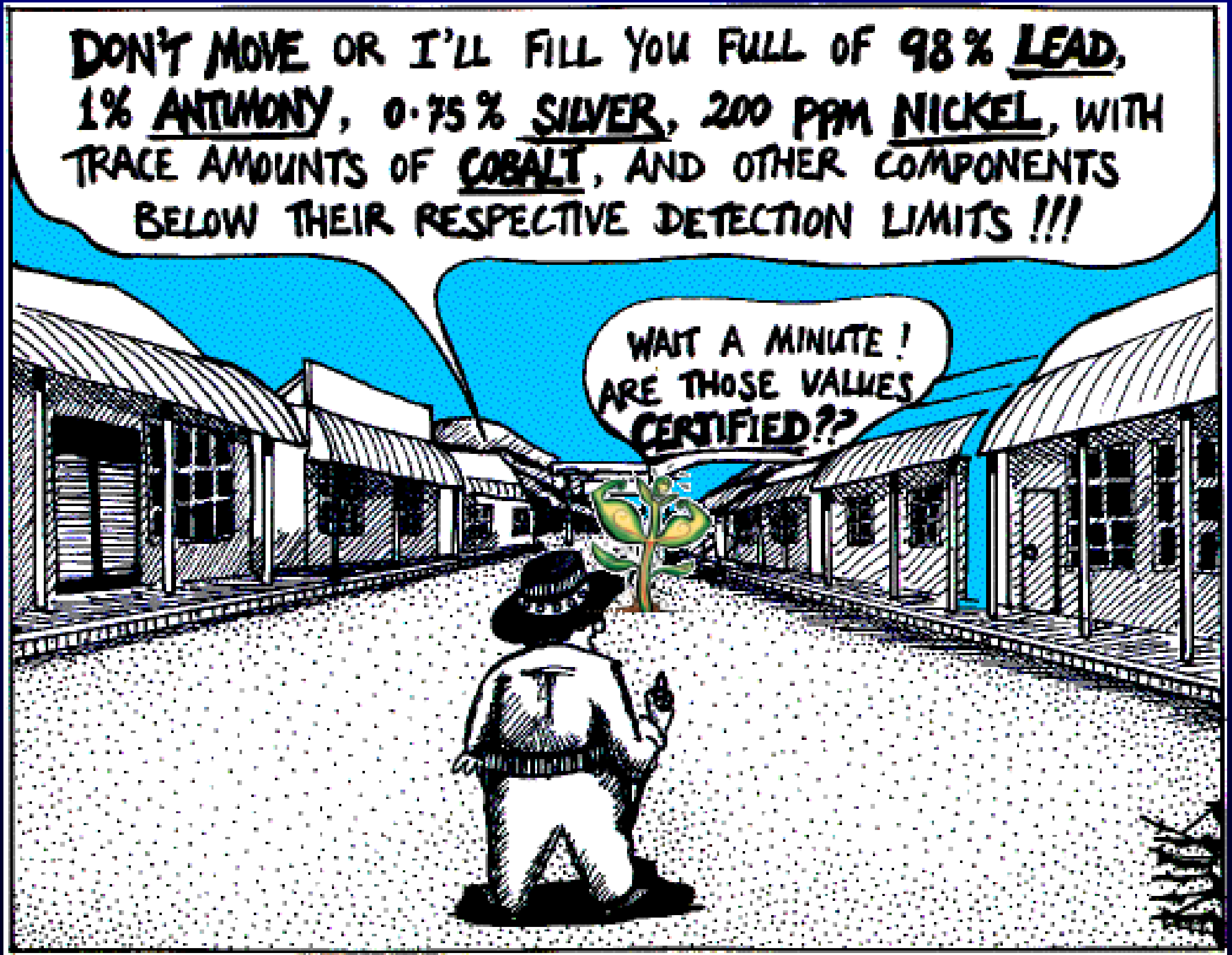
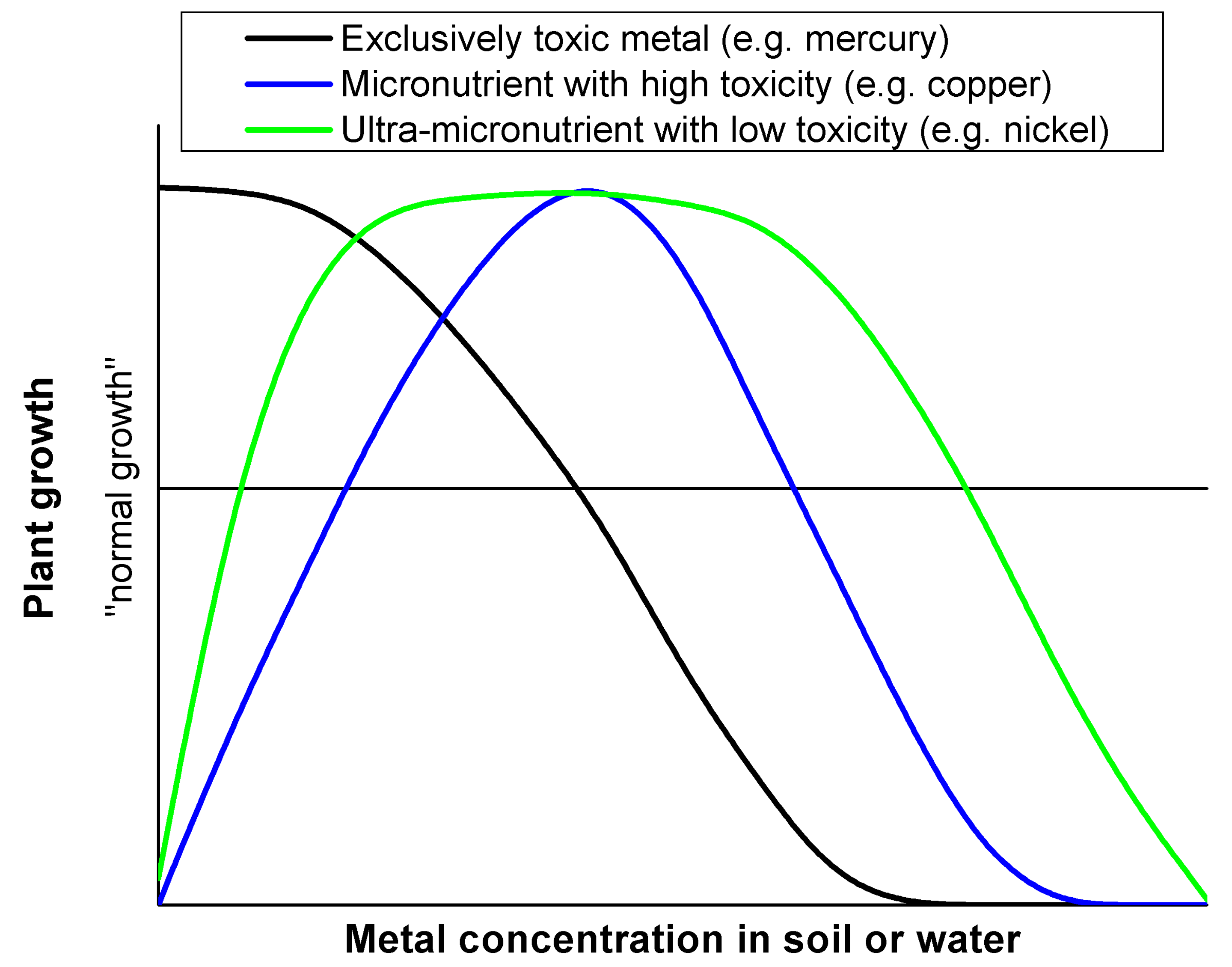
Heavy Metals and Plants - a complicated relationship
→ Metals in Photosynthesis



Heavy metal hyperaccumulation in the Wild West

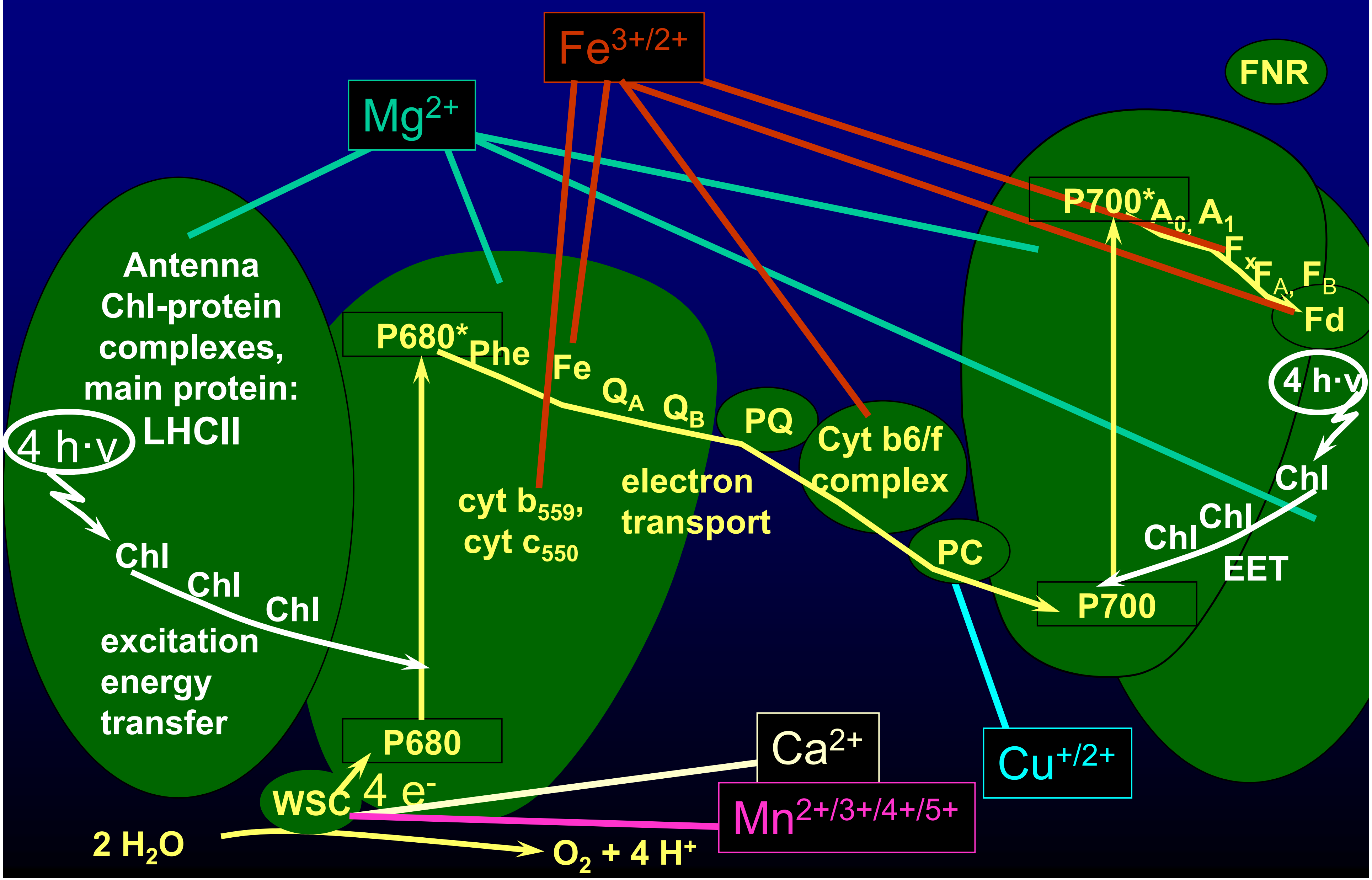
modified from: http://strangematter.sci.waikato.ac.nz/

Dose-Response principle for heavy metals



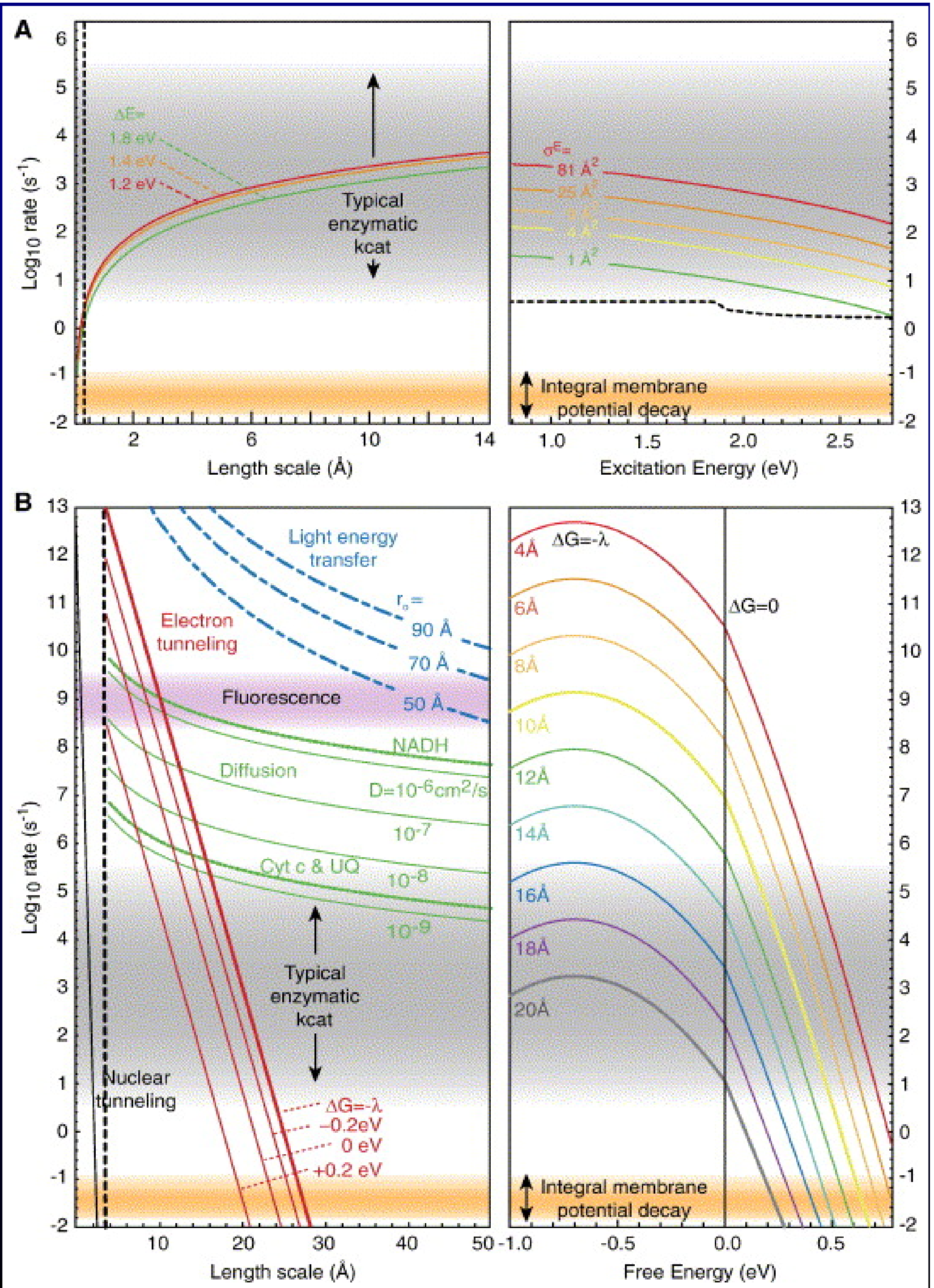
Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

Metal sites in photosynthetic proteins



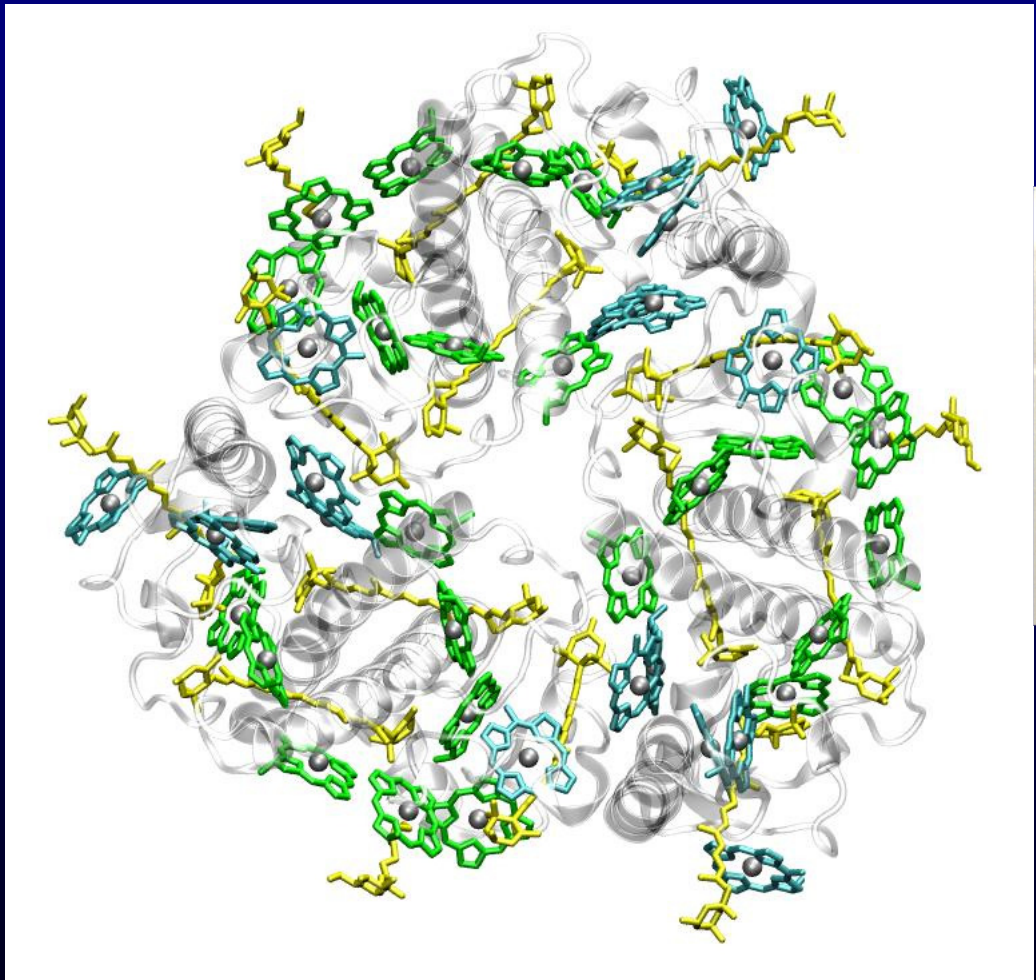
Comparison of energy transfer mechanisms

- ➢ For all processes, speed of energy transfer decreases with increasing distance.
- ➢ This limits the rate and efficiency of enzymatic and non-enzymatic processes. The longer the transfer time, the higher the risk of energy loss by unwanted processes
- ➢ Electron tunnelling is fast for very short distances, but very slow for longer distances → most relevant <10Å.
- ➢ Diffusion speed decreases less with increasing distances, therefore it becomes faster than tunnelling at more than 10-20Å.



From: Noy D, Moser CC, Dutton PL (2006) BBA Bioen. 1757, 90-105

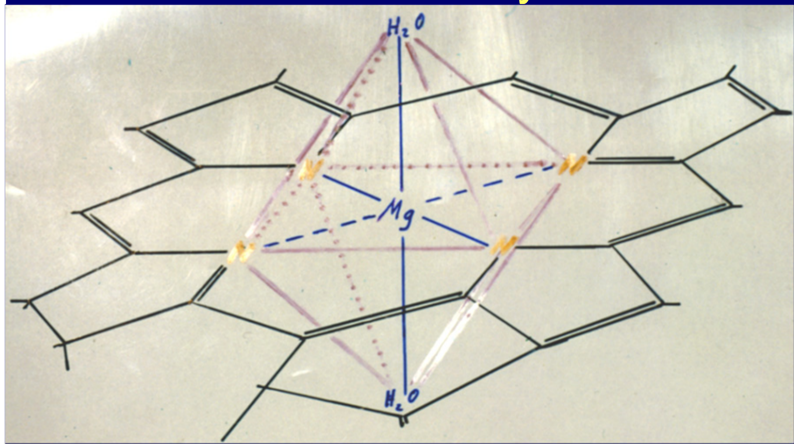
Photosynthesis related proteins with metal centres
1. Light harvesting complexes



From: commons.wikimedia.org

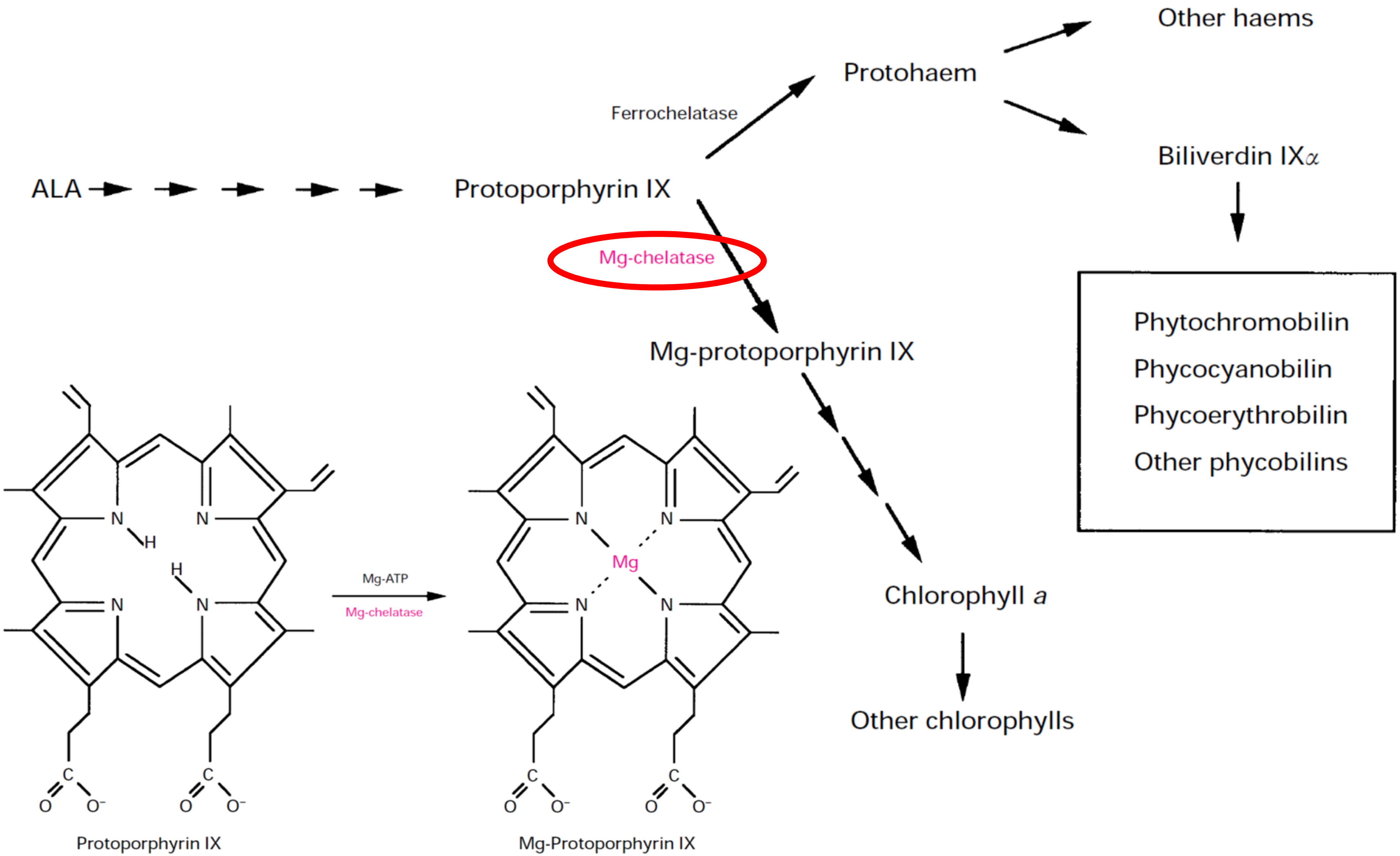
LHCII structure

- usually trimers
- structure stabilised by Chl



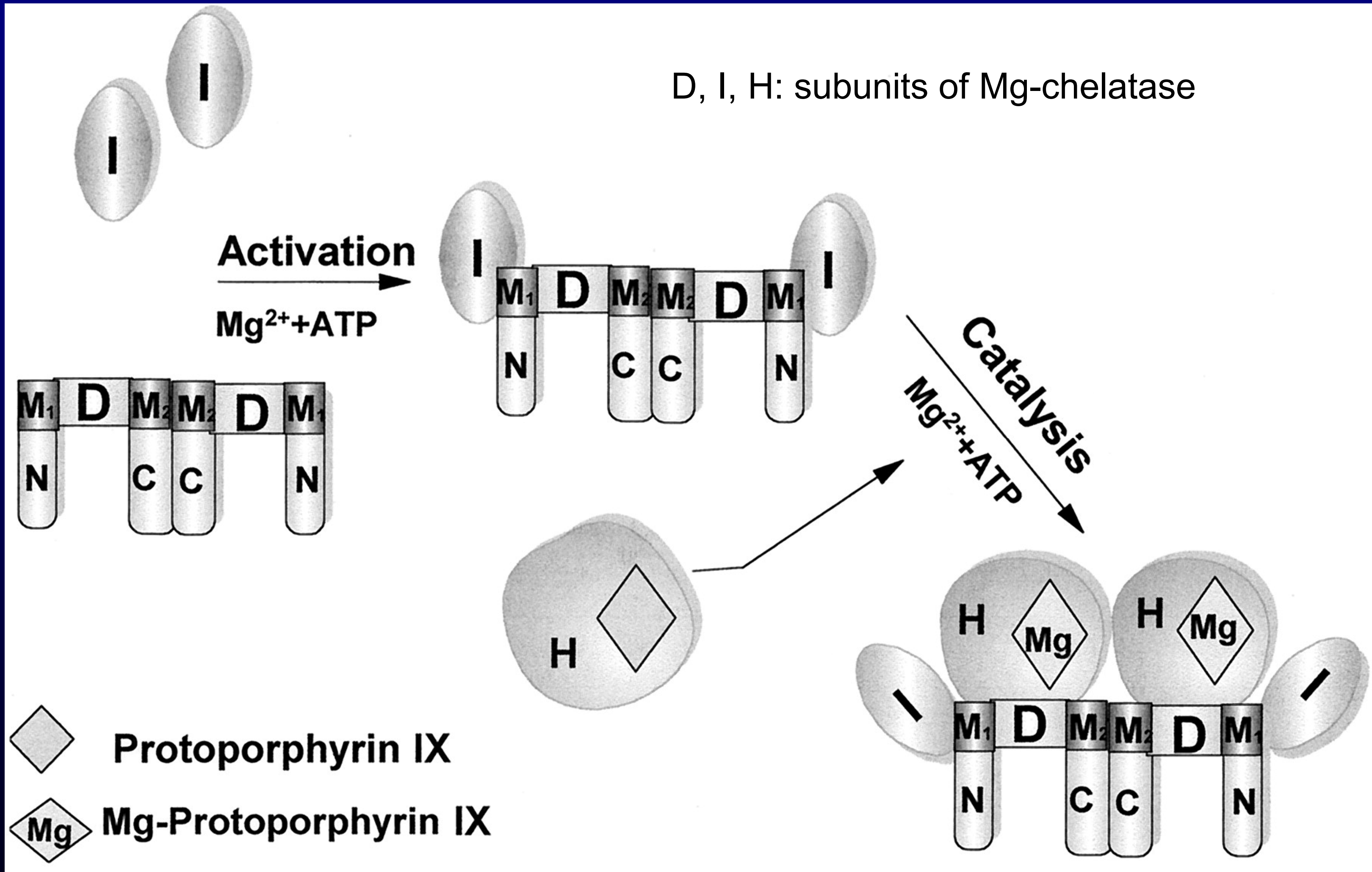
- binding via axial ligands on **Mg^{2+}**
- energy transfer depends on $1/r^6$

Photosynthesis related proteins with metal centres
1. Light harvesting complexes → Chl biosynthesis

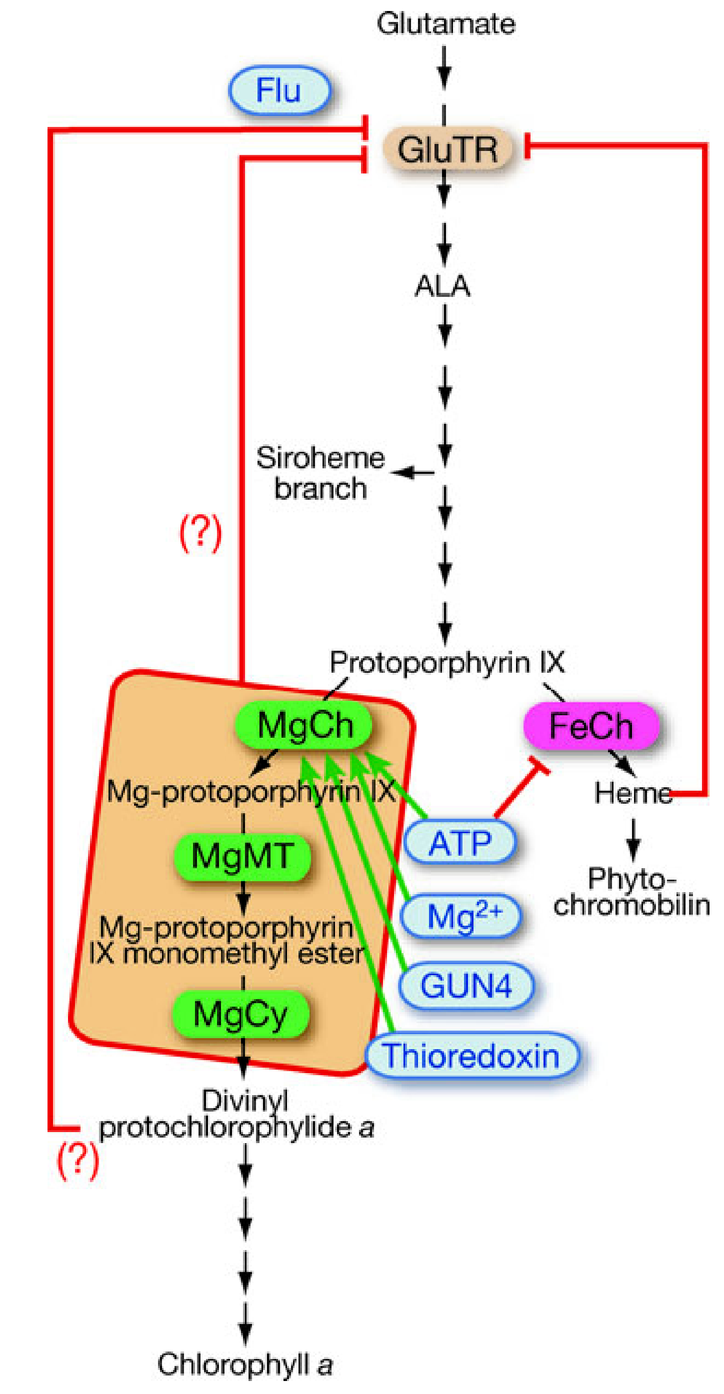


From: Walker CJ, Willows RD (1997) Mechanism and regulation of Mg-chelatase. Biochem J 327, 321-33

Photosynthesis related proteins with metal centres
1. Light harvesting complexes → Chl biosynthesis



From: Gräfe S et al. PNAS 1999;96:1941-1946



Photosynthesis related proteins with metal centres
1. Light harvesting complexes

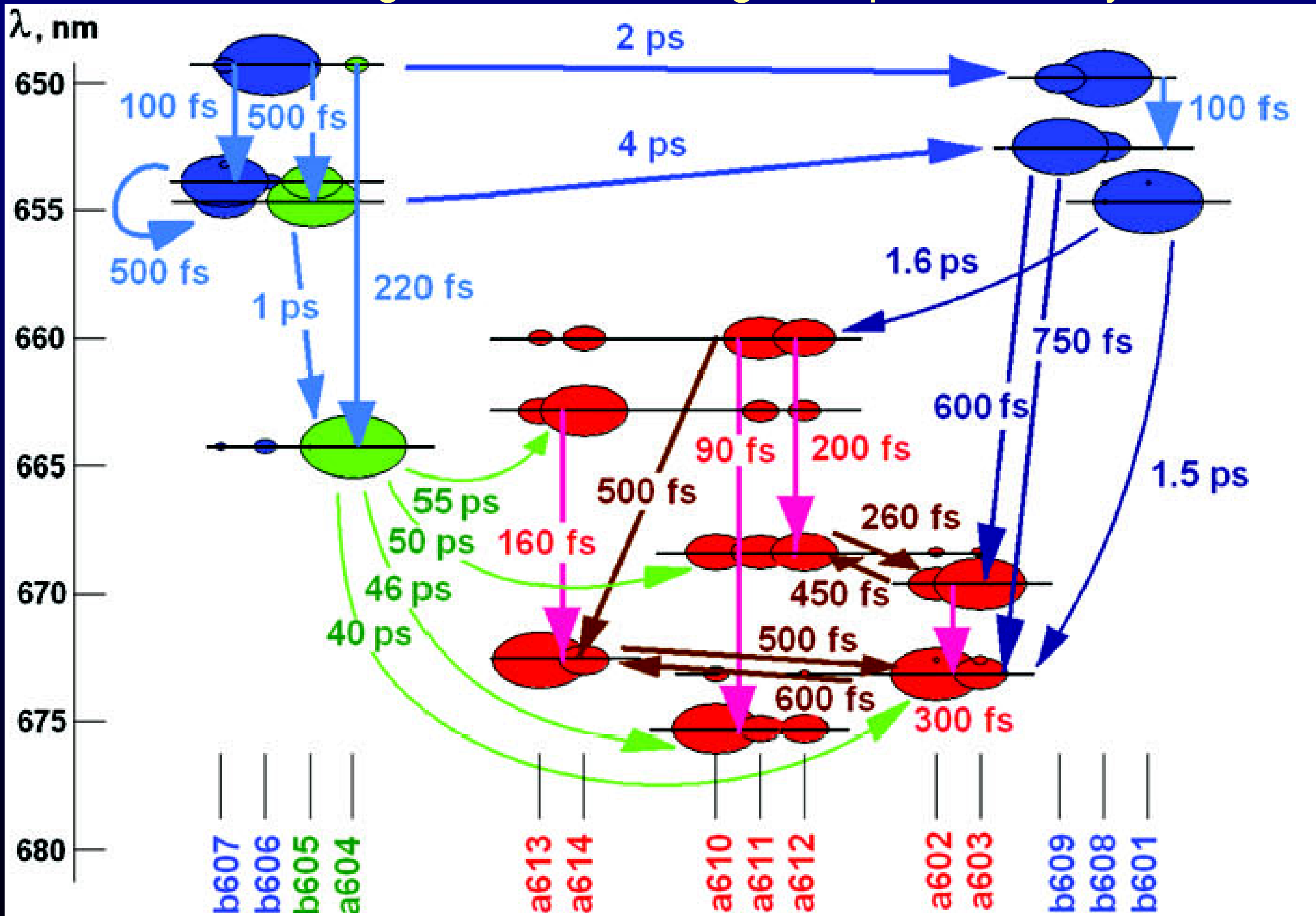
Chl biosynthesis regulation

- activation by substrates
- inhibition by intermediate products

From: Tanaka R, Tanaka A (2007) Ann Rev Plant Biol 58, 321-46

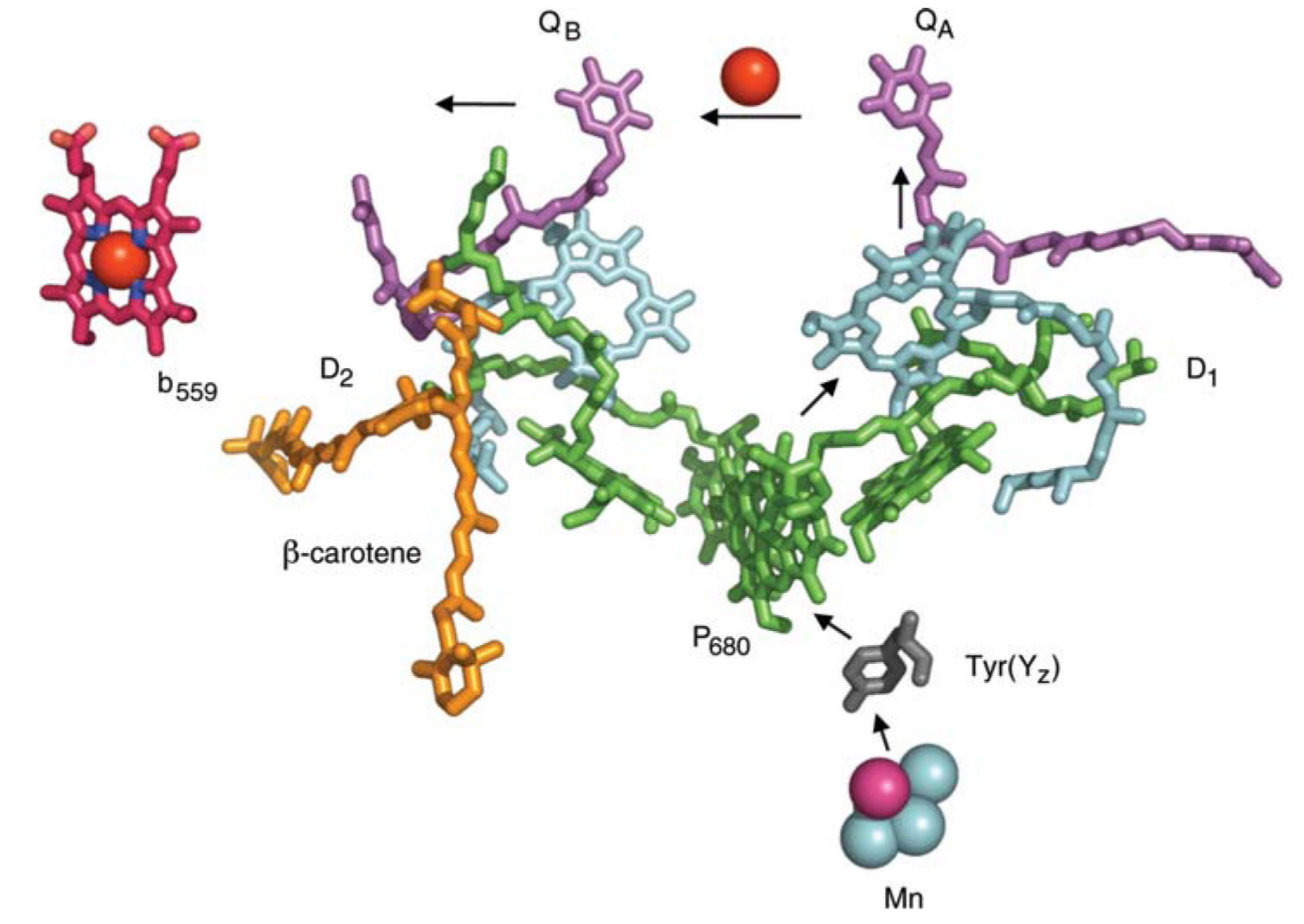
Photosynthesis related proteins with metal centres
1. Light harvesting complexes: Transfer times between Chls towards & in PSIIRC

→ fast, but still too slow for singlet excited state lifetimes of most Chl complexes with central ions other than Mg^{2+}! → choice of Mg^{2+} despite instability of the complex



From: vanGrondelle R, Novoderezhkin VI, 2006, PCCP8, 793-807

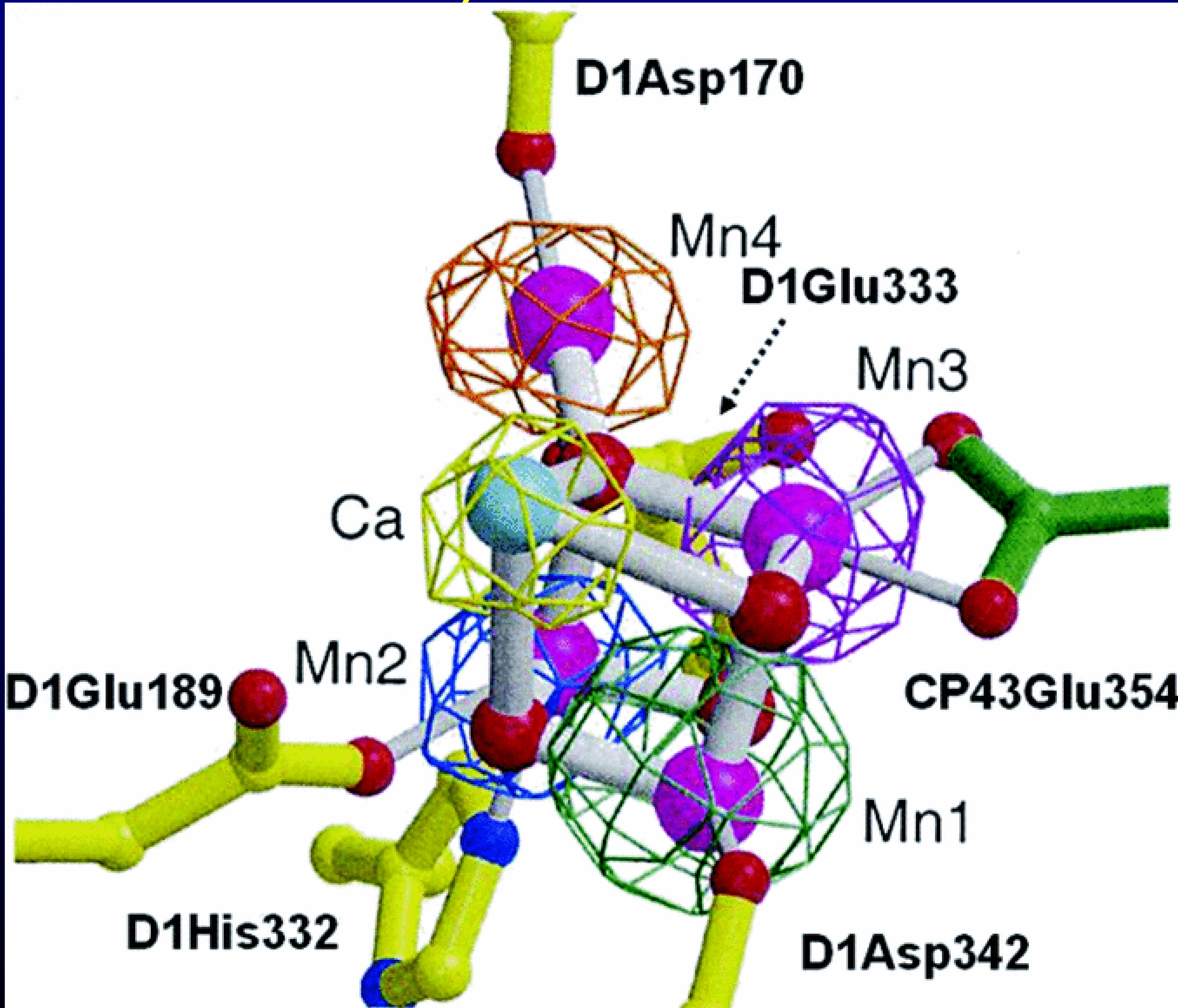
Photosynthesis related proteins with metal centres
2. Photosystem II reaction centre – electron transport



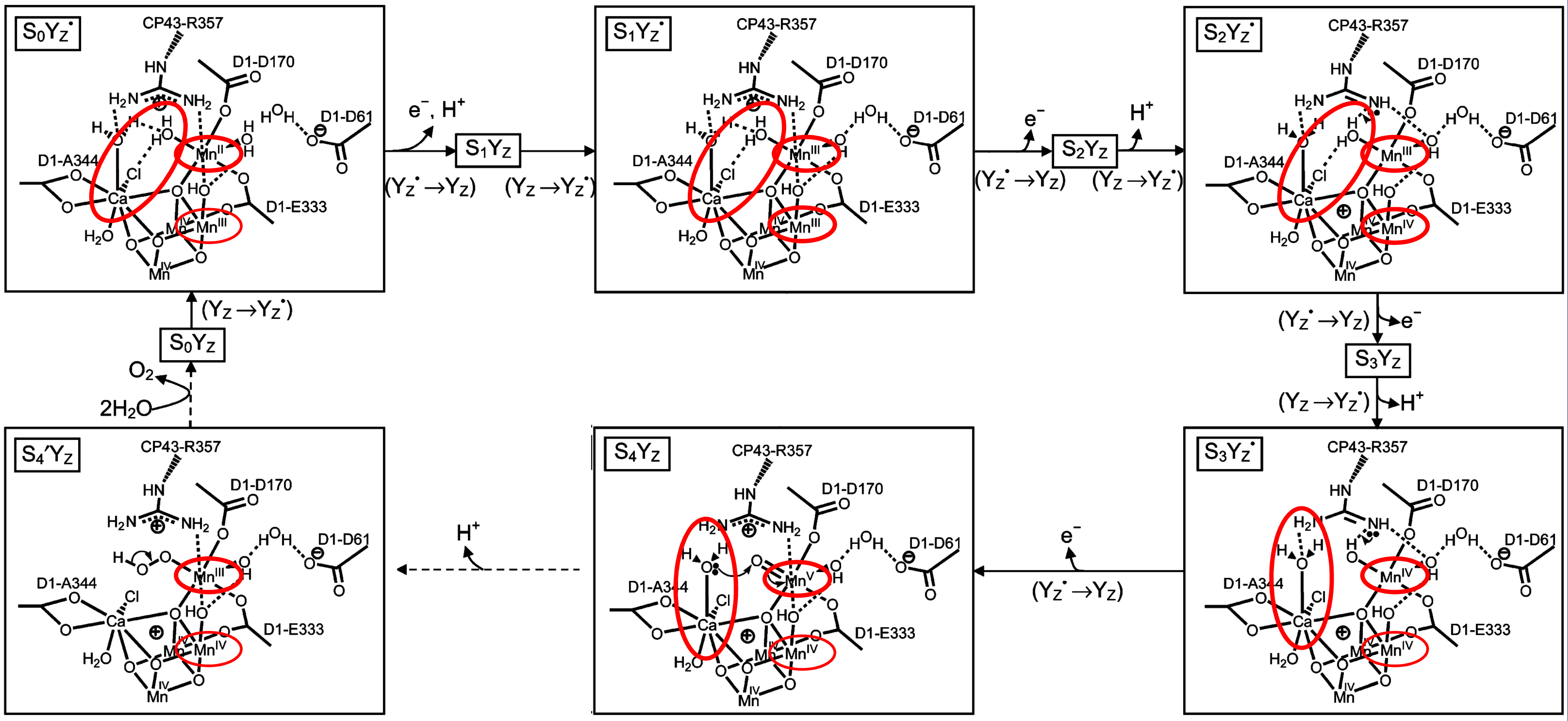
From: Nelson N, Yocum CF, 2006, AnnRevPlantBiol 57, 521-65

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

Most important manganese enzyme: Water splitting complex of PSII
a) structure



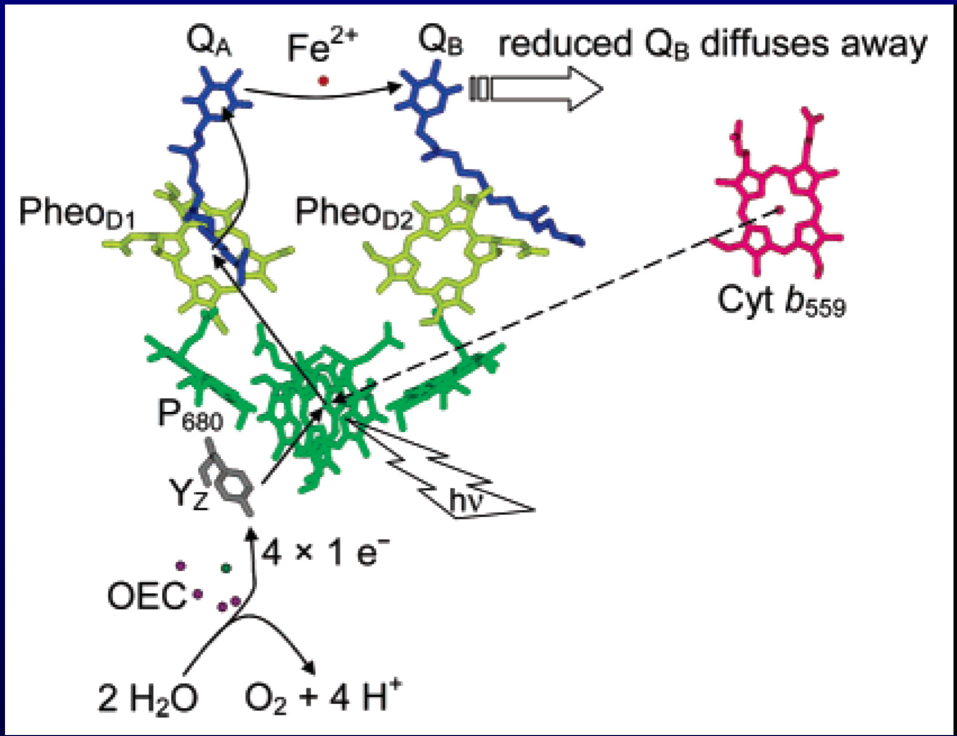
Water splitting complex of the photosystem II reaction centre (b) proposed mechanism



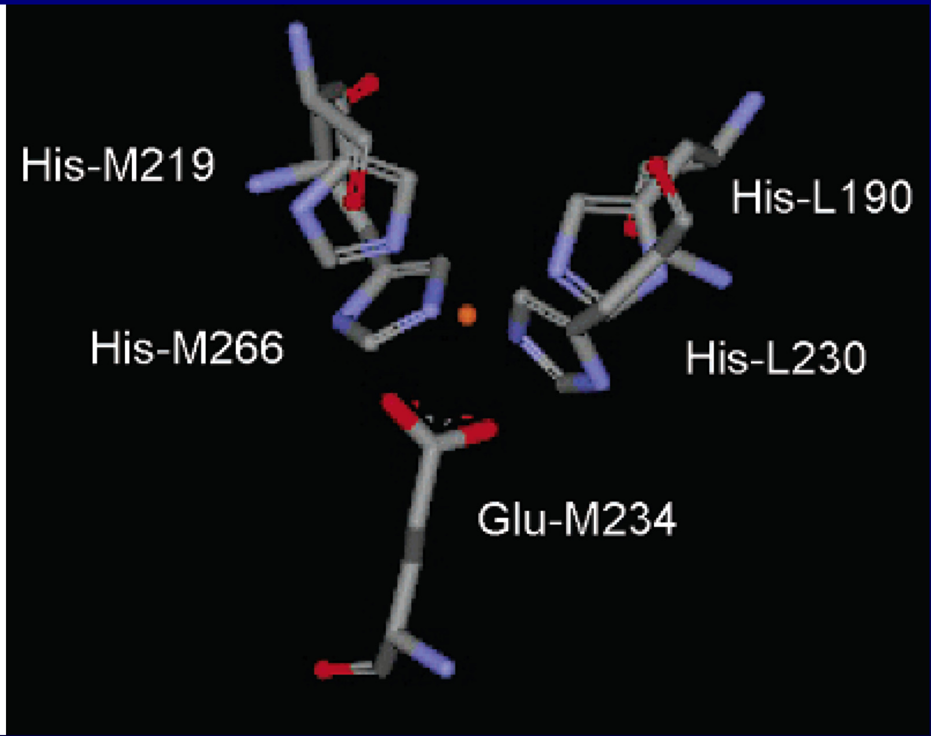
From: McEvoy JP, Brudvig GW, 2006, Chemical Reviews 106, 4455-83

- 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

Non-heme iron in the photosystem II reaction centre



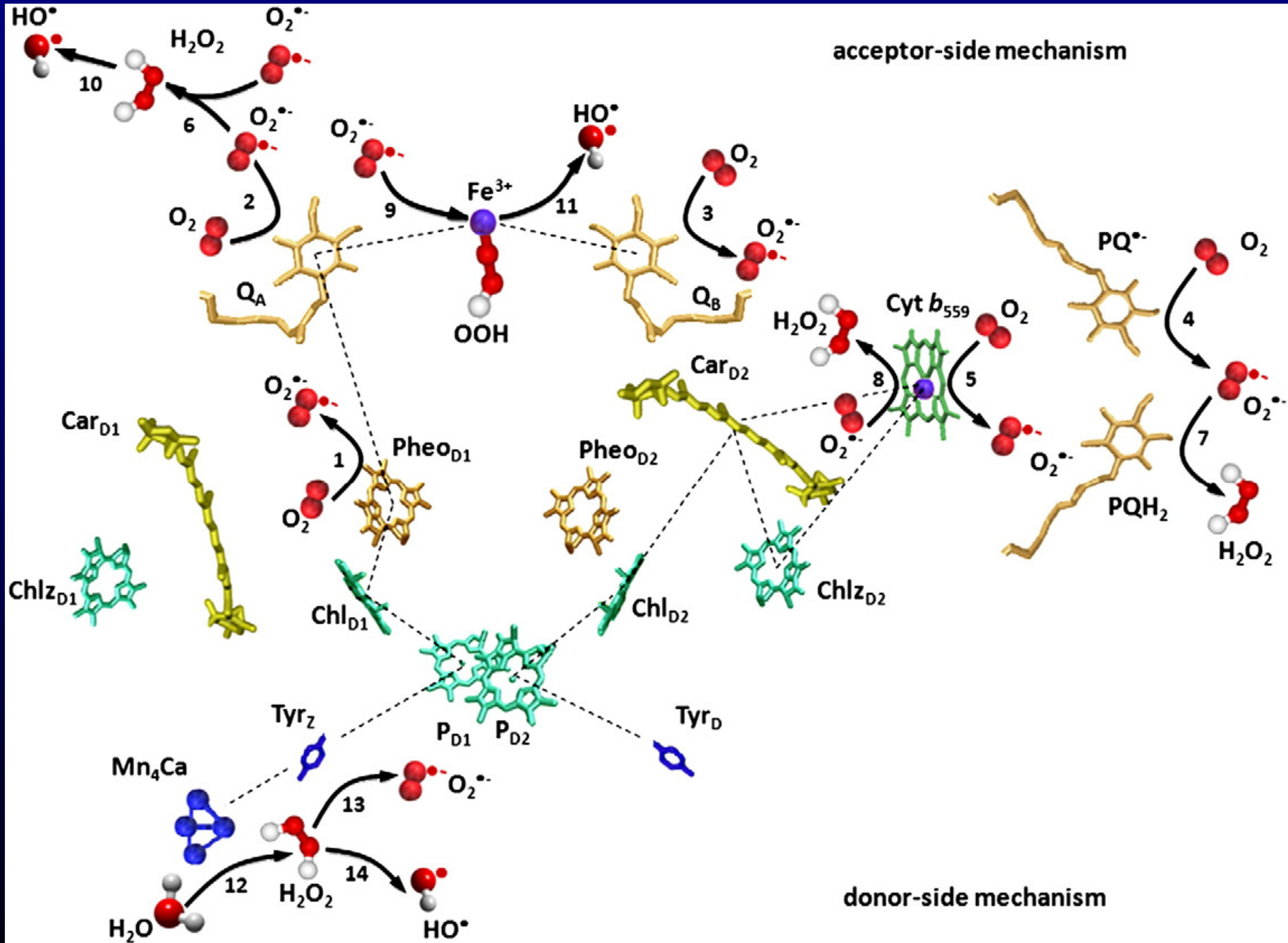
From: McEvoy JP, Brudvig GW, 2006, Chemical Reviews 106, 4455-83



From: Utschig LM, Thurnauer MC, 2004, AccChemRes37, 439-47

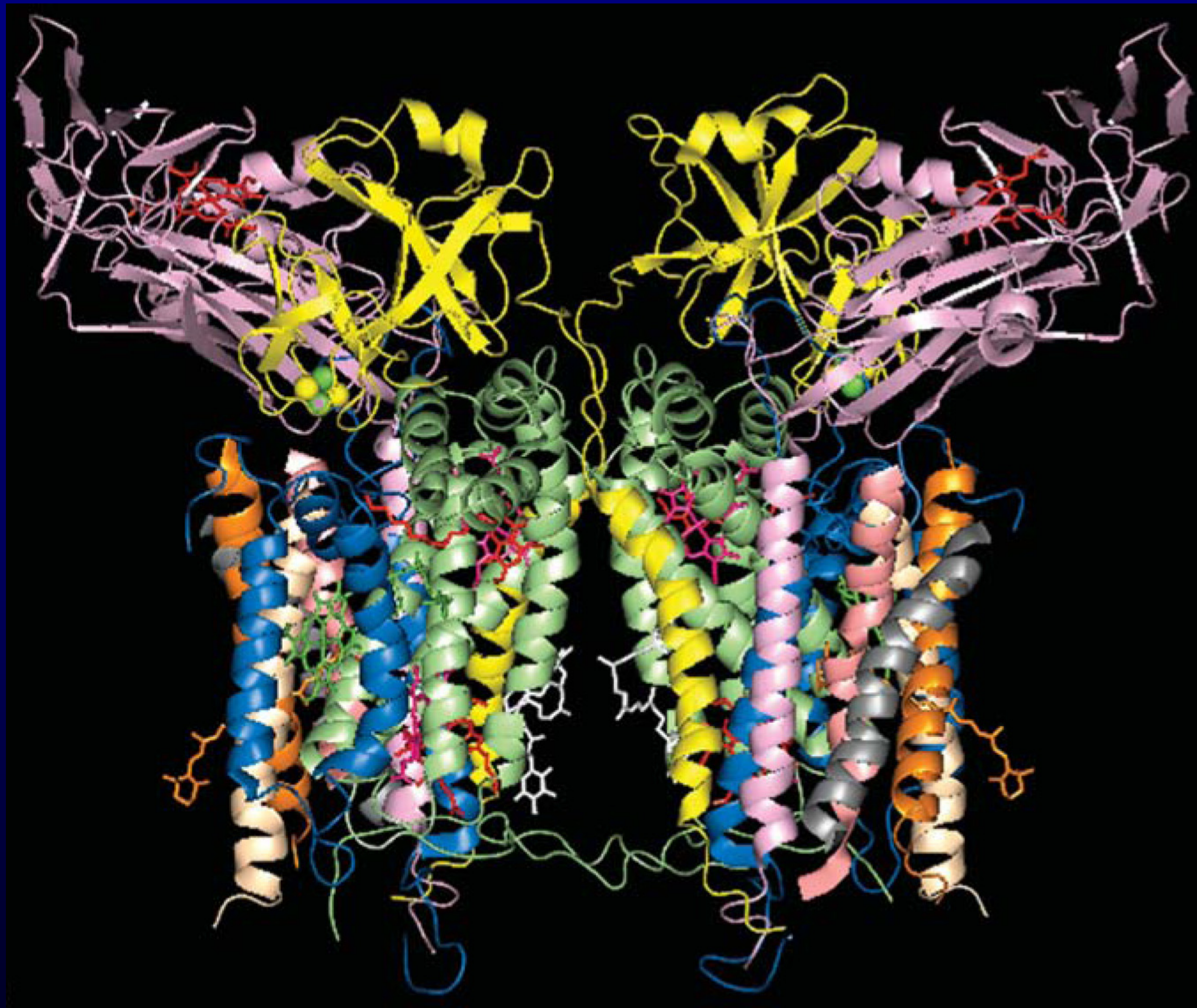
- bound by 4 histidines and 1 glutamate near the stroma surface of the PSIIRC
- guides/helps electrons tunnelling from Q_A to Q_B

Photosynthesis related proteins with metal centres
PSIIRC: generation of ROS



From: Pospisil P (2012) Biochimica et Biophysica Acta 1817, 218-31

Photosynthesis related proteins with metal centres
3. Cyt b_6f complex: Structure



From: Cramer WA, Zhang H, Yan J, Kurisu G, Smith JL, 2006, AnnRevBiochem75, 769-90

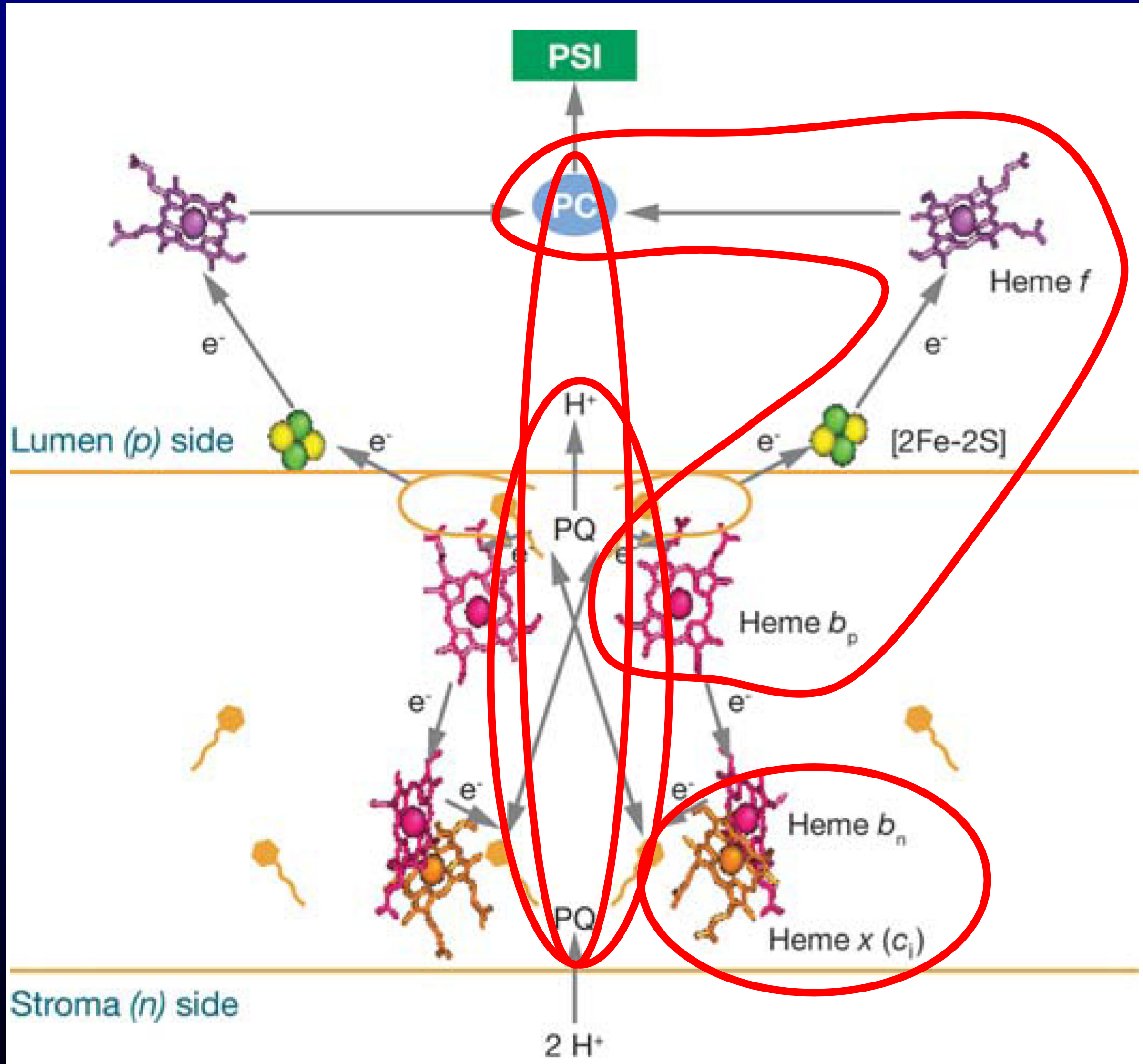
Structural characteristics

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

Photosynthesis related proteins with metal centres
3. Cytb$_6$f complex: Mechanism

Functional characteristics

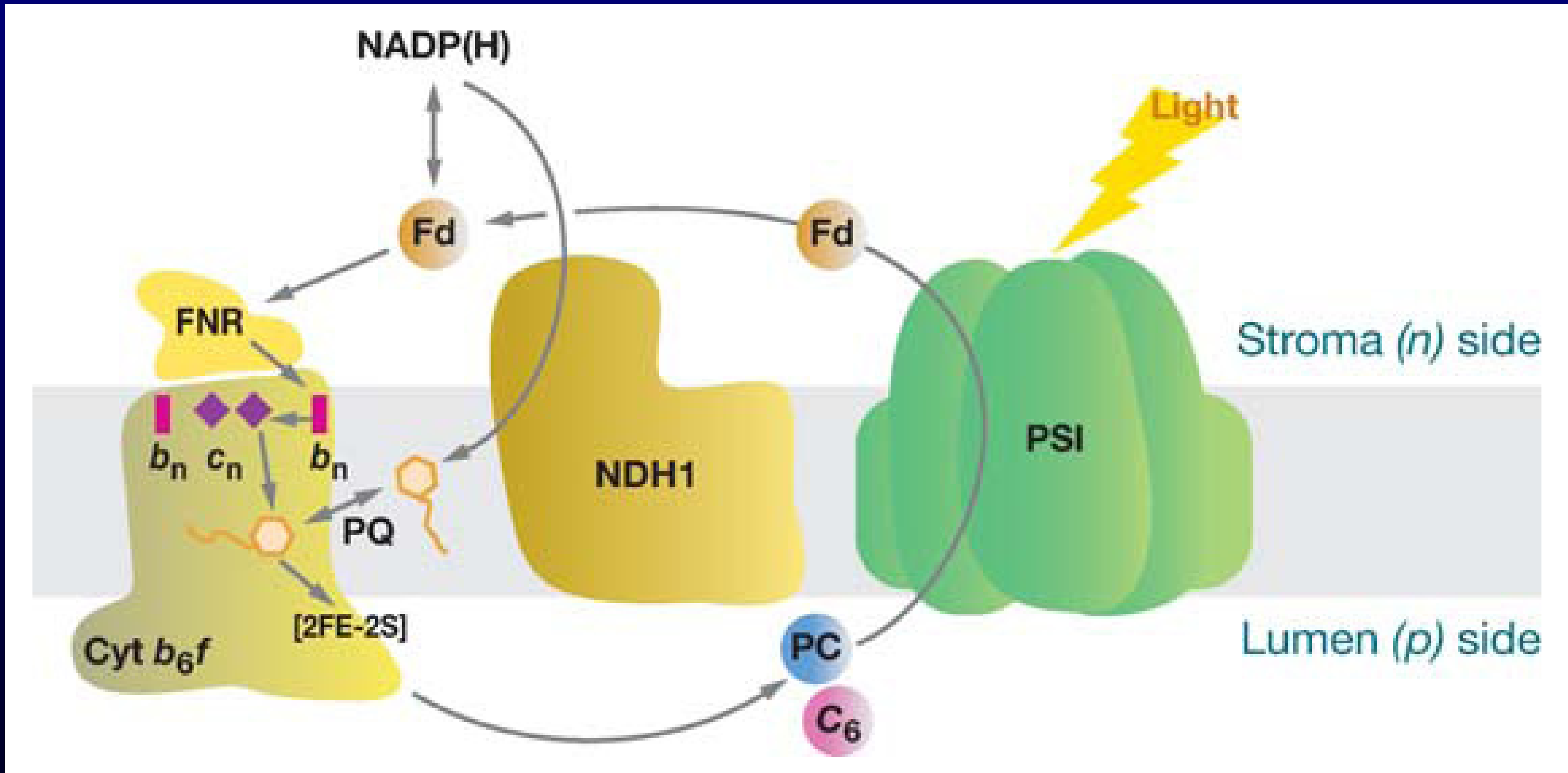
- transfers e- from PQ to plastocyanin (PC),
- It uses the difference in potential between Q_B and PC for translocating a proton via 2x2 heme *b* groups and 2x1 heme *x* group
- Electrons are transferred from the heme b groups to PC via a "Rieske" [2Fe2S]-cluster and a heme *f* group
- Cyclic electron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



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

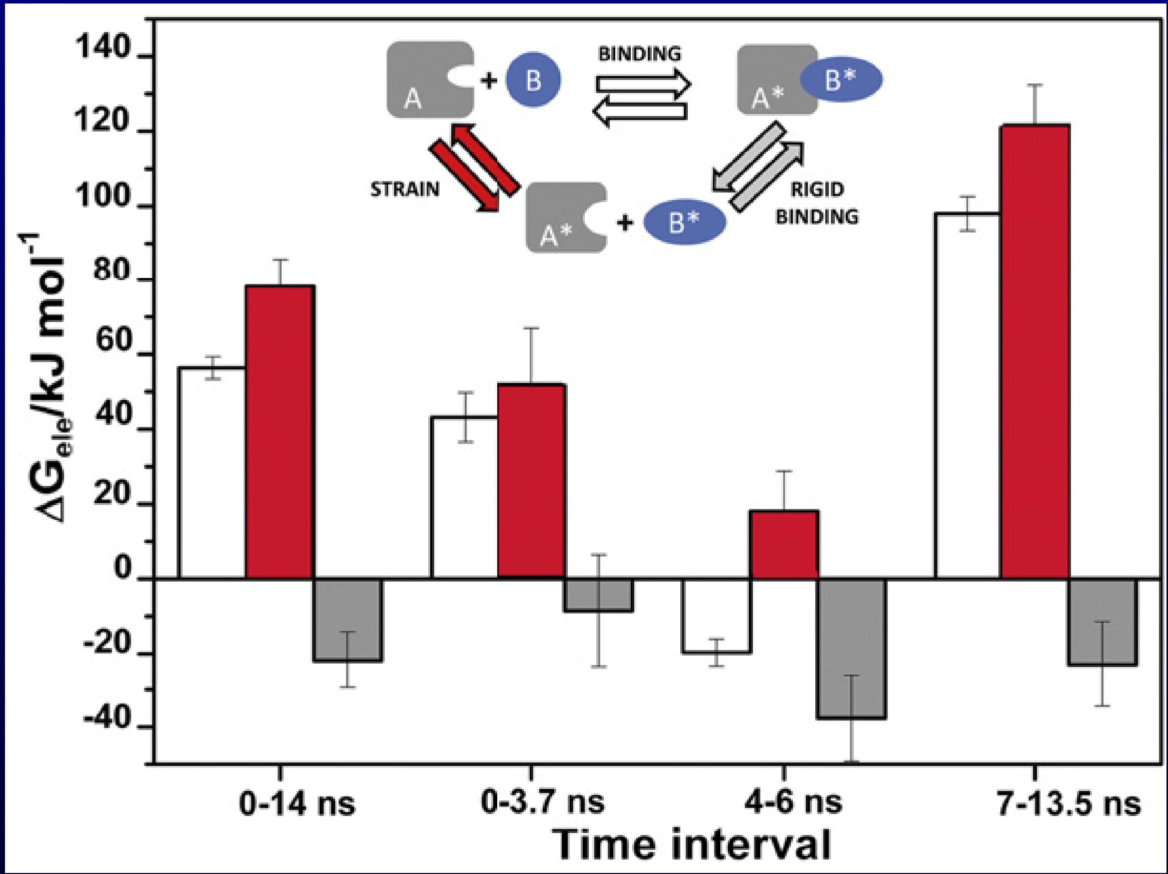
Photosynthesis related proteins with metal centres
3. Cytb$_6$f complex: Mechanism

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

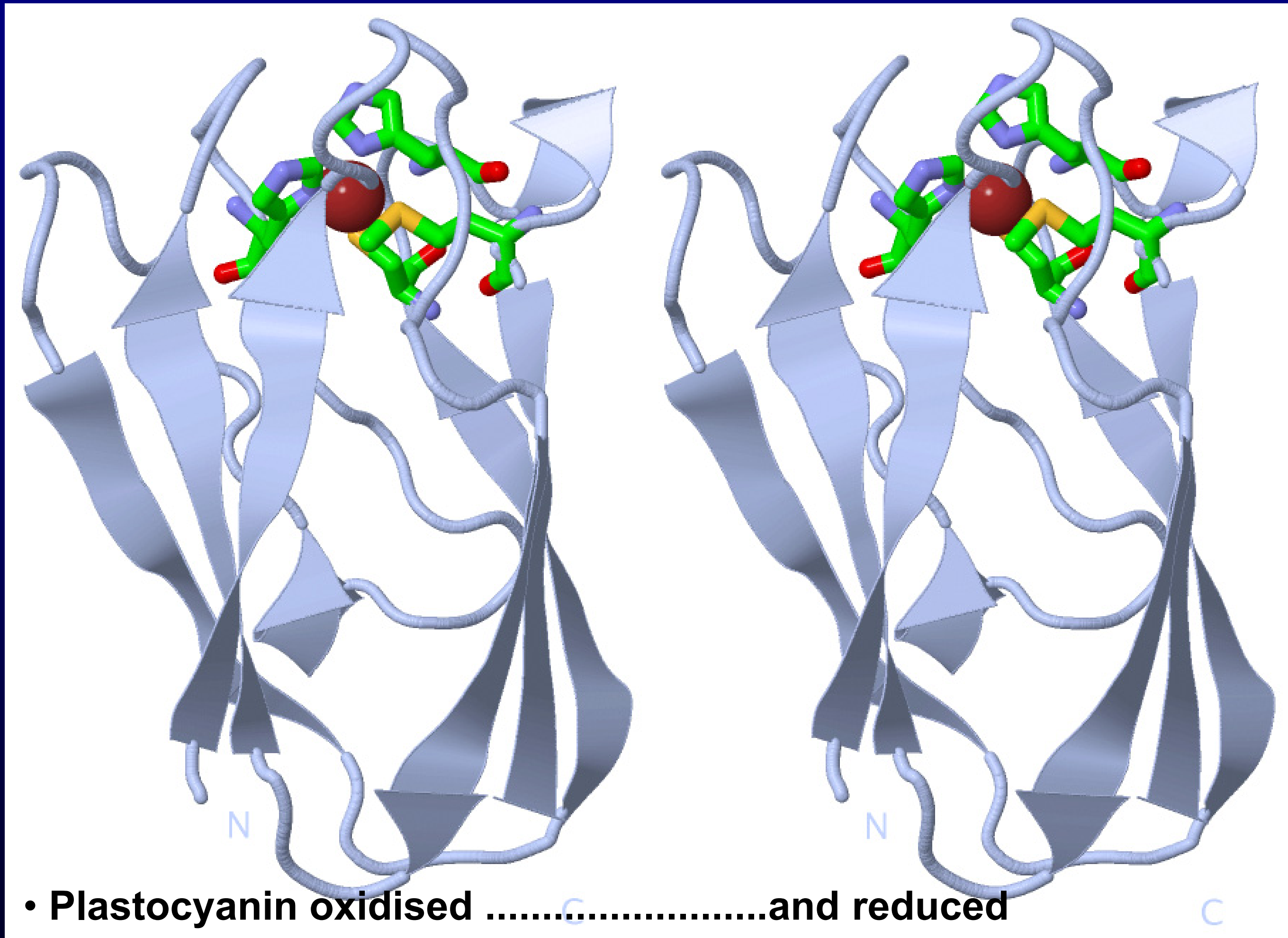
Photosynthesis related proteins with metal centres
Interaction between cytochrome f and plastocyanin



From: Cruz-Gallardo I, et al. (2012) FEBS Lett 586, 646-52

- very short-lived interaction: (ns range), conformational changes make binding transiently favourable (→ negative ΔG)
- both pathways (strain→ rigid binding and direct binding) occur

Photosynthesis related proteins with metal centres
4. 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

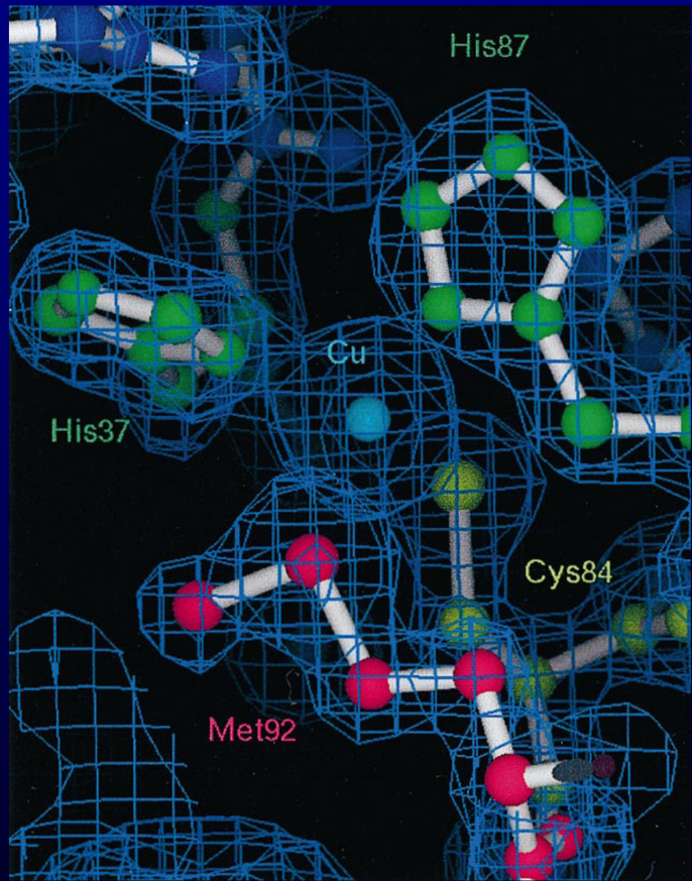
Structural characteristics

- 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

Photosynthesis related proteins with metal centres
4. Plastocyanin

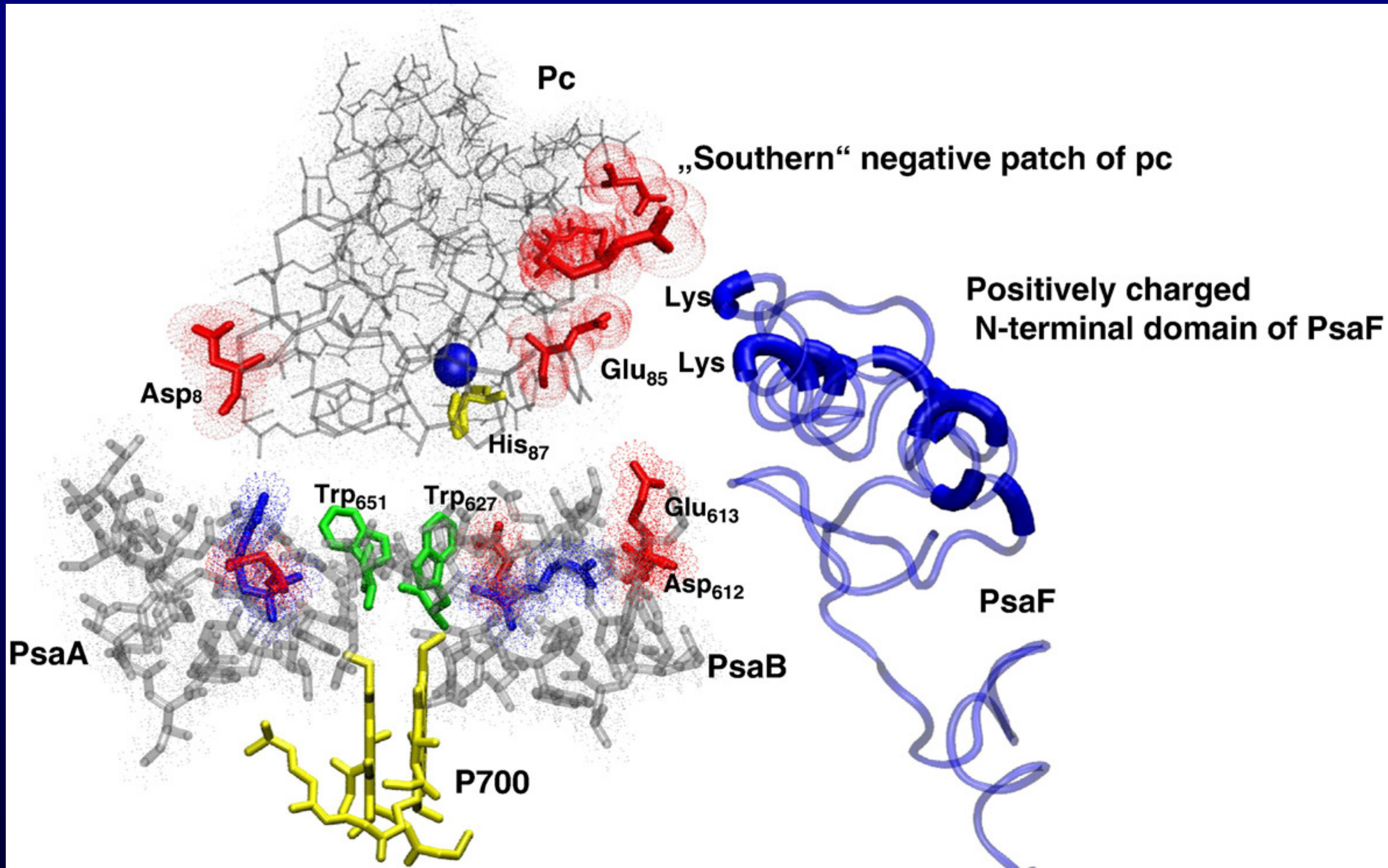
Functional characteristics

- Oxidised (Cu^{2+}) plastocyanin accepts electron from Cyt_{b6f} complex,
- Reduced (→ Cu^{+}) plastocyanin diffuses to the PSIRC
- Plastocyanin releases the electron (Cu^{+} → 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

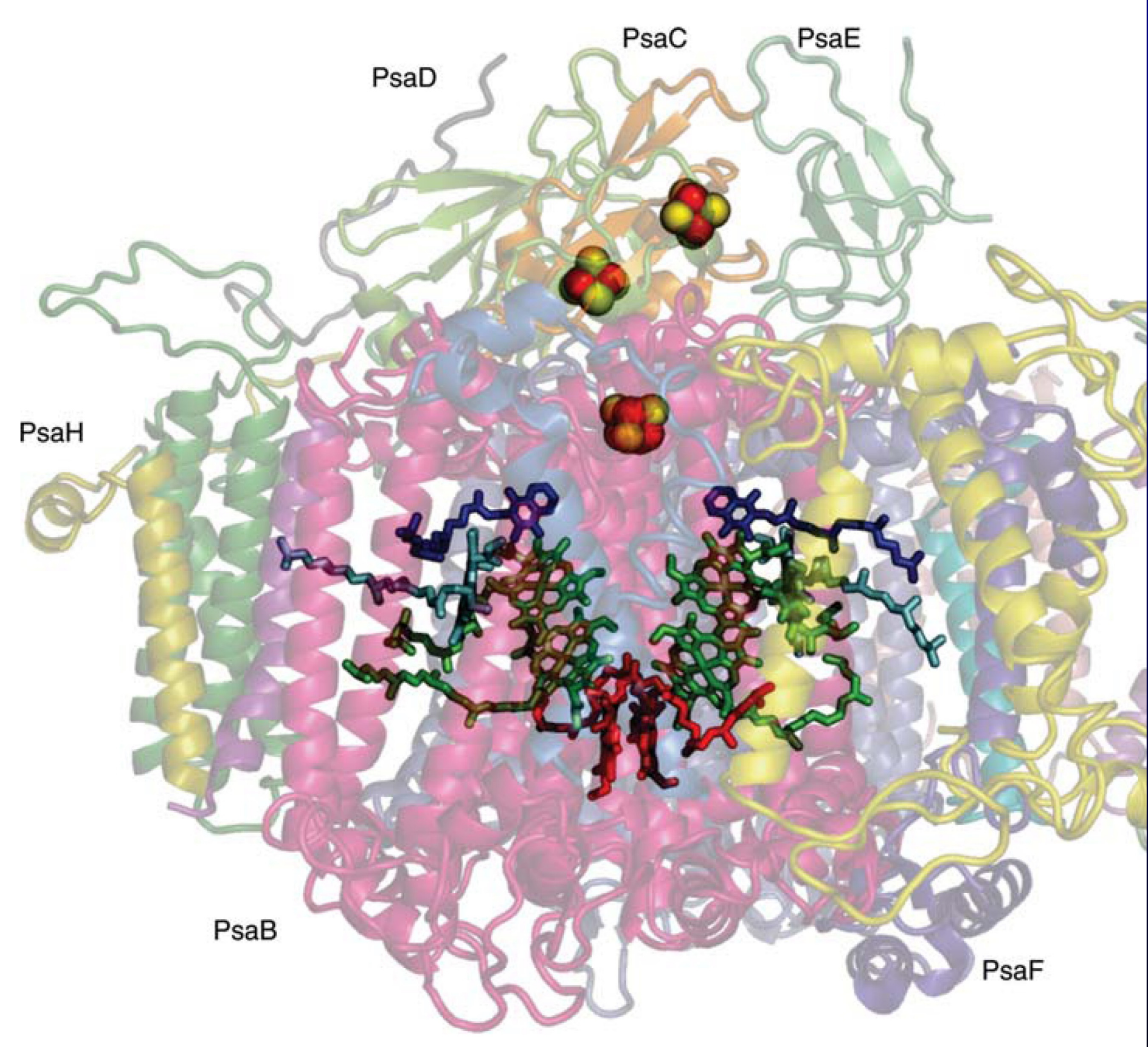
Photosynthesis related proteins with metal centres
4. Plastocyanin coupling to PSI



From: Busch A, Hippler M (2011) BBA1807, 864-77

- coupling to PSI via "southern negative patch) of PC to positively charged N-terminal domain of PsaF
- copper centre of PC near double Trp acting as electron channel towards P700

Photosynthesis related proteins with metal centres
5. Photosystem I reaction centre
(a) Overview



Structural characteristics

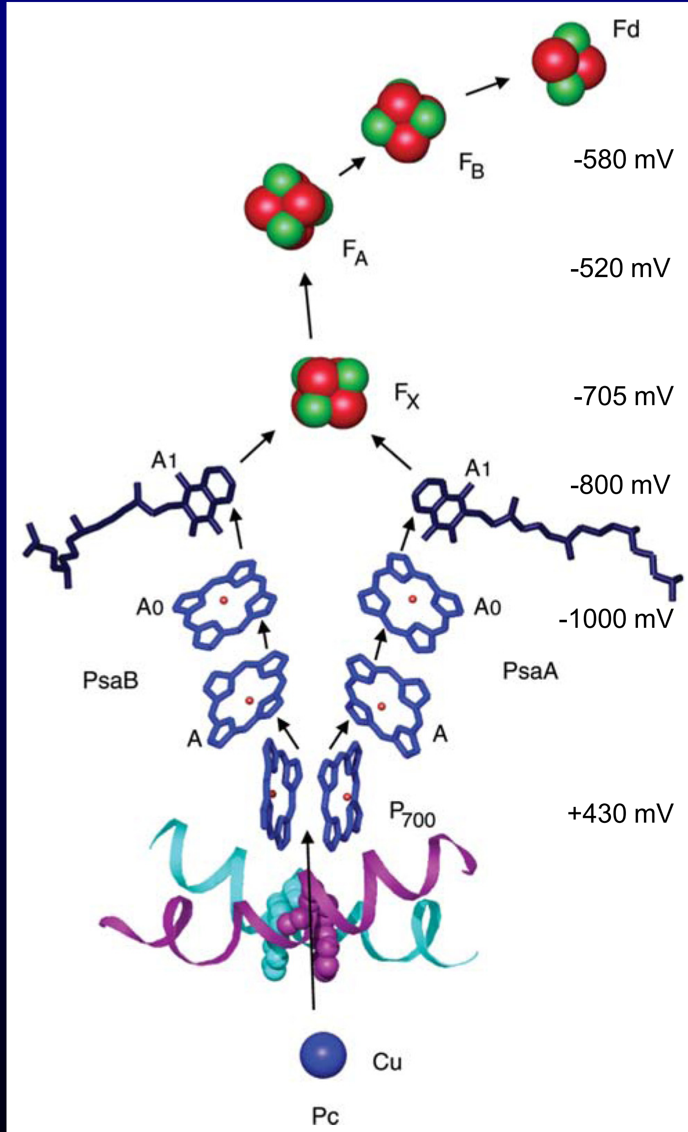
- forms trimers
- 12 subunits per monomer
- 127/133 cofactors per monomer (cyanos/plants):
  96/102 chlorophylls
  22 carotenoids
  2 phylloquinones
  3 [Fe4S4] clusters
  4 lipids

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

Photosynthesis related proteins with metal centres
5. Photosystem I reaction centre
(a) Overview

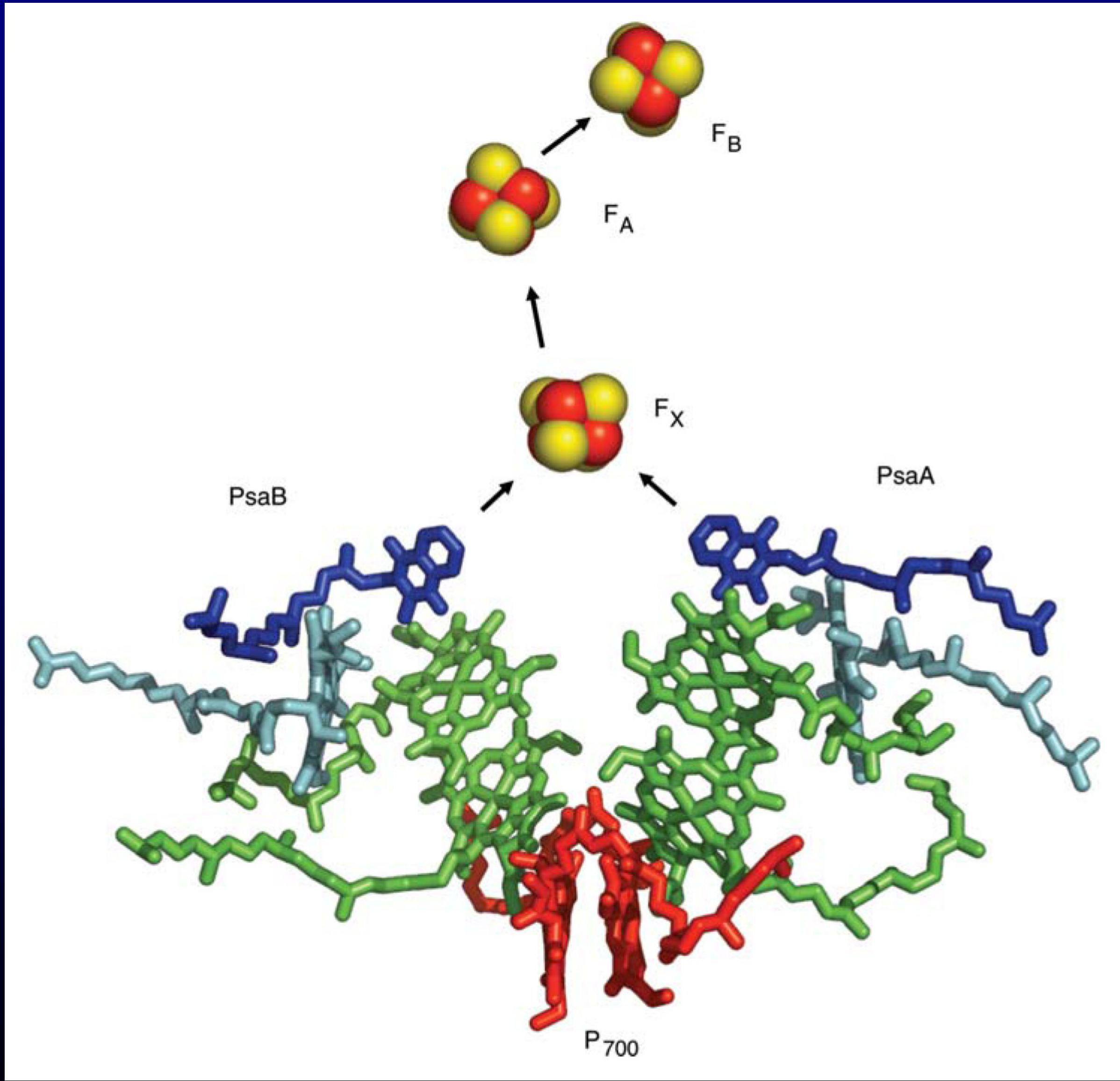
Funtional characteristics:

- 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

Photosynthesis related proteins with metal centres
5. Photosystem I reaction centre
(b) iron-sulphur clusters

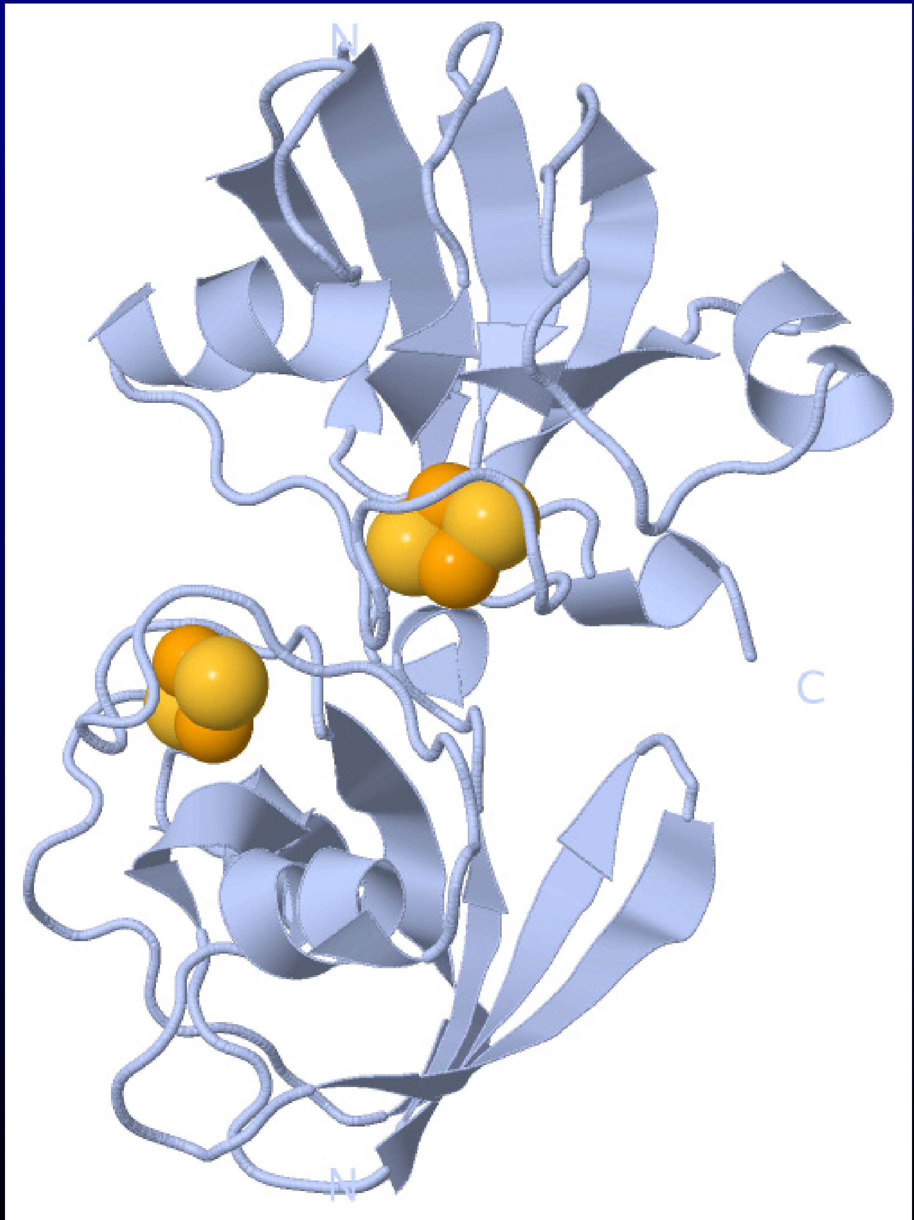


Function of the 4Fe4S-clusters in PSIRC

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

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

Photosynthesis related proteins with iron centres
Ferredoxin

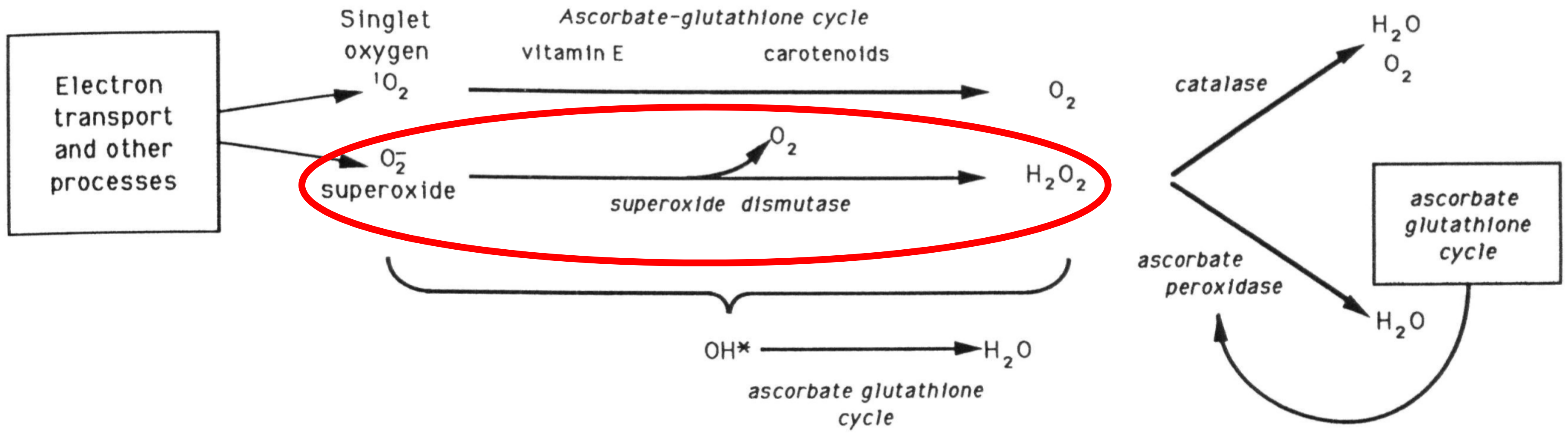


Structure and function

- 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

One of the most important copper enzymes: Superoxide dismutase (SOD), in plants a Cu/Zn enzyme (a) function

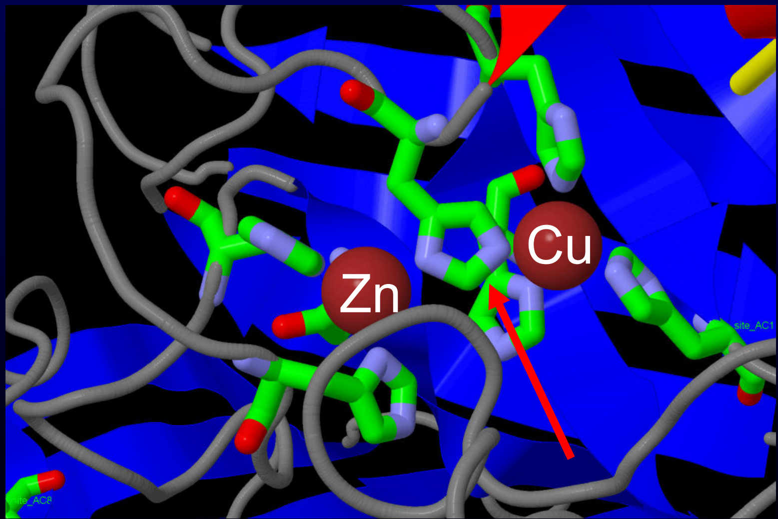


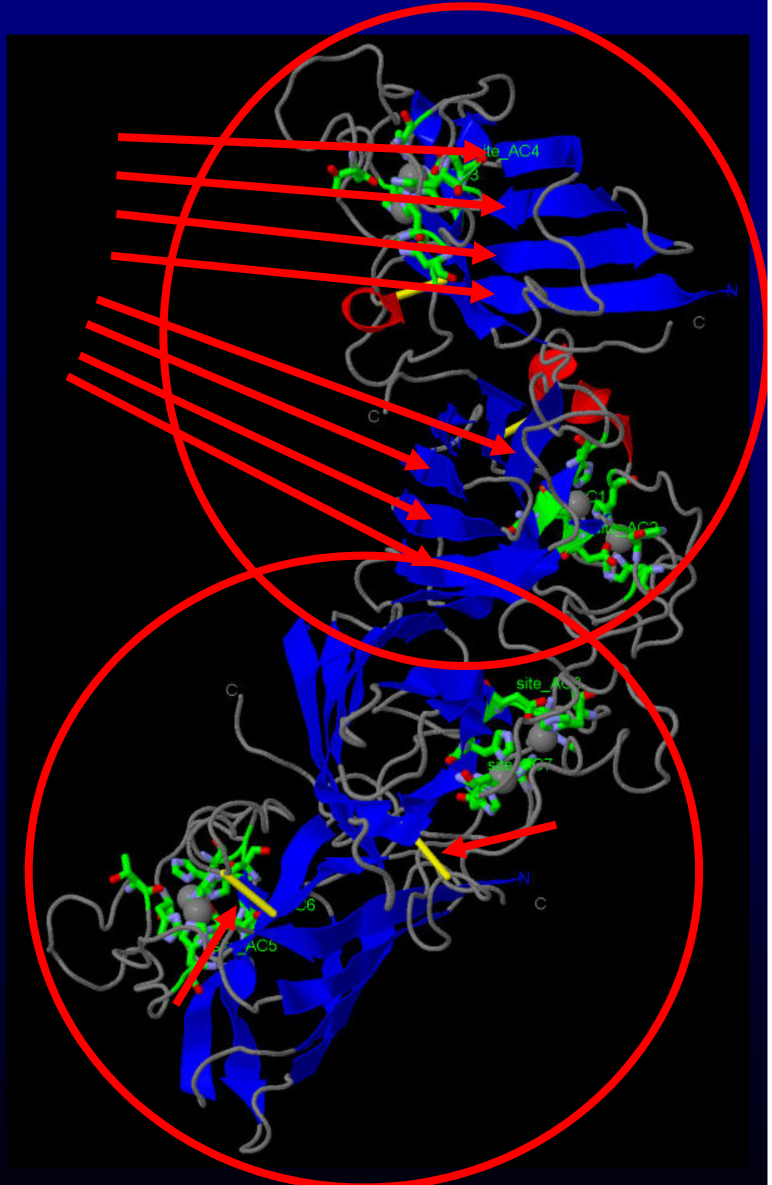
From: Foyer CH et al., 1994, PlantCellEnvi17_507-23

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

One of the most important copper enzymes: Superoxide dismutase (SOD), in plants a Cu/Zn enzyme (b) structure

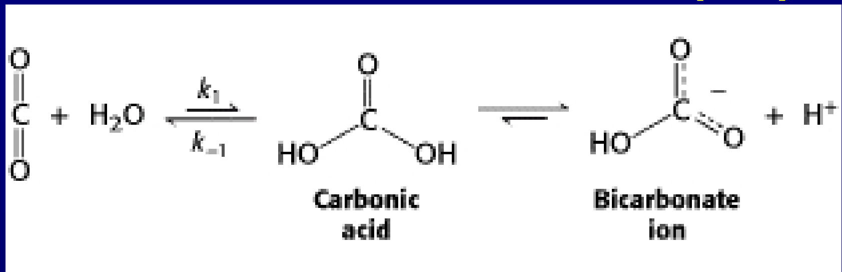
- Dimer of two identical subunits, in crystals 2 dimers together
- Each subunit consists of:
  - 8 anti-parallel β-strands forming a flattened cylinder,
  - 3 external loops
- 1 Cys-Cys disulfide bond stabilises loops
- 1 Cu^{2+} and 1 Zn^{2+} per subunit
- Cu^{2+} bound by 4 His, Zn^{2+} by 3 His + 1 Aspartate
- His-63 bridges Cu^{2+} and Zn^{2+}
- : K_D of copper: 10^{-15} M





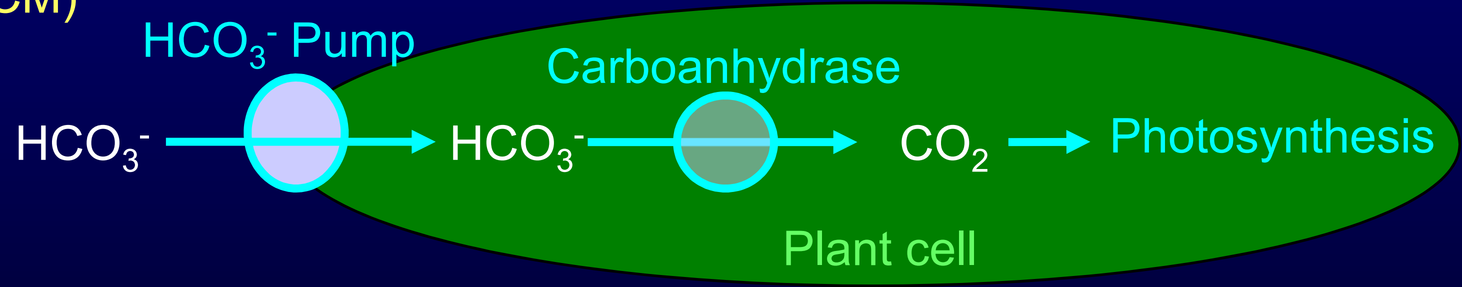
Spinach SOD, From: Kitagawa Y et al., 1991, J Biochem 109, 477-85, images generated with Jena 3D viewer

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

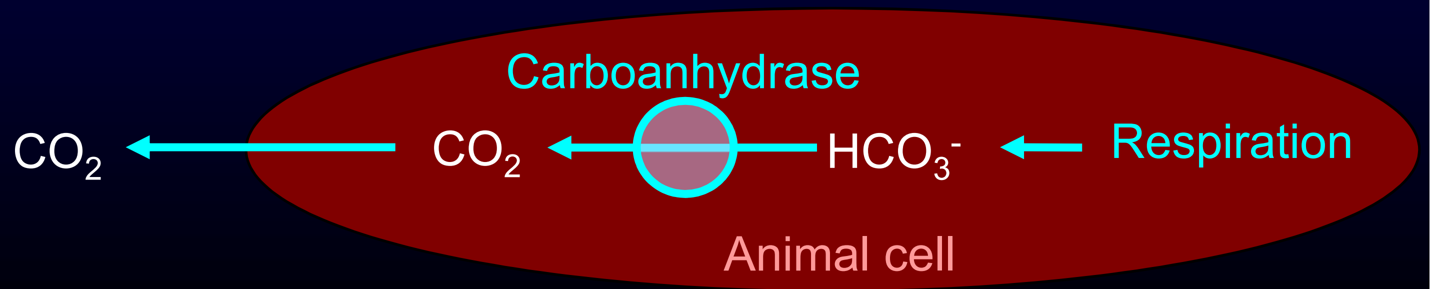


function of carboanhydrases (from: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer&part=A1199)

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

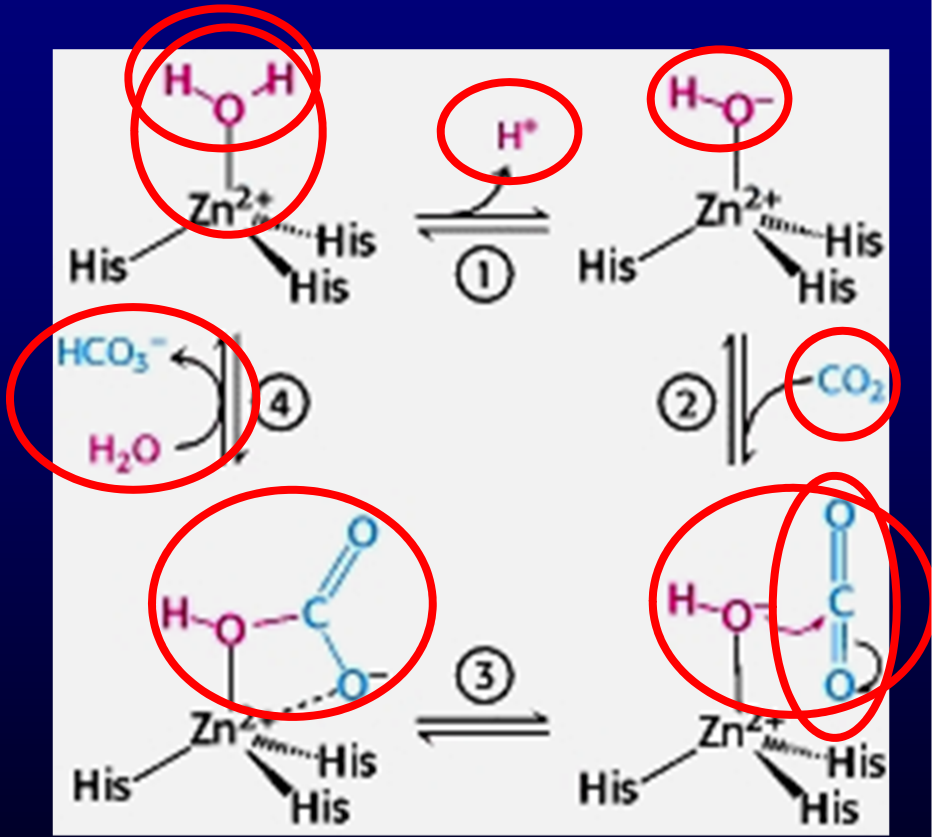


- Present in most respiratory organisms (incl. animals like us!) for removing CO_2 from the body by exhalation



Photosynthesis related Enzymes with metal centres
CO_2 delivery: Cd- and Zn- carboanhydrases
(a2) reaction mechanism from CO_2 to bicarbonate

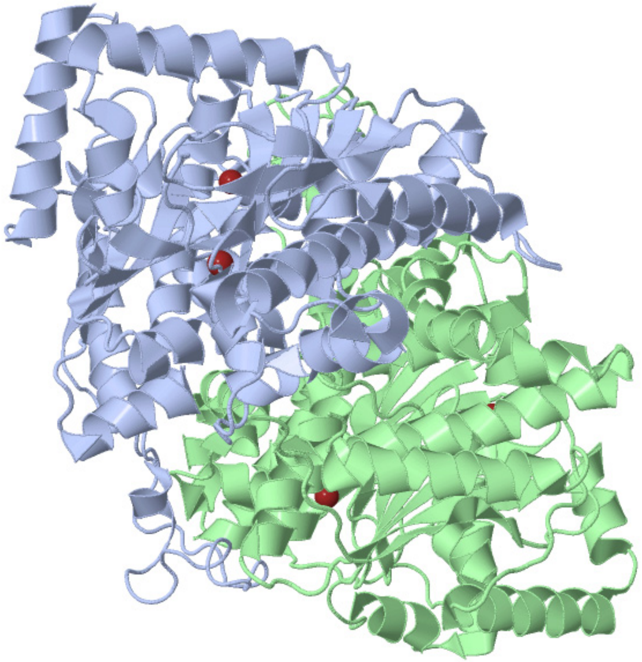
- By lowering the pK_a of water from 15.7 to 7, the binding of water to Zinc facilitates the release of a proton, which generates a hydroxide ion.
- Carbon dioxide binds to the active site of the enzyme and is positioned to react with the hydroxide ion.
- The hydroxide ion attacks the carbon dioxide, converting it into a bicarbonate ion.
- The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another molecule of water.

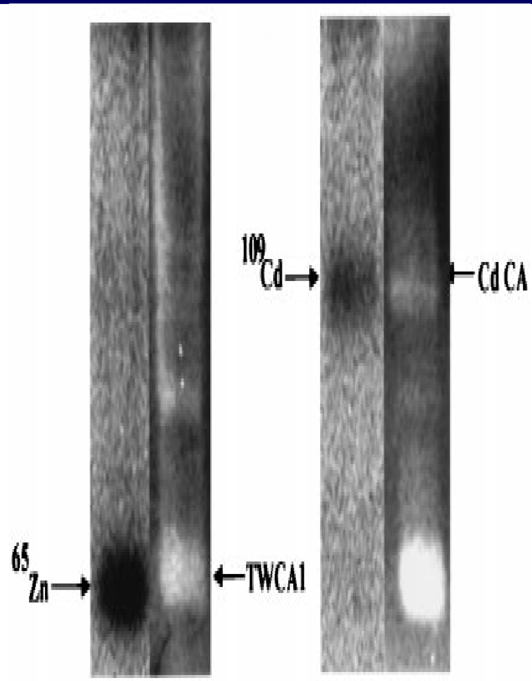


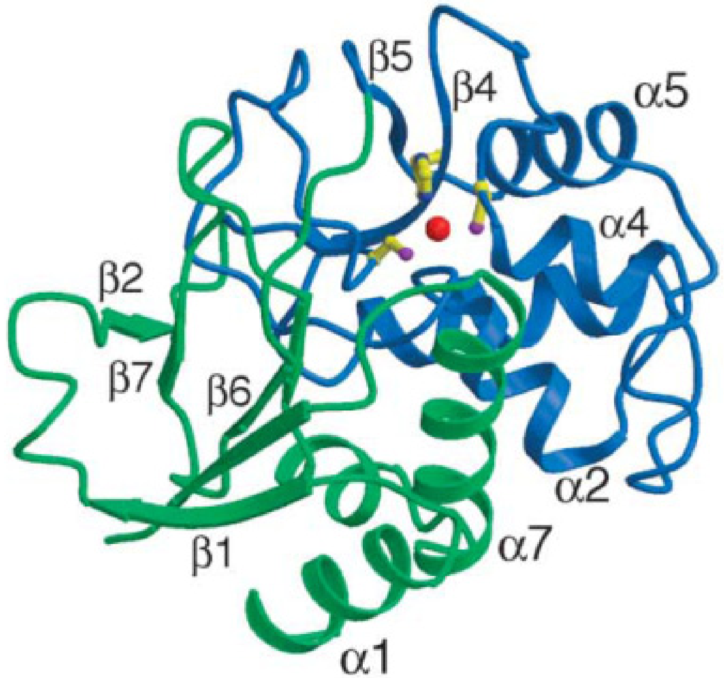
Reaction mechanism of Zn-carboanhydrases (from: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer&part=A1199)

Photosynthesis related Enzymes with metal centres
CO_2 delivery: Cd- and Zn- carboanhydrases
(b) comparison of Cd- and Zn-Carboanhydrases

- Cd-CA is much larger than Zn-CA
- Cd-CA can bind both Cd and Zn. Activity with Zn slightly, but Activity with Cd much higher than in regular Zn-Carboanhydrases.







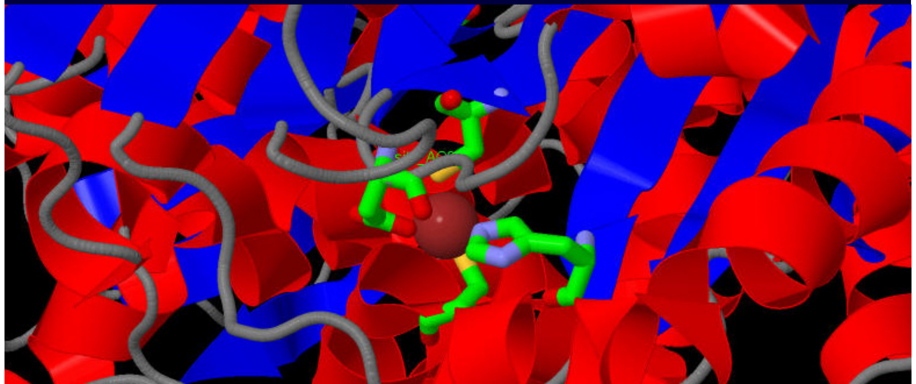
Porphyridium Zn-CA (Mitsuhashi S et al., 2000, JBC 275, 5521-6)

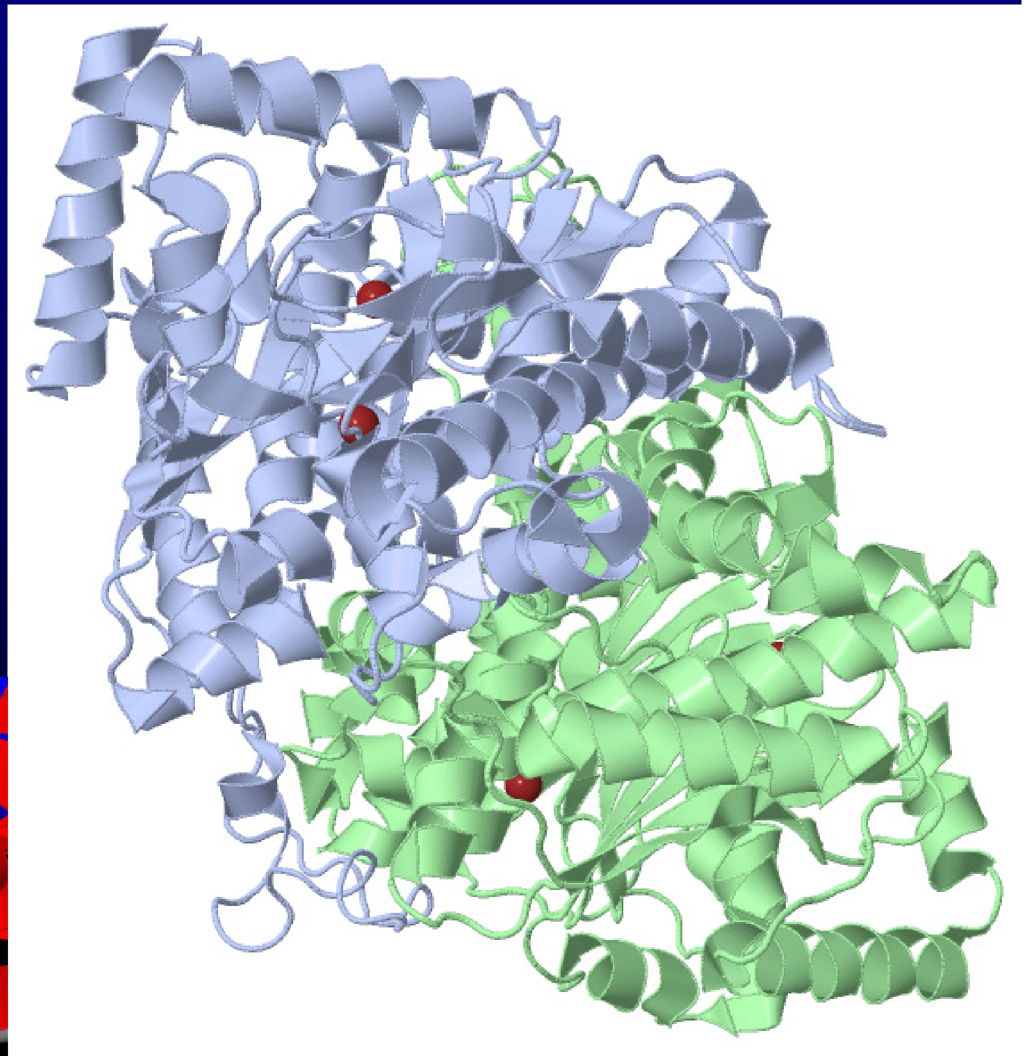
From: Lane and Morel, 2000, PNAS Vol. 97

Thalassiosira Cd-Carboanhydrase (Xu et al., 2008, Nature 452, 56-61)

Photosynthesis related Enzymes with metal centres
CO_2 delivery: Cd- and 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

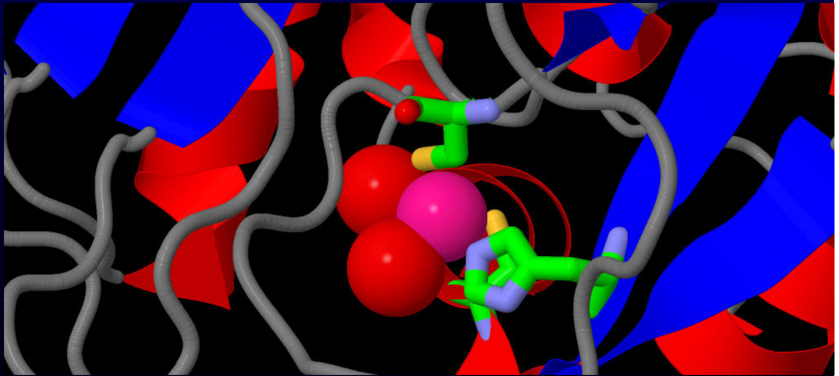


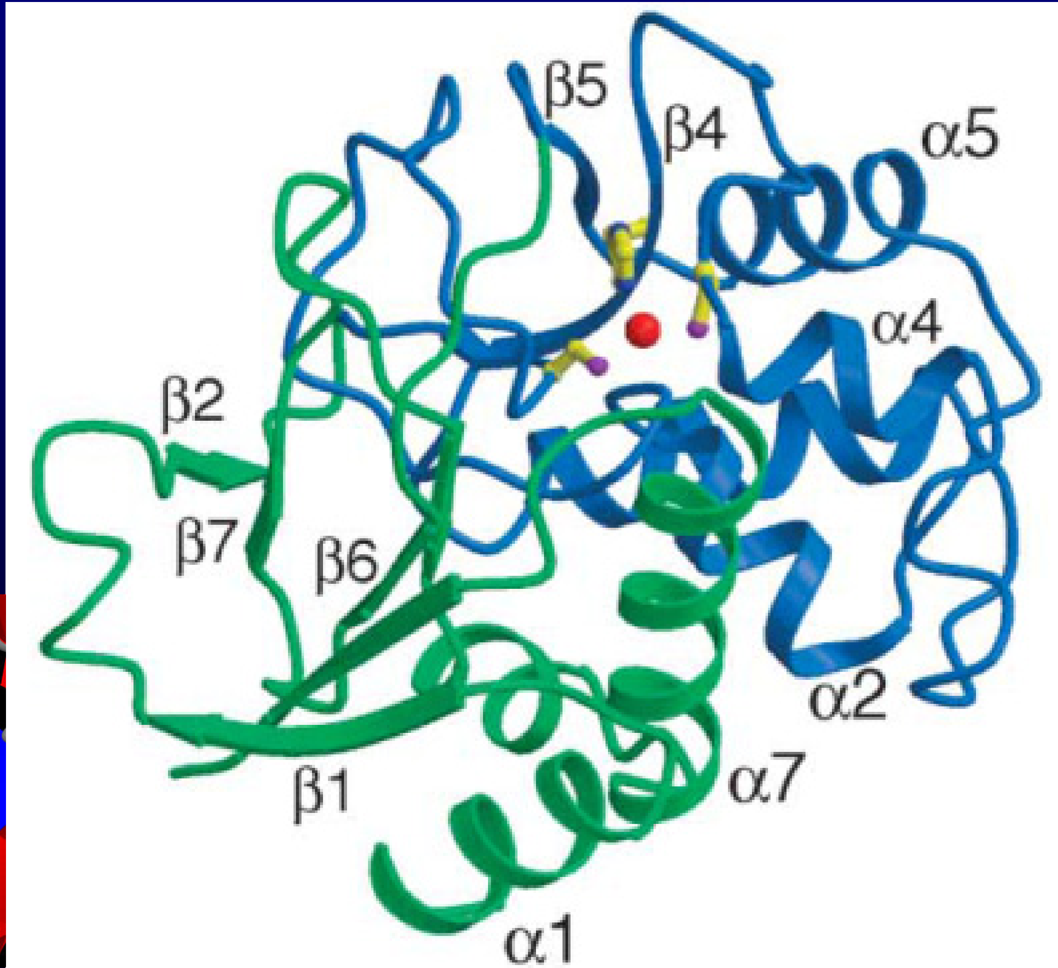


Zn-Carboanhydrase from the marine red alga Porphyridium (Mitsuhashi S et al., 2000, JBC 275, 5521-6)

Photosynthesis related Enzymes with metal centres
CO_2 delivery: Cd- and Zn- carboanhydrases
(b2) Properties and Structure of the Cd-Carboanhydrase

- Cd-CA has 7 α-helices and 9 β-sheats,
- Cd is at the lower End of a funnel-like substrate binding pocket
- Cd^{2+} is bound by 2x Cys and 1x His, plus 1x Water (→ tetrahedral coordination).





Cd-Carboanhydrase from the marine diatom Thalassiosira weissflogii, Xu et al., 2008, Nature 452, pp 56-61

All slides of my lectures can be downloaded from my workgroup homepage

Biology Centre CAS → Institute of Plant Molecular Biology → Departments → Department of Plant Biophysics and Biochemistry,

or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html