Photosynthesis- role of metals

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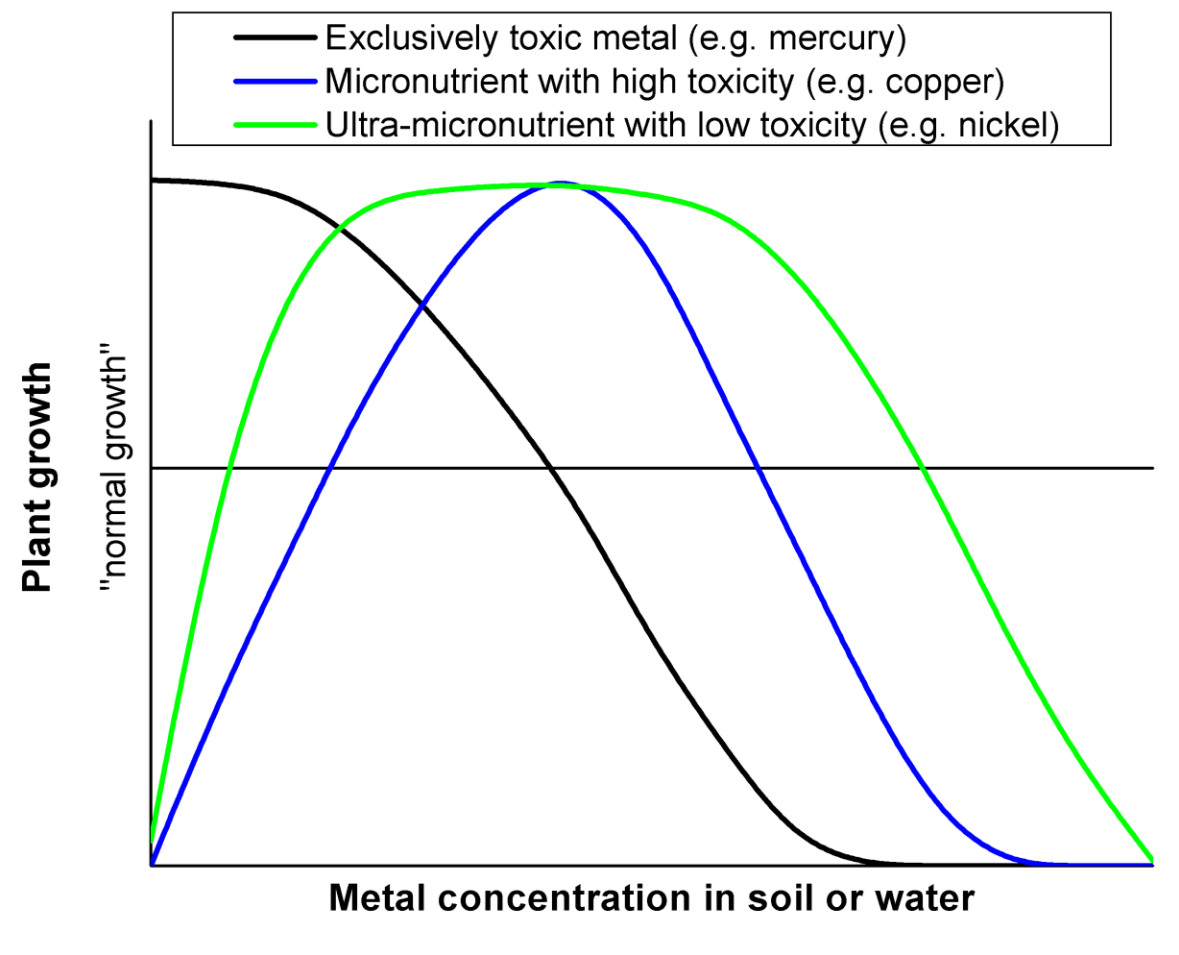
Training school for working with metalloproteins
Trace metal metabolism in plants

CA 19116 PLANTMETALS

České Budějovice, 17-19.07.2023.

Dose-Response principle for heavy metals



Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

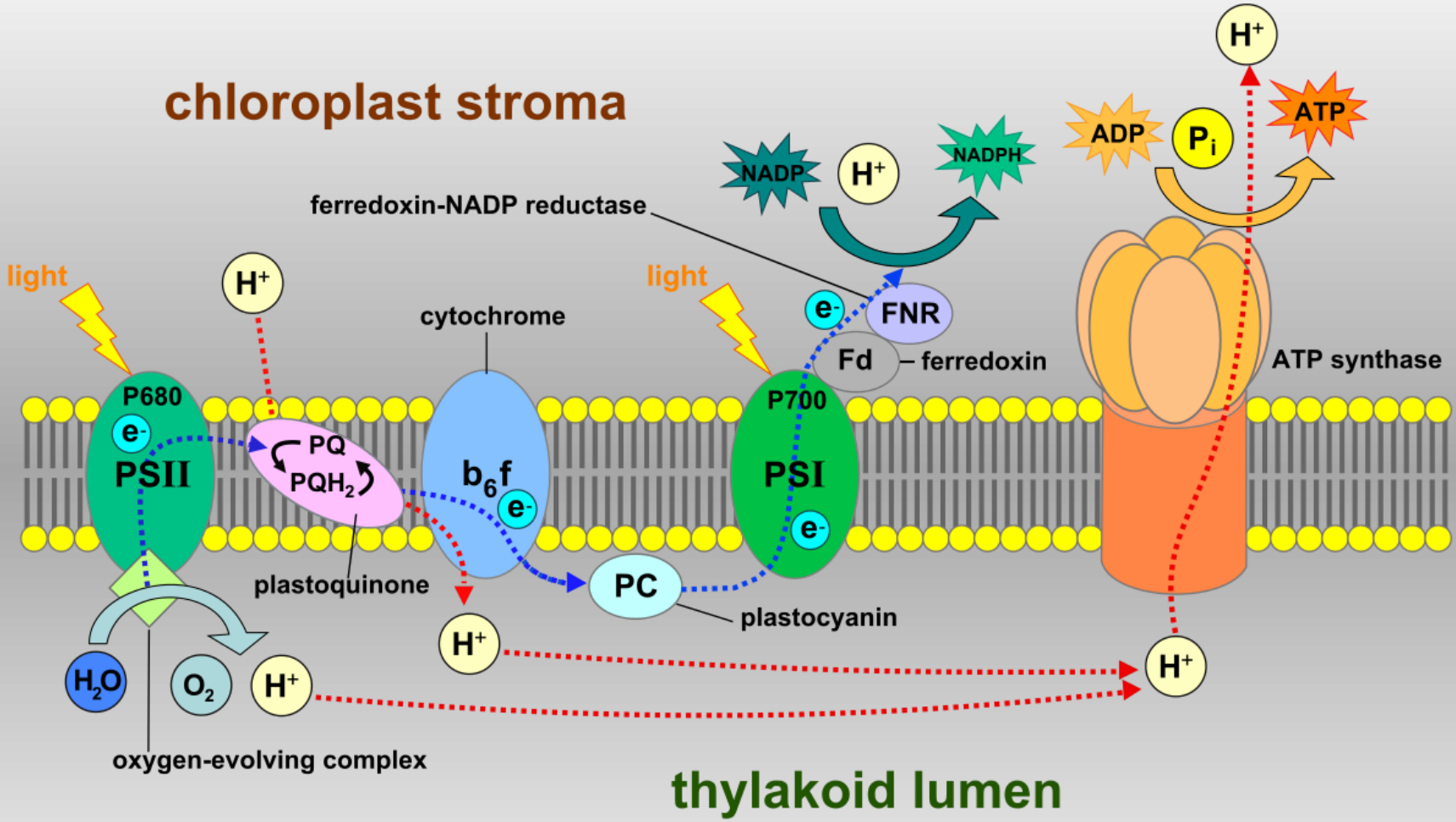
Photosynthesis - main target of metal deficiency/toxicity

- The essential role of metals in photosynthesis (Ca, Cu, Fe, Mg, Mn, Zn) as enzyme cofactors, part of clusters that maintain the function and structure of proteins and form stable metal complexes.

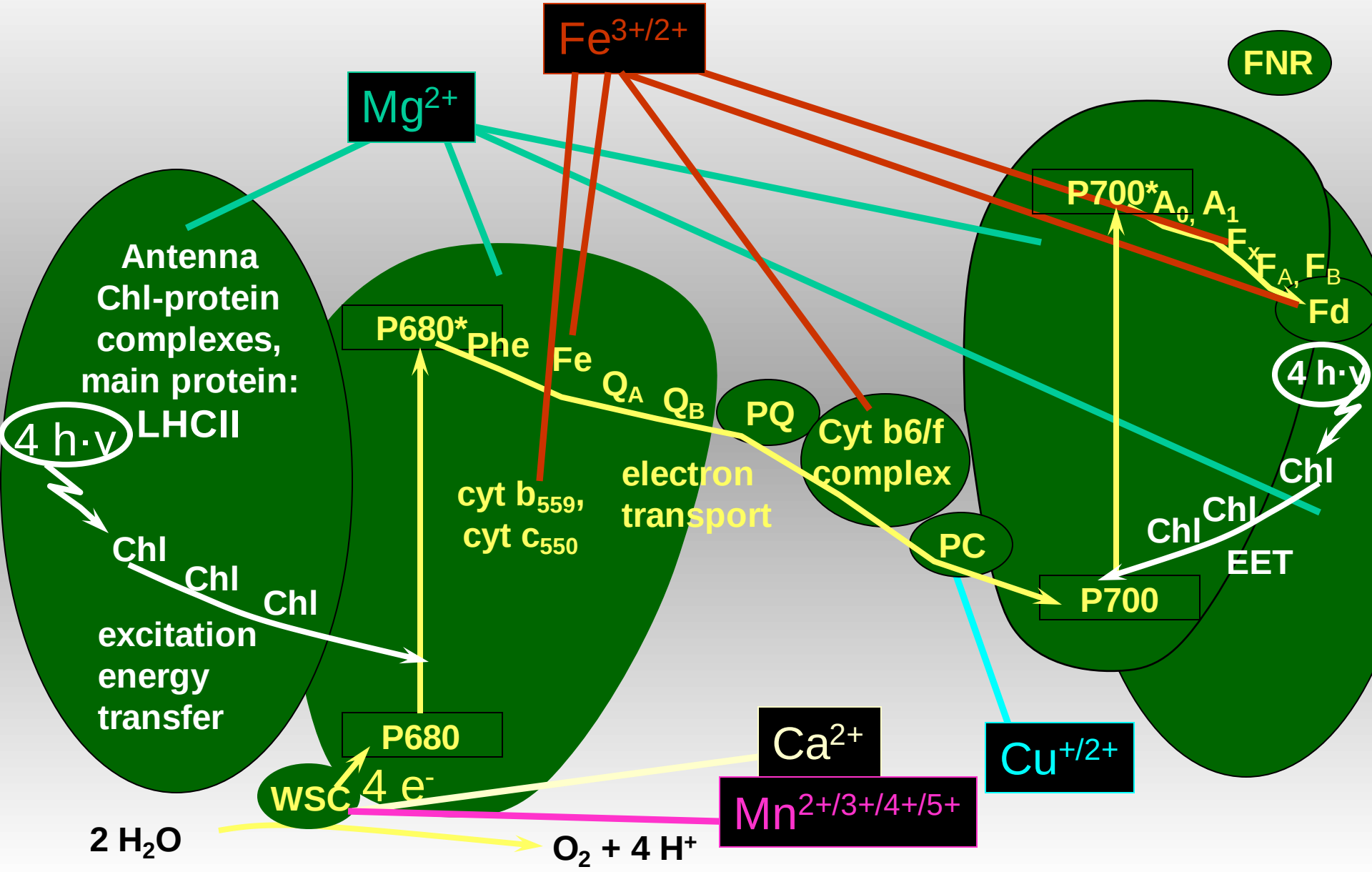
* Up to 80% of total leaf Fe and about 30% of leaf Cu is allocated to the chloroplasts (review by Schmidt et al., 2020).

- Chloroplasts as primary target of metal toxicity in photosynthetic tissues leading to redox imbalance and oxidative stress

Overview of photosynthetic el. transport chain

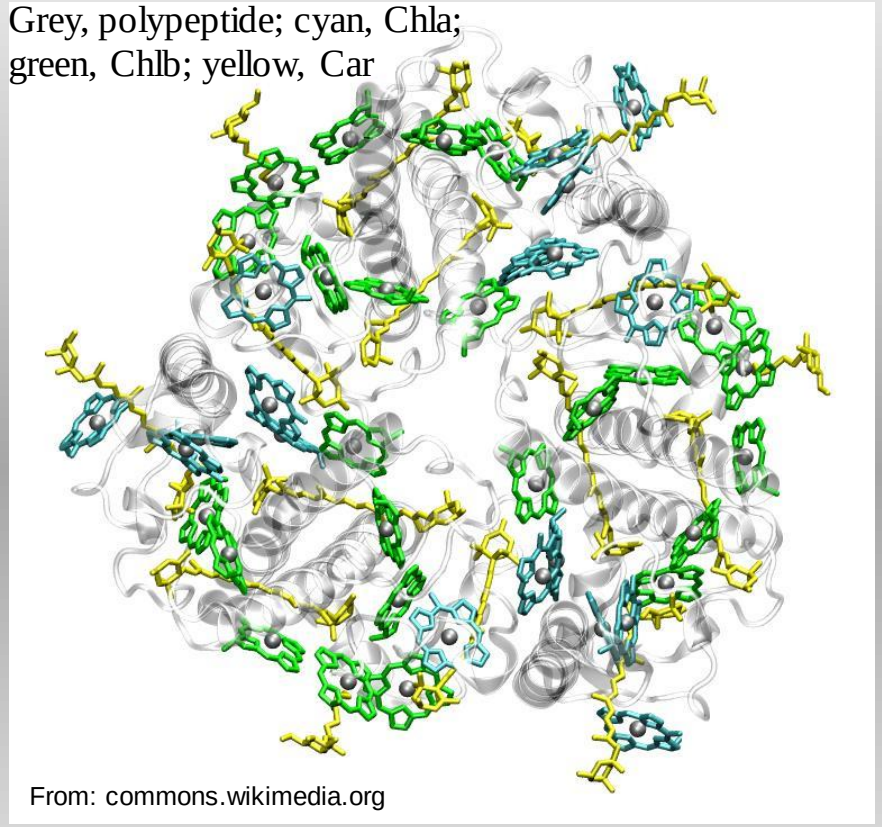


Metal sites in photosynthetic proteins



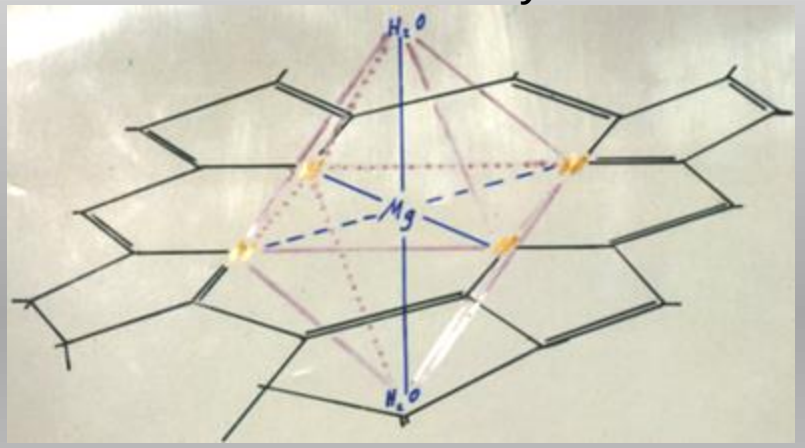
Photosynthesis related proteins with metal centres

LHCII structure



LHCII structure

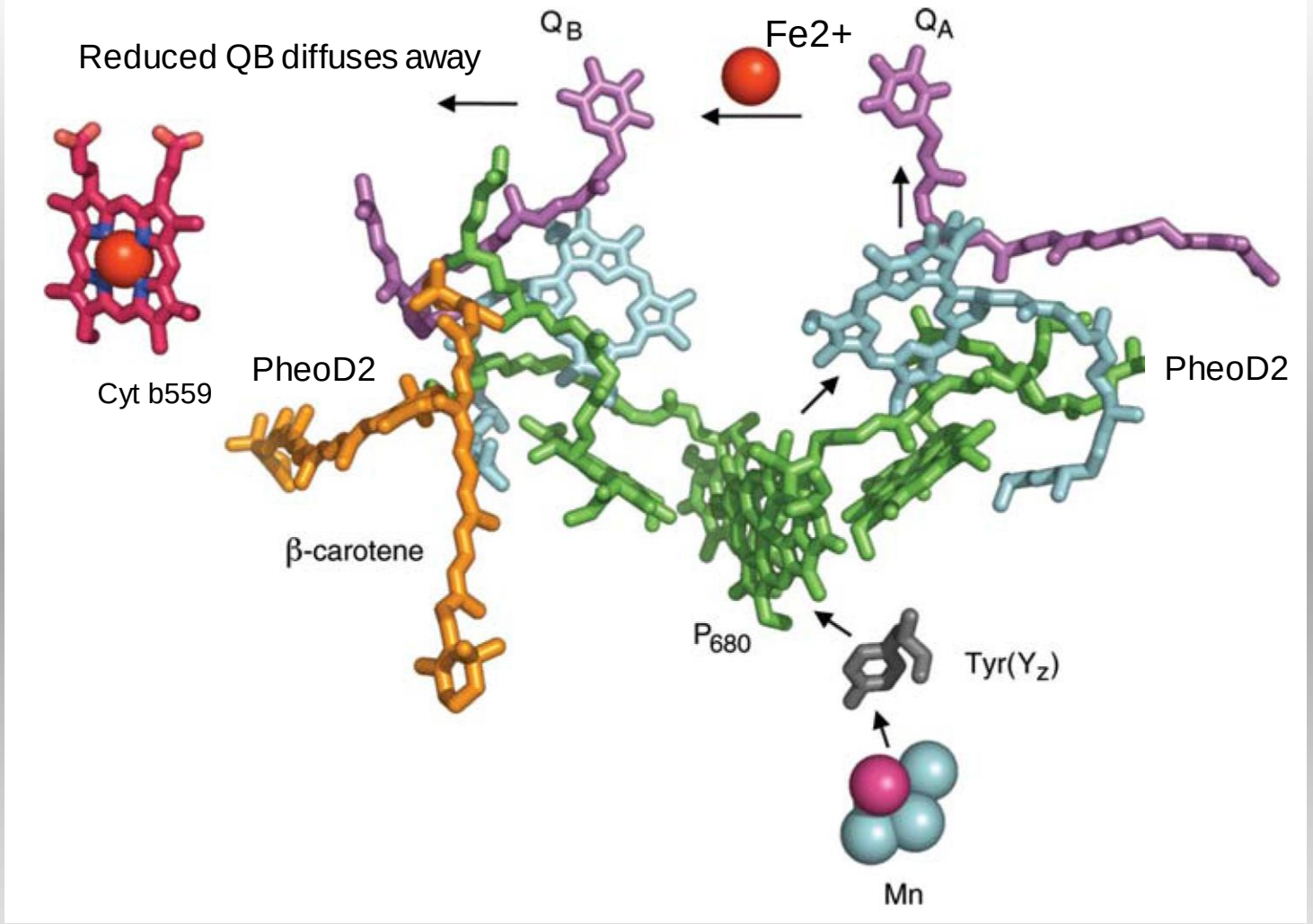
- usually trimers
- structure stabilised by Chl



- binding via axial ligands on **Mg^{2+}**

Pigment-protein systems responsible for photon absorption and transfer of the excitation energy to the reaction center, where charge separation occurs

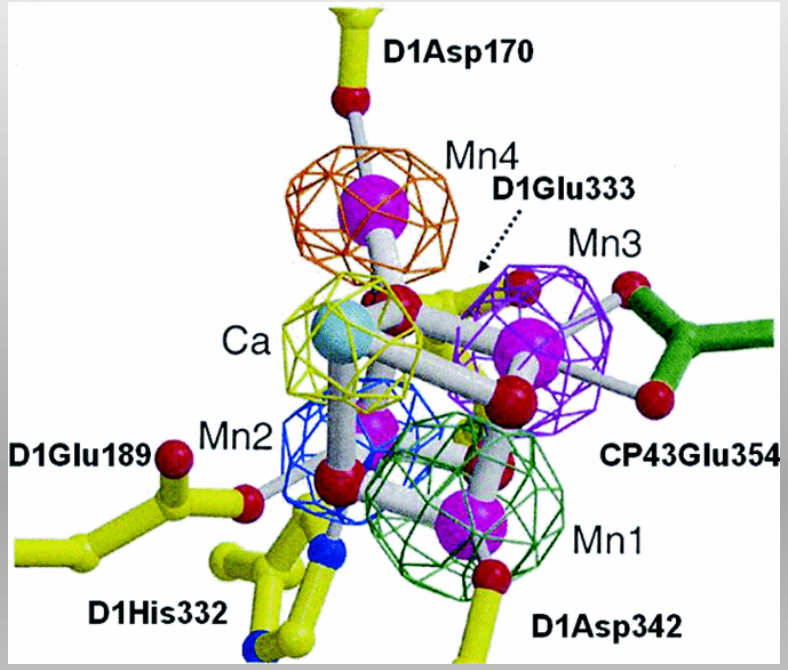
Photosynthesis related proteins with metal centres
Photosystem II reaction centre – electron transport



From: Nelson N, Yocum CF, 2006, AnnRevPlantBiol 57, 521-65

- electrons are transferred from water to plastoquinone b (Q_B)
- Manganese / calcium, magnesium and iron centres involved in e^- transport

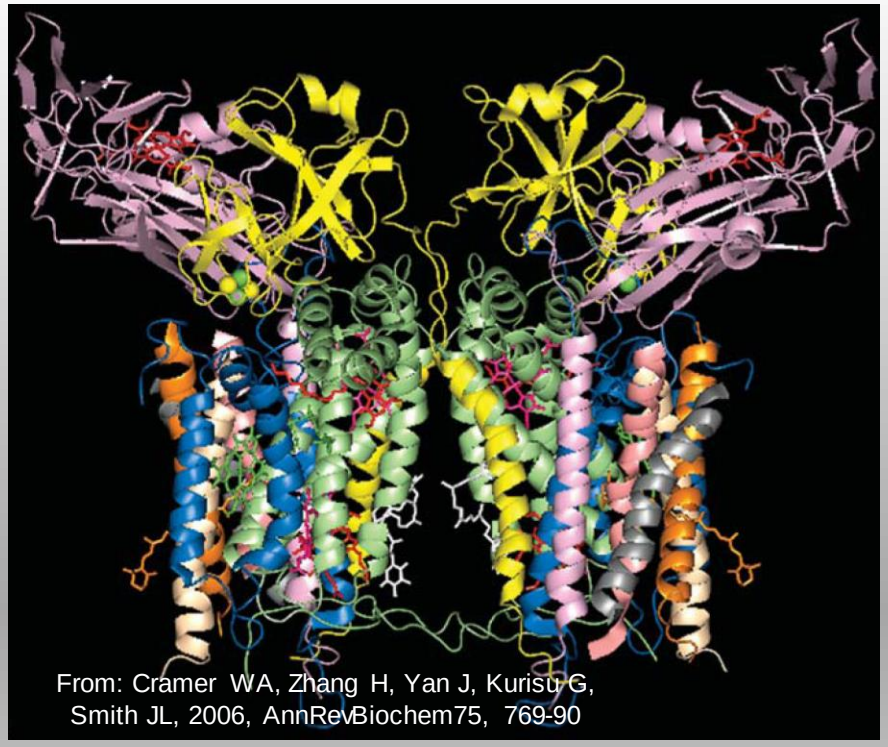
Most important manganese function: Water splitting complex of PSII structure



Four Mn ions constitute the catalytic center in the Mn4CaO5 cluster of the oxygen-evolving complex in PSII. Mn ions cycle through different oxidation states (Mn^{3+}, Mn^{4+}), the so-called S states, driven by the successive absorption of photons to extract electrons from H2O.

- 2 of the 4 Mn ions are redox-active ($^{3+/4+}$), accepting electrons from water and transferring them to P680
- Ca^{2+} helps in binding the water

Cyt b_6f complex



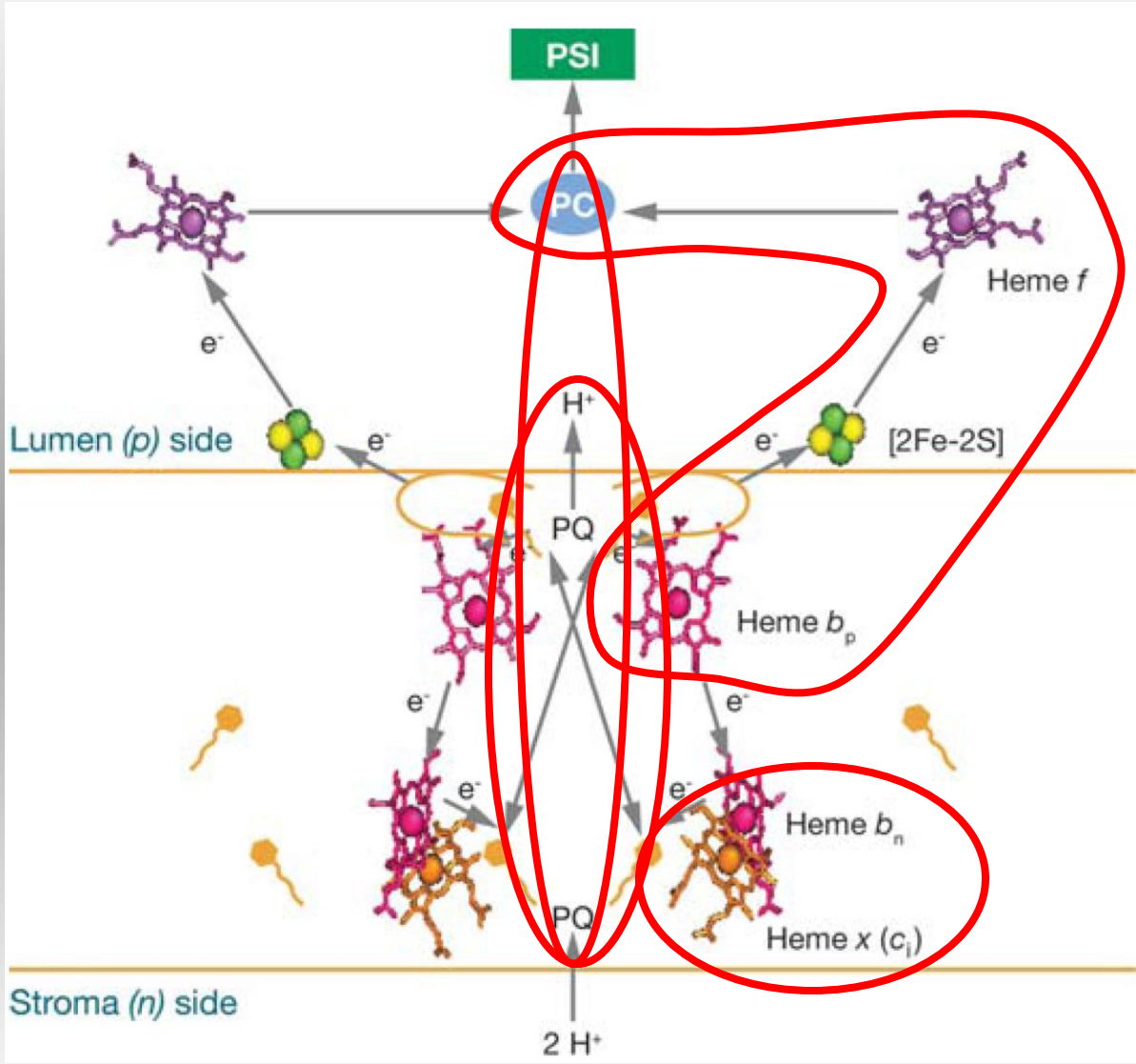
Structural characteristics

- Homodimer, each monomer consisting of 8 subunits totalling about 109 kDa
- Each monomer contains 13 transmembrane helices, and beta sheets in the Rieske subunit

Cytb$_6$f complex- mechanism

Functional characteristics

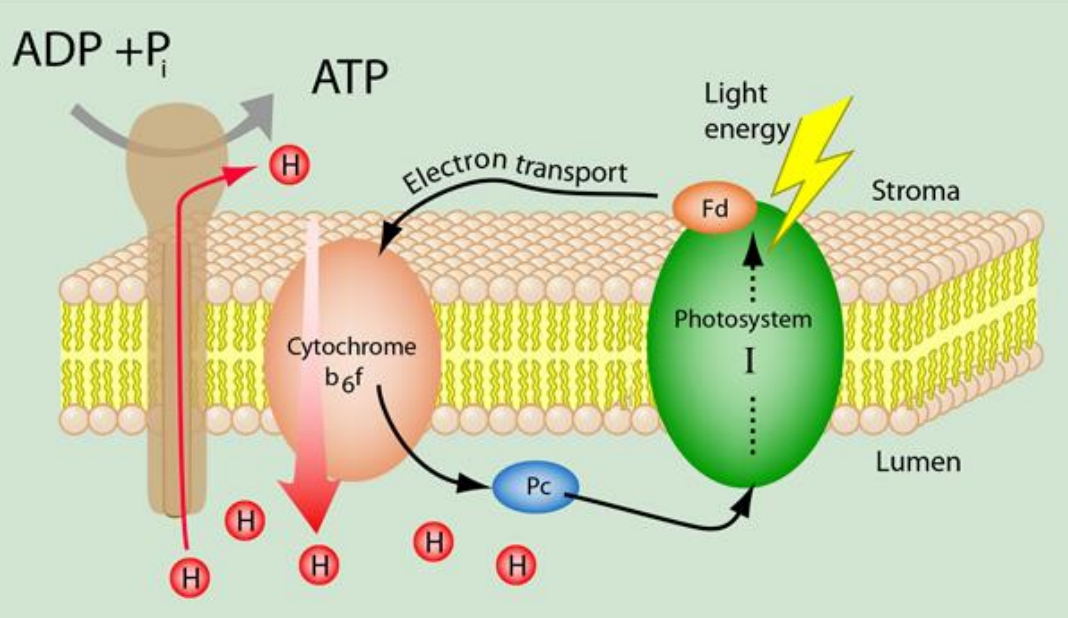
- transfers e- from PQ to plastocyanin (PC),
- It uses the difference in potential betwen Q_B and PC for translocating a proton via 2x2 heme *b* groups and 2x1 heme *x* group
- Electrons are transferred from the heme b groups to PC via a “Rieske” [2Fe2S]-cluster and a heme *f* group
- Cyclic electron transport occurs via coupling of ferredoxin to heme *x*



From: Cramer WA, Zhang H, Yan J, Kurisu G, Smith JL, 2006, AnnRevBiochem75_769-90

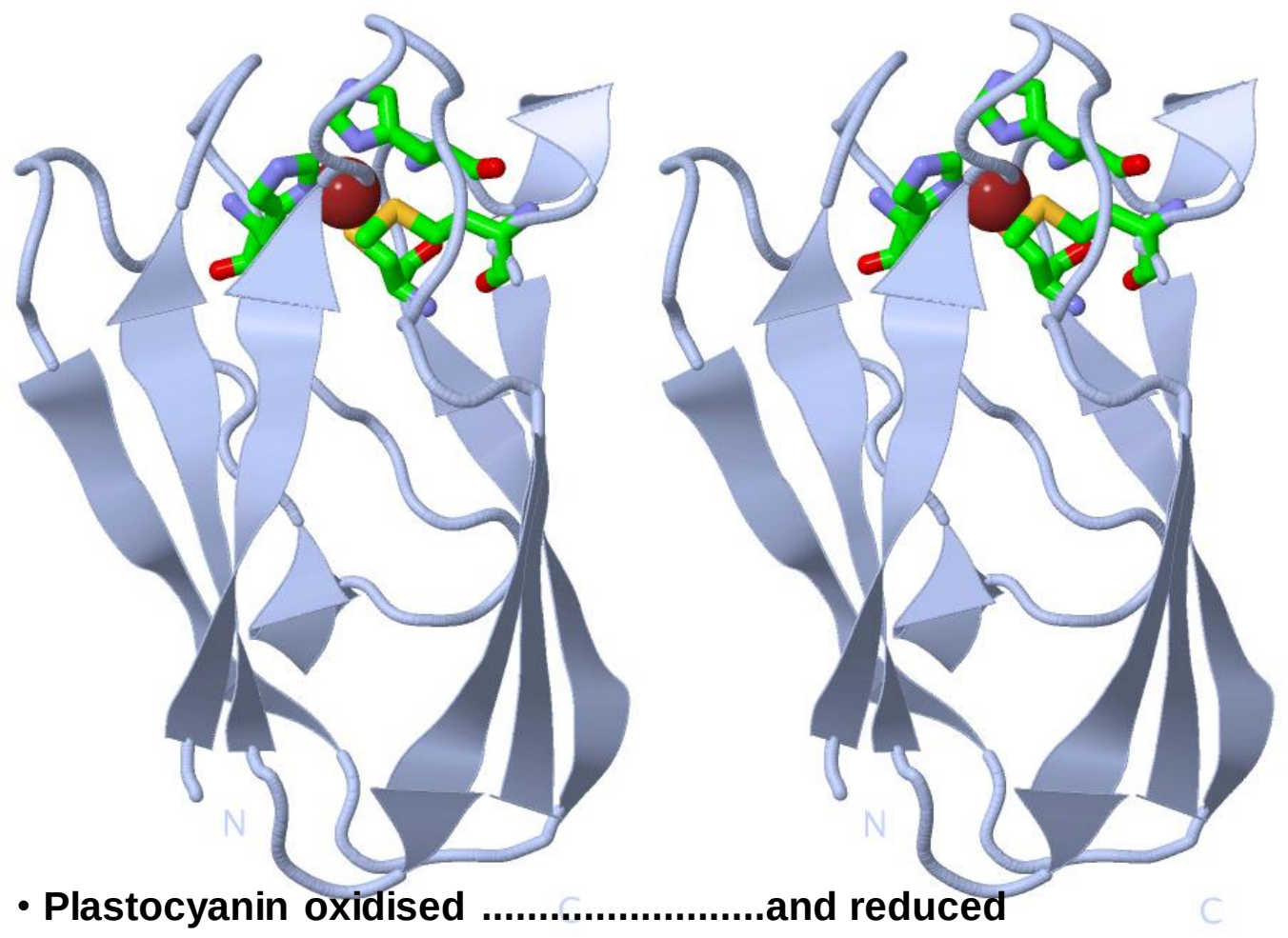
$Cytb_6f$ complex: Mechanism

Cyclic electron transport occurs via coupling of ferredoxin to heme *x*



From: Cramer WA, Zhang H, Yan J, Kurisu G, Smith JL, 2006, AnnRevBiochem75_769-90

Plastocyanin



• **Plastocyanin oxidised .....and reduced**

From: www.fli-leibniz.de with reference to data of Inoue T, Sugawara H, Hamanaka S, Tsukui H, Suzuki E, Kohzuma T, Kai Y, 1999, Biochemistry 38, 6063-9

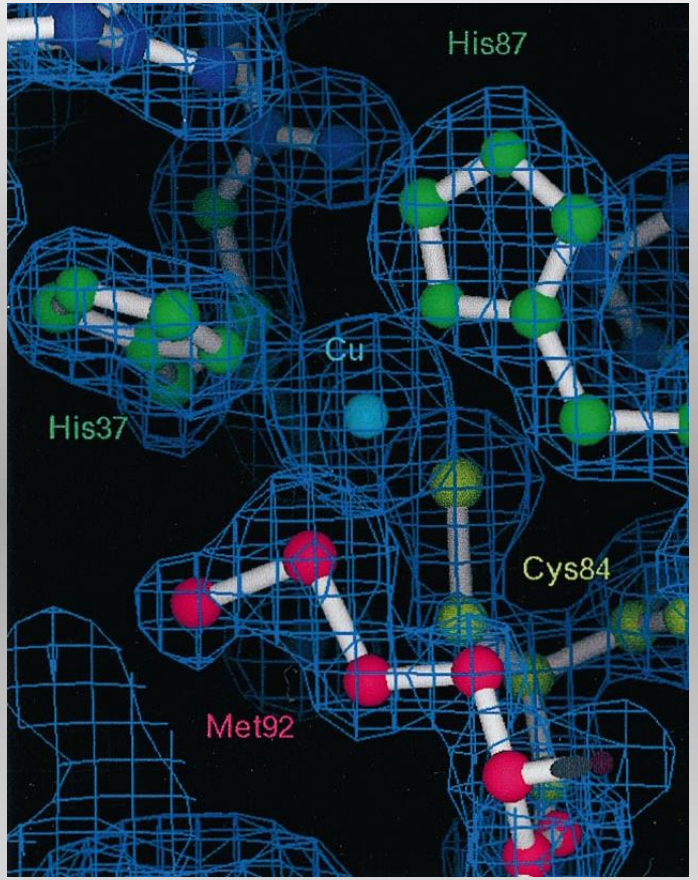
Structural characteristics

- about 100 amino acids, soluble protein
- type 1 (“blue”) copper protein
- copper bound by 2 His, 1 Cys, and 1 Met residue in distorded tetrahedral geometry

Plastocyanin

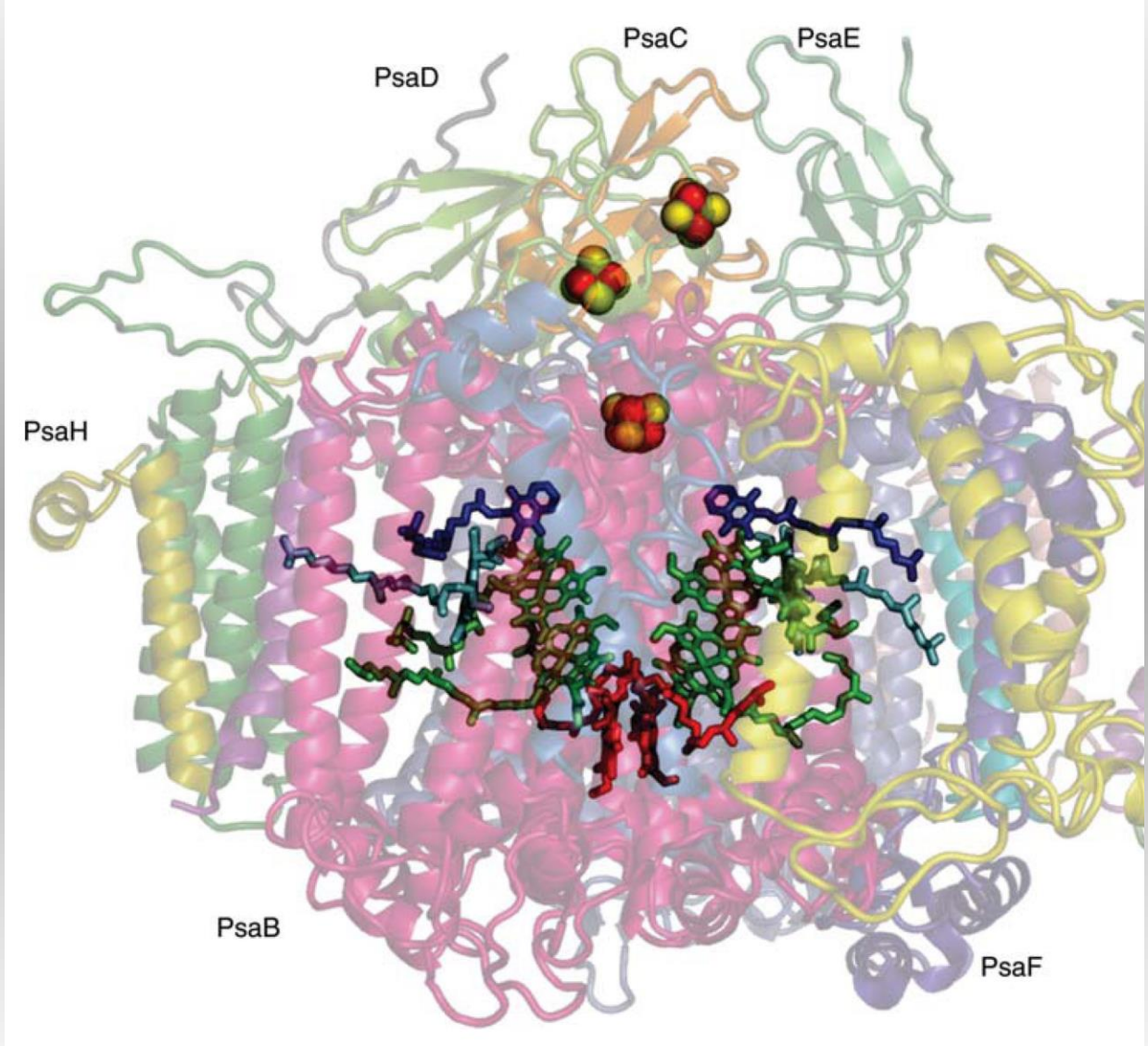
Functional characteristics

- Oxidised (Cu^{2+}) plastocyanin accepts electron from Cyt_{b6f} complex,
- Reduced ($\rightarrow Cu^{+}$) plastocyanin diffuses to the PSIRC
- Plastocyanin releases the electron ($Cu^{+} \rightarrow Cu^{2+}$)
- Rigid protein structure facilitates fast red/ox-changes, but recent data show that copper binding still causes changes in structure ("induced rack" rather than "entatic state")



From: Shibata N, Inoue T, Nagano C, Nishio N, Kohzuma T, Onodera K, Yoshizaki F, Sugimura Y, Kai Y, 1999, J Biol Chem. 274: 4225-30

Photosystem I reaction centre
(a) Overview



Structural characteristics

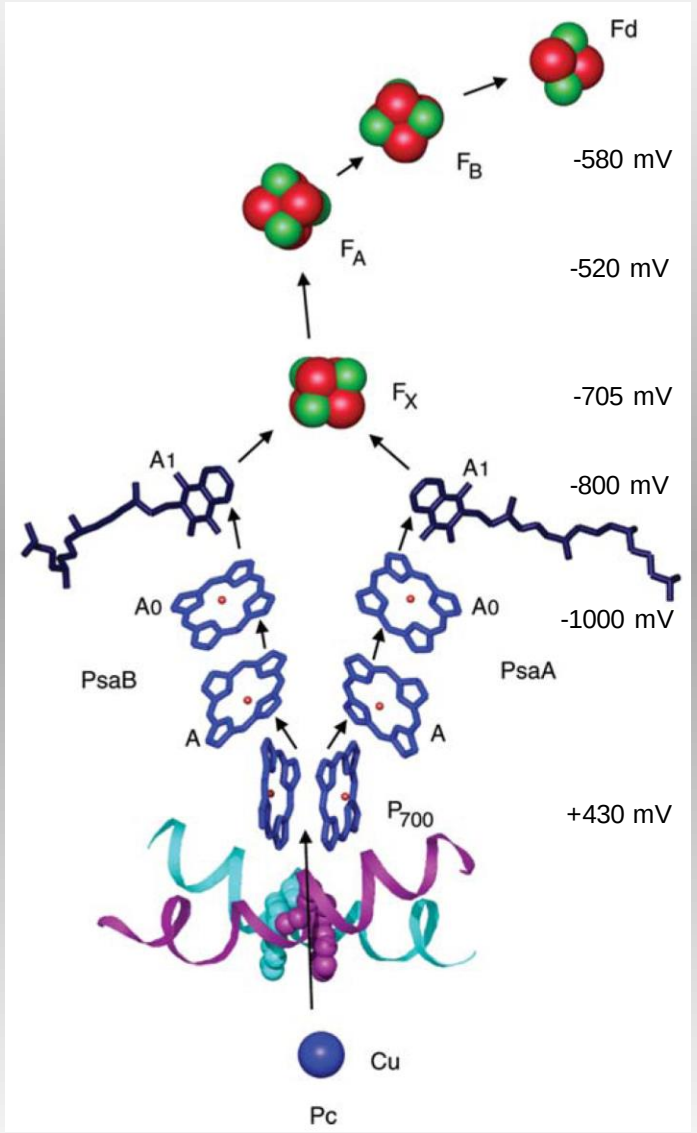
- forms trimers
- 12 subunits per monomer
- 127/133 cofactors per monomer (cyanos/plants):
  96/102 chlorophylls
  22 carotenoids
  2 phylloquinones
  3 [Fe4S4] clusters
  4 lipids

From: Nelson N, Yocum CF, 2006, AnnRevPlantBiol 57, 521-65

Photosystem I reaction centre
(a) Overview

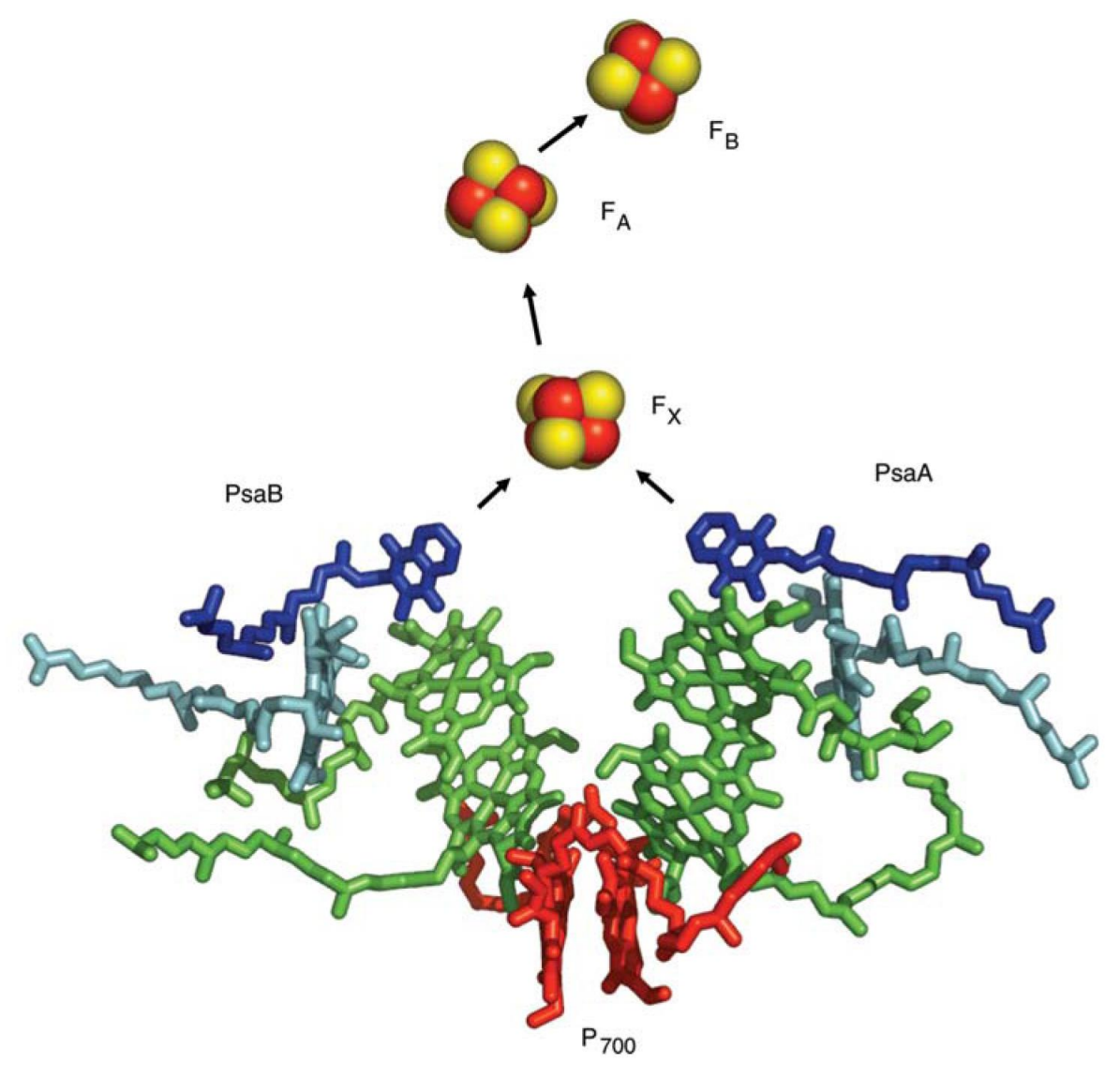
Funtional characteristics:

- primary charge separation:
  special pair (=P700, Chl a / Chl a' heterodimer),
  releases e^- to A_0 via A (both Chl a)
- e^- transport via A1 (phylloquinone) and the [4Fe4S]-clusters F_X, F_A and F_B to the [4Fe4S]-cluster of ferredoxin
- P700 is re-reduced by plastocyanin



From: Nelson N, Yocum CF, 2006, AnnRevPlantBiol 57, 521-65

Photosystem I reaction centre
(b) iron-sulphur clusters

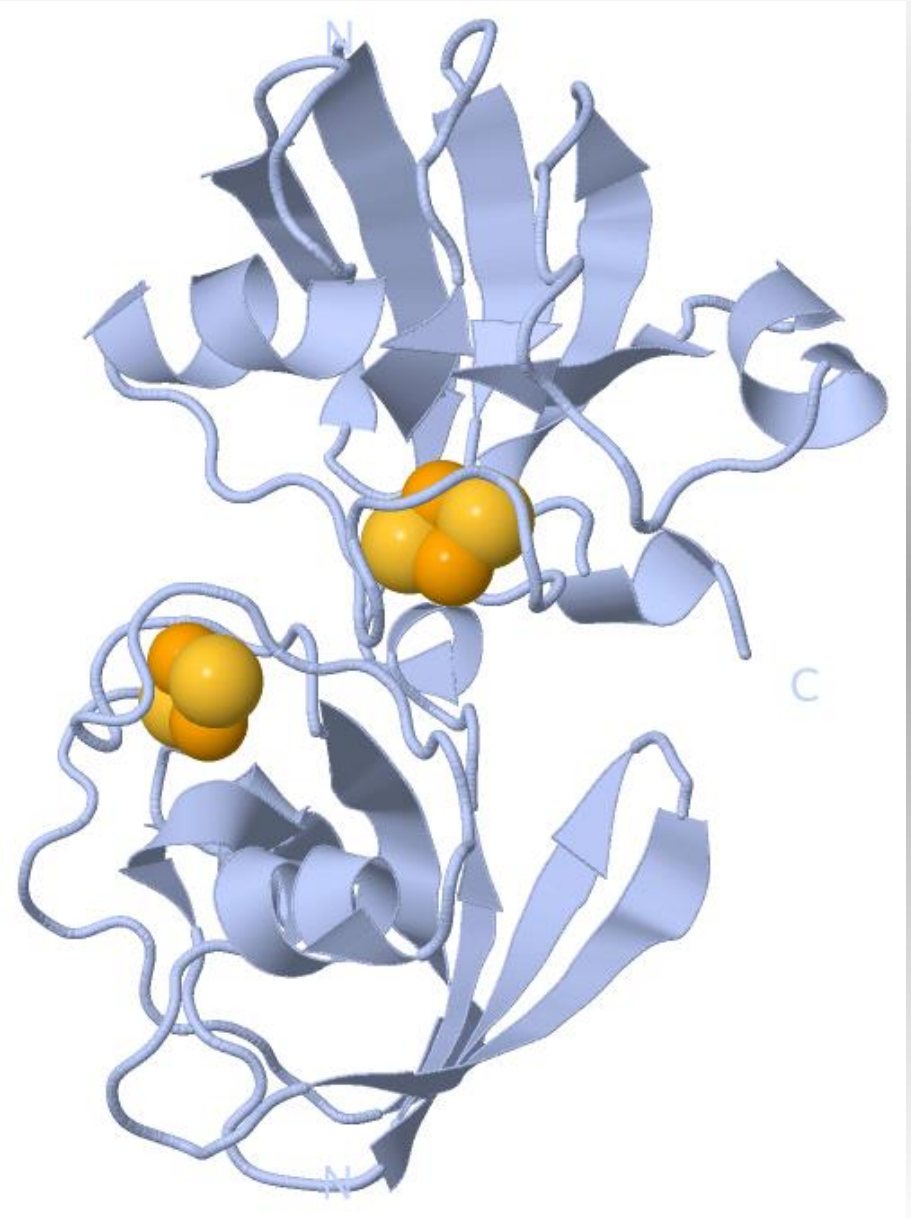


Function of the 4Fe4S-clusters in PSIRC

- accept electrons from the phylloquinones ("A_1")
- transfer the electrons to ferredoxin

From: Nelson N, Yocum CF, 2006, AnnRevPlantBiol 57, 521-65

Ferredoxin



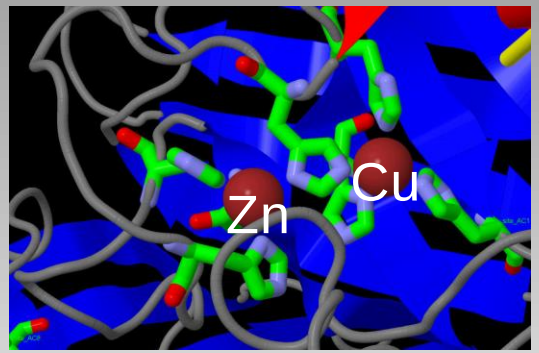
Structure and function

- usually dimer
- soluble protein with one [2Fe2S]-cluster per monomer
- transfers electrons from PSIRC to ferredoxin reductase (→ linear electron transport) or to the Cyt b6f complex (→ cyclic electron transport)

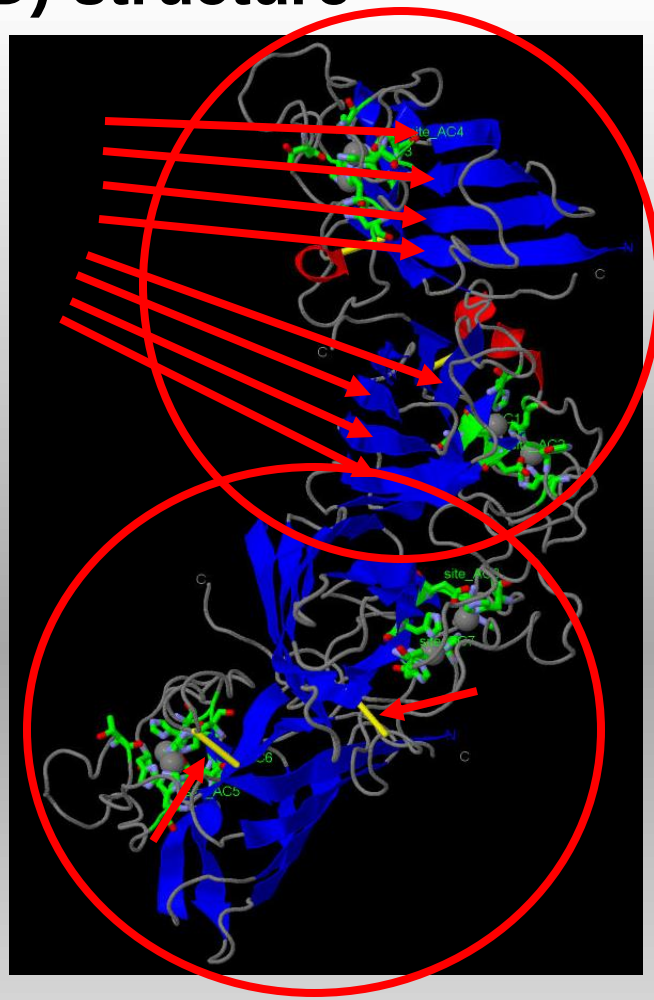
From: www.fli-leibniz.de with reference to data of Bes MT, Parisini E, Inda LA, Saraiva LM, Peleato ML, Sheldrick GM, 1999, Structure, 15;7(10):1201-11

Superoxide dismutase (SOD) structure

- Dimer of two identical subunits
- Each subunit consists of:
  - 8 anti-parallel β-strands forming a flattened cylinder,
  - 3 external loops
- 1 Cys-Cys disulfide bond stabilises loops
- 1 Cu^{2+} and 1 Zn^{2+} per subunit
- Cu^{2+} bound by 4 His, Zn^{2+} by 3 His + 1 Aspartate
- His-63 bridges Cu^{2+} and Zn^{2+}

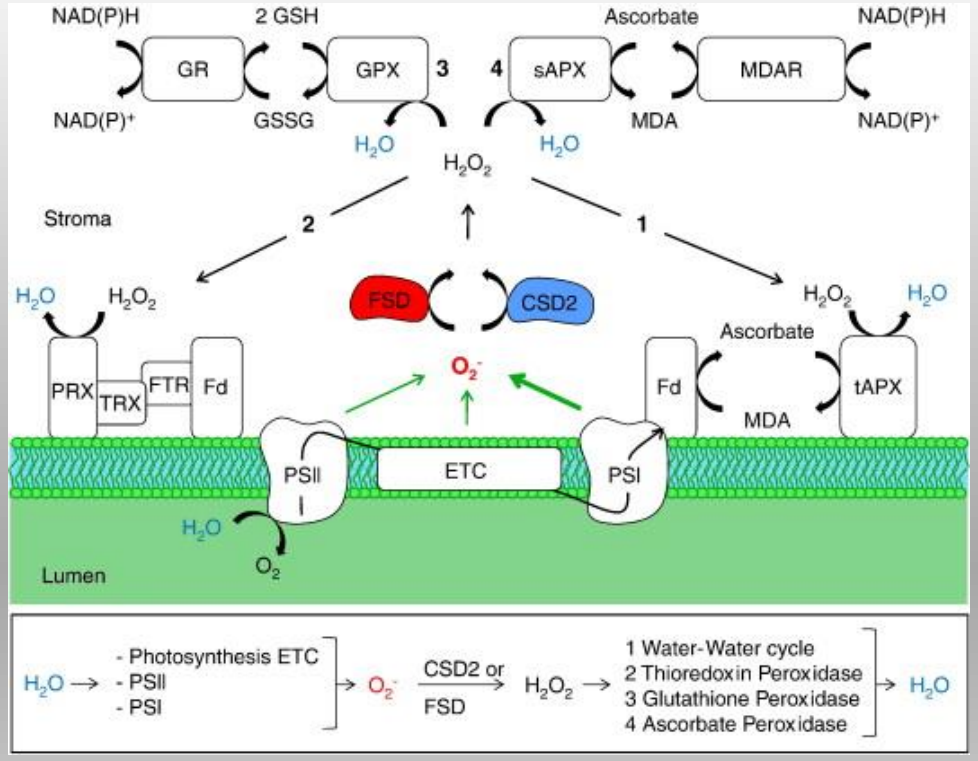


Spinach SOD, From: Kitagawa Y et al., 1991, J Biochem 109, 477-85, images generated with Jena 3D viewer



- SOD-Present in all aerobic organisms, particularly important in photosynthetic organisms
- Detoxifies superoxide that was generated e.g. by photosynthesis or respiration

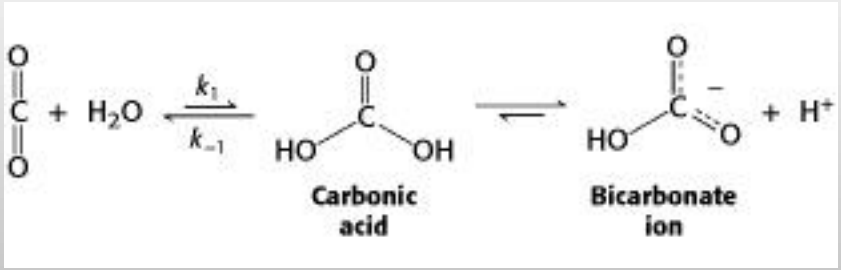
Water water cycle



From: Foyer CH et al., 1994, PlantCellEnvi17_507-23

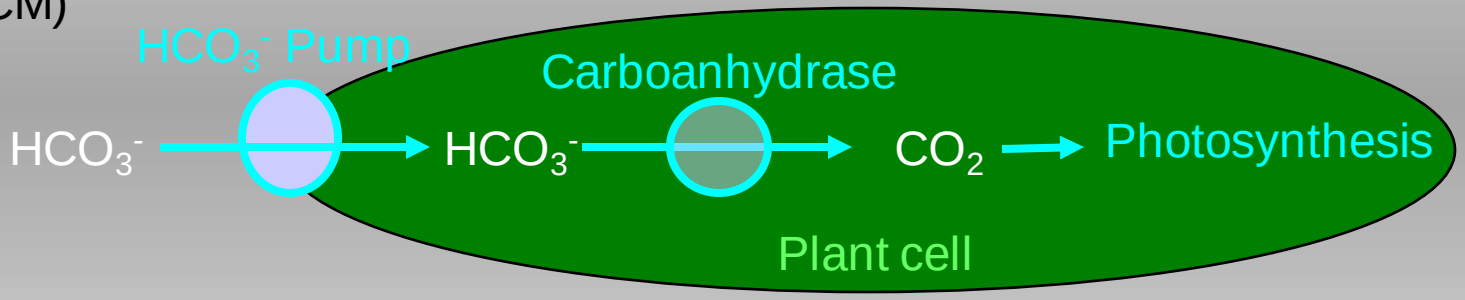
The water–water cycle and detoxification of superoxide in the chloroplast stroma. Hydrogen peroxide produced by SOD can be removed via four possible pathways labeled 1–4. Abbreviations: sAPX, stromal ascorbate peroxidase; tAPX, thylakoid-bound ascorbate peroxidase; ETC, electron transport chain; Fd, ferredoxin; FTR, Fd-Trx-reductase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, monodehydroascorbate; MDAR, MDA reductase; PRX, peroxiredoxin; PSI, photosystem I; PSII, photosystem II; TRX, thioredoxin

Enzymes with metal centres
CO_2 delivery: and Zn- carboanhydrases
(a1) function



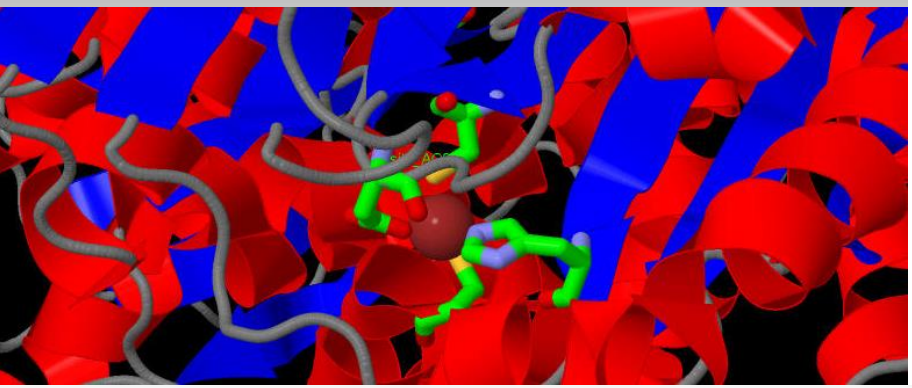
function of carboanhydrases (from: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer&part=A1199)

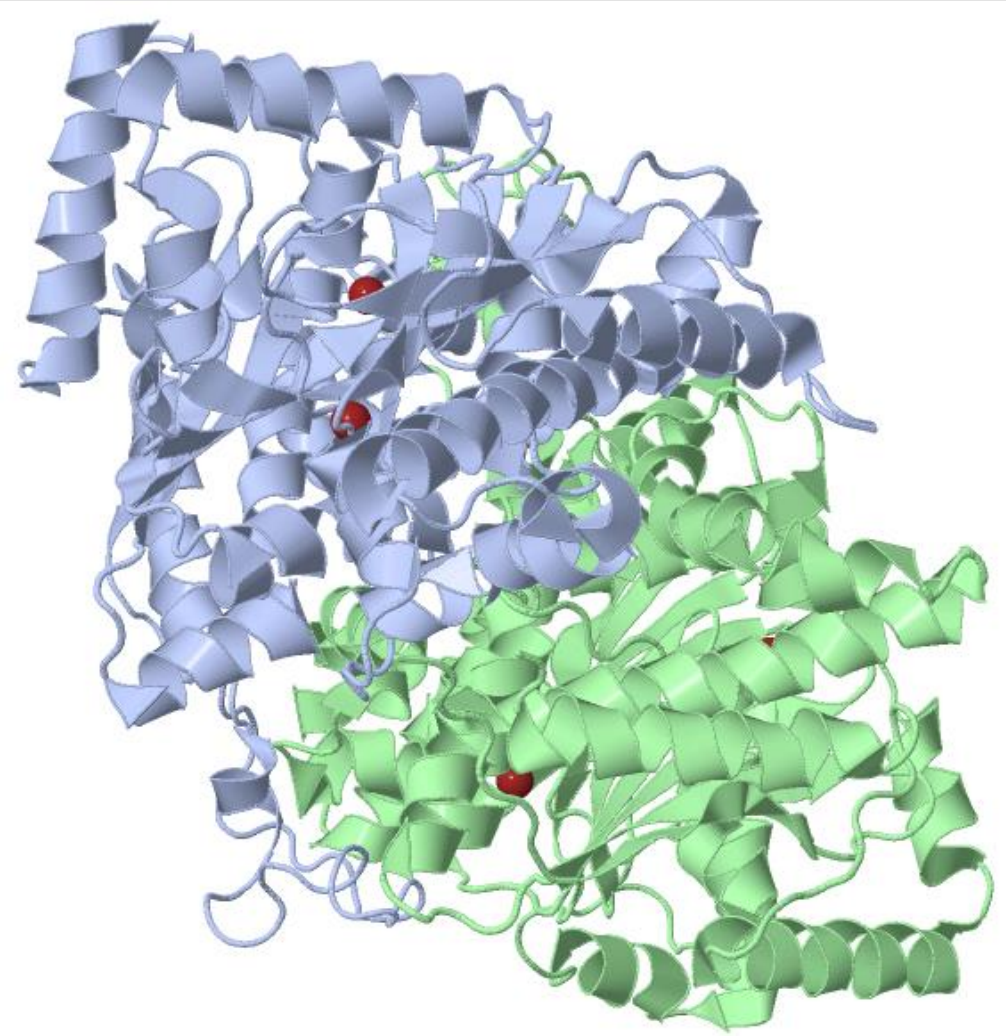
- Convert carbon dioxide to bicarbonate and vice versa
- Present in all aquatic photosynthetic organisms as part of the Carbon Concentrating Mechanism (CCM)



Photosynthesis related Enzymes with metal centres
CO_2 delivery: Zn- carboanhydrases
(b1) structure and properties of a typical Zn-CA

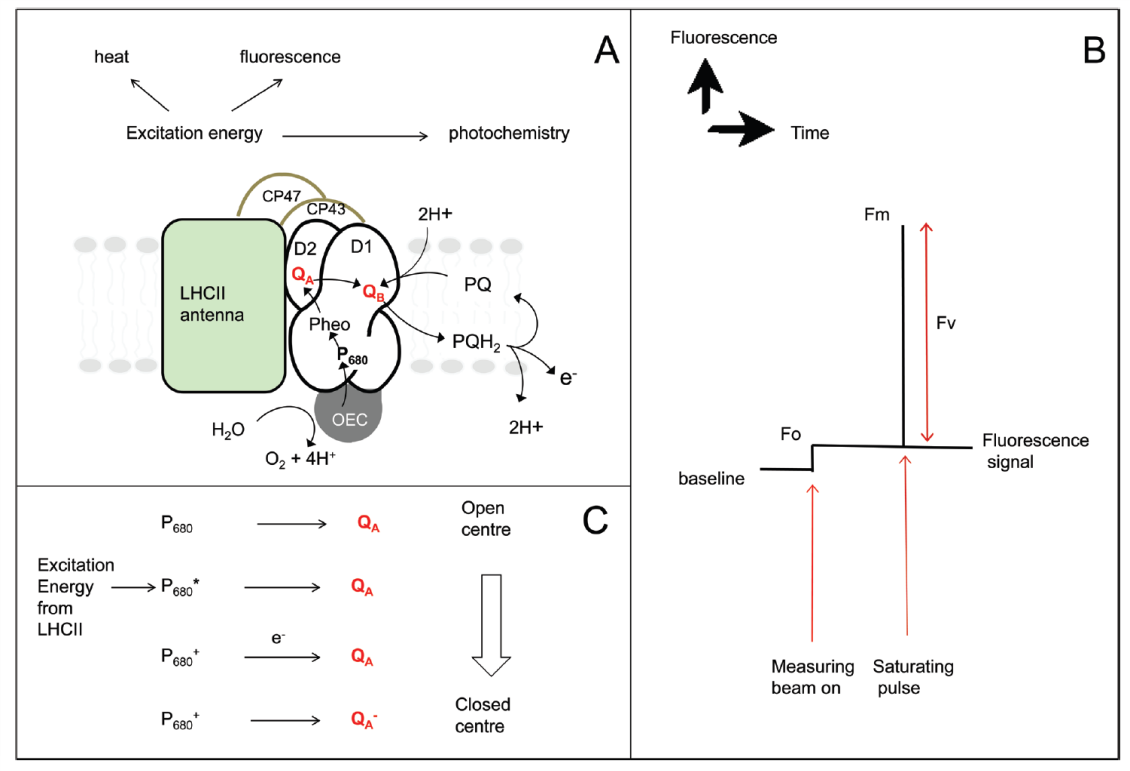
- Zn-CA is a homodimer
- Each monomer consists of and α/β-domain and 3 α-helices
- Zn^{2+} is coordinated by 2 Cys, 1 Asp and 1 His





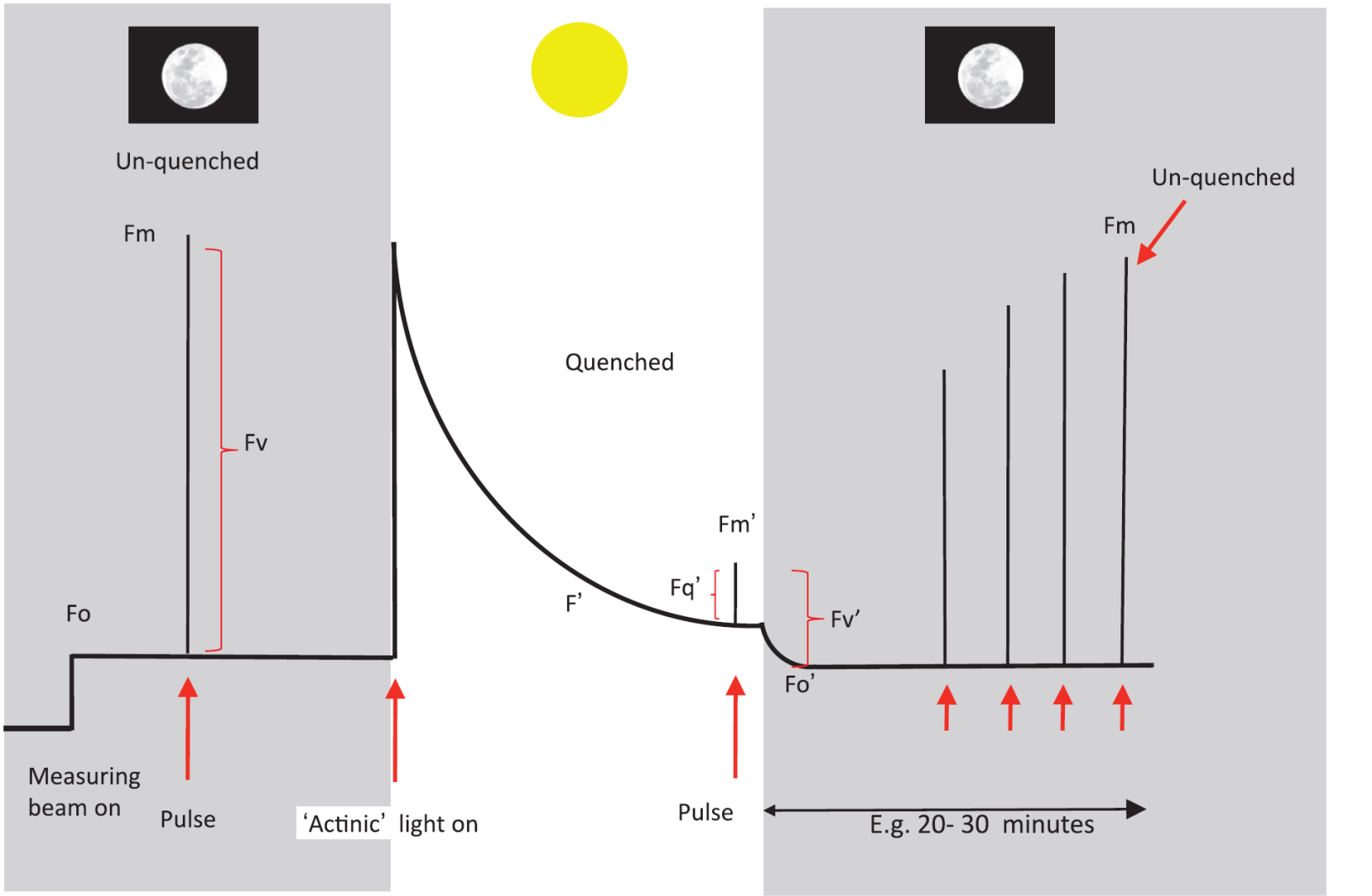
Zn-Carboanhydrase from the marine red alga Porphyridium (Mitsuhashi S et al., 2000, JBC 275, 5521-6)

Measurements of chlorophyll fluorescence kinetics

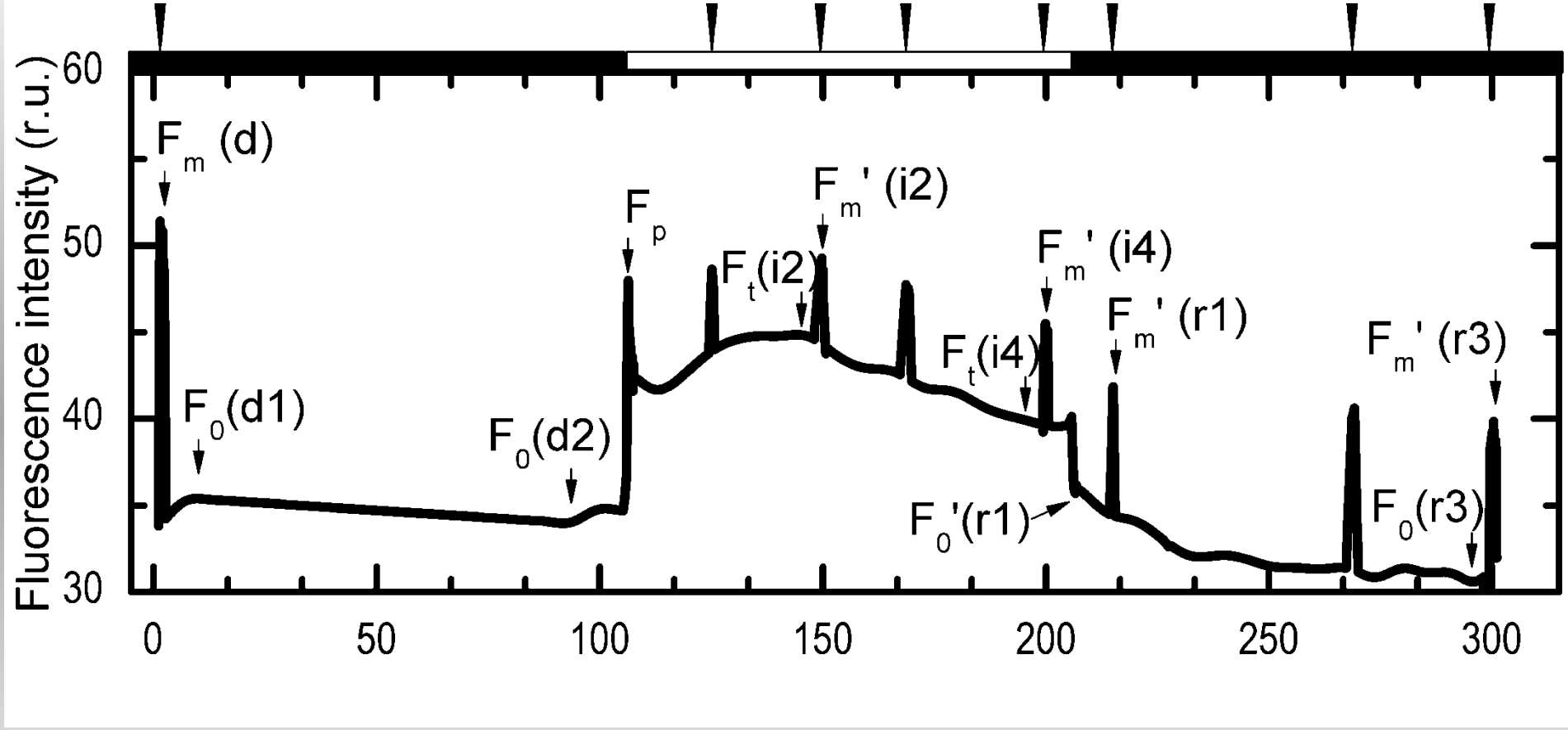


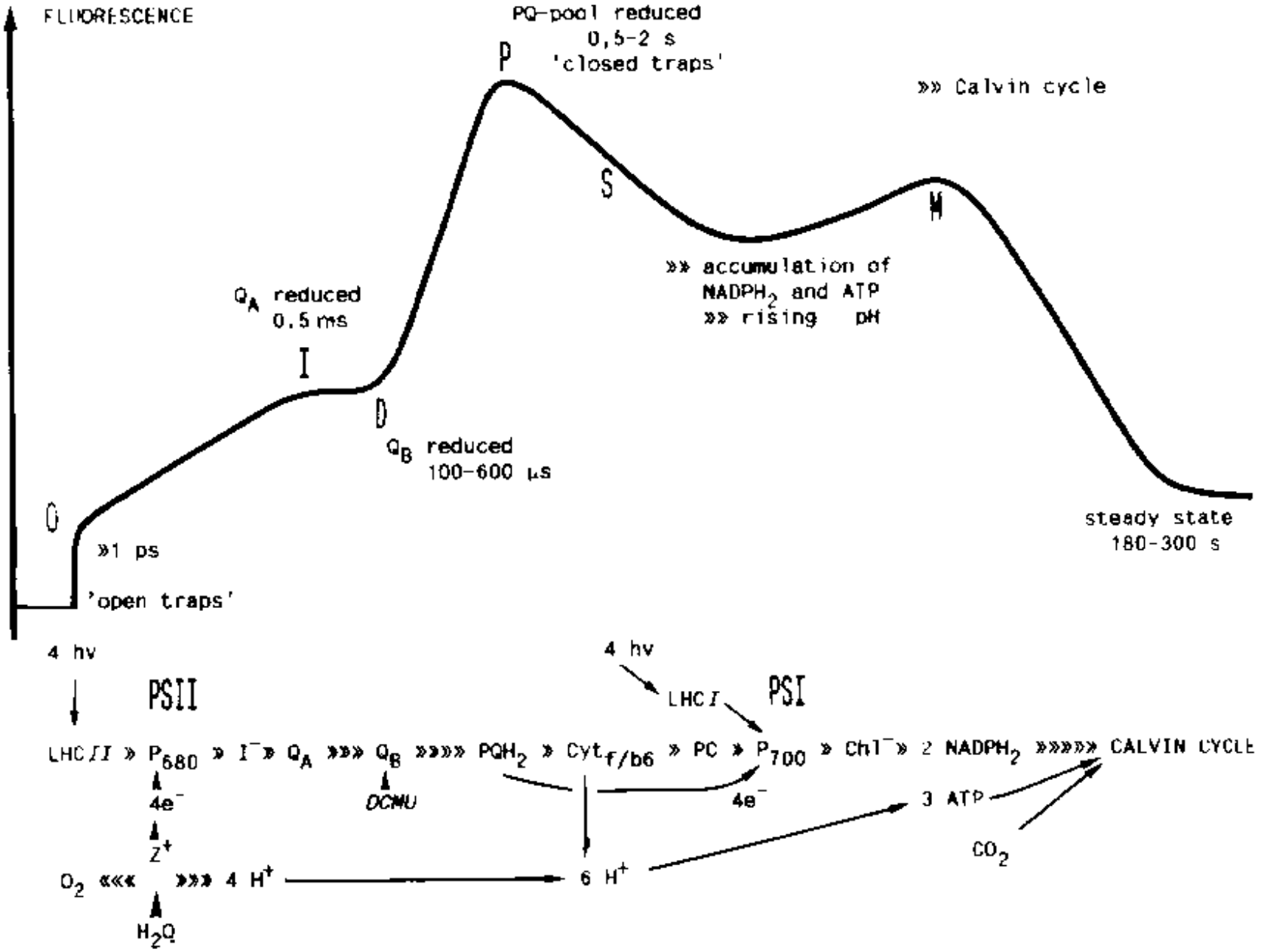
Murchie and Lawson, 2013, JExpBot

(A) electron transport within the PSII reaction centre complex. Energy absorbed by chlorophyll within the light-harvesting complex can be dissipated via photochemistry, by heat (non-photochemical quenching), or as fluorescence. The competition between these processes allows us to resolve the efficiency of PSII. (B) A typical fluorescence trace made on dark-adapted leaf material The measuring beam excites Chl but does not induce electron transport through PSII (C) A schematic figure explaining the transfer of energy and electrons within PSII that result in open and closed centres and the formation of *F*o and *F*m states, respectively. The excited state P680* and subsequent transfer of an electron to the primary acceptor QA gives rise to a closed centre. QA – cannot accept another electron until it has passed its electron onto the next electron acceptor, QB.

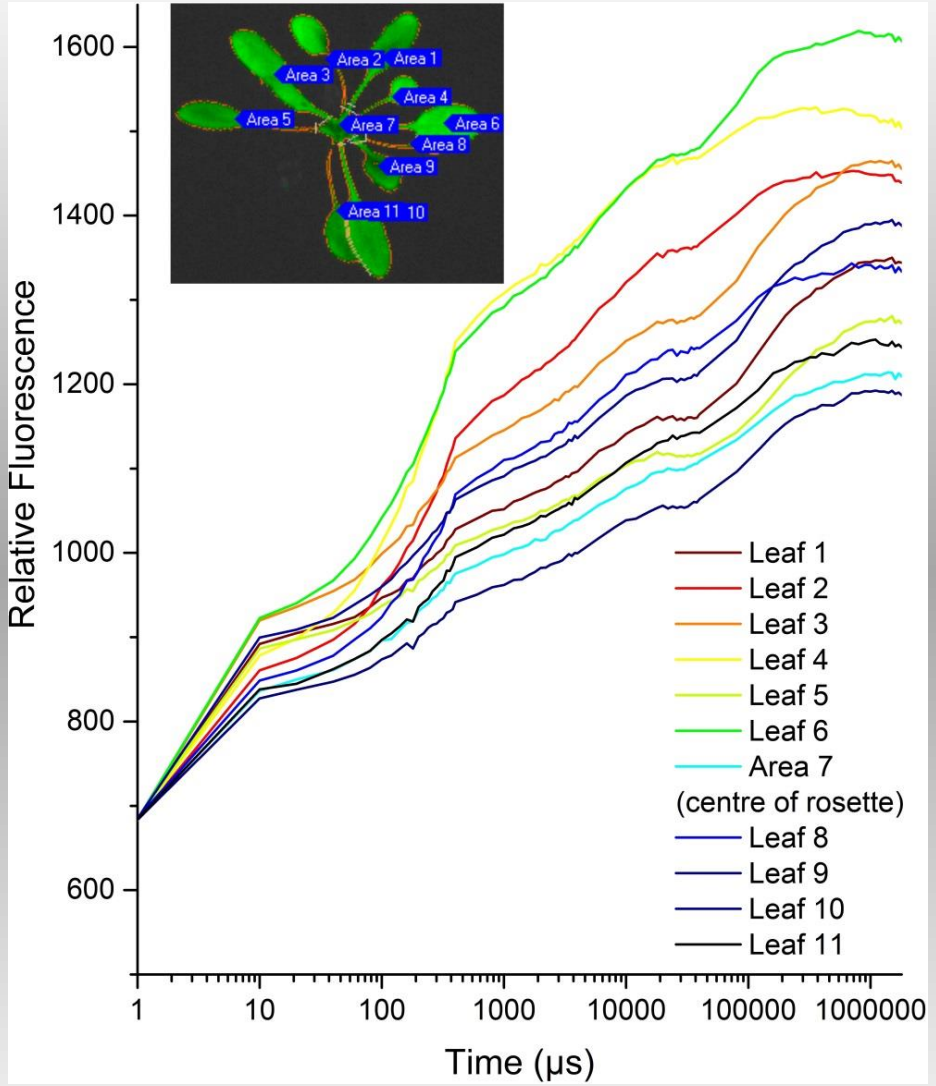


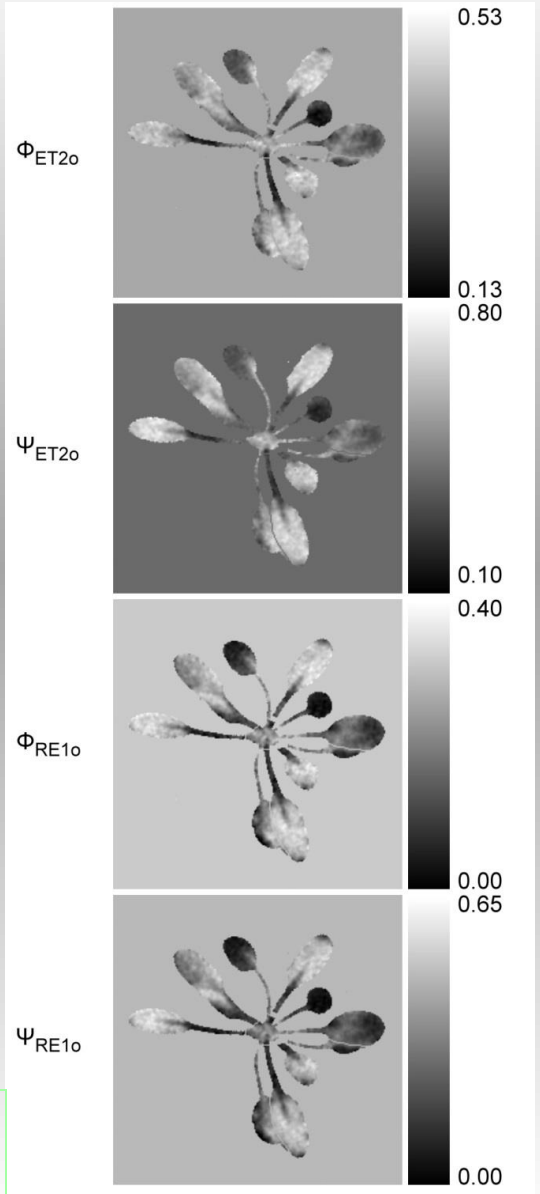
A stylized fluorescence trace of a typical experiment using dark-adapted leaf material to measure photochemical and nonphotochemical parameters


Fluorescence intensity (r.u.)
60
50
40
30
0
50
100
150
200
250
300
F m (d)
F 0(d1)
F 0(d2)
F p
F t(i2)
F m ' (i2)
F t(i4)
F m ' (i4)
F m ' (r1)
F 0'(r1)
F m ' (r3)
F 0(r3)


FLUORESCENCE
PQ-pool reduced
0,5-2 s
'closed traps'
P
»» Calvin cycle
S
M
»» accumulation of
NADPH2 and ATP
»» rising pH
QA reduced
0.5 ms
I
D
QB reduced
100-600 µs
O
»1 ps
'open traps'
steady state
180-300 s
4 hv
PSII
4 hv
LHC I
PSI
LHC II » P680 » I⁻» QA »»» QB »»»» PQH2 » Cytf/b6 » PC » P700 » Chl⁻» 2 NADPH2 »»»»» CALVIN CYCLE
4e⁻
DCMU
4e⁻
3 ATP
Z+
CO2
O2 «««
»»» 4 H+
6 H+
H2O

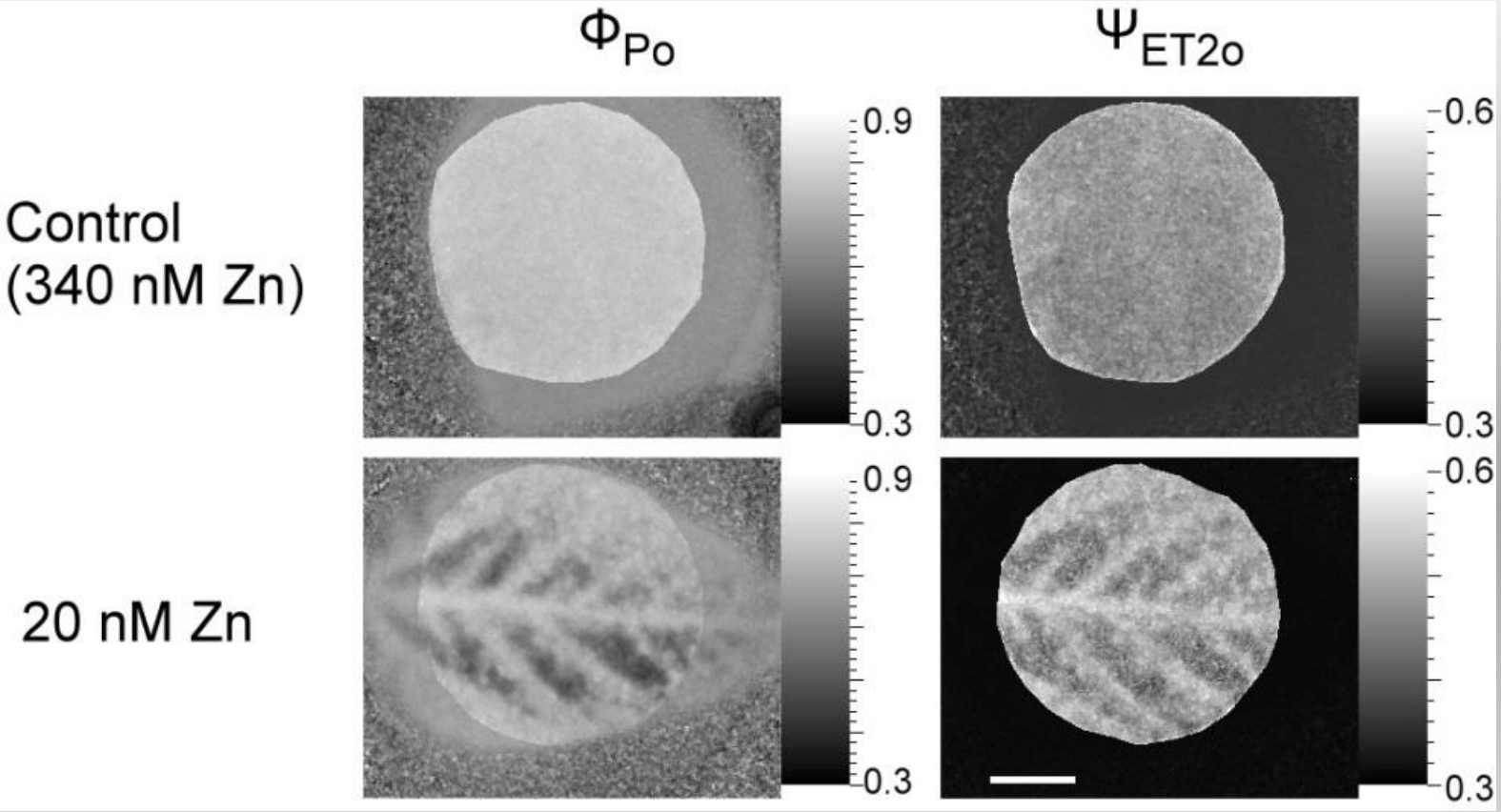
Differences in OJIP kinetics between leaves of an *A. thaliana* plant measured by direct fast imaging





Küpper H, Benedikty Z, Morina F, Andresen E, Mishra A, Trtílek M (2019) Plant Physiology 179, 369-381, DOI: https://doi.org/10.1104/pp.18.00953

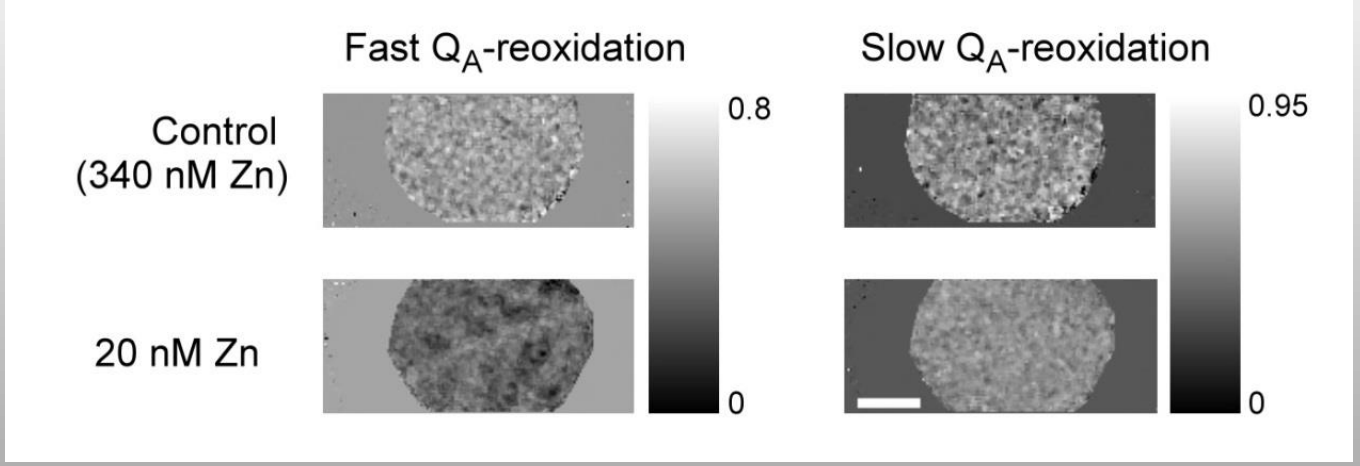
Changes of Ψ_{ET2o} and Φ_{Po} in response to zinc deficiency stress



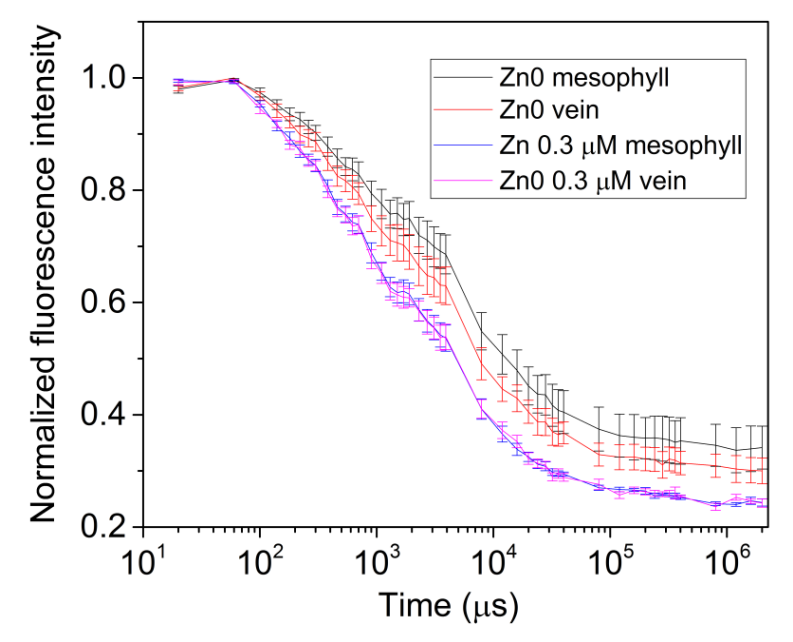
Soybeans treated with "0" Zn addition (20 nM residue from chemicals and water) compared to "control" (340 nM Zn) for 6 weeks. Scale bar is 1 cm.

Küpper H, Benedikty Z, Morina F, Andresen E, Mishra A, Trtílek M (2019) Plant Physiology 179, 369-381, DOI: https://doi.org/10.1104/pp.18.00953

Differences in Q_A re-oxidation kinetics of veins (bundle sheath cells) and regular mesophyll cells in response to zinc deficiency stress



Soybeans treated with "0" Zn addition (20 nM residue from chemicals and water) compared to "control" (340 nM Zn) for 6 weeks.
Scale bar is 1 cm.

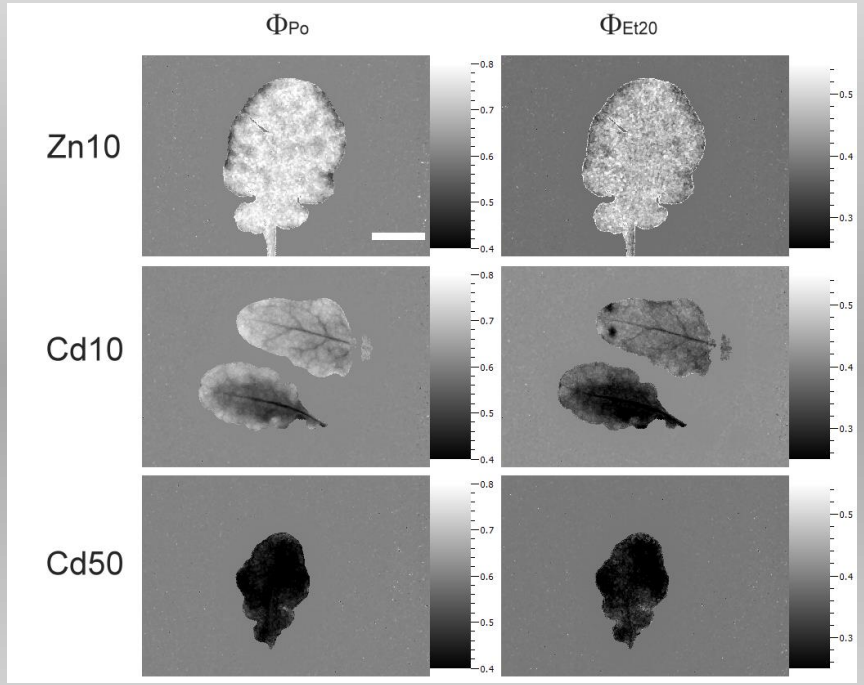


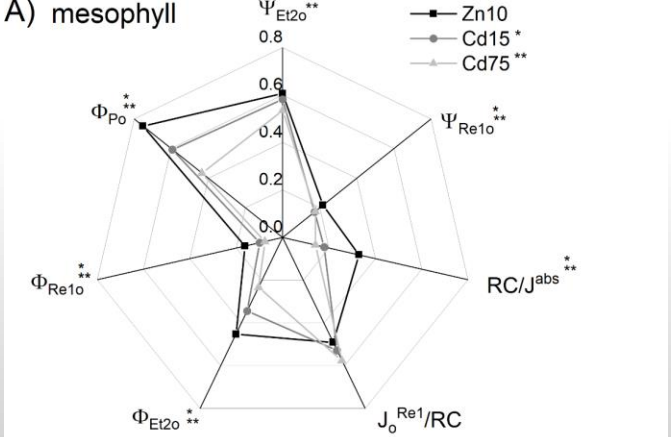
Küpper H, Benedikty Z, Morina F, Andresen E, Mishra A, Trtílek M (2019) Plant Physiology 179, 369-381, DOI: https://doi.org/10.1104/pp.18.00953

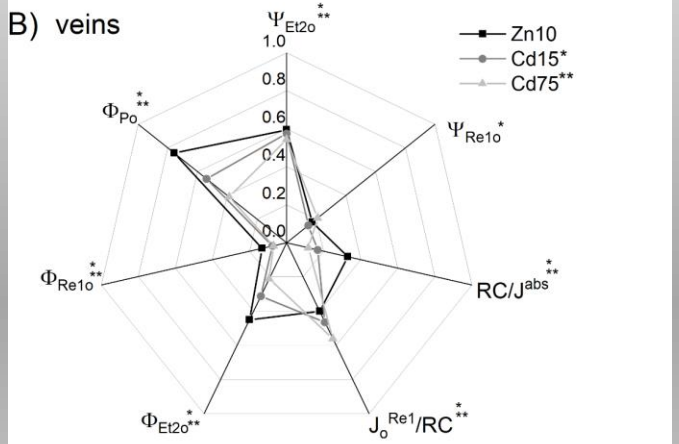
Information selected from the fast OJIP fluorescence induction (data necessary for the calculation of the so-called JIP parameters)	
$F_o = F_{20\mu s \text{ or } 50\mu s}$	First reliable fluorescence value after the onset of actinic illumination; used as initial value of the fluorescence
$F_{300\mu s}$	Fluorescence value at 300 μs
$F_J \equiv F_{2ms}$	Fluorescence value at 2 ms (J-level)
$F_I \equiv F_{30ms}$	Fluorescence value at 30 ms (I-level)
$F_P (\equiv F_M)$	Fluorescence value at the peak of OJIP curve; maximum value under saturating illumination
t_{Fmax}	Time to reach the maximum fluorescence value F_M
Area	Area between OJIP curve and the line $F = F_M$
Technical flurescence parameters	
$V_V = F_t - F_o$	Variable Chl fluorescence
$F_V = F_M - F_o$	Maximum variable Chl fluorescence
$V_t = (F_t - F_o)/(F_M - F_o)$	Relative variable Chl fluorescence
$M_o = (\Delta V/\Delta t)_o = 4\ ms^{-1} \cdot (F_{300\mu s} - F_o)/(F_M - F_o)$	Approximate value of the initial slope of relative variable Chl fluorescence curve V_t (for $F_o = F_{50\mu s}$)
$S_m \equiv Area/F_V$	Normalized area (assumed proportional to the number of reduction and oxidation of one Q_A-molecule during the fast OJIP transient, and therefore related to the number of electron carriers per electron transport chain)
Definitions of energy fluxes	
$J^{ABS} = J^{TR} + J^{DI}$	Rate of photon absorption by total PSII antenna-denoted *as absorbed photon flux*
J^{TR}	Rate of exciton trapping (leading to Q_A reduction) by all PSII RCs-denoted *trapped exciton flux*
J_o^{TR}	Maximum (initial) trapped exciton flux
J^{DI}	Rate of energy dissipation in all the PSIIs, in processes other than trapping – denoted as *dissipated energy flux*
J_o^{ET2}	Electron transport flux from Q_A to Q_B
J_o^{RE1}	Electron transport flux until PSI acceptors (defined at $t = 30$ ms, corresponding to the I-level)
Quantum yields and efficiencies/probabilities	
$\varphi_{Po} \equiv J_o^{TR}/J^{ABS} = 1 - F_o/F_M$	Maximum quantum yield of primary PSII photochemistry
$\varphi_{Pt} \equiv J^{TR}/J^{ABS} = 1 - F_t/F_M = \varphi_{Po} \cdot (1 - V_t)$	Quantum yield of primary PSII photochemistry
$\varphi_{ET2o} \equiv J_o^{ET2}/J^{ABS} = 1 - F_J/F_M = \varphi_{Po} \cdot (1 - V_J)$	Quantum yield of the electron transport flux from Q_A to Q_B
$\varphi_{RE1o} \equiv J_o^{RE1}/J^{ABS} = 1 - F_I/F_M = \varphi_{Po} \cdot (1 - V_I)$	Quantum yield of the electron transport flux until the PSI electron acceptors
$\psi_{ET2o} \equiv J_o^{ET2}/J_o^{TR} = 1 - V_J$	Efficiency/probability with which a PSII trapped electron is transferred from Q_A to Q_B
$\psi_{RE1o} \equiv J_o^{RE1}/J_o^{TR} = 1 - V_I$	Efficiency/probability with which a PSII trapped electron is transferred until PSI acceptors
$\delta_{RE1o} \equiv J_o^{RE1}/J_o^{ET2} = (1 - V_I)/(1 - V_J)$	Efficiency/probability with which an electron from Q_B is transferred until PSI acceptors

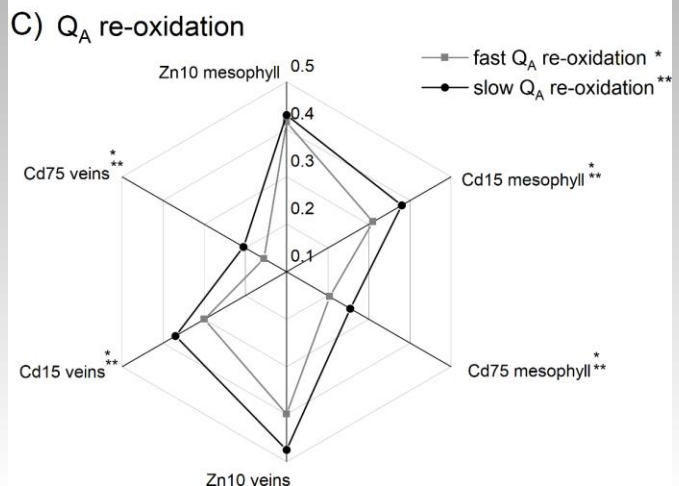
Stirbet, Govindjee 2011

Arabidopsis halleri- effects of Cd on photosynthetic performance









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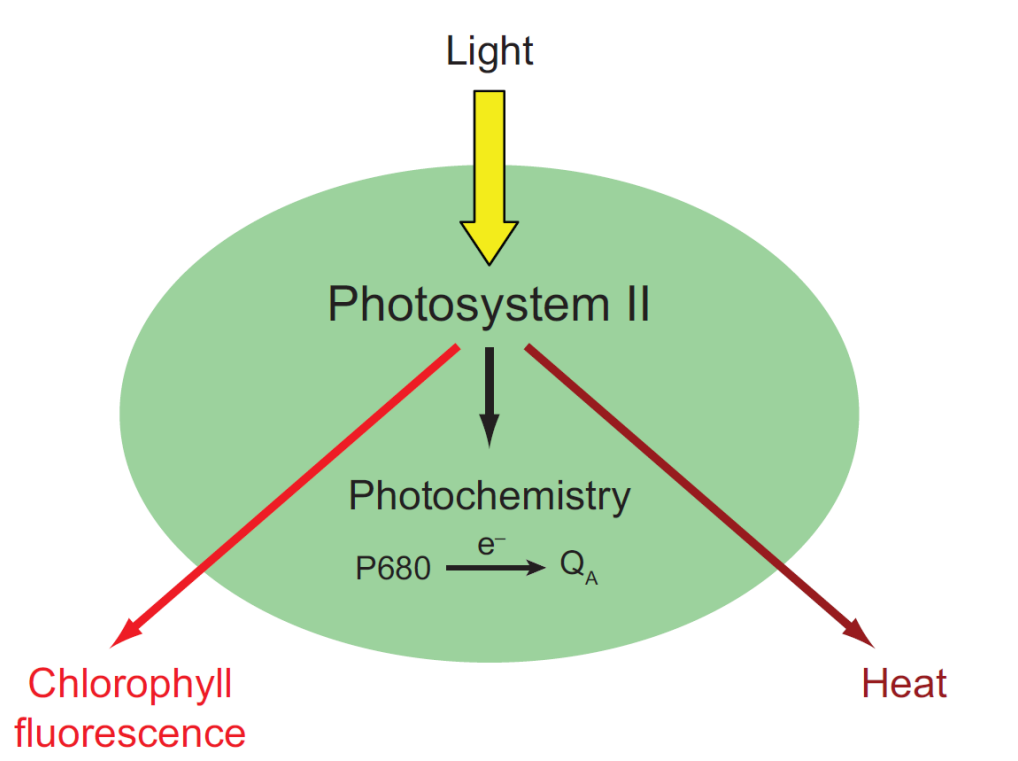
Non-photochemical quenching



Baker, 2008

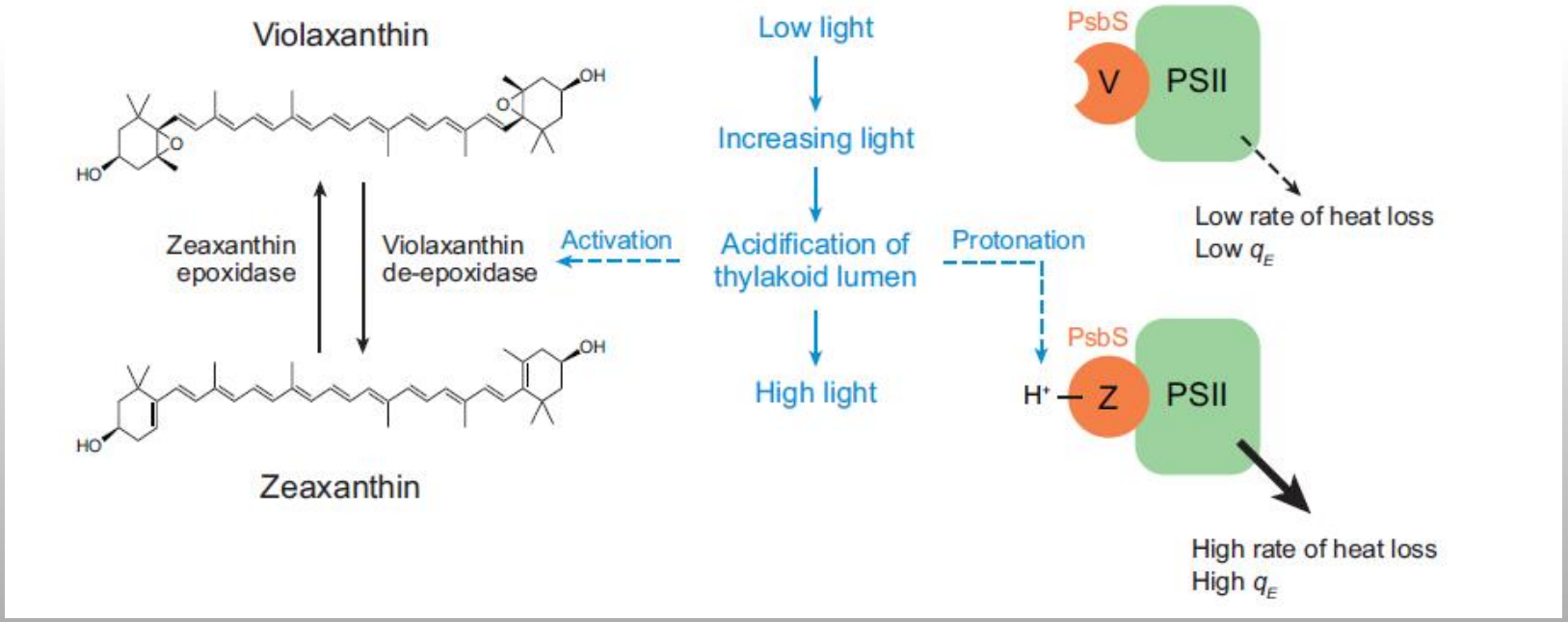
Simple model of the possible fate of light energy absorbed by PSII. Light energy absorbed by chlorophylls associated with PSII can be used to drive photochemistry in which an electron (e−) is transferred from the reaction center chlorophyll, P680, to the primary quinone acceptor of PSII, QA. Alternatively, absorbed light energy can be lost from PSII as chlorophyll fluorescence or heat. The processes of photochemistry, chlorophyll fluorescence, and heat loss are in direct competition for excitation energy. If the rate of one process increases the rates of the other two will decrease

Parameter	Definition	Physiological relevance
F, F'	Fluorescence emission from dark- or light-adapted leaf, respectively.	Provides little information on photosynthetic performance because these parameters are influenced by many factors. F' is sometimes referred to as F_s' when at steady state
F_o, F_o'	Minimal fluorescence from dark- and light-adapted leaf, respectively	Level of fluorescence when Q_A is maximally oxidized (PSII centers open)
F_m, F_m'	Maximal fluorescence from dark- and light-adapted leaf, respectively	Level of fluorescence when Q_A is maximally reduced (PSII centers closed)
F_v, F_v'	Variable fluorescence from dark- and light-adapted leaves, respectively	Demonstrates the ability of PSII to perform photochemistry (Q_A reduction)
F_q'	Difference in fluorescence between F_m' and F'	Photochemical quenching of fluorescence by open PSII centers.
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by PSII is used for reduction of Q_A.
F_q'/F_m'	PSII operating efficiency	Estimates the efficiency at which light absorbed by PSII is used for Q_A reduction. At a given photosynthetically active photon flux density (PPFD) this parameter provides an estimate of the quantum yield of linear electron flux through PSII. This parameter has previously been termed $\Delta F/F_m'$ and ϕ_{PSII} in the literature.
F_v'/F_m'	PSII maximum efficiency	Provides an estimate of the maximum efficiency of PSII photochemistry at a given PPFD, which is the PSII operating efficiency if all the PSII centers were 'open' (Q_A oxidized).
F_q'/F_v'	PSII efficiency factor	Relates the PSII maximum efficiency to the PSII operating efficiency. Nonlinearly related to the proportion of PSII centers that are 'open' (Q_A oxidized). Mathematically identical to the coefficient of photochemical quenching, q_P.
NPQ	Nonphotochemical quenching	Estimates the nonphotochemical quenching from F_m to F_m'. Monitors the apparent rate constant for heat loss from PSII. Calculated from $(F_m/F_m') - 1$.
q_E	Energy-dependent quenching	Associated with light-induced proton transport into the thylakoid lumen. Regulates the rate of excitation of PSII reaction centers.
q_I	Photoinhibitory quenching	Results from photoinhibition of PSII photochemistry.
q_L	Fraction of PSII centers that are 'open'	Estimates the fraction of 'open' PSII centers (with Q_A oxidized) on the basis of a lake model for the PSII photosynthetic apparatus. Given by $(F_q'/F_v')(F_o'/F')$
q_T	Quenching associated with a state transition	Results from phosphorylation of light-harvesting complexes associated with PSII
ϕ_F	Quantum yield of fluorescence	Number of fluorescent events for each photon absorbed

Non-photochemical quenching

NPQ in leaves can consist of three components, based on their relaxation kinetics in the dark:

Energy-dependent quenching, q_E, (major component under moderate light in non-stressed leaves)
Photoinhibitory quenching, q_I, (excess light)
State transition quenching, q_T (low light)

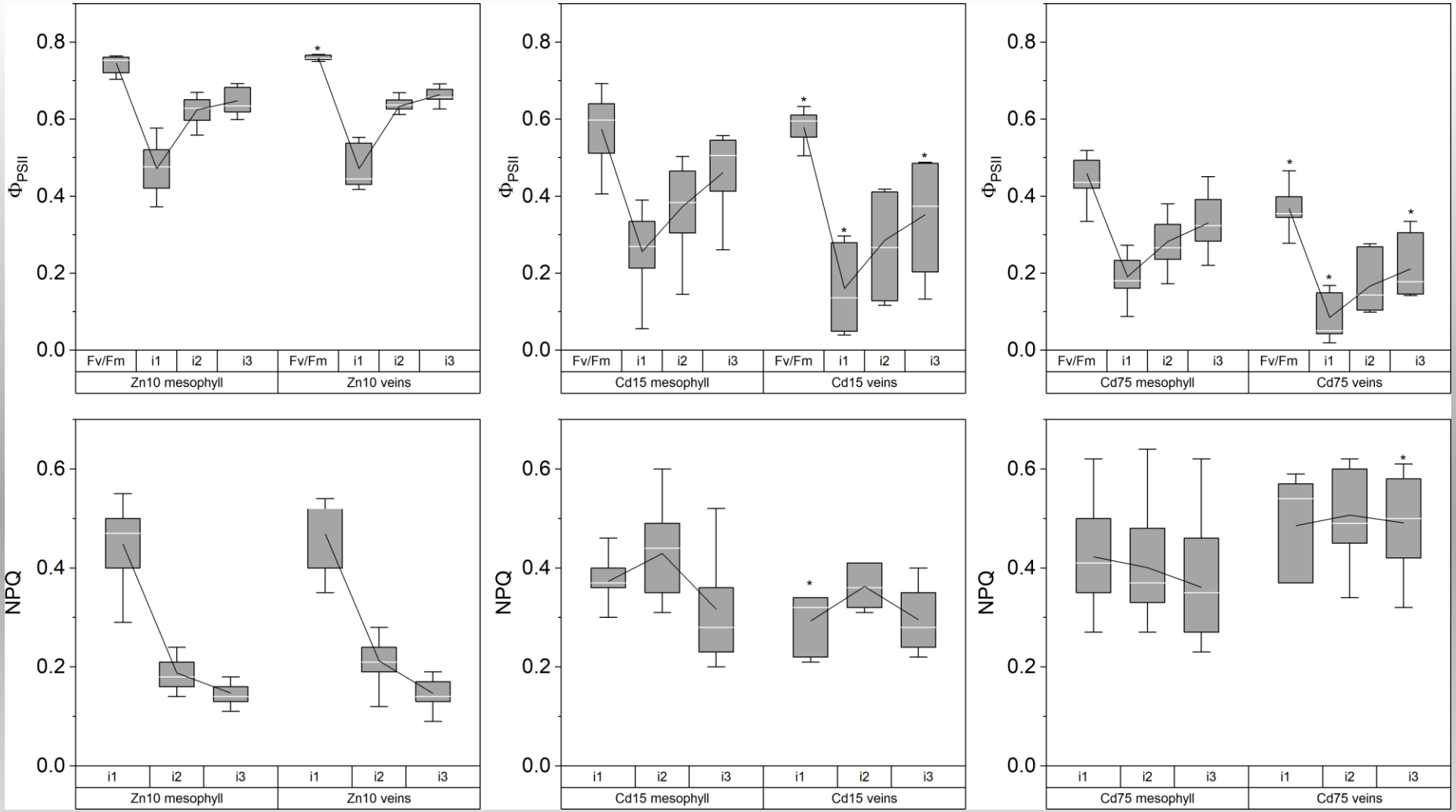


Baker, 2008

qE is developed with increased acidification of the thylakoid lumen resulting from electron transport. This
acidification results in the activation of violaxanthin de-epoxidase and protonation of some carboxylic acid residues of the PsbS, a protein associated with the PSII antennae

Protonation of PsbS and binding of zeaxanthin to PSII produces conformational changes in the antennae that result in increases in the quantum yield of thermal dissipation of excitation.

Higher sensitivity of veins to Cd toxicity than the mesophyll in *A. halleri*



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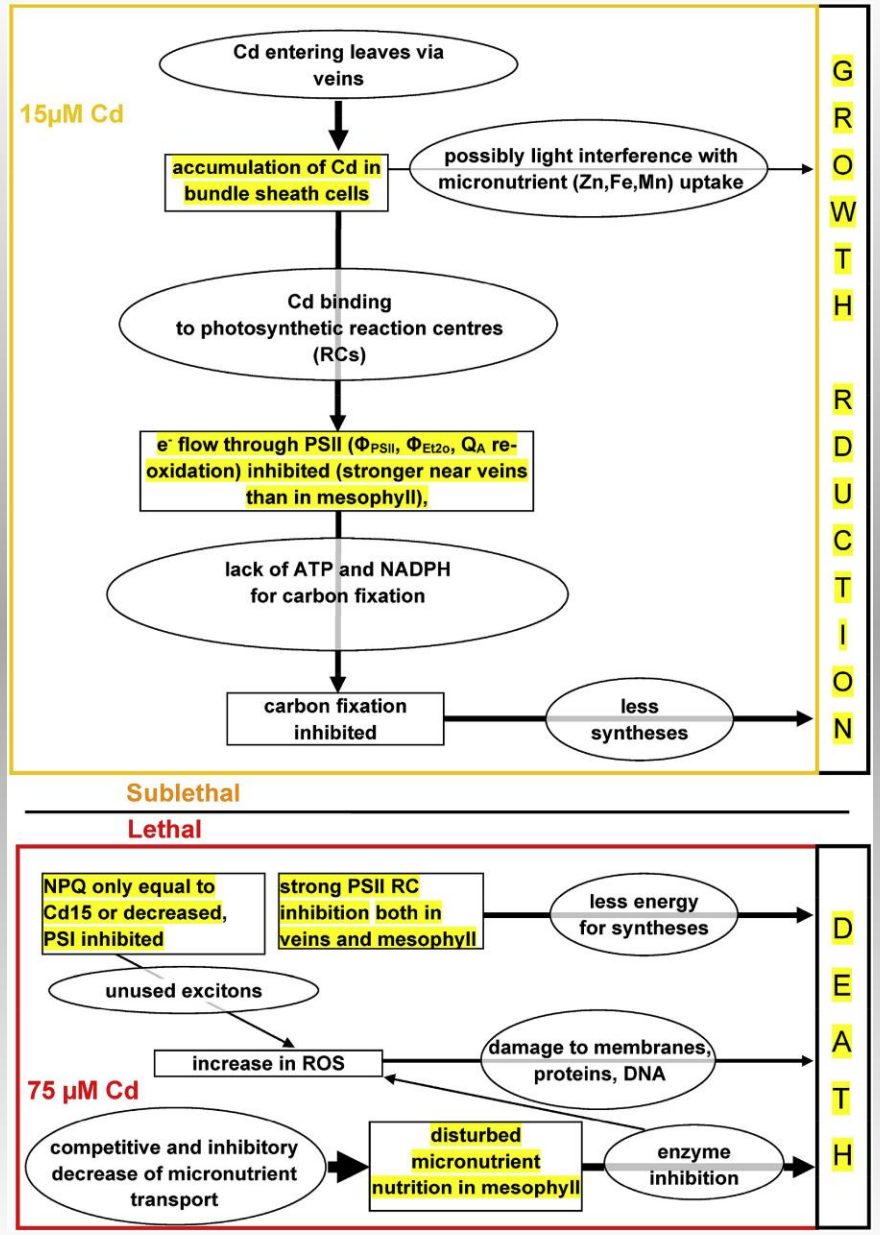
Scheme of pathways of cadmium toxicity in photosynthetic tissues.



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Concluding remarks

- Photosynthesis is a sensitive parameter of plant physiological state in response to stress (nutrient deficiency, toxicity)
- Fluorescence imaging can identify spatial heterogeneity of photosynthetic performance
- Fluorescence imaging offers new possibilities for understanding the operation and regulation of photosynthesis (in response to different factors) in different tissues/cells

Thank you for your attention!