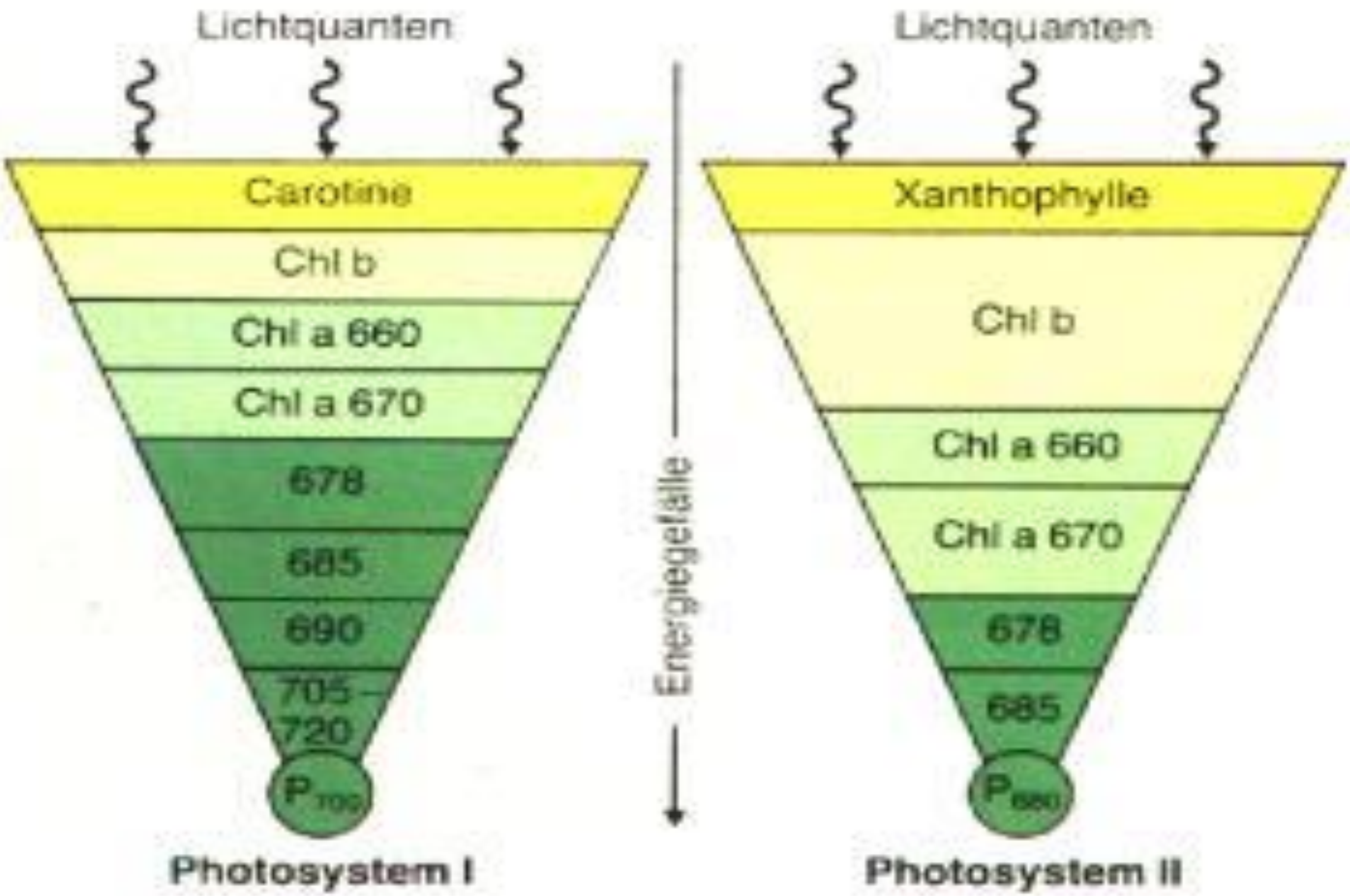
Stethoscope

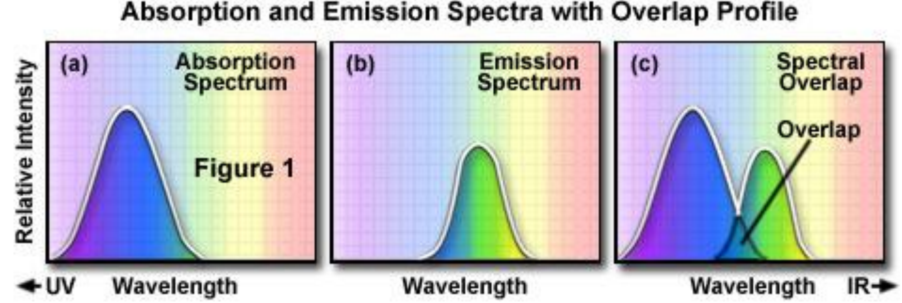
Fluorometer

Electrocardiograph

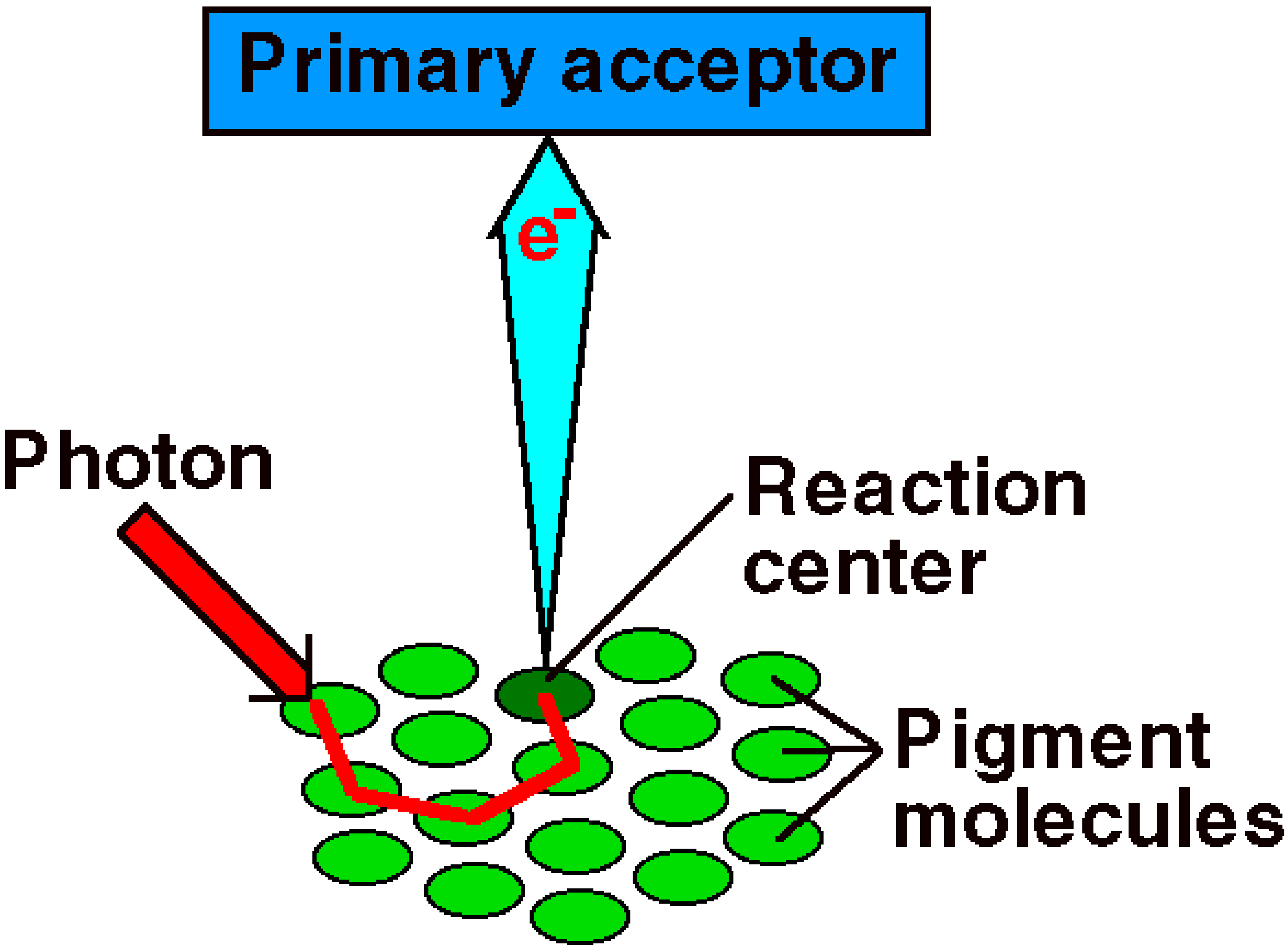


Ch F curve


Lichtquanten
Carotine
Chl b
Chl a 660
Chl a 670
678
685
690
705 –
720
P700
Photosystem I
Energiegefälle
Lichtquanten
Xanthophylle
Chl b
Chl a 660
Chl a 670
678
685
P680
Photosystem II

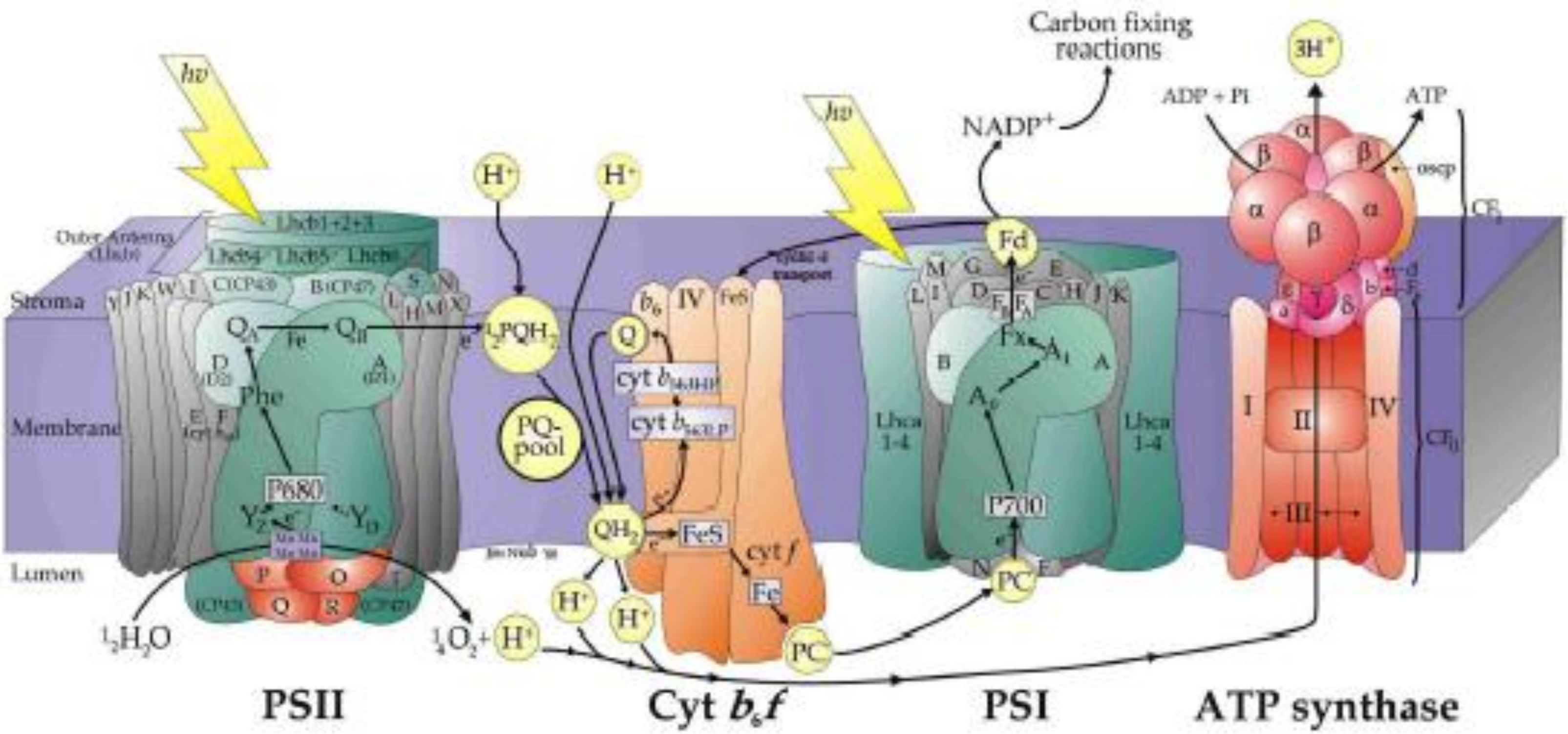


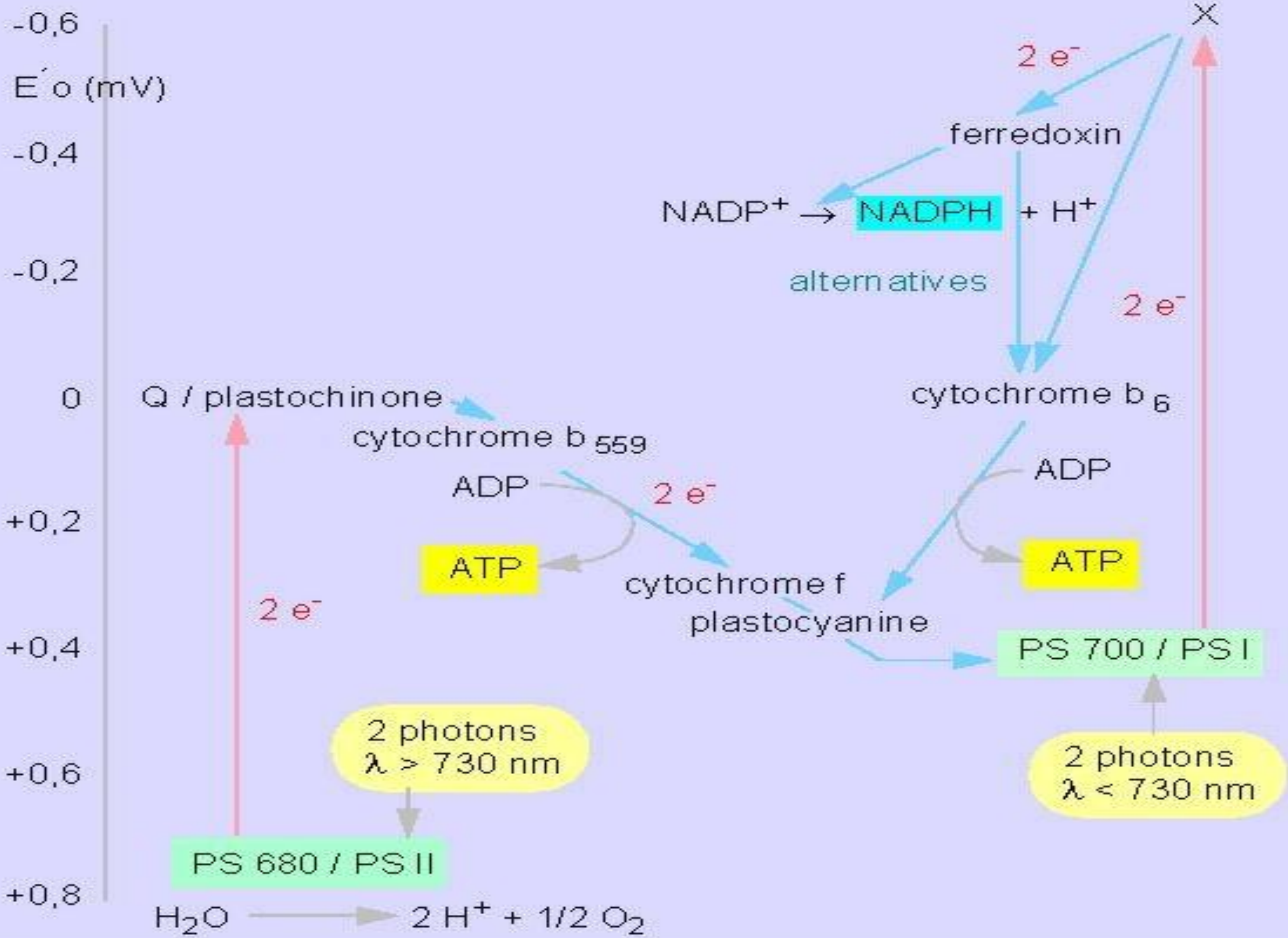
Absorption and Emission Spectra with Overlap Profile
(a)
Absorption Spectrum
Figure 1
(b)
Emission Spectrum
(c)
Spectral Overlap
Overlap
Relative Intensity
UV
Wavelength
Wavelength
Wavelength
IR

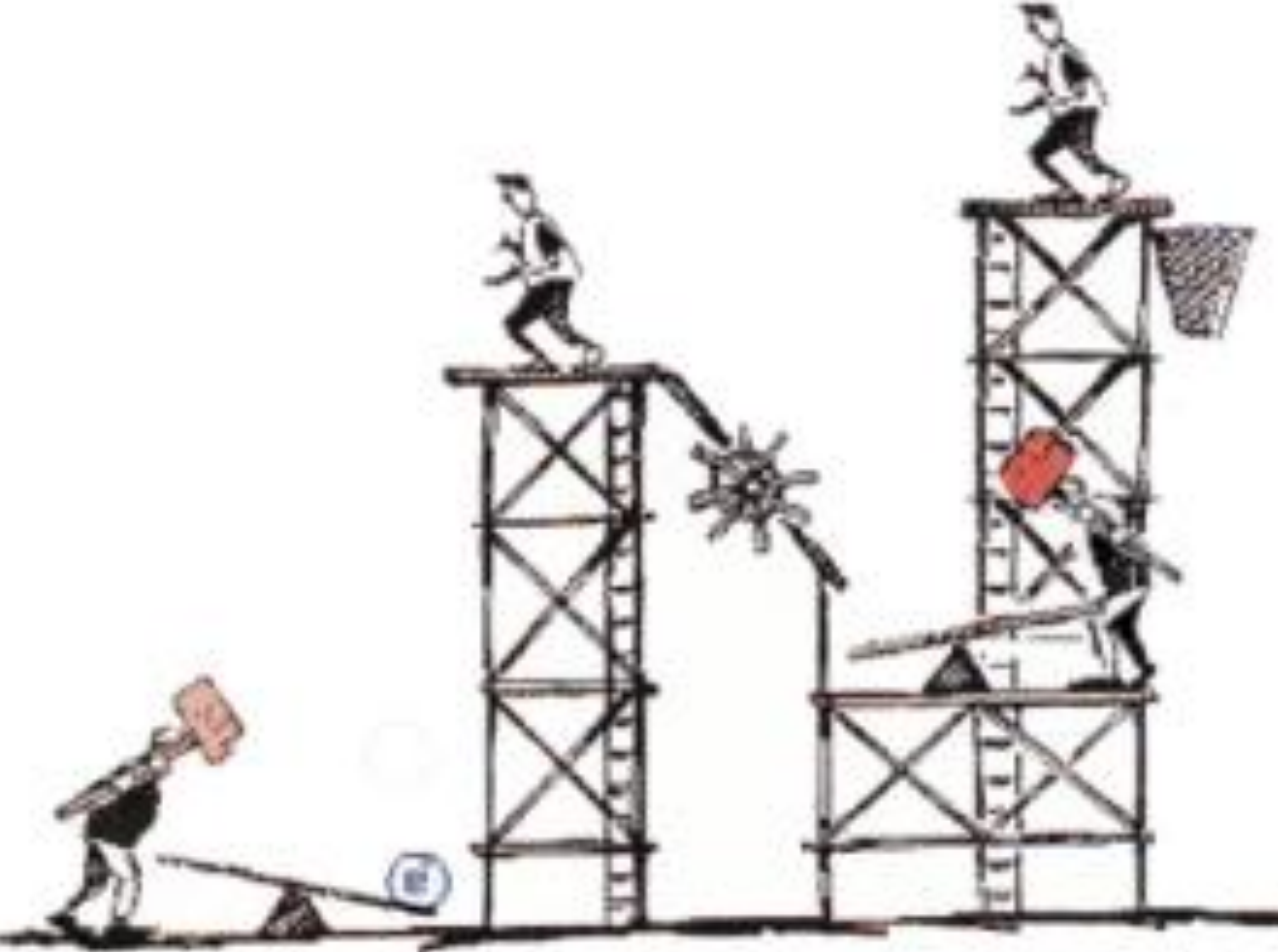


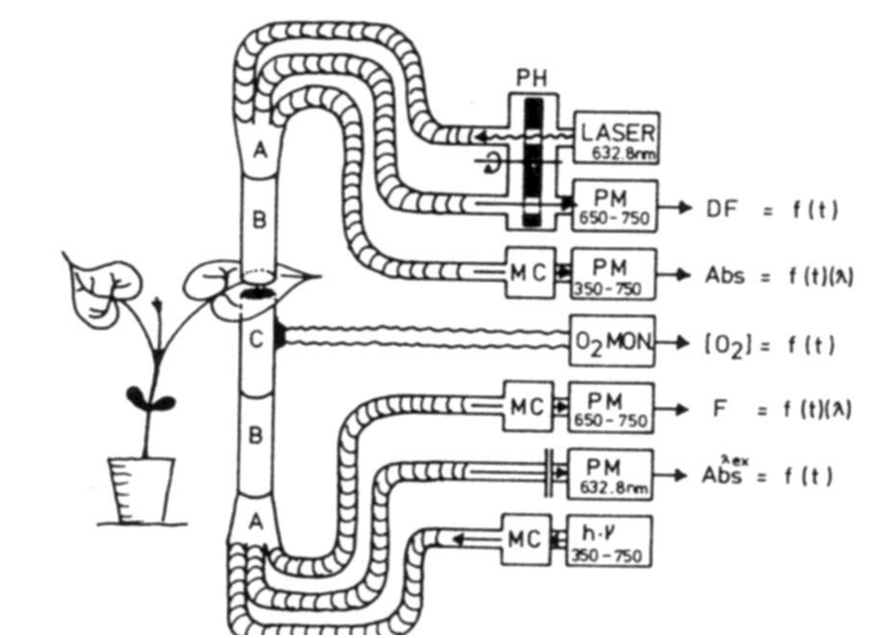
Primary acceptor
e-
Photon
Reaction center
Pigment molecules

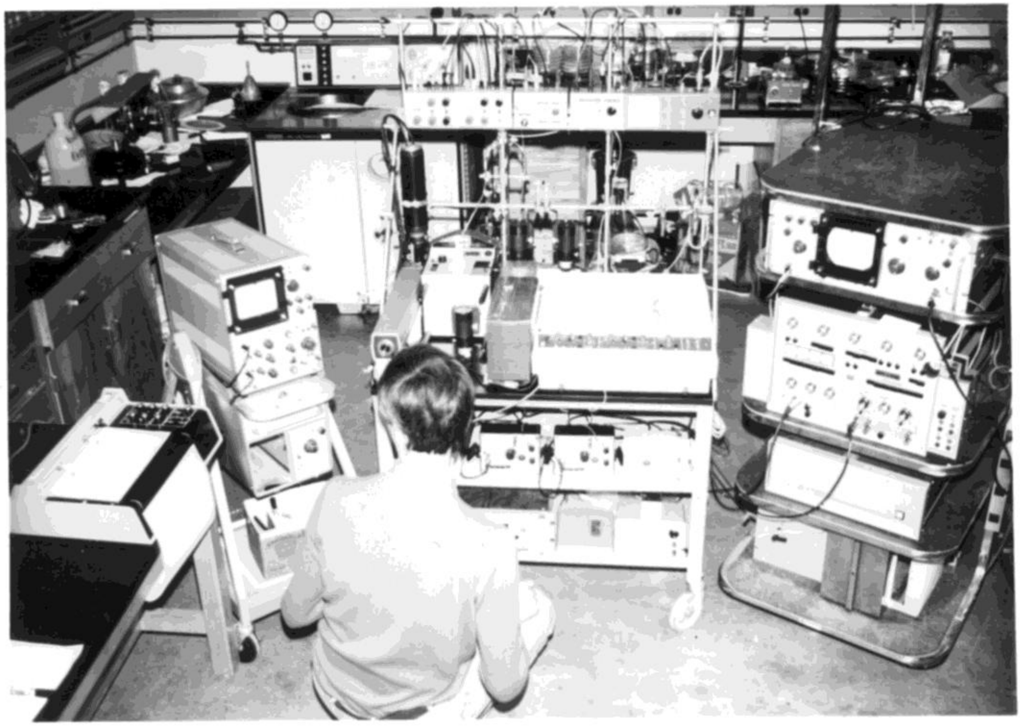
...embedded in a membrane.

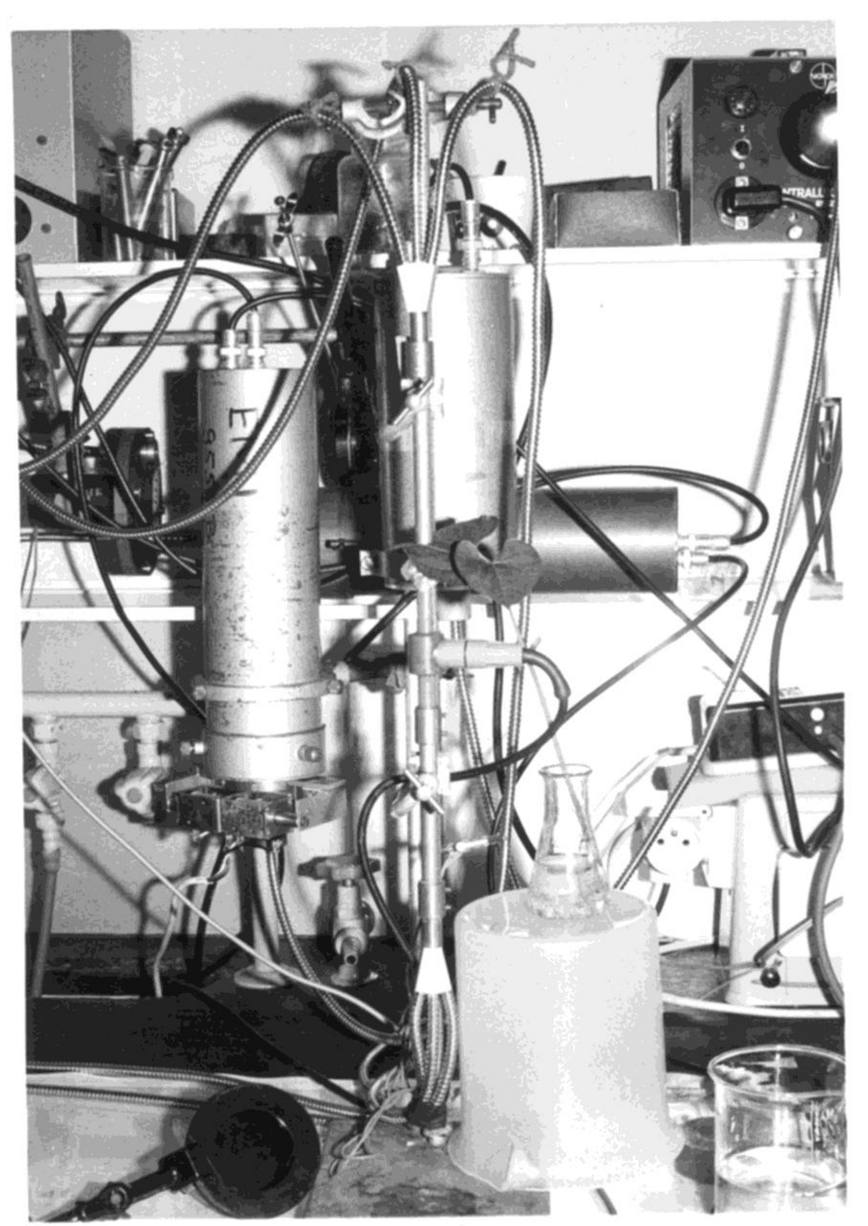



-0,6
E´o (mV)
-0,4
-0,2
0
+0,2
+0,4
+0,6
+0,8
X
2 e⁻
ferredoxin
NADP⁺ → NADPH + H⁺
alternatives
2 e⁻
cytochrome b 6
Q / plastochinone
cytochrome b 559
ADP
2 e⁻
ATP
cytochrome f
plastocyanine
ADP
ATP
2 e⁻
PS 700 / PS I
2 photons
λ > 730 nm
2 photons
λ < 730 nm
PS 680 / PS II
H2O → 2 H⁺ + 1/2 O2



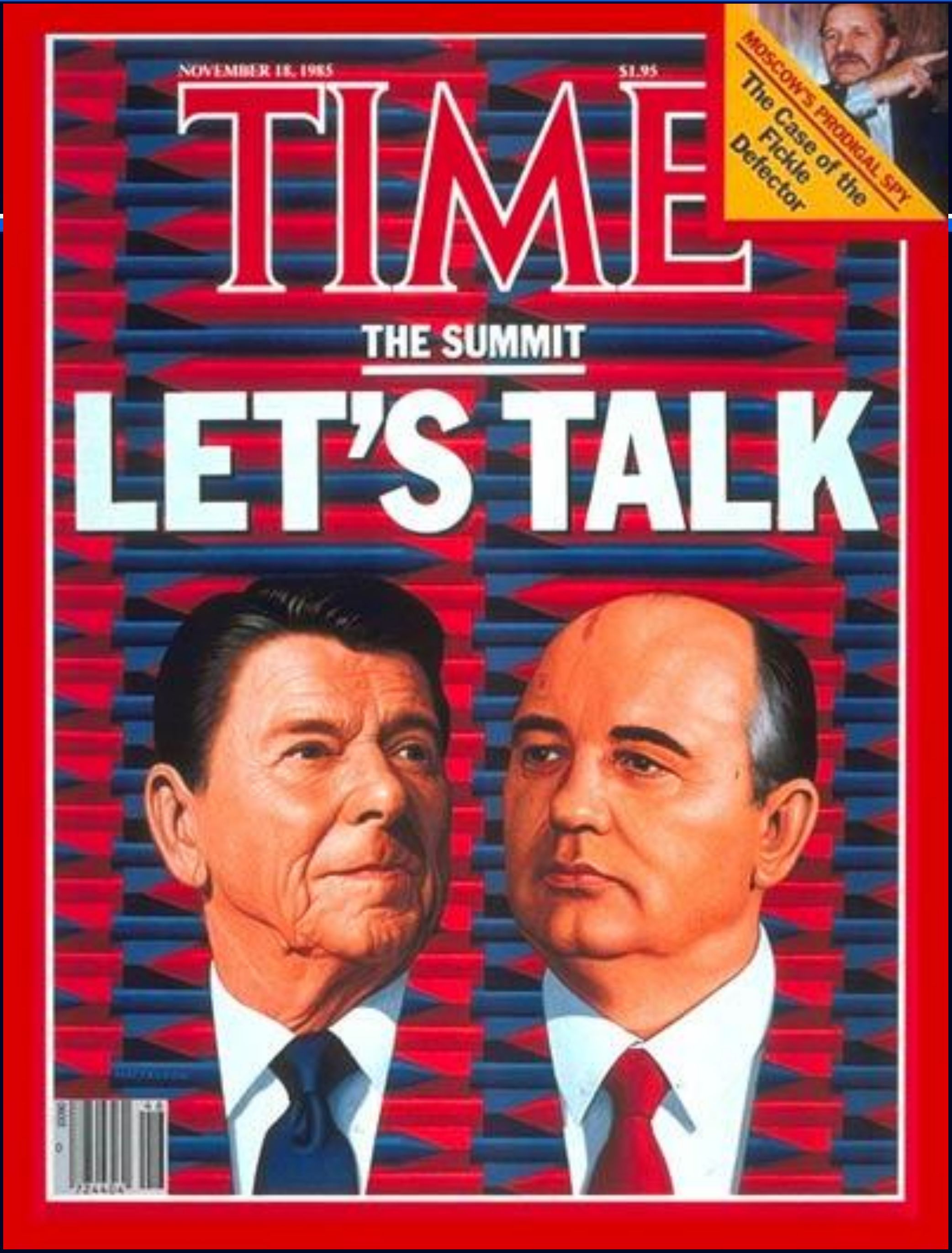

PH
LASER 632.8nm
PM 650-750
DF = f(t)
MC
PM 350-750
Abs = f(t)(λ)
O2 MON
[O2] = f(t)
MC
PM 650-750
F = f(t)(λ)
PM 632.8nm
Abs^λex = f(t)
MC
h·ν 350-750
A
B
C
B
A






ЧС 1100 универсален брояч 80MHz
LINEARVERSTARKER-ANALYSATOR VA-V-100
HOCHSPANNUNG


7 Fr 87 Ra 88 Ac 89 Ku 104 105
U 92
лантаноиды
Ce 58 Pr 59 Nd 60 Pm 61 Sm 62 Eu 63 Gd 64 Tb 65 Dy 66 Ho 67 Er 68 Tm 69 Yb 70 Lu 71
актиноиды
Th 90 Pa 91 U 92 Np 93 Pu 94 Am 95 Cm 96 Bk 97 Cf 98 Es 99 Fm 100 Md 101 (No) 102 (Lr) 103
DAEWOO


NOVEMBER 18, 1985
$1.95
TIME
MOSCOW'S PRODIGAL SPY
The Case of the Fickle Defector
THE SUMMIT
LET'S TALK

Fluorometer (*Stress meter*)

Continuous Excitation Fluorescence Measurement System

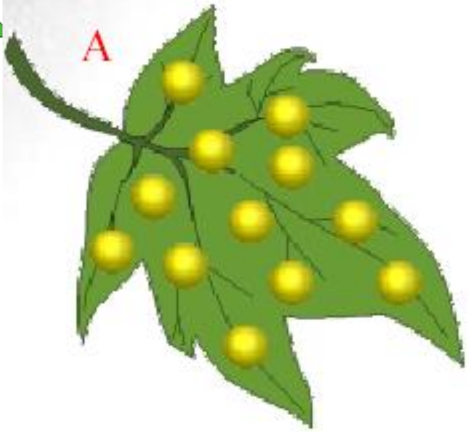
Pulse Modulated Fluorescence Measurement System

Which technique to be used ??

- After dark adaptation

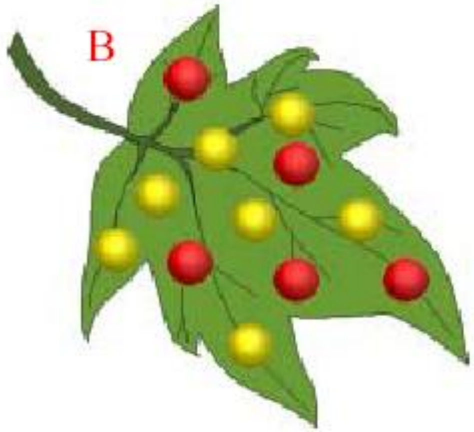
- After light adaptation

km/h
x1000r/min
TARMAC
GRAVEL
SNOW
H
F
E
ANCER



Dark Adapted State.

All electron acceptors fully oxidised & available to receive light energy.

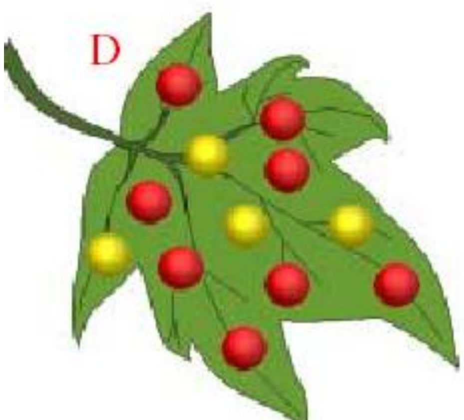


Intermediate State.

Some electron acceptors Reduced by light & no longer available for photochemistry.

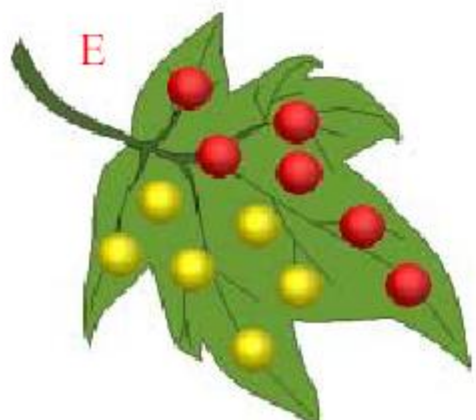
Light Saturated State.

All electron acceptors reduced by light & no longer available for photochemistry.



Quenching State.

Re-reduction of electron acceptors occurs as energy proceeds to photochemistry, acceptors are again available for photochemistry.



Steady State.

Equilibrium is established between energy input / dissipation processes and photochemistry.

Oxidised electron acceptors

Reduced electron acceptors

After dark adaptation

Fo - fluorescence level when plastoquinone electron acceptor pool (Qa) is fully oxidised.

Fm - fluorescence level when Qa is transiently fully reduced.

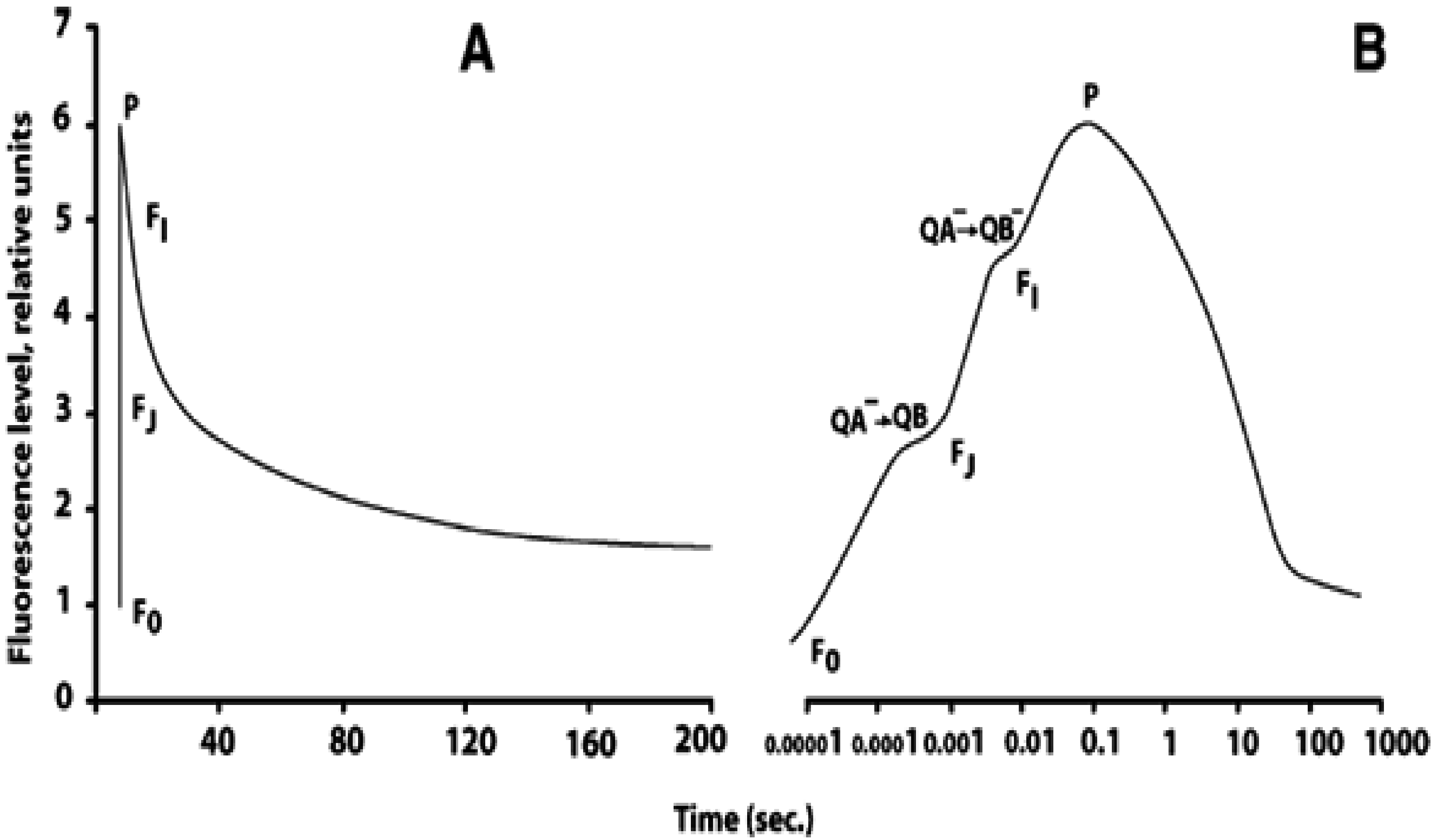
Fv - variable fluorescence (Fm-Fo).

Fv/Fm - maximum quantum efficiency of Photosystem II.

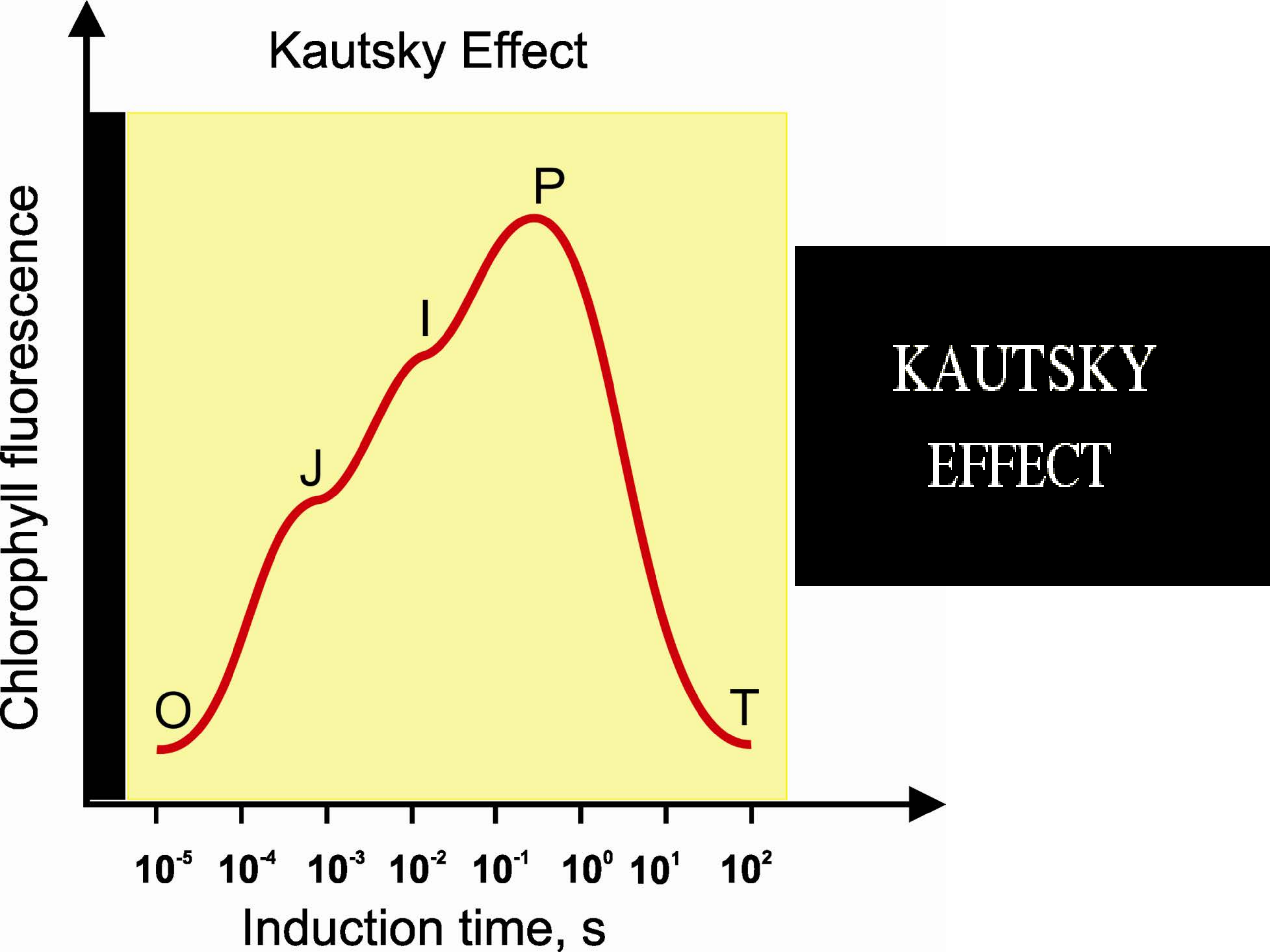
Tfm - time at which Fm occurs.

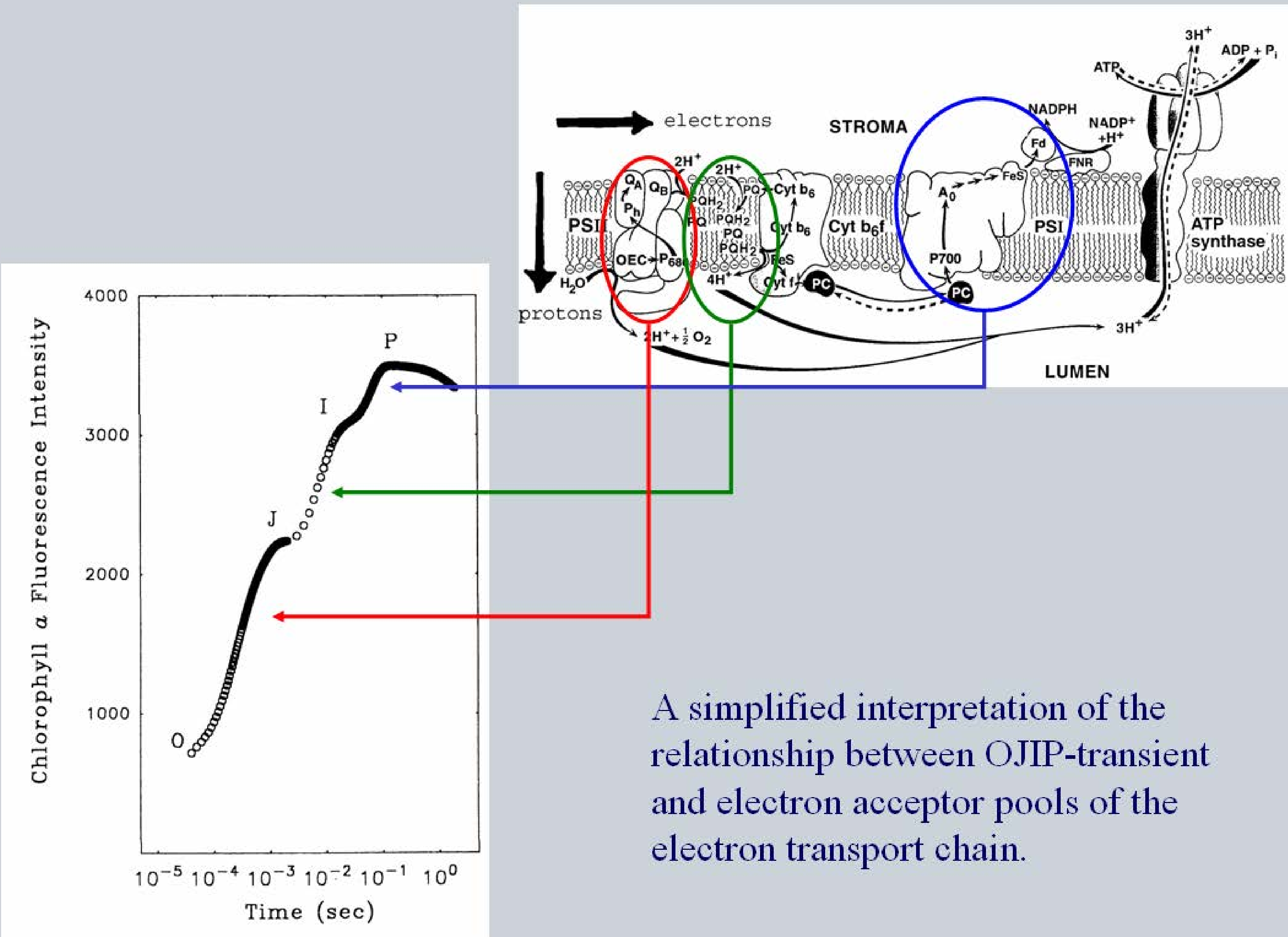
Area - area over the curve between Fo and Fm, relates to the pool size of PSII electron transport acceptors.

OJIP analysis (Strasser R.J., Srivasatava A. and Govindjee,1995 Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria, Photochemistry and Photobiology, 61, 32-34.).



A
B
Fluorescence level, relative units
7
6
5
4
3
2
1
0
P
F_I
F_J
F_0
40
80
120
160
200
$Q_A^- \rightarrow Q_B^-$
$Q_A^- \rightarrow Q_B$
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Time (sec.)


Kautsky Effect
Chlorophyll fluorescence
P
I
J
O
T
10^-5 10^-4 10^-3 10^-2 10^-1 10^0 10^1 10^2
Induction time, s
KAUTSKY
EFFECT



A simplified interpretation of the relationship between OJIP-transient and electron acceptor pools of the electron transport chain.

JIP-Test fluxes of R.J. STRASSER, placed into any Z-Scheme

PS II **PS I**

ABS $_{total}$

β_{abs} α_{abs}

ABS2 ABS1

TR2 TR1

(P 680) (P 700)

(H_2O) (Mn) ED Thyr (RC II) (Q_A^-) ET (PQ) (Cyt bf) (PC) (RC I) ($A_o A_1$) RE (Fd^-) (NADP)

OE (b) (ROS) (a) (MV) CF

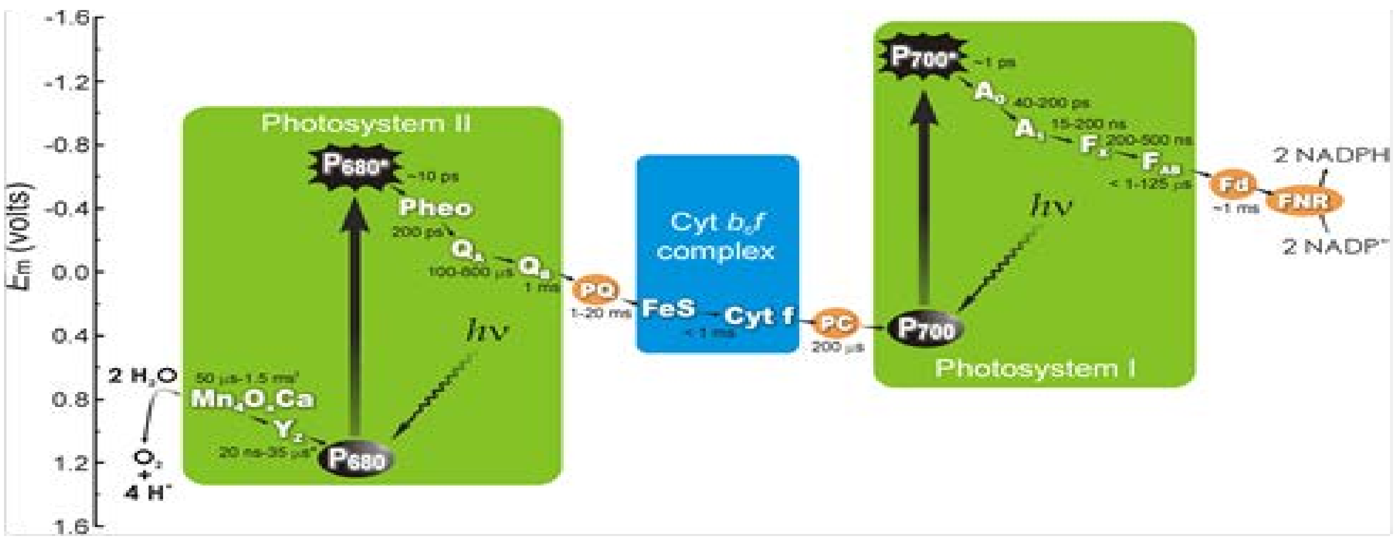
CE2 CE1

ABS =Absorption,

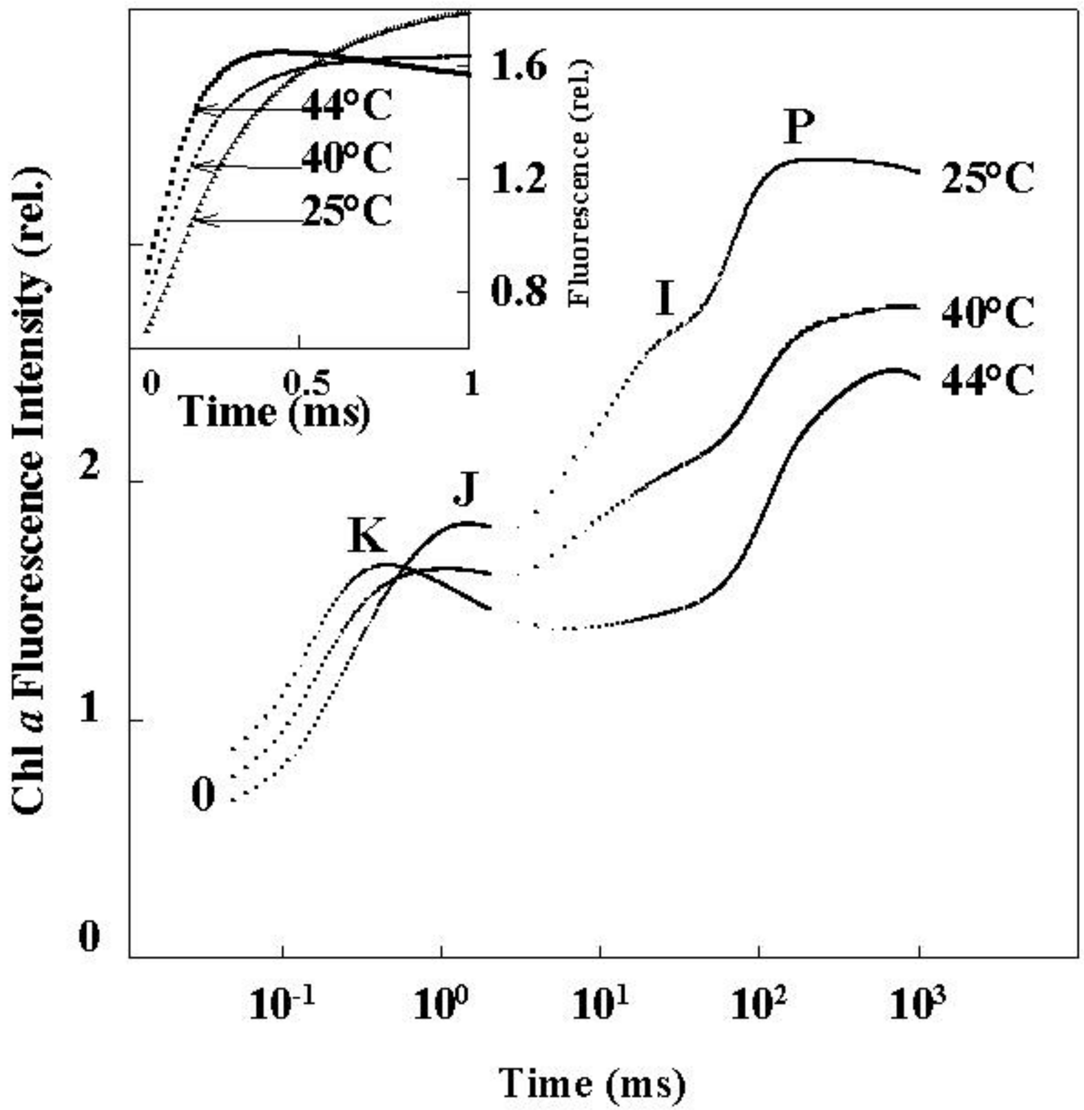
TR = Trapping,

ED=Electron Donation, ET = Electron Transport, RE=Reduction of End-acceptors

OE=Oxygen Evolution, CE =Cyclic Electron Transport CF= Carbon Fixation



The Z-Scheme (Courtesy of Dmitriy Shevela)

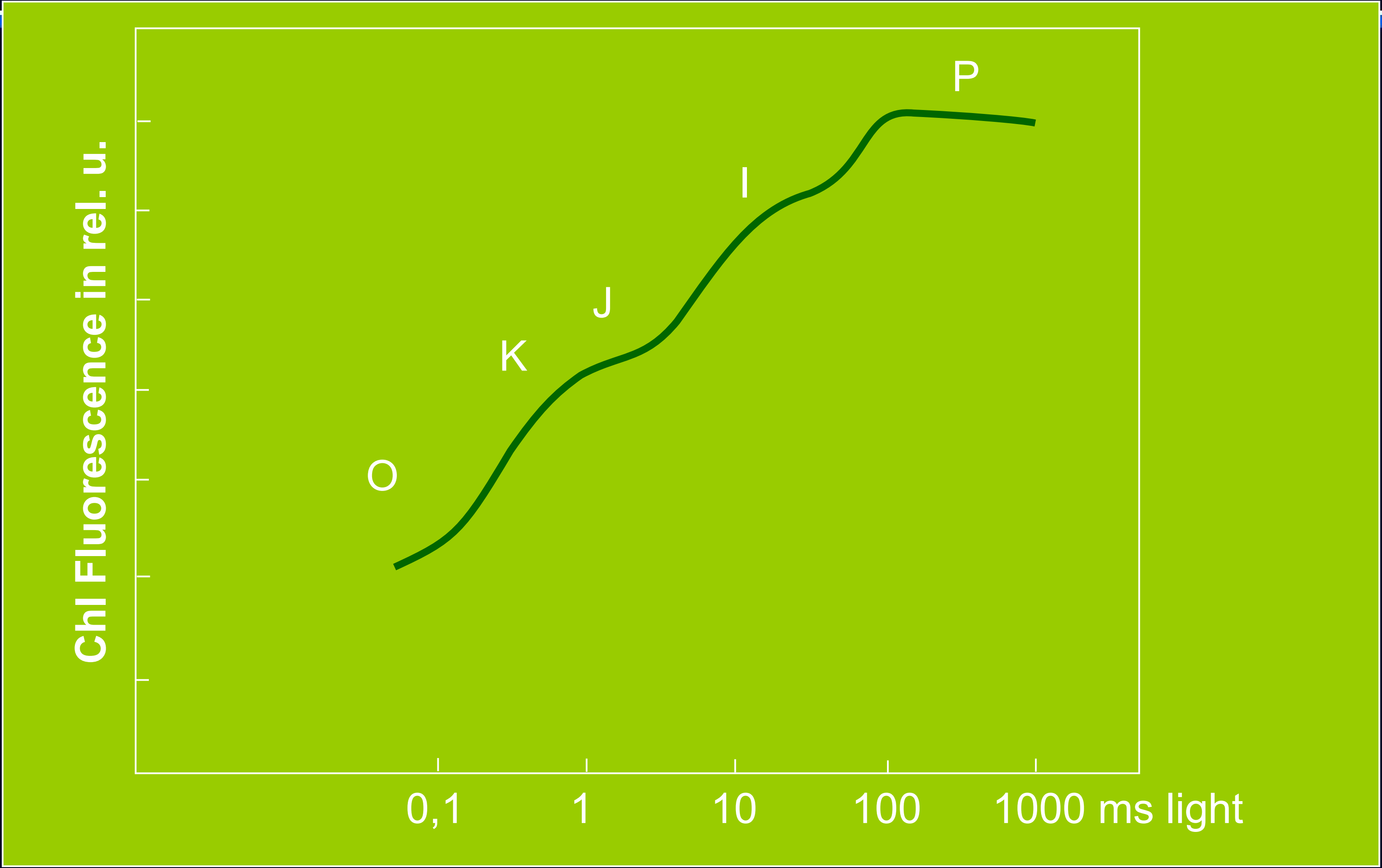


(O-J) phase corresponds to a complete reduction of the primary electron acceptor QA of PSII,

the release of fluorescence quenching during the (J-I) phase is controlled by the PSII donor side (water splitting activity).

(I-P) corresponds to the release of fluorescence quenching by the oxidised plastoquinone pool

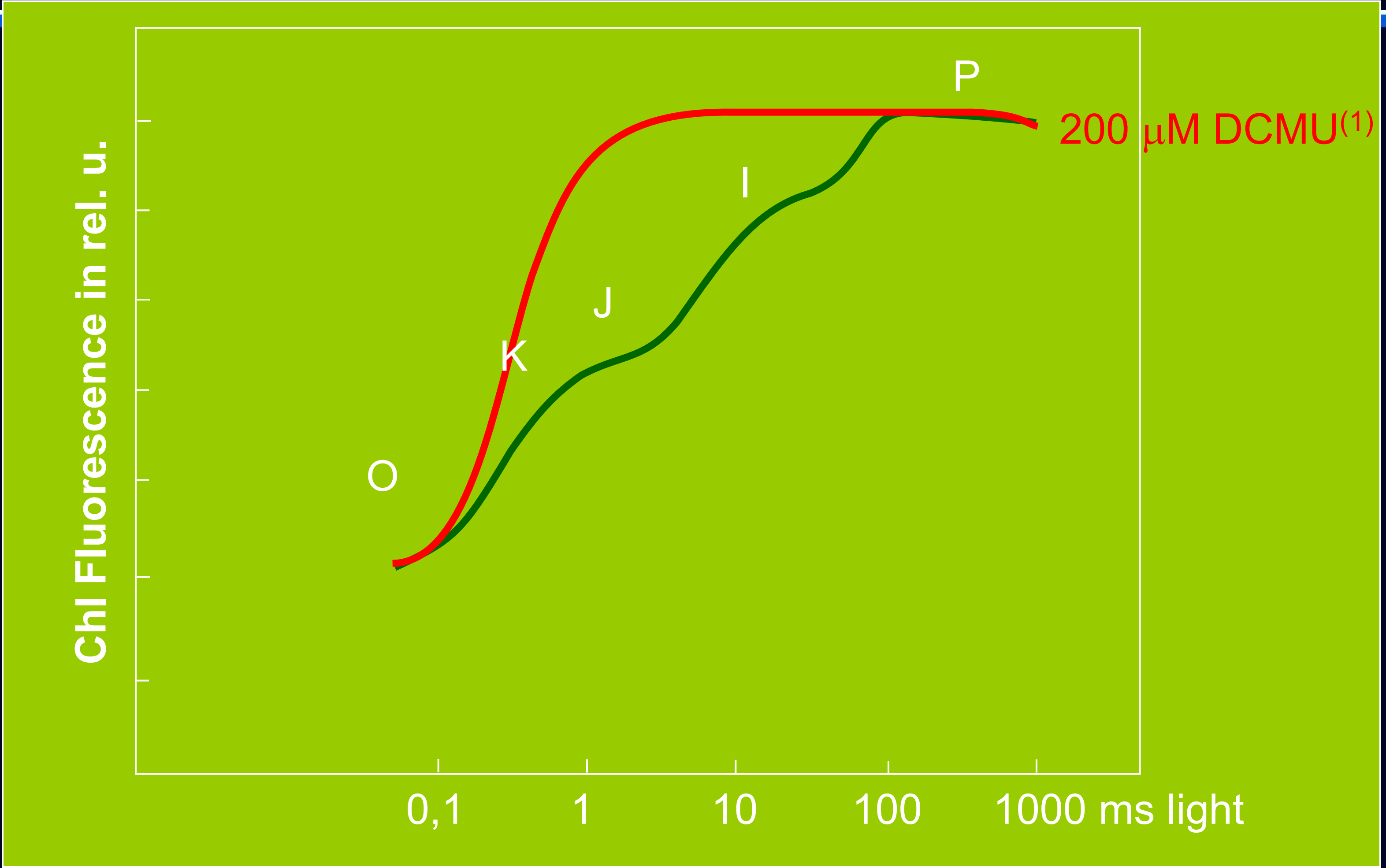
Fluorescence Rise



Srivastava A, Strasser RJ (1999) in: Crop Improvement for Food Security (Behl RK et al. eds.) SSARM, HISAR, pp 60-71

(1)Haldimann P, Strasser RJ (1999) Photosynthesis Research 62: 67-83

Fluorescence Rise



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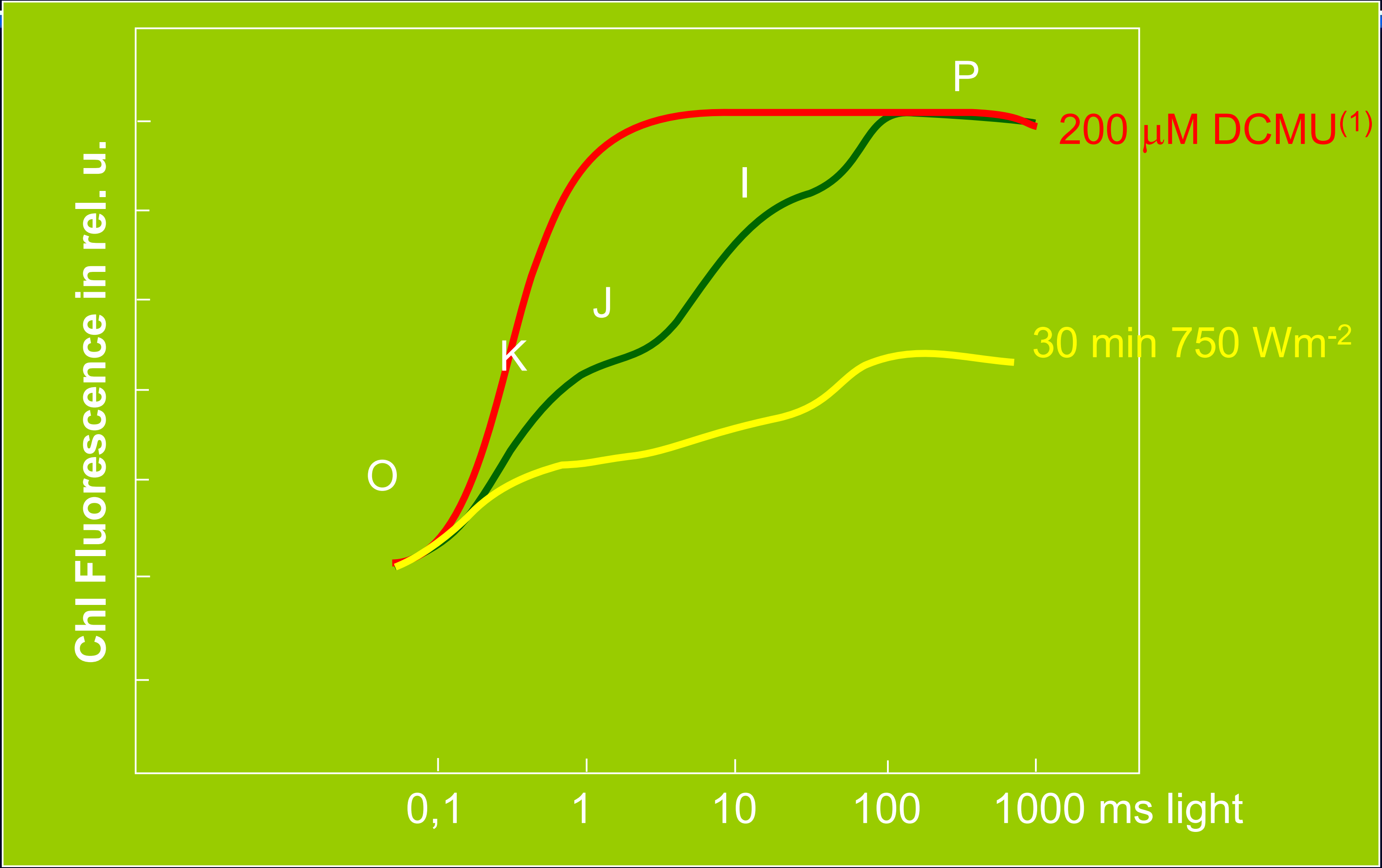
Fluorescence Rise



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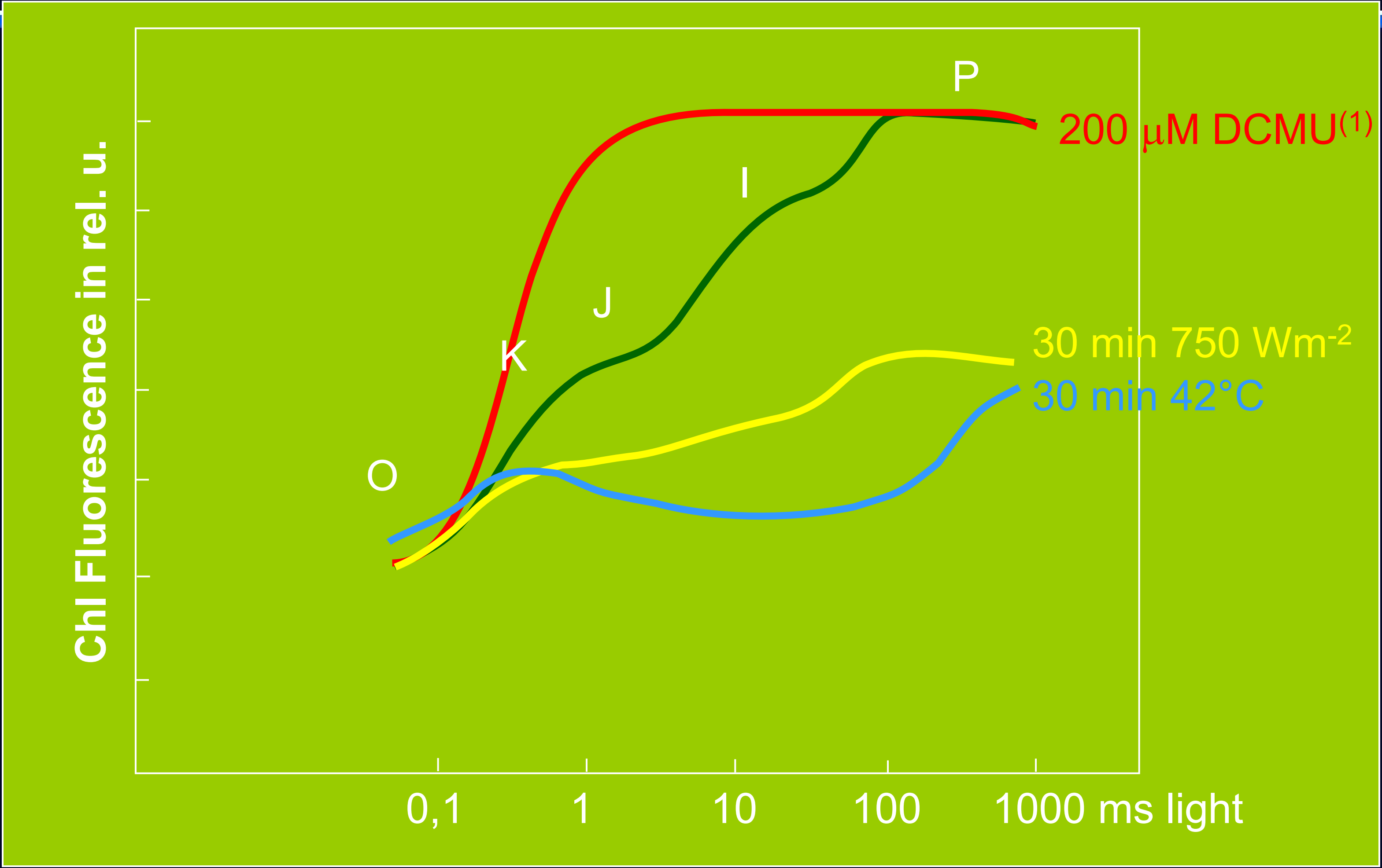
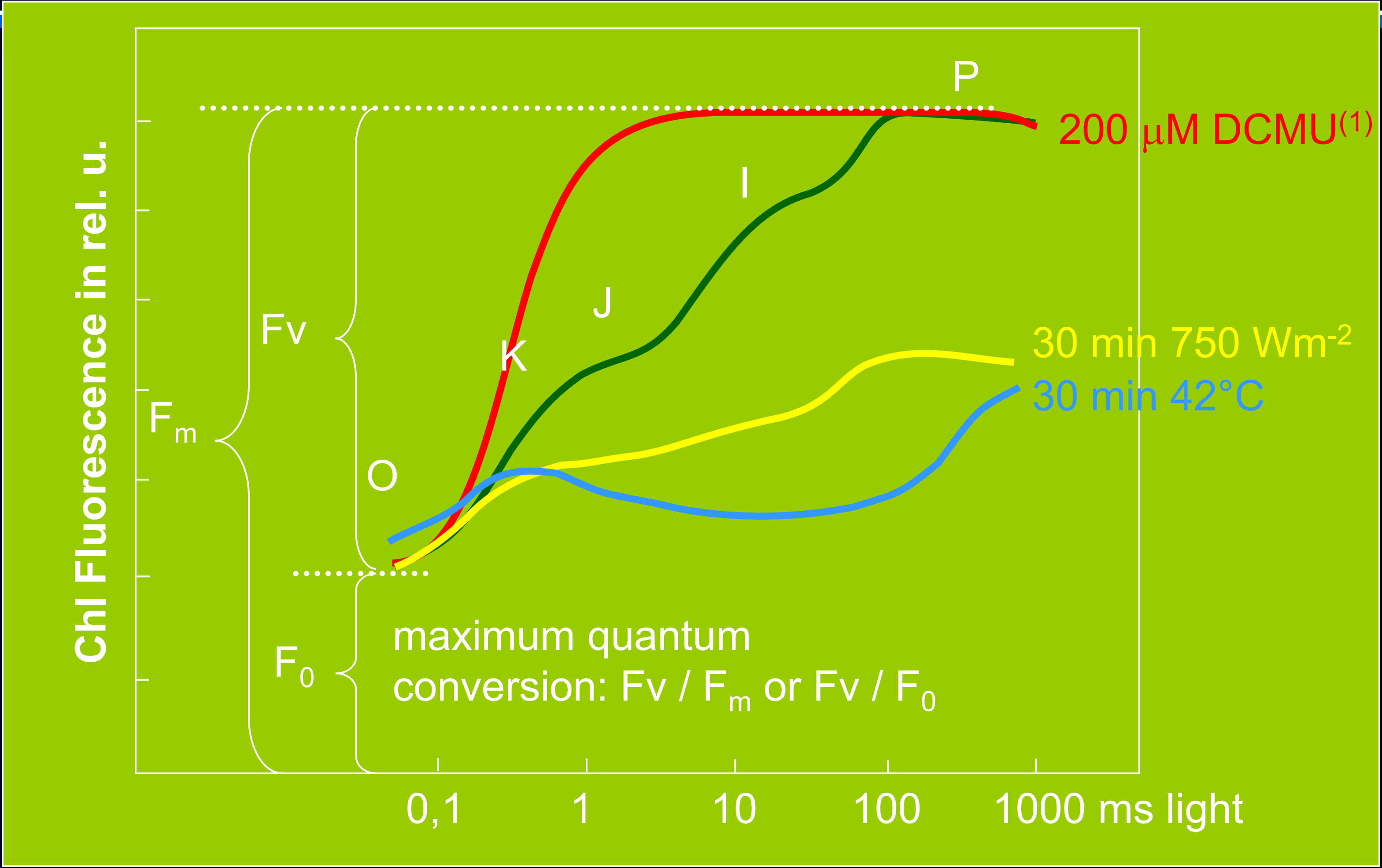
Fluorescence Rise



Srivastava A, Strasser RJ (1999) in: Crop Improvement for Food Security (Behl RK et al. eds.) SSARM, HISAR, pp 60-71

[1]Haldimann P, Strasser RJ (1999) Photosynthesis Research 62: 67-83

Fluorescence Rise



Srivastava A, Strasser RJ (1999) in: Crop Improvement for Food Security (Behl RK et al. eds.) SSARM, HISAR, pp 60-71

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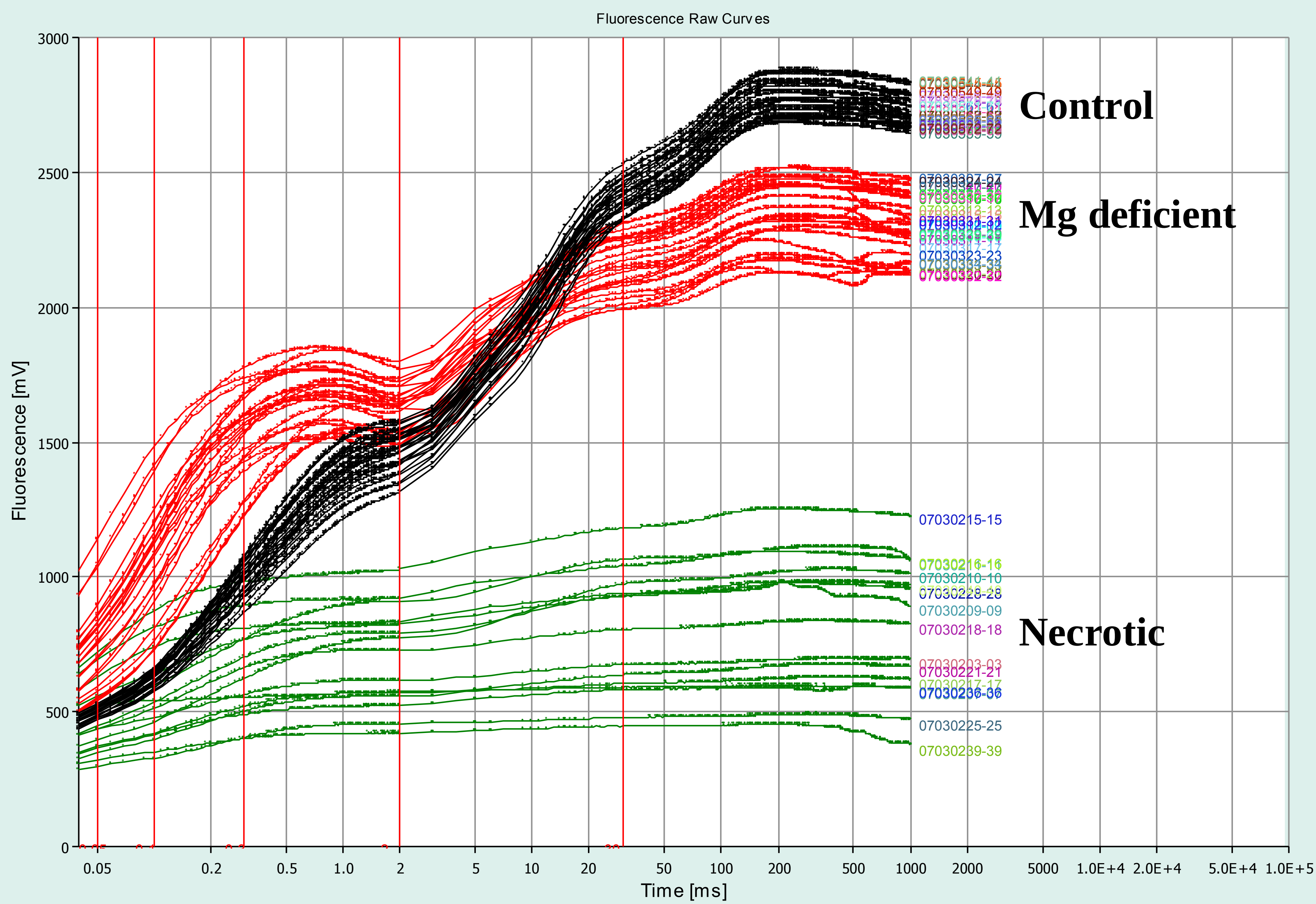
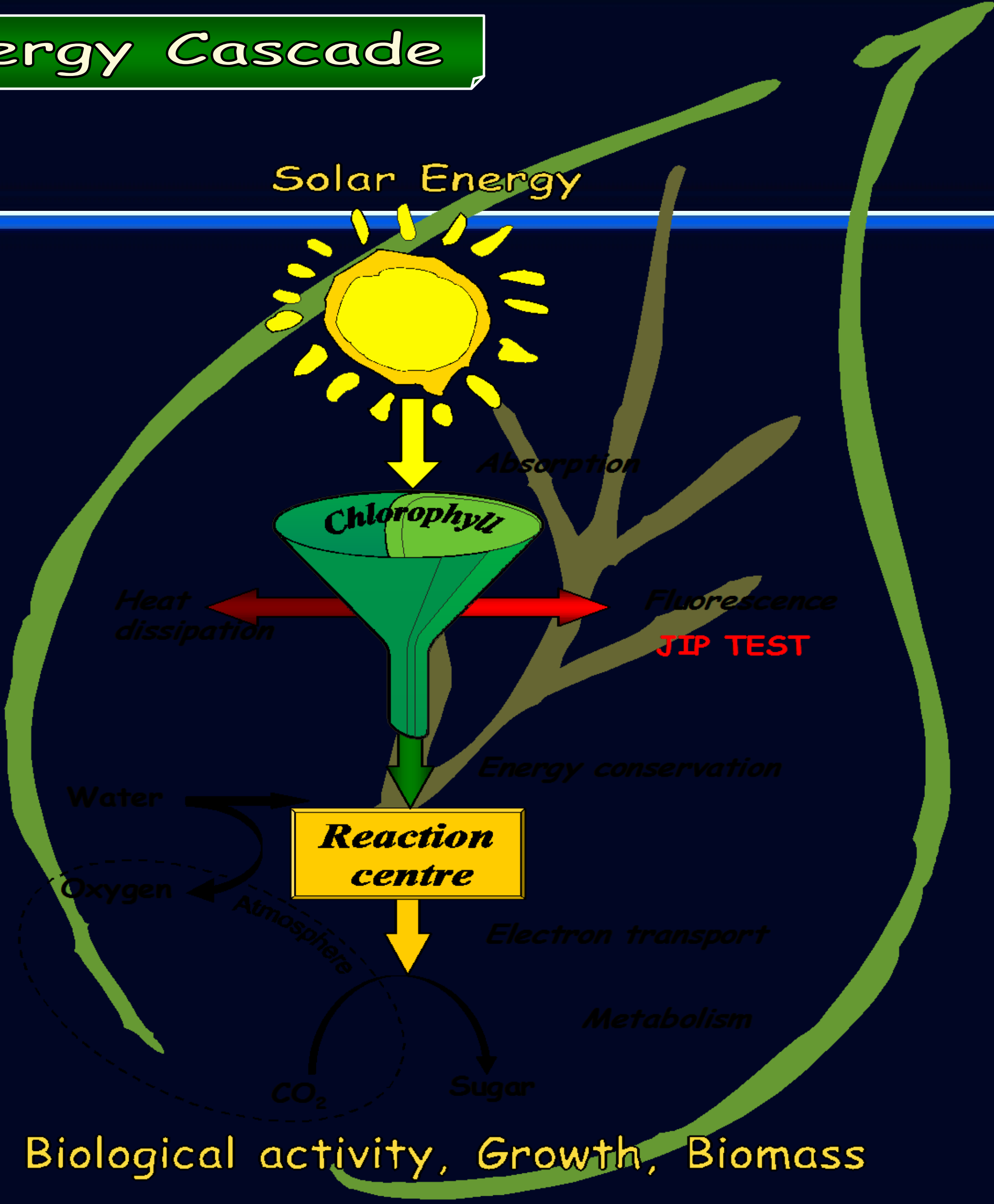
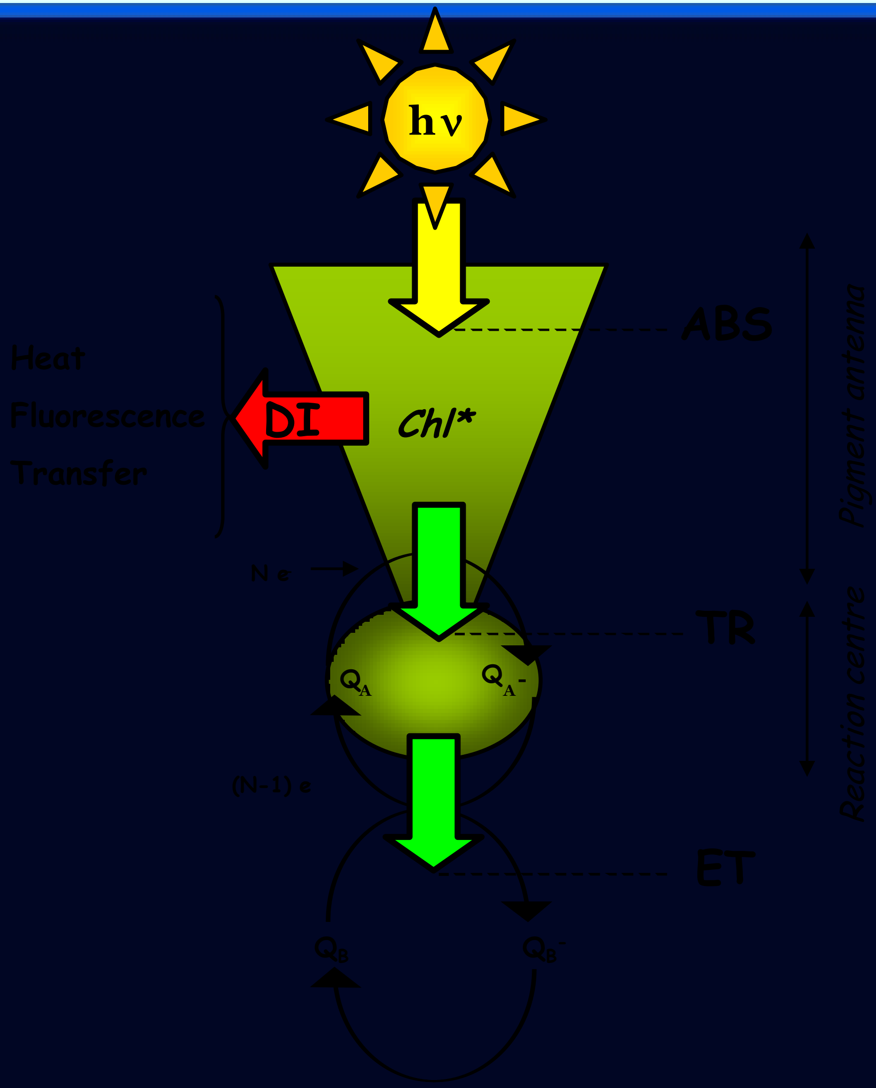
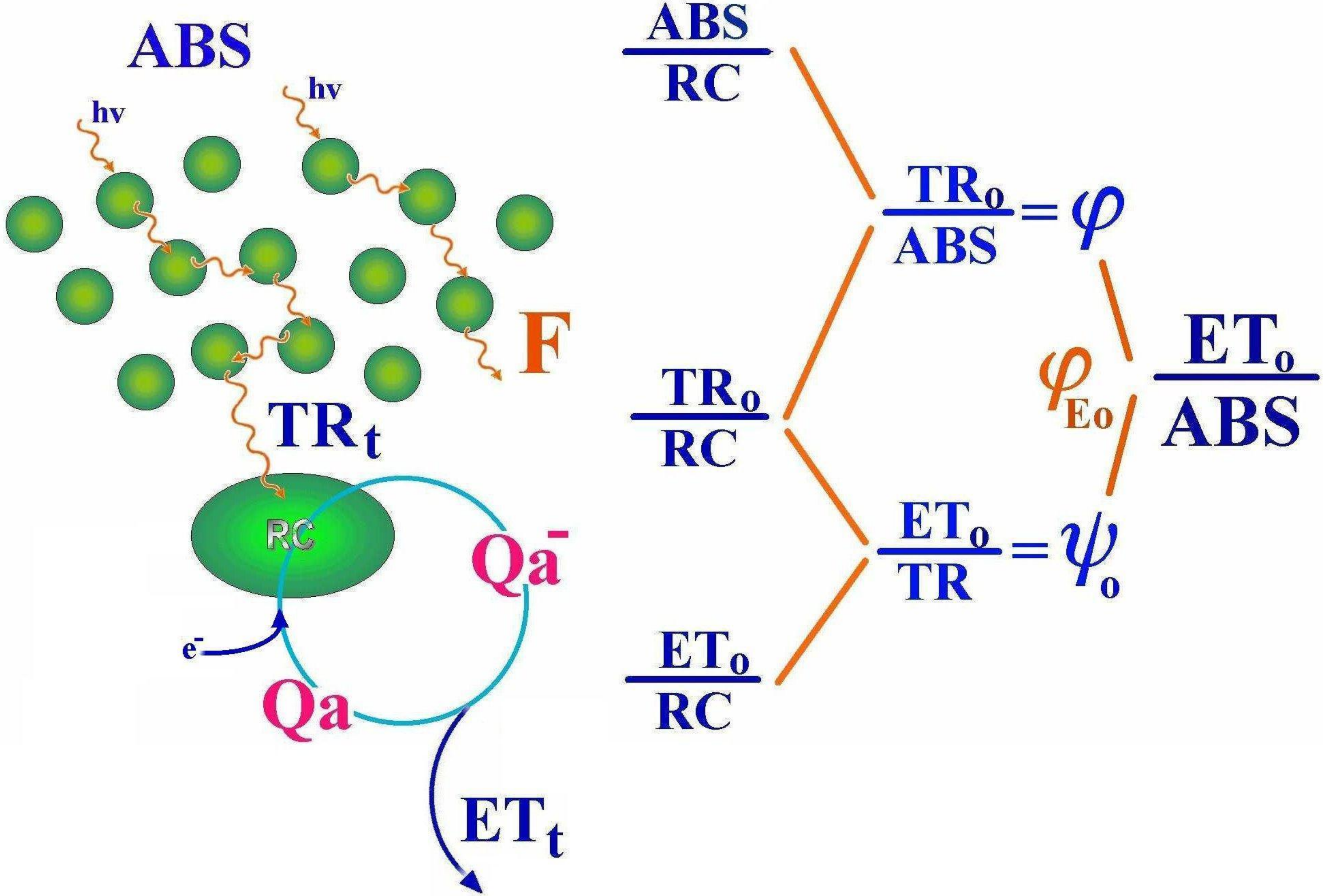

Fluorescence Raw Curves
Fluorescence [mV]
3000
2500
2000
1500
1000
500
0
Time [ms]
0.05
0.2
0.5
1.0
2
5
10
20
50
100
200
500
1000
2000
5000
1.0E+4
2.0E+4
5.0E+4
1.0E+5
Control
Mg deficient
Necrotic
07030323-23
07030215-15
07030210-10
07030209-09
07030218-18
07030203-03
07030221-21
07030225-25
07030239-39

The Energy Cascade





hν
ABS
Heat
Fluorescence
Transfer
DI
Chl*
Pigment antenna
N e
TR
Q_A
Q_A^-
Reaction centre
(N-1) e
ET
Q_B
Q_B^-



ABS
hv
hv
F
TR_t
RC
Qa^-
e^-
Qa
ET_t
$\frac{ABS}{RC}$
$\frac{TR_0}{ABS} = \varphi$
$\frac{TR_0}{RC}$
φ_{Eo}
$\frac{ET_0}{ABS}$
$\frac{ET_0}{TR} = \psi_0$
$\frac{ET_0}{RC}$

Derived JIP-test parameters table

Technical parameters

Slope at the origin of the fluorescence rise	**M_O**	$= (F_{300\mu s}-F_O) / (F_M-F_O)$
Relative variable fluorescence at 2 ms	**V_J**	$= (F_{2ms}/F_O) / (F_M-F_O)$

The specific fluxes (expressed per RC - reaction center)

Absorption, per RC	**ABS/RC**	$= (M_O/V_J) / ((1-F_O/F_M))$
Trapping at time zero, per RC	**TRo/RC**	$= M_O/V_J$
Dissipation at time zero, per RC	**DIo/RC**	= (ABS/RC) - (TRo/ABS)
Electron transport at time zero, per RC	**ETo/RC**	$= (M_O/V_J)\ (1-V_J)$

The phenomenological fluxes (expressed per CS – cross section of the leaf tissue)

Absorption, per CS	**ABS/CS**	$= (TR_O/ABS) / (ABS/CS)$
Trapping at time zero, per CS	**TRo/CS**	= (TRo/ABS) (ABS/CS)
Dissipation at time zero, per CS	**DIo/CS**	= (ABS/CS) - (TRo/CS)
Electron transport at time zero, per CS	**ETo/CS**	$= (M_O/V_J)\ (1-V_J)$

The yields (or fluxes ratios)

Maximum quantum yield of primary photochemistry	φ_{Po}	$= TRo/ABS = (F_M-F_O) / F_M$
Probability that a traped exciton moves an electron further than Q_A^-	Ψ_o	$= ETo/TRo = 1 - V_J$
Probability that an absorbed photon moves an electron further than Q_A^-	$\varphi_{Eo} = \varphi_{Po}\,\Psi_o$	= (TRo/ABS) (ETo/TRo) $= ETo/ABS = (1-F_O/F_M)\ (1-V_J)$

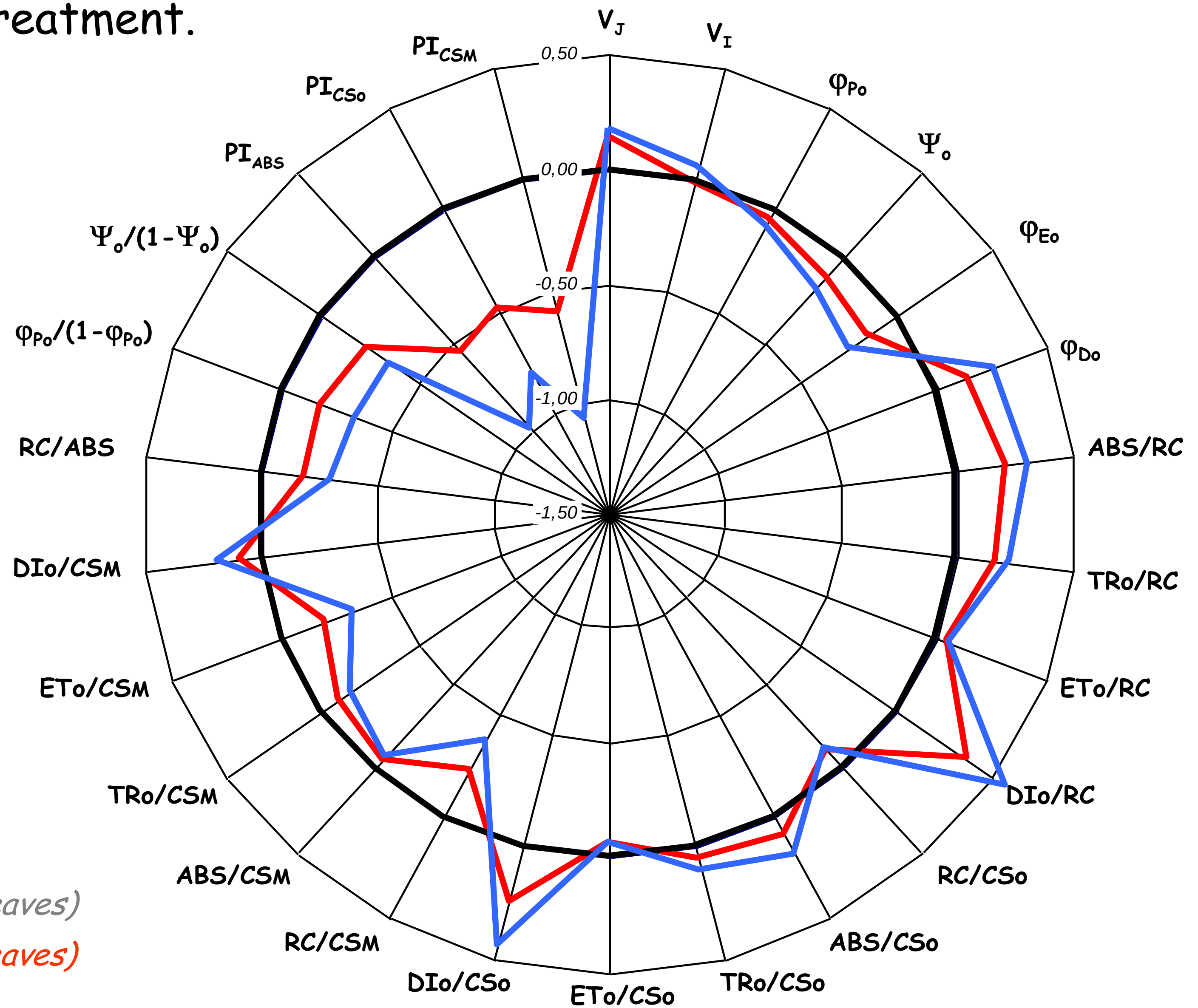
Vitality Indexes

Density RCs per chlorophyll	**RC/ABS**
Conformation term for primary photochemistry	**$(\varphi_{Po}/(1 - \varphi_{Po}))$** $= TRo/DIo = F_O/F_M$
Conformation term for the thermal reactions (non light depending reactions)	**$(\Psi_o /(1 - \Psi_o))$** $= ETo/(dQ_A^-/dt_0)$
Performance Index	$PI_{ABS} = [RC/ABS]\ [\varphi_{Po}/(1 - \varphi_{Po}]\ [\Psi_o /(1 - \Psi_o]$
Driving force on a chlorophyll basis	$\Delta F_{ABS} = \log\ [PI_{ABS}]$

These graphics present the constellation of selected JIP-test parameters which quantify the behaviour of plants exposed to different stress treatment.

Siper-plot representation. Variations of the normalised JIP-test parameters by the respective control. More precisely, the nutritional stress linked to a lack of B and Mg is regarded as a deviation of the reference state and considered as non stress (for which the control values turn on a circle with a radius of 100%).

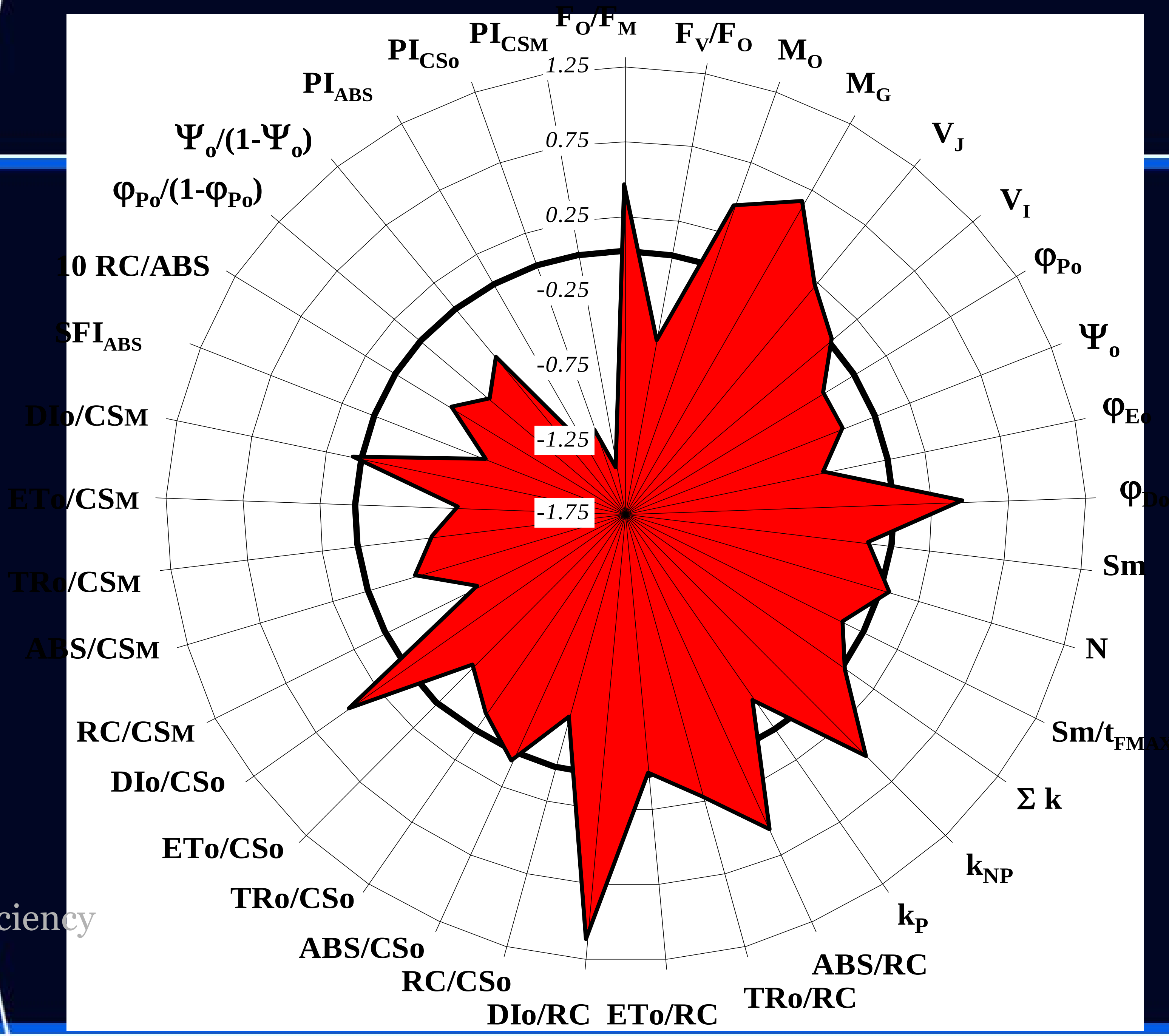
Boron deficiency first appears on the youngest leaves whereas magnesium deficiency can be detected on the oldest leaves.



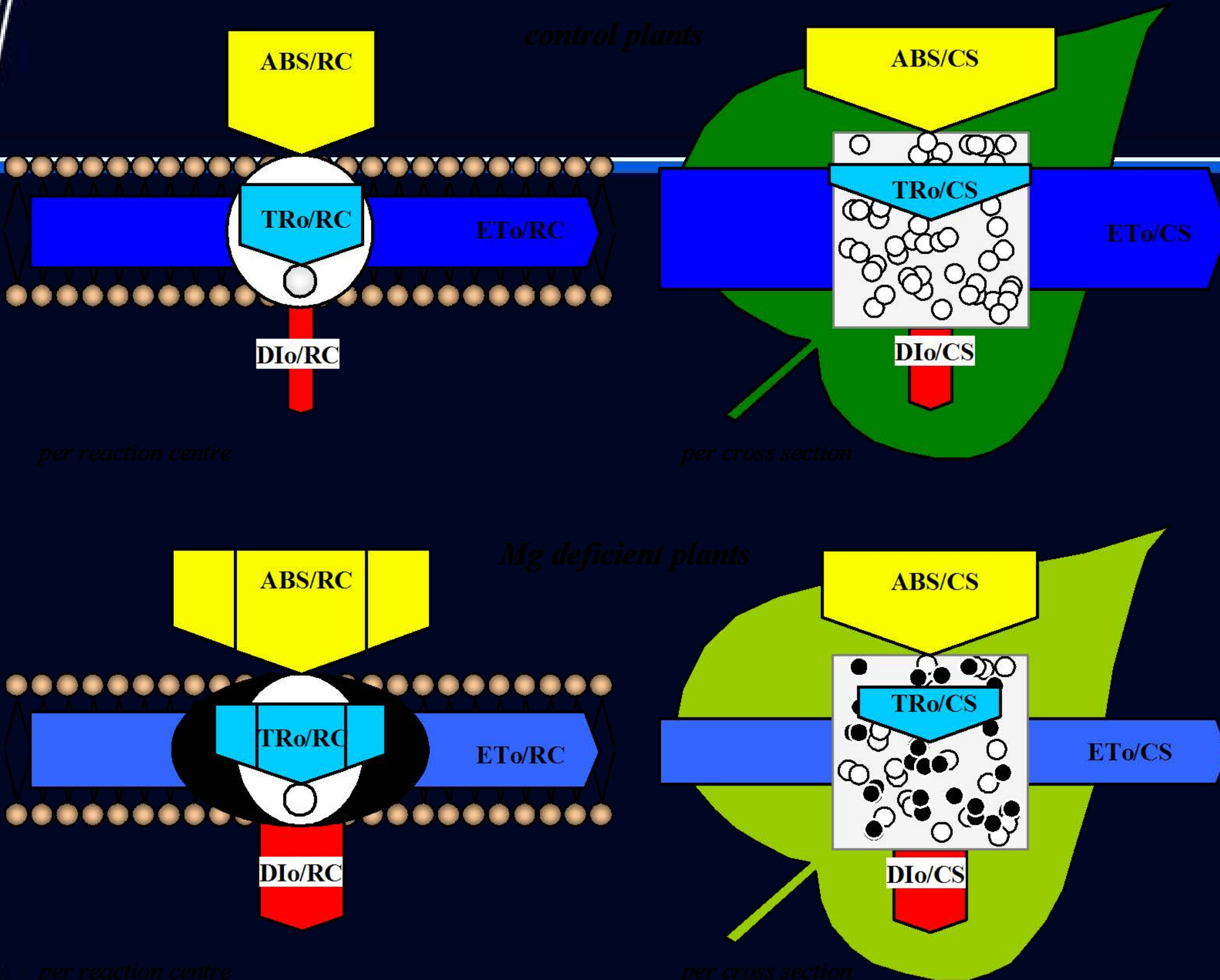
Respective control

B deficiency (youngest leaves)

Mg deficiency (mature leaves)

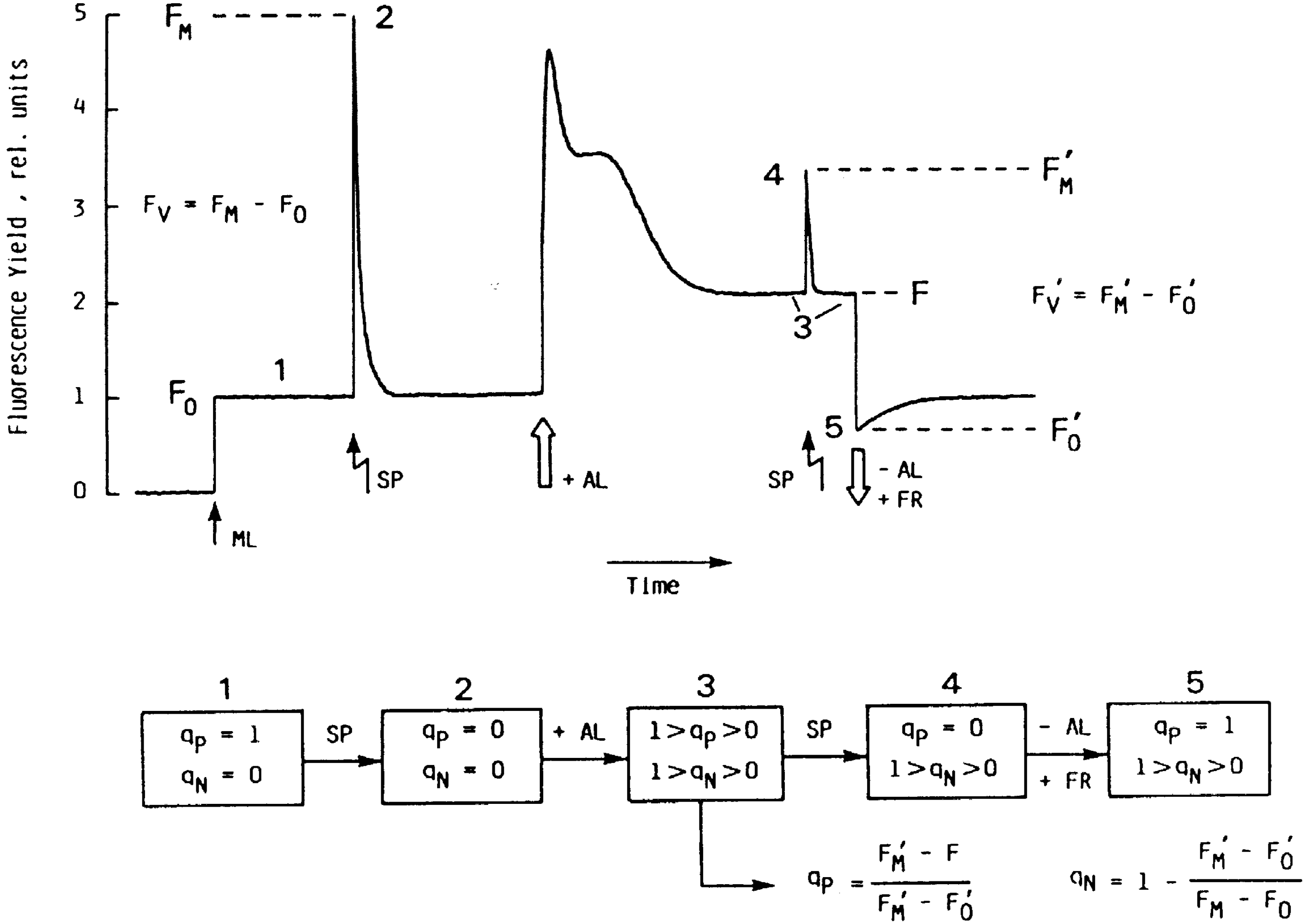


Mg deficiency



Pipe line model

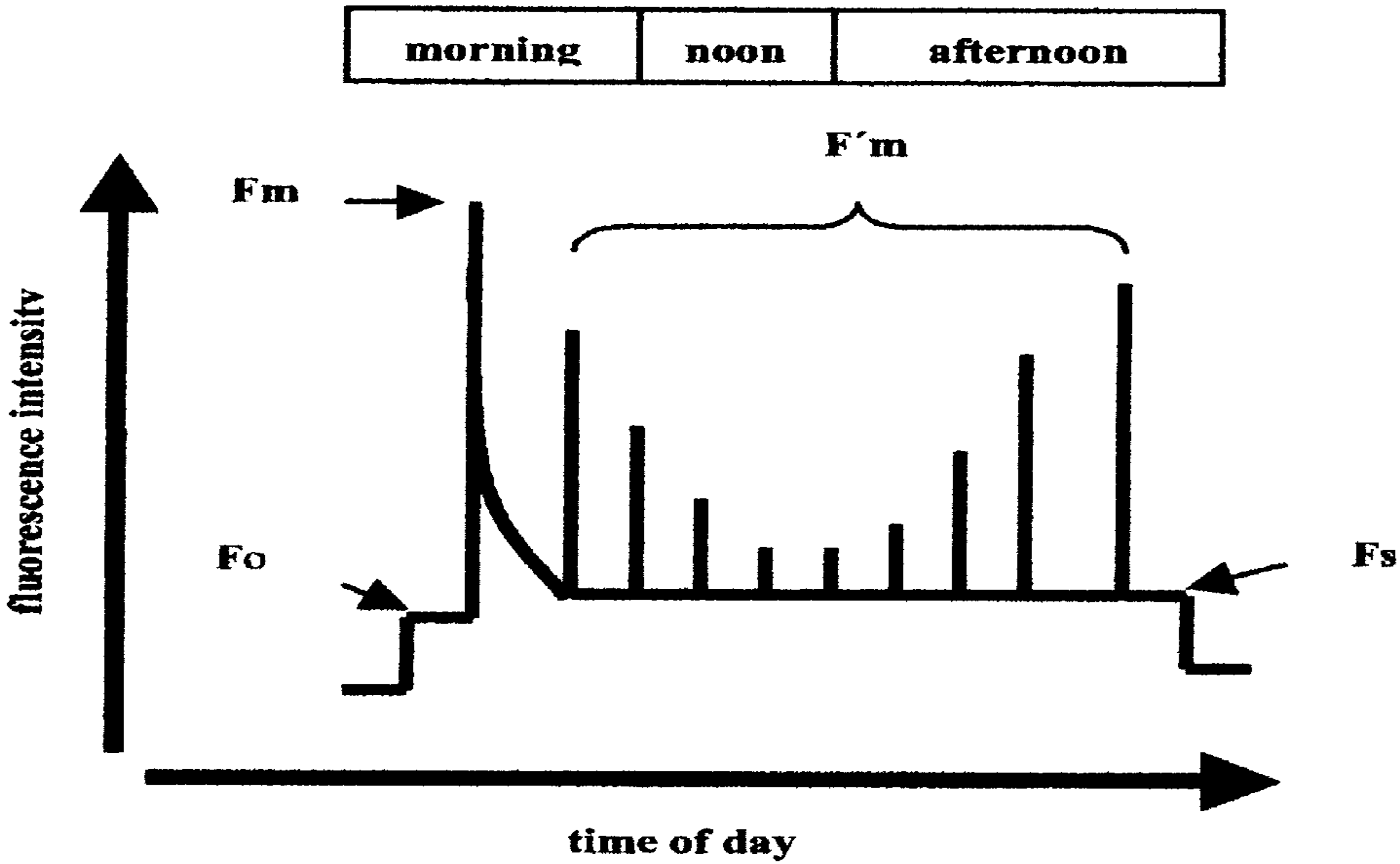
Leaf model



. Principles of quenching analysis by the saturation pulse method. Fluorescence yield is measured with a modulation fluorometer. Depending on the light conditions 5 different states are distinguished and the corresponding points in the induction curve characterized by fluorescence yield notations (e.g., F_0, F_m) and quenching coefficients (q_P and q_N). Fluorescence quenching at a given time following the onset of actinic illumination (at point 3) is evaluated by comparison with a dark-adapted reference state (1). which is characterized by $q_P = 1$ and $q_N = 0$. In both cases a pulse of saturating light is applied to close all PS II reaction centers. thus eliminating photochemical quenching ($q_P = 0$) (points 2 and 4). It is assumed that non-photochemical quenching is not affected during a saturation pulse. q_P and q_N are quenching *coefficients*, designating the *relative* decrease in *variable* fluorescence yield. The fluorescence yield F'_0, i.e., in the energized state with all centers open, is determined briefly after switching-off actinic light in the presence of weak far-red illumination (point 5). ML, weak modulated measuring light (approx. 6 nmol m^{-2} s^{-1} at 660 nm); SP, saturating light pulse (approx. 10 000 μmol m^{-2} s^{-1}, 400 nm $< \lambda <$ 700 nm, applied for 0.5–2 s); AL. continuous actinic light; FR, far-red light (approx. 6 μmol m^{-2} s^{-1}, $\lambda >$ 700 nm).

Measured Parameters

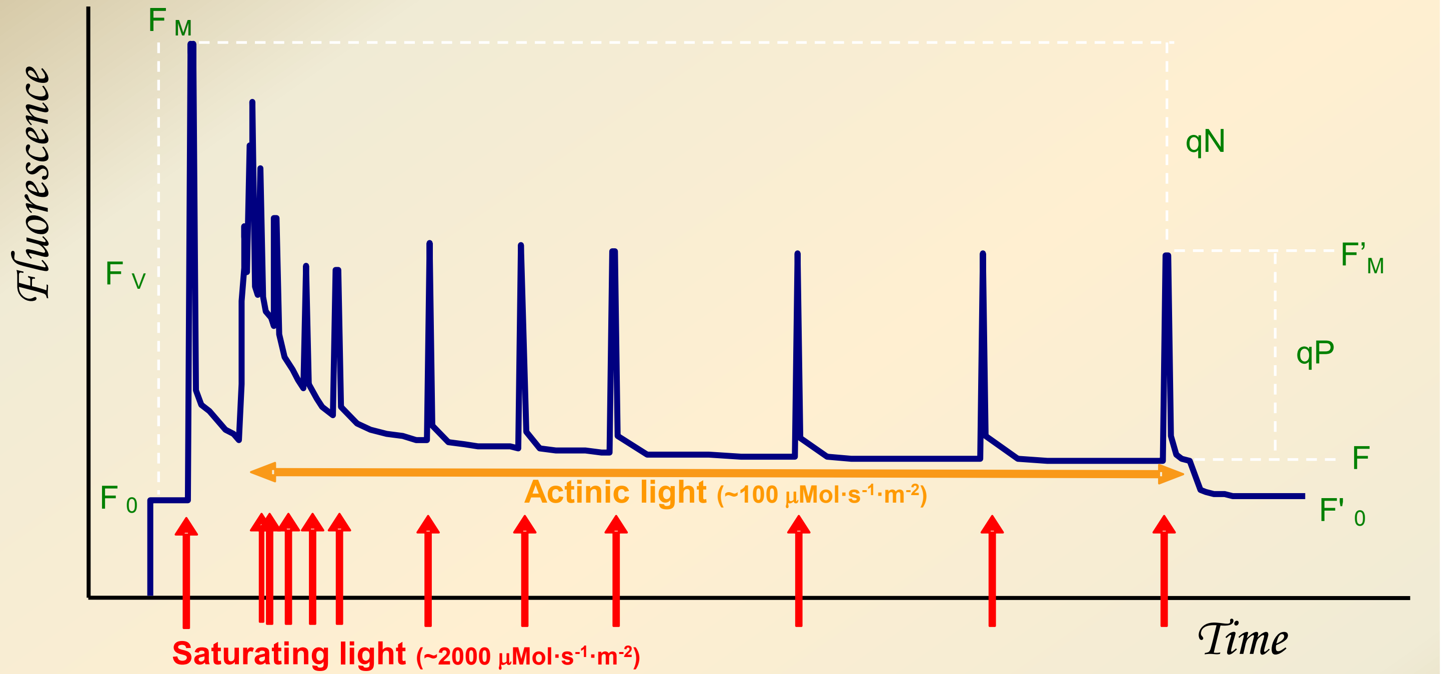
Parameter:	Measurement:	Units:	Derivation:
PAR	Incident photosynthetically active radiation	$\mu molm^{-2}s^{-1}$	
Temp	Temperature	°C	
Fs	Steady state fluorescence yield	Bits	
Fm'	light-adapted fluorescence maximum	Bits	
Fv'	Light-adapted variable fluorescence	Bits	= Fm'-Fo'
Fv'/Fm'	Antennae efficiency of PSII	No units	= (Fm'-Fo') / Fm'
ϕ_{PSIIR}	quantum efficiency of PSII	No units	= (Fm'-Fs) / Fm' (Genty *et al.* 1989)
qP	photochemical quenching co-efficient	No units	= (Fm'-Fs) / (Fm'- Fo')
qNP	Non-photochemical quenching co-efficient	No units	= (Fm-Fm') / (Fm-Fo')
NPQ	Alternative definition of non-photochemical quenching	No units	= (Fm-Fm') / Fm'
ETR	Electron Transport Rate	No units	= PAR * 0.5 * 0.84 * ϕ_{PSII}



Sequence of the determination of various chlorophyll *a* fluorescence parameters in barley leaves in the course of the natural day using the MiniPam.

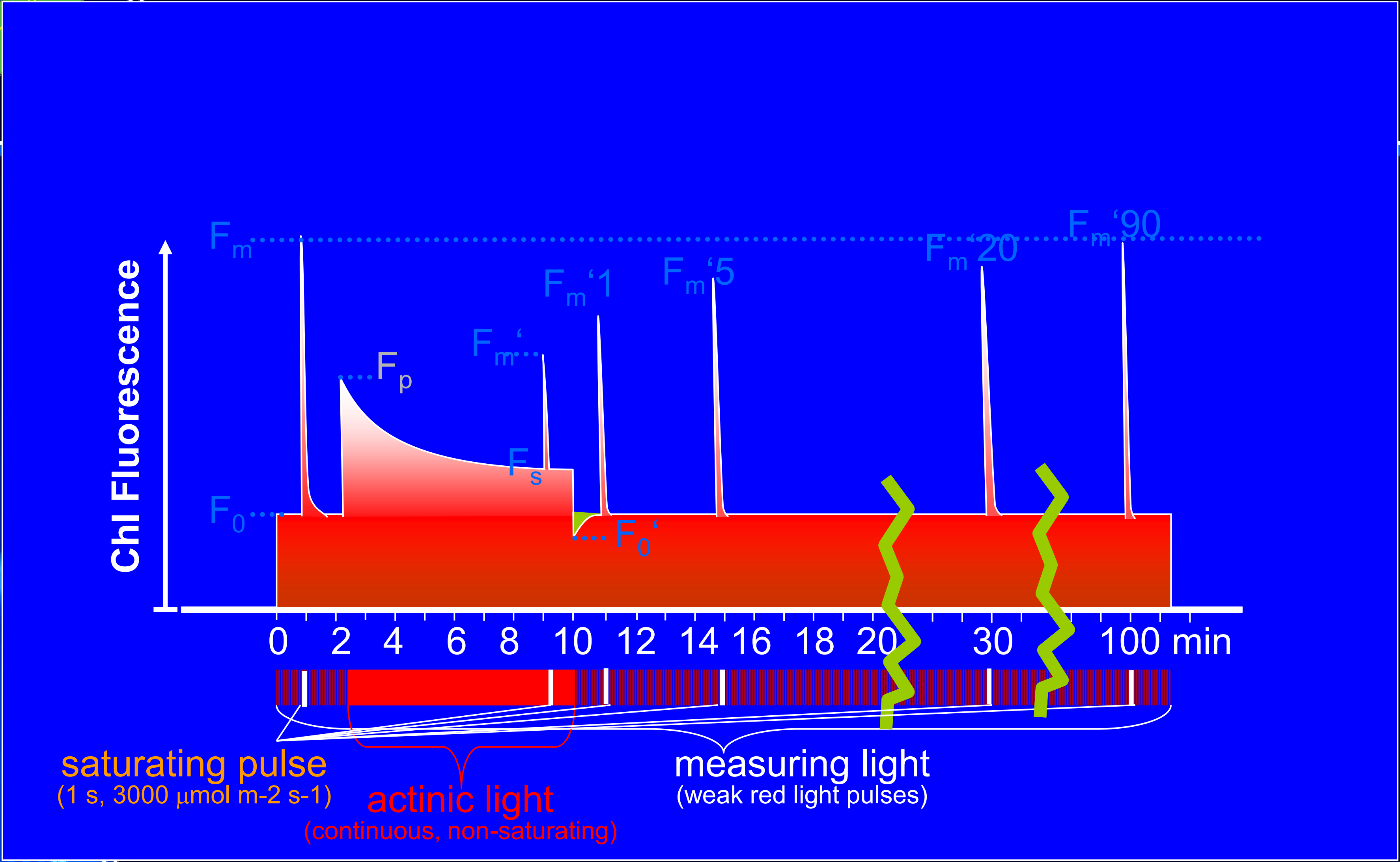
Red chlorophyl fluorescence kinetic and quenching factors

Light adapted plant



$$F_V / F_M = \frac{F_M - F_0}{F_M}$$

$$NPQ = \frac{F_M - F_M^{'}}{F_M^{'}}$$



F_0 = ‚dead' fluorescence

F_p = kinetic peak

F_s = steady state

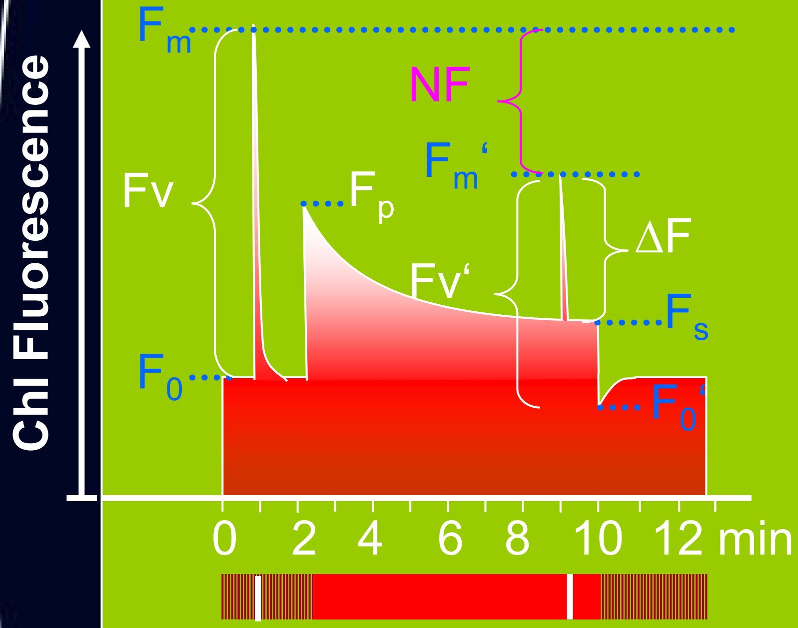
F_m = maximum (dark adapted)

F_m' = maximum (with actinic light)

F_m'X = maximum (X min after actinic light)

Photochemical activity of PS II

from Lichtenthaler and Buschmann 2004



$Fv = Fm - Fo$

$Fv' = Fm' - Fo'$

$NF = Fm - Fm'$

$\Delta F = Fm' - F$

optimal quantum conversion: Fv / Fm or Fv / Fo (Kitajima and Butler 1975)

effective quantum efficiency: $\Delta F / Fm'$ (Genty et al. 1989)

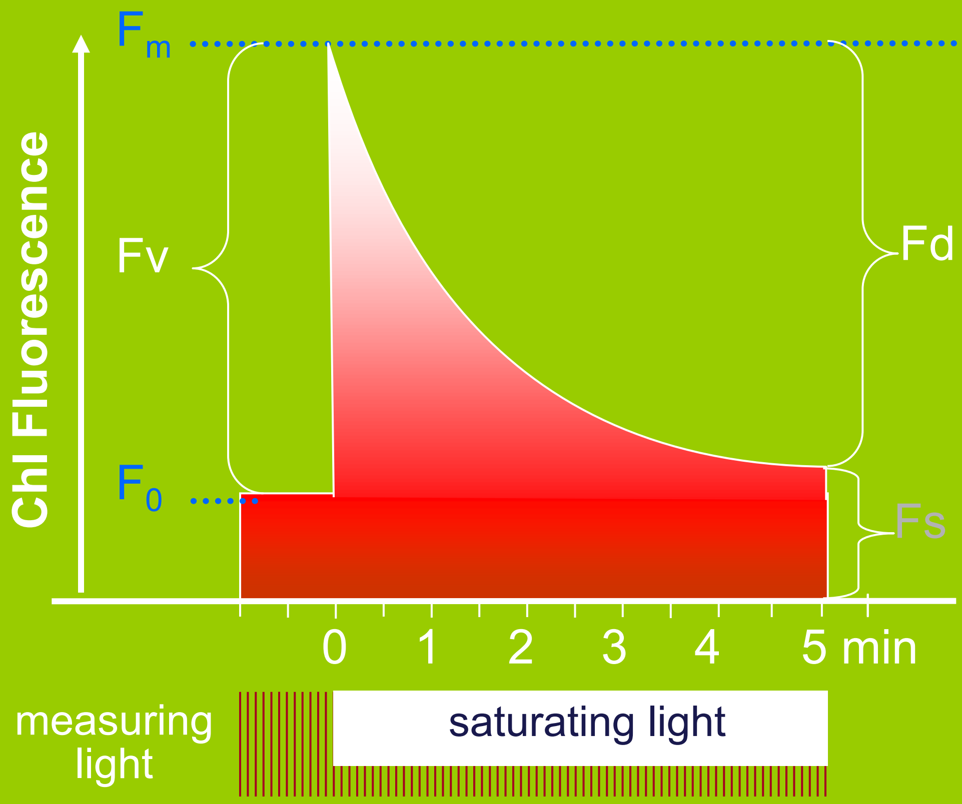
photochemical quench: $q_P = \Delta F / Fv'$ (Bilger and Schreiber 1986)

non-photochemical quench: $q_N = NF / Fv$ (Bilger and Schreiber 1986)

$NPQ = NF / Fm$ (Bilger and Björkman 1990)

RFd-measurement

from Lichtenthaler 1988



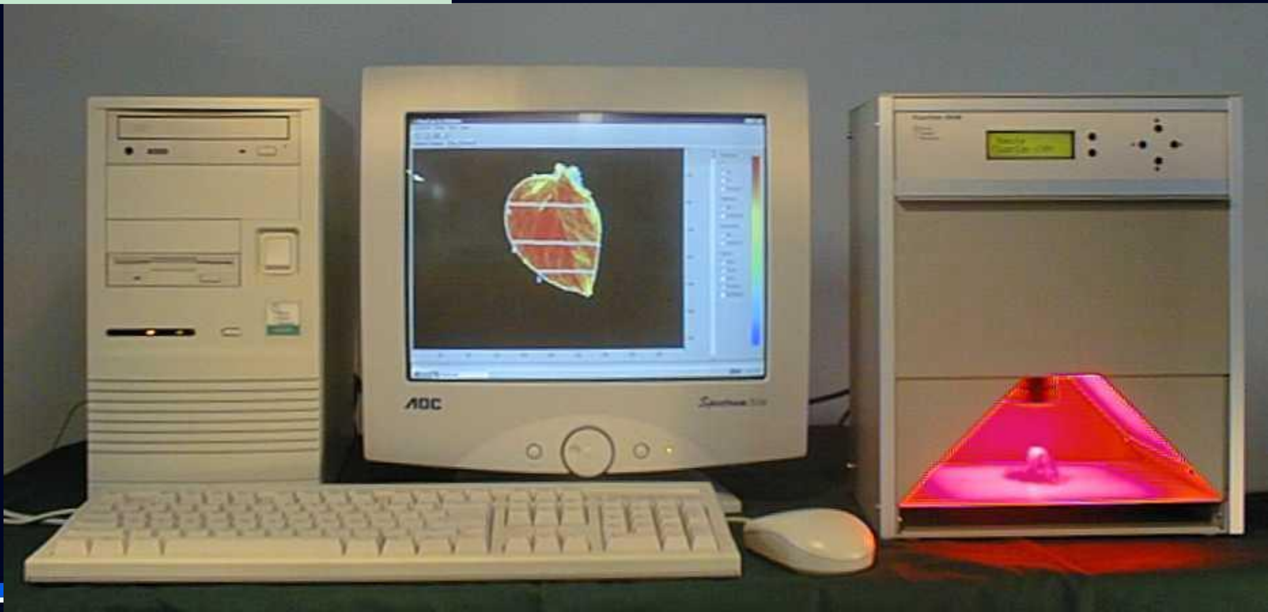
Parameters obtained:

- RFd = Fd / Fs
- Fv / Fm
- Fv / Fo

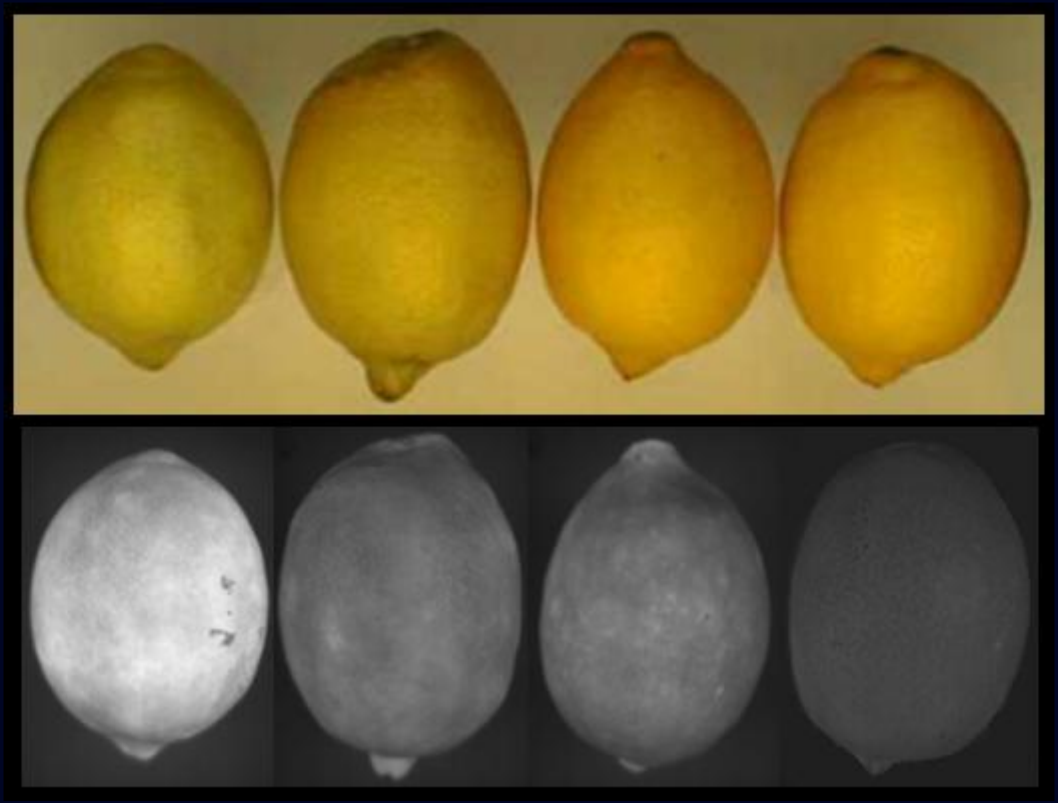

Mean (2)
DA:1.41
Last
DA:1.49
DA meter 2008

Kamery fluorescencji

(systemy obrazowania fluorescencji chlorofilu)



http://www.psi.cz/



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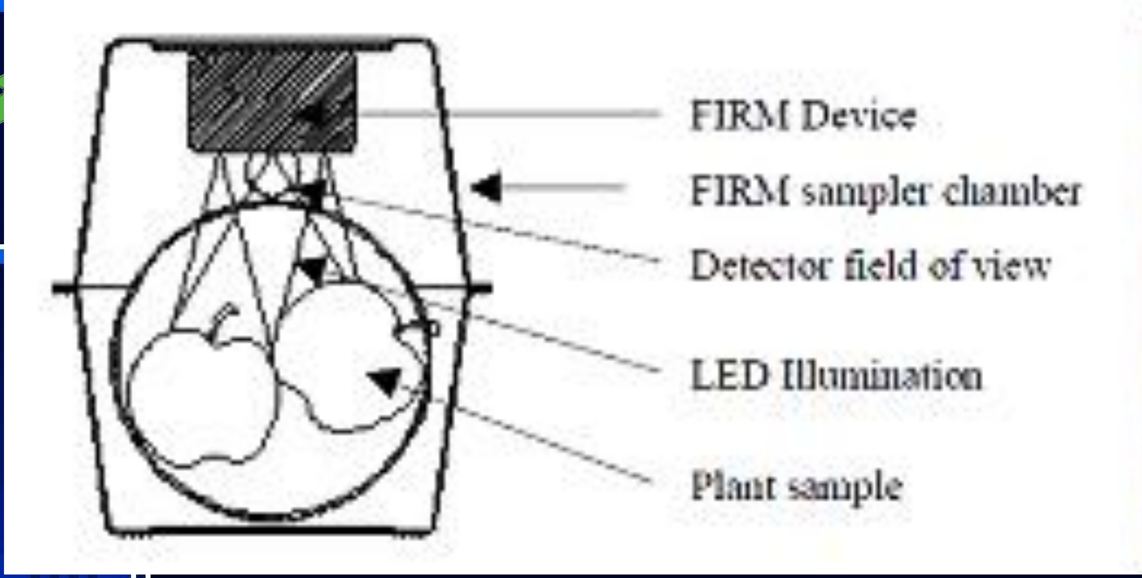

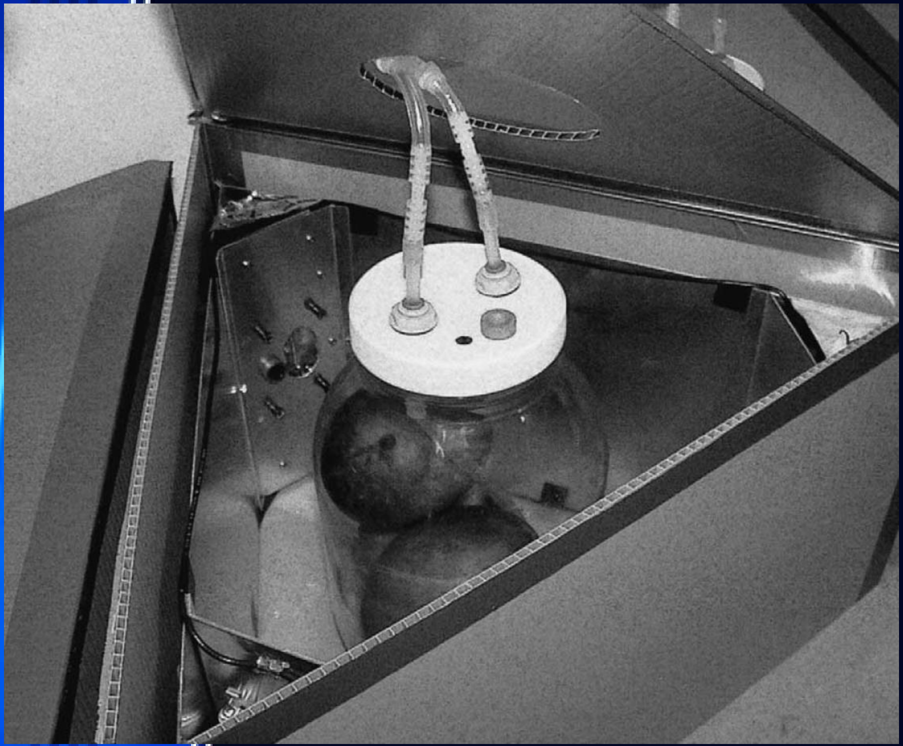

Satlantic_Fruit FL

Isolcell


2
3

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Postharvest Biology and Technology 24 (2002) 201–205

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Research note

Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit☆

Robert K. Prange [1]*, John M. DeLong [2], Jerry C. Leyte [3], Peter A. Harrison [4]

Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, 32 Main Street, Kentville, NS, Canada B4N 1J5

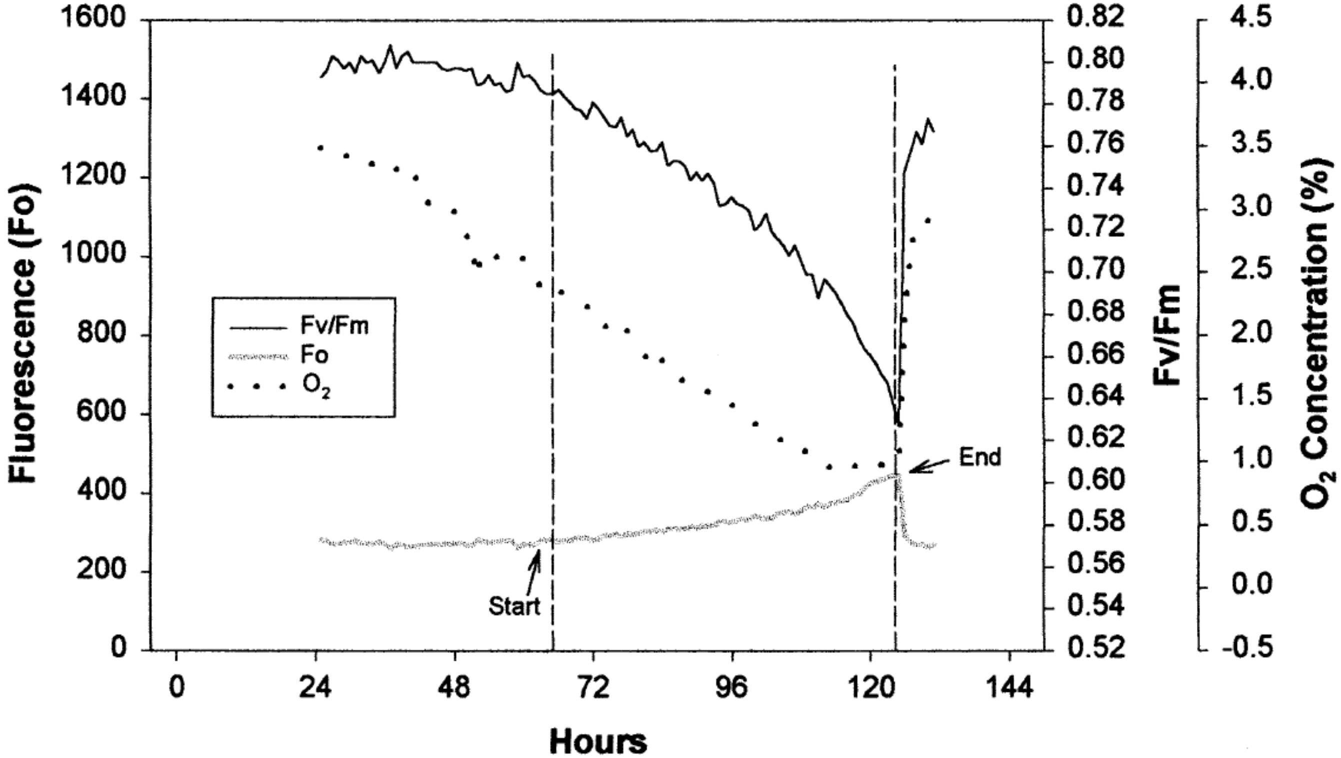
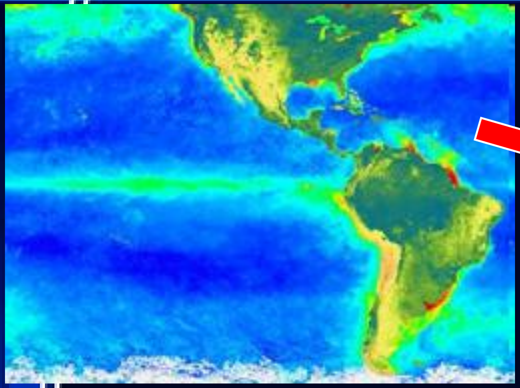
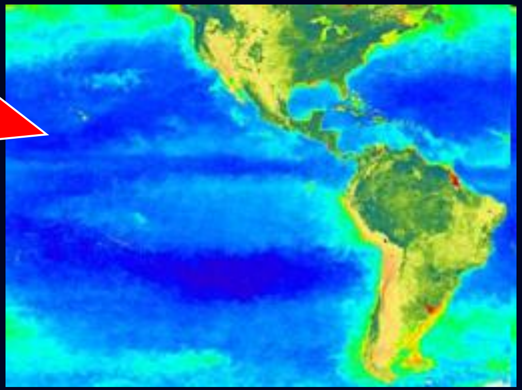


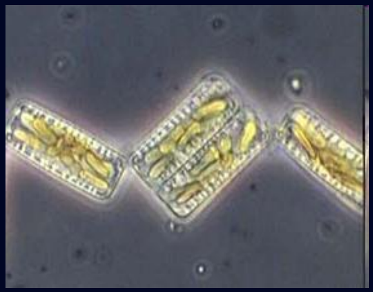
Fig. 2. Changes in apple fruit F_o and F_v/F_m chlorophyll fluorescence and O_2 concentration over time. 'Start' indicates a change in both F_o and F_v/F_m due to low O_2. 'End' indicates an increase in O_2 concentration and a corresponding reversal in F_o and F_v/F_m values.

Leopold Park, Brussels - Belgium

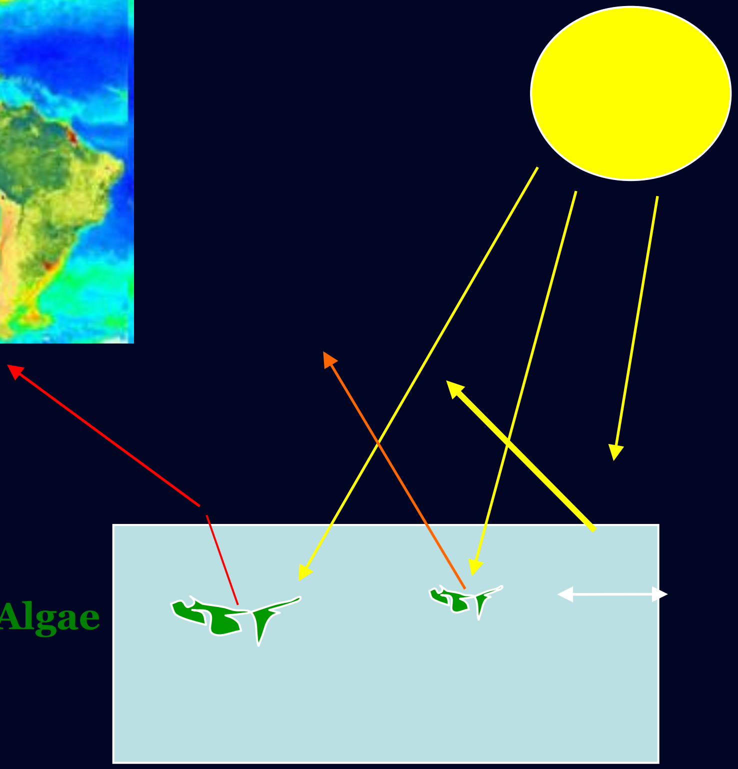
MODIS - NASA







Algae



http://daac.gsfc.nasa.gov/oceancolor/scifocus/oceanColor/warming.shtml
http://oceancolor.gsfc.nasa.gov/

Stress-´Detection Lenses