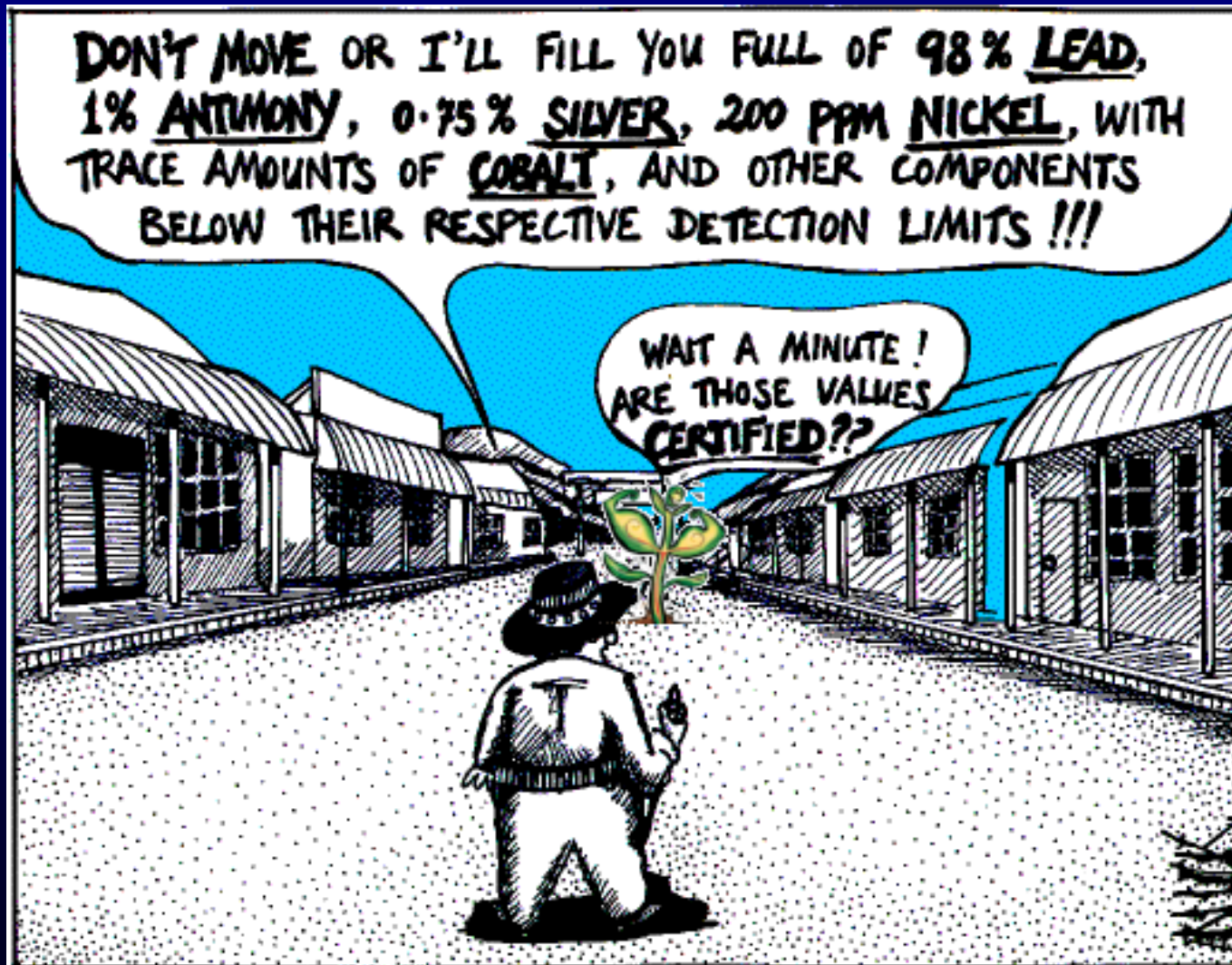


Heavy Metals and Plants - a complicated relationship

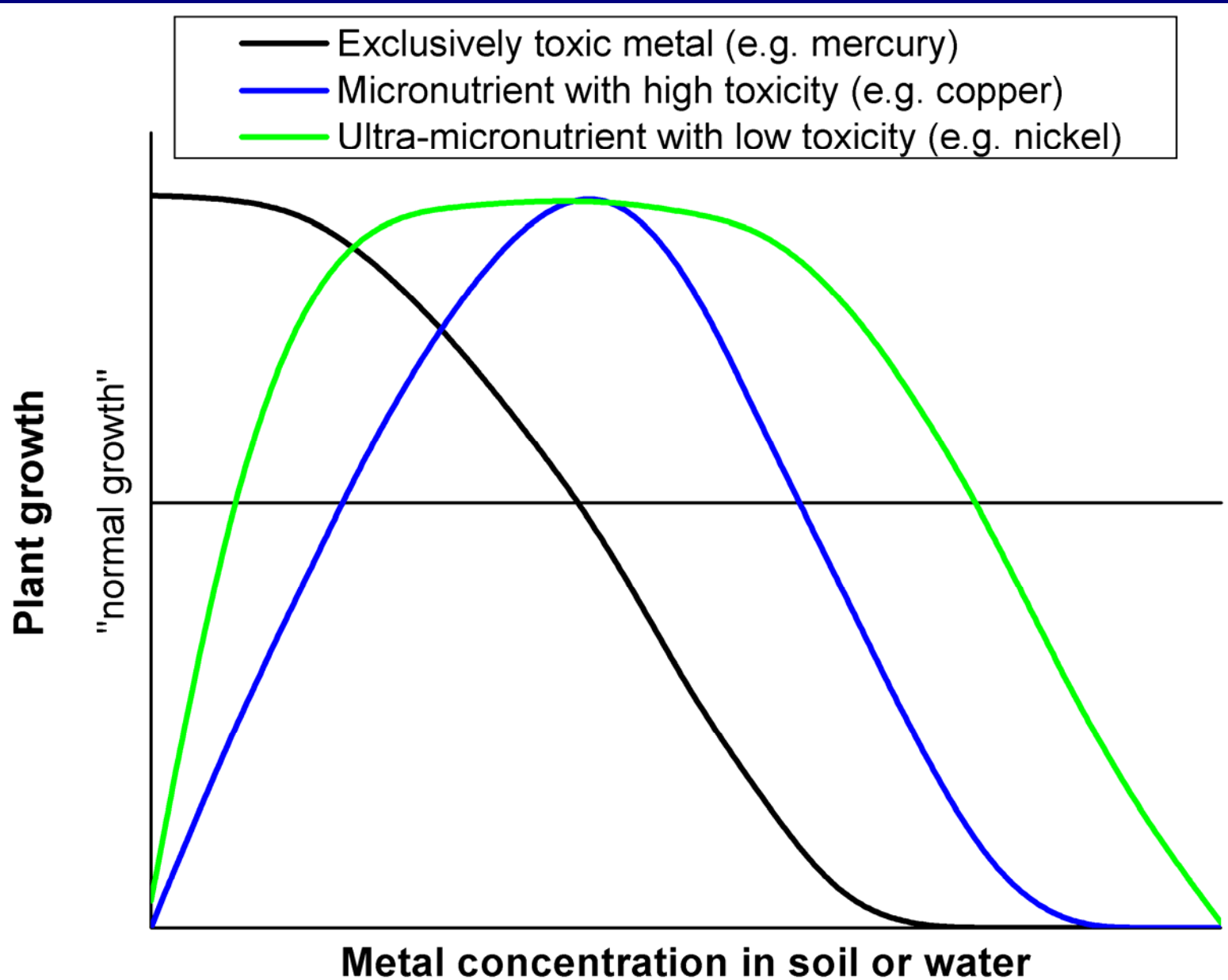
→ Metals in Photosynthesis



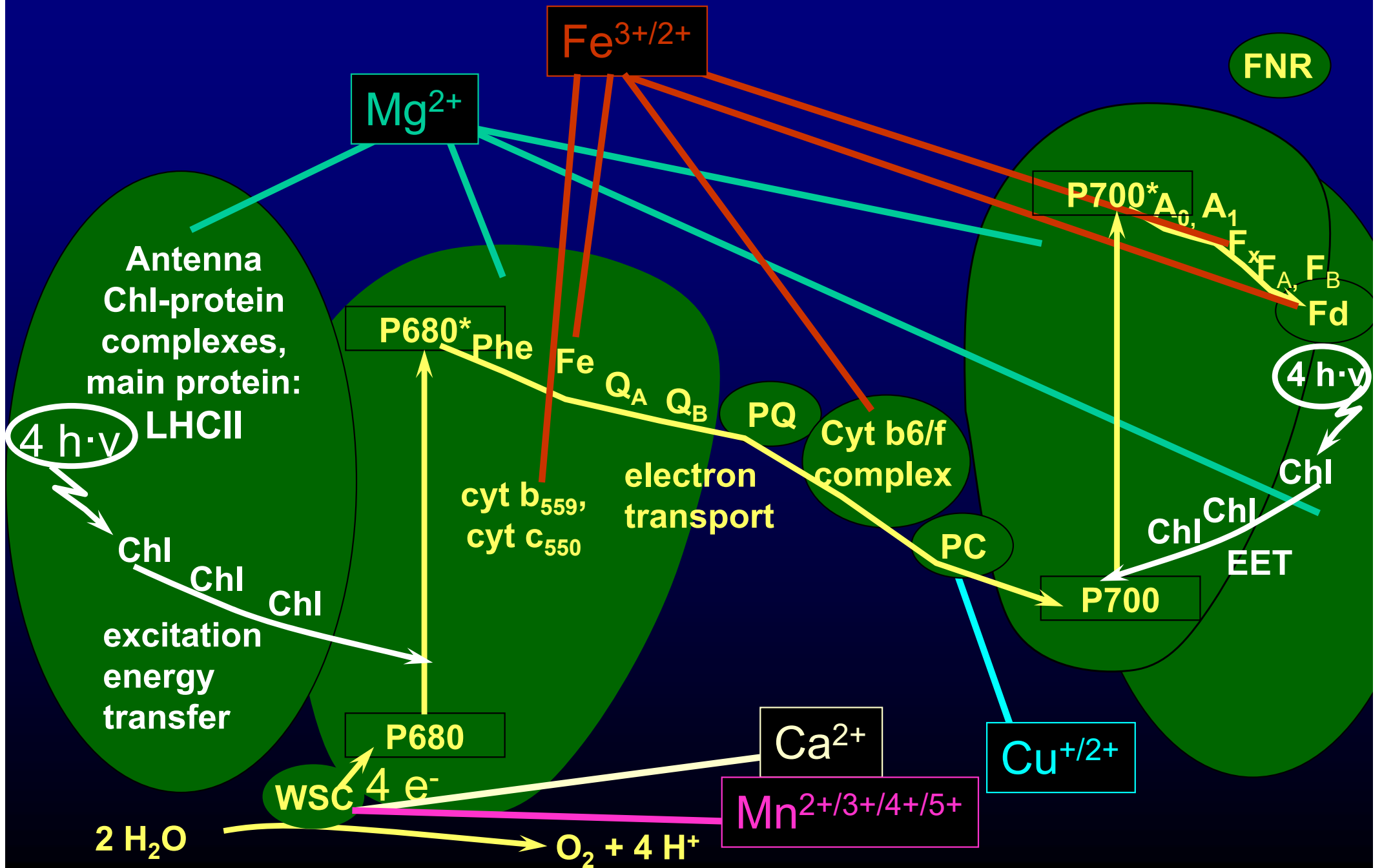
Heavy metal hyperaccumulation in the Wild West

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

Dose-Response principle for heavy metals

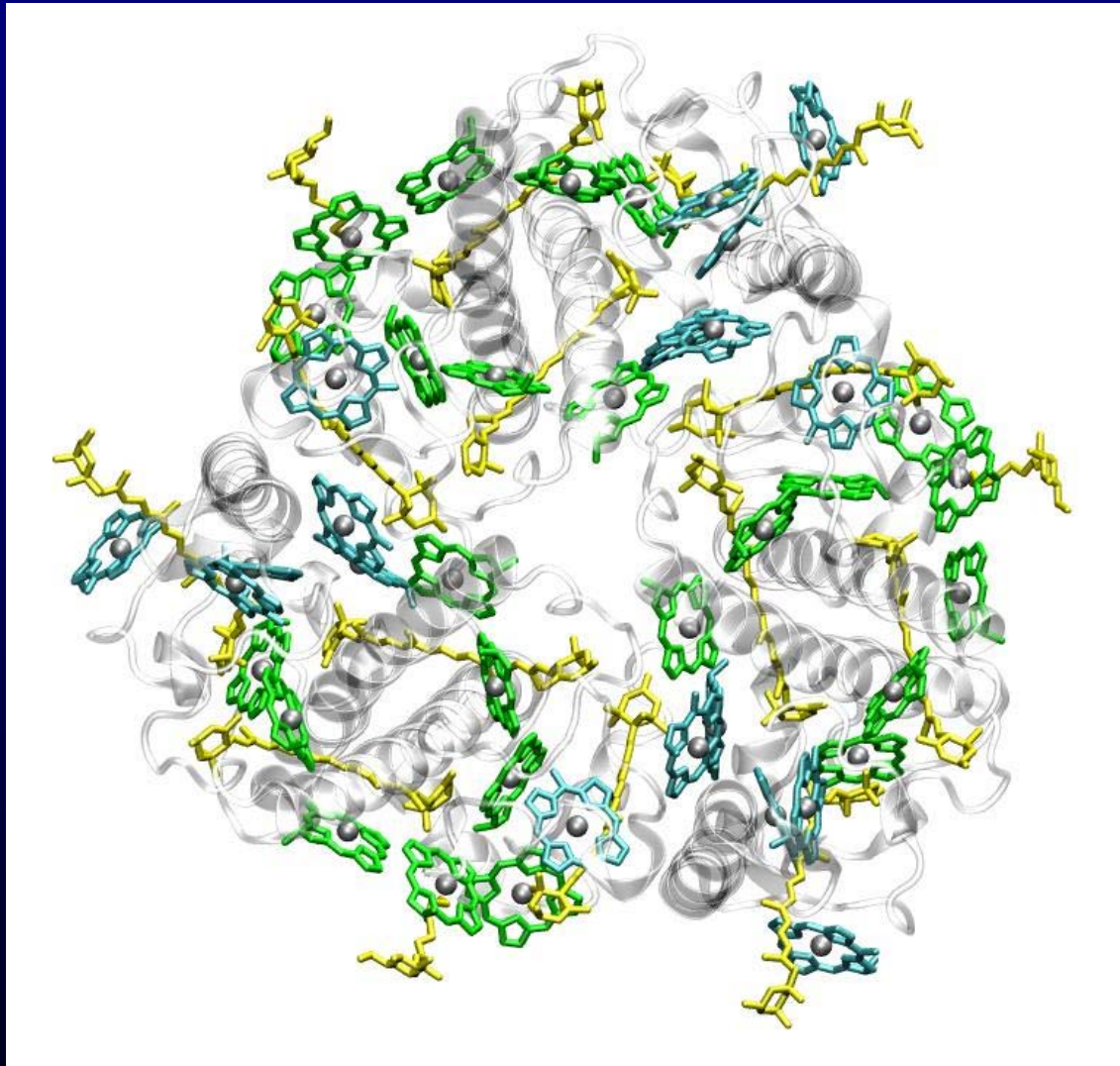


Metal sites in photosynthetic proteins



Photosynthesis related proteins with metal centres

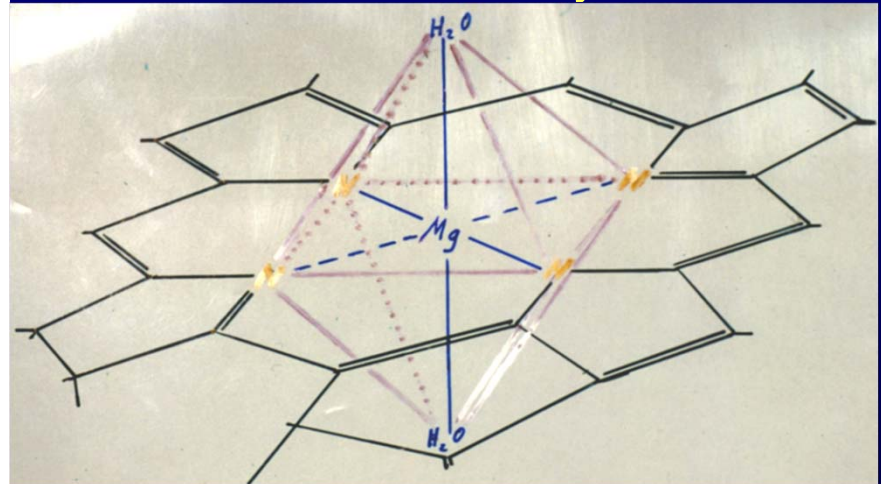
1. Light harvesting complexes



From: commons.wikimedia.org

LHCII structure

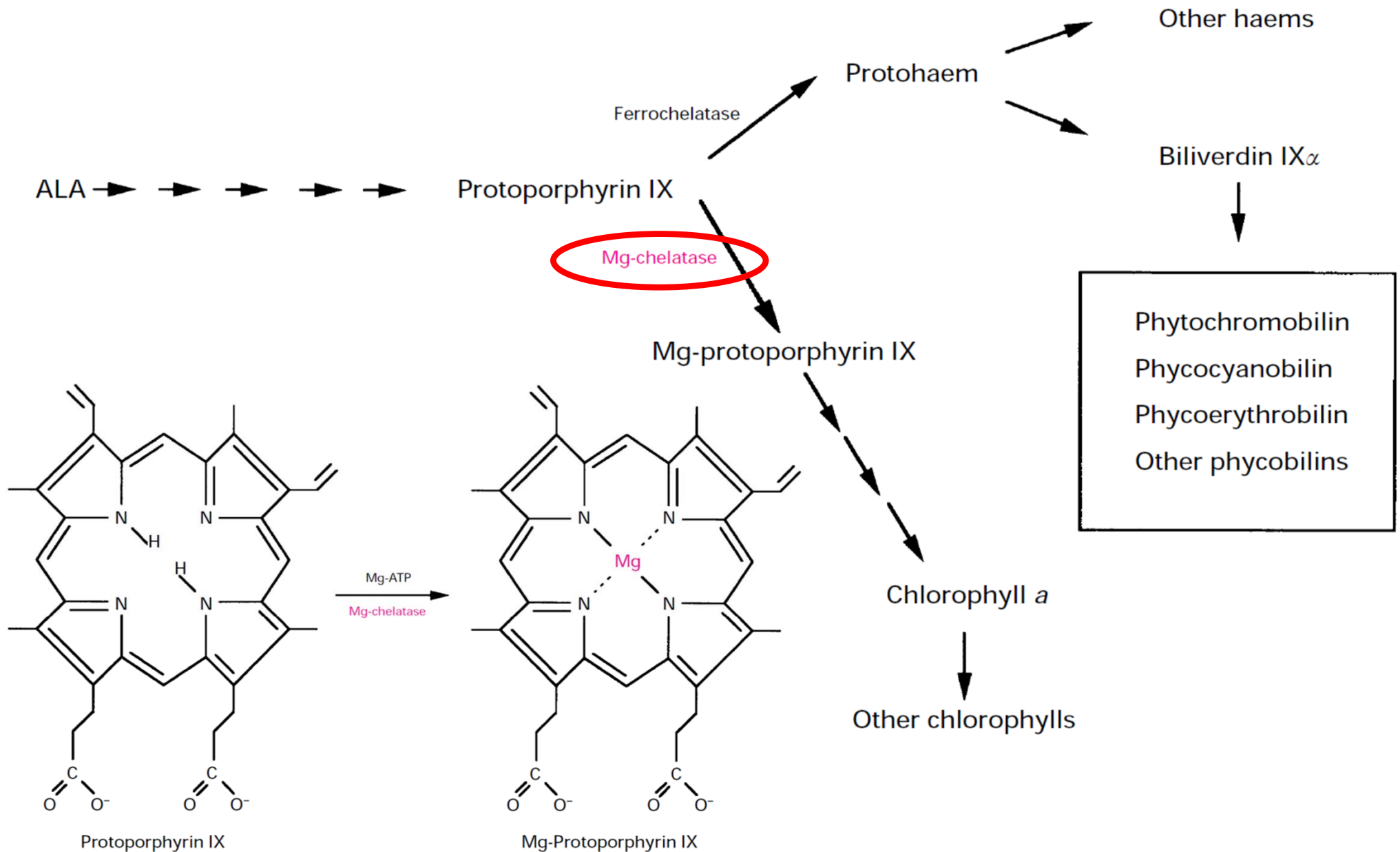
- usually trimers
- structure stabilised by Chl



- binding via axial ligands on **Mg²⁺**
- energy transfer depends on $1/r^6$

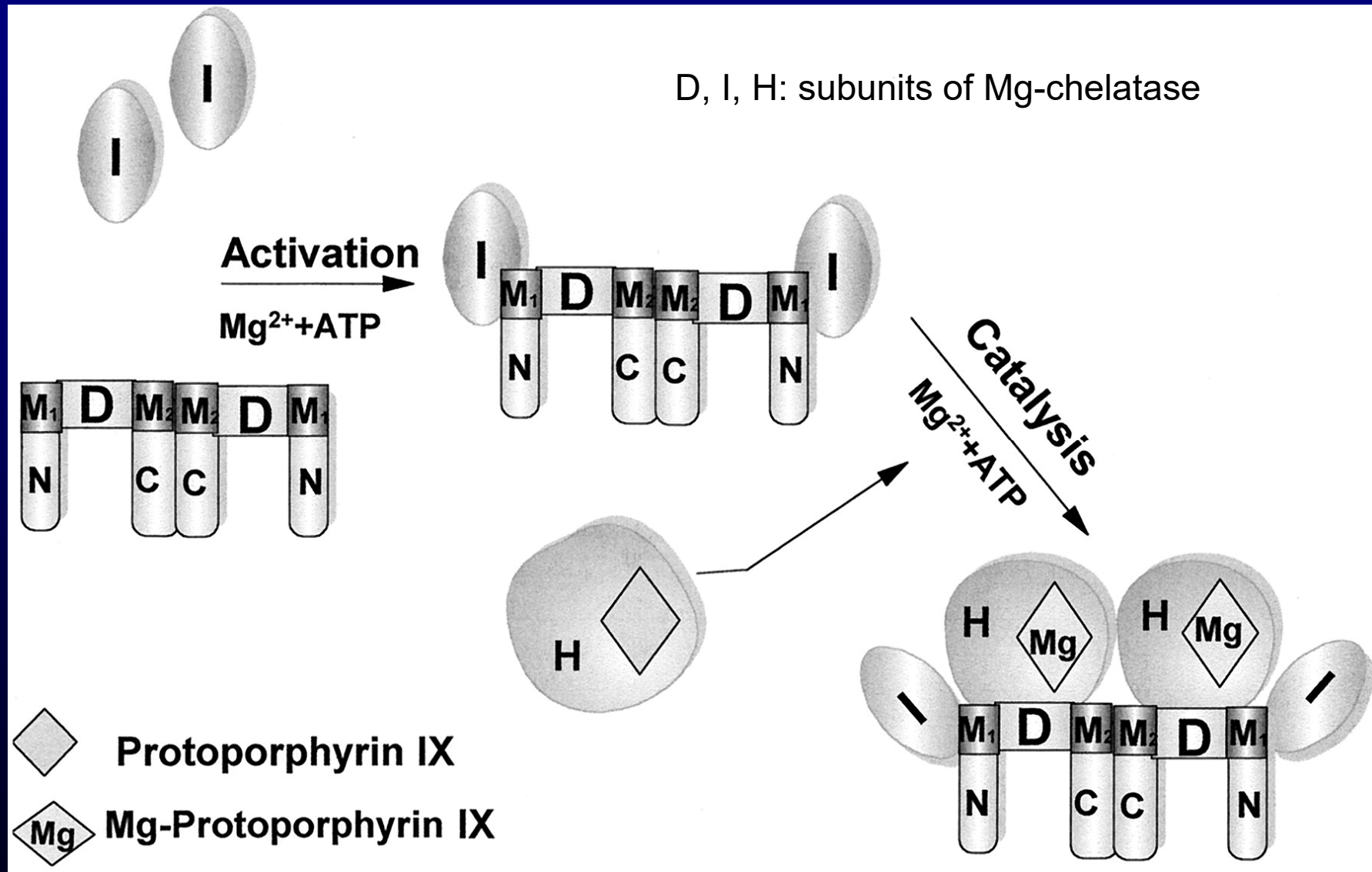
Photosynthesis related proteins with metal centres

1. Light harvesting complexes → Chl biosynthesis



Photosynthesis related proteins with metal centres

1. Light harvesting complexes → Chl biosynthesis

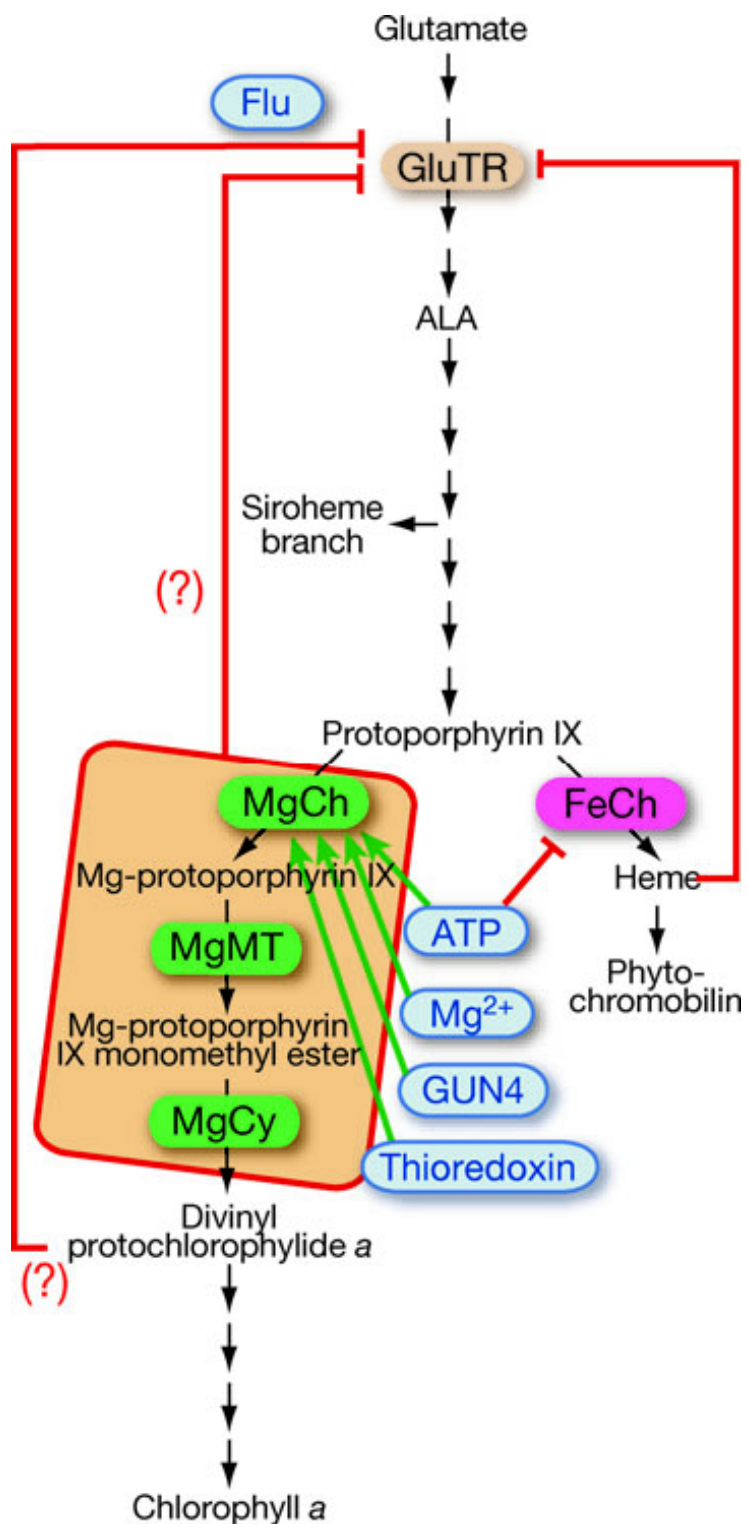


Photosynthesis related proteins with metal centres

1. Light harvesting complexes

Chl biosynthesis regulation

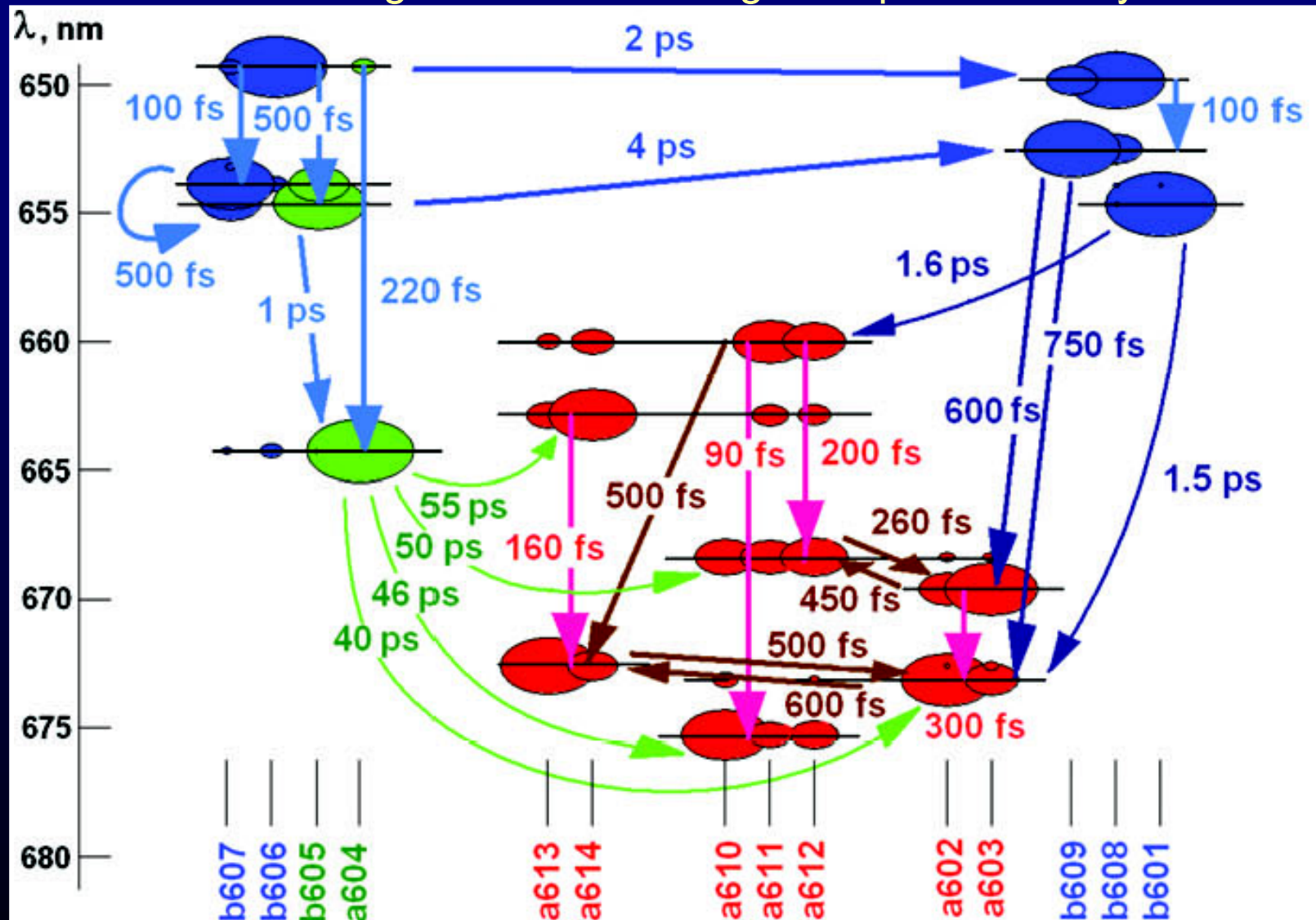
- activation by substrates
- inhibition by intermediate products



Photosynthesis related proteins with metal centres

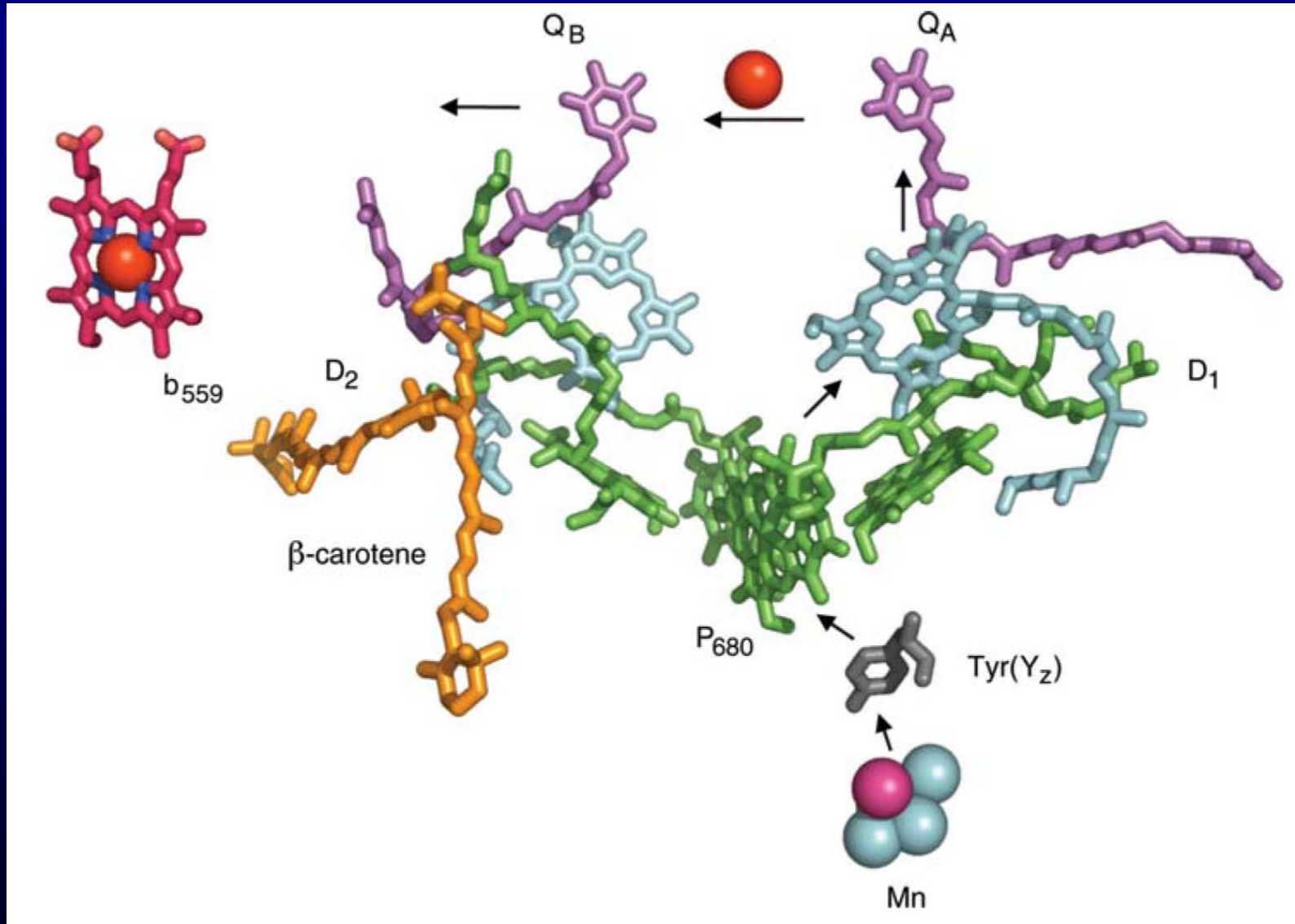
1. Light harvesting complexes: Transfer times between Chls towards & in PSIIRC

→ 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



Photosynthesis related proteins with metal centres

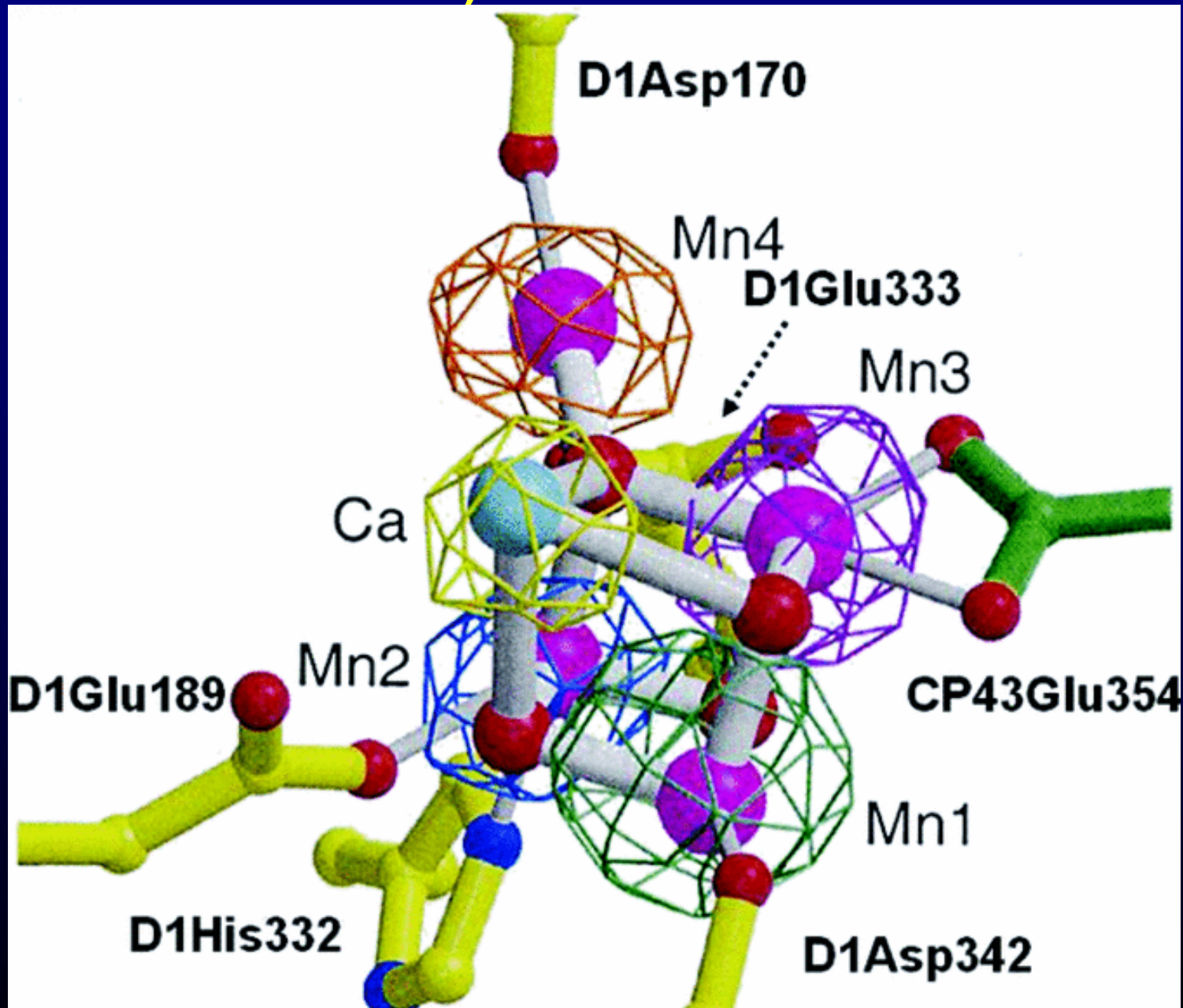
2. Photosystem II reaction centre – electron transport



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

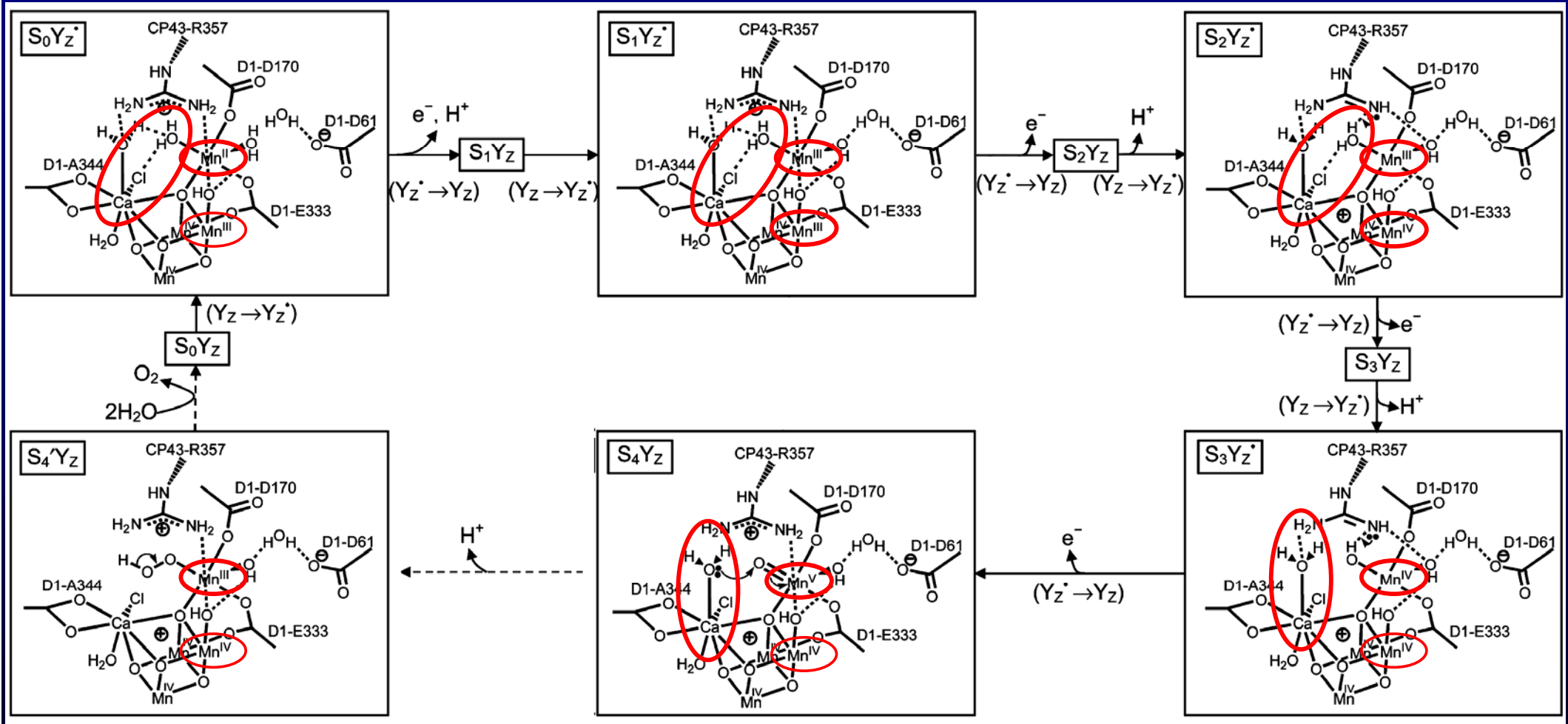
- electrons are transferred from water to plastoquinone b (Q_B)
- Manganese / calcium, magnesium and iron centres involved in e⁻ transport

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



Water splitting complex of the photosystem II reaction centre

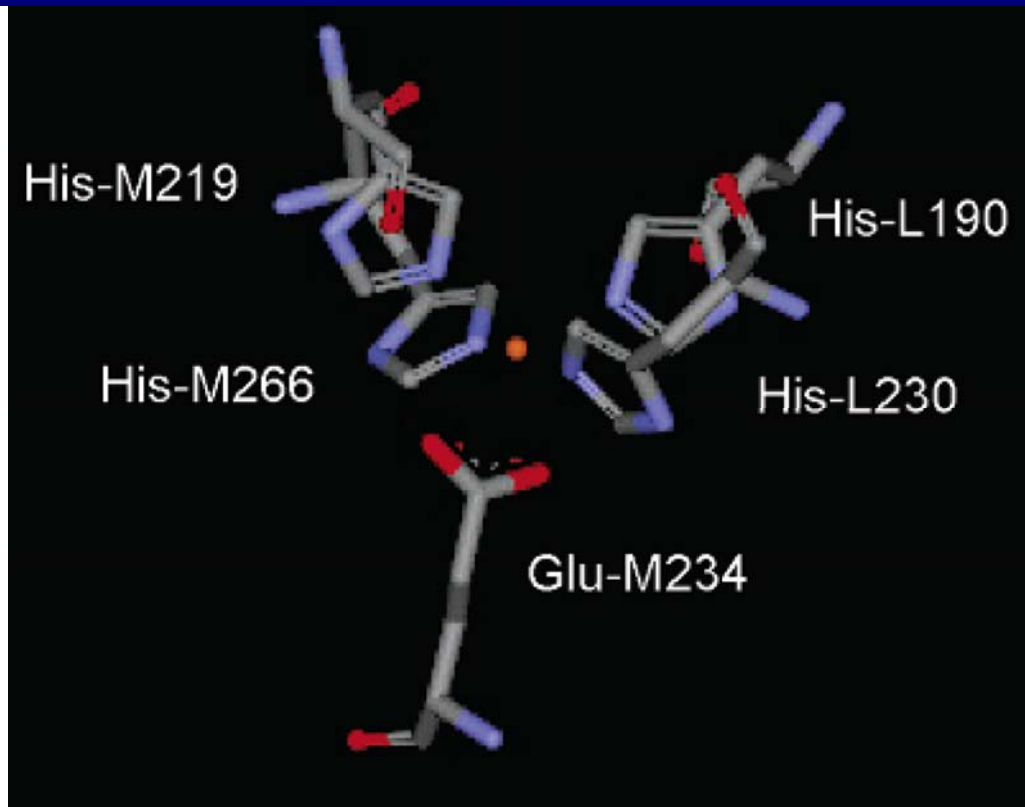
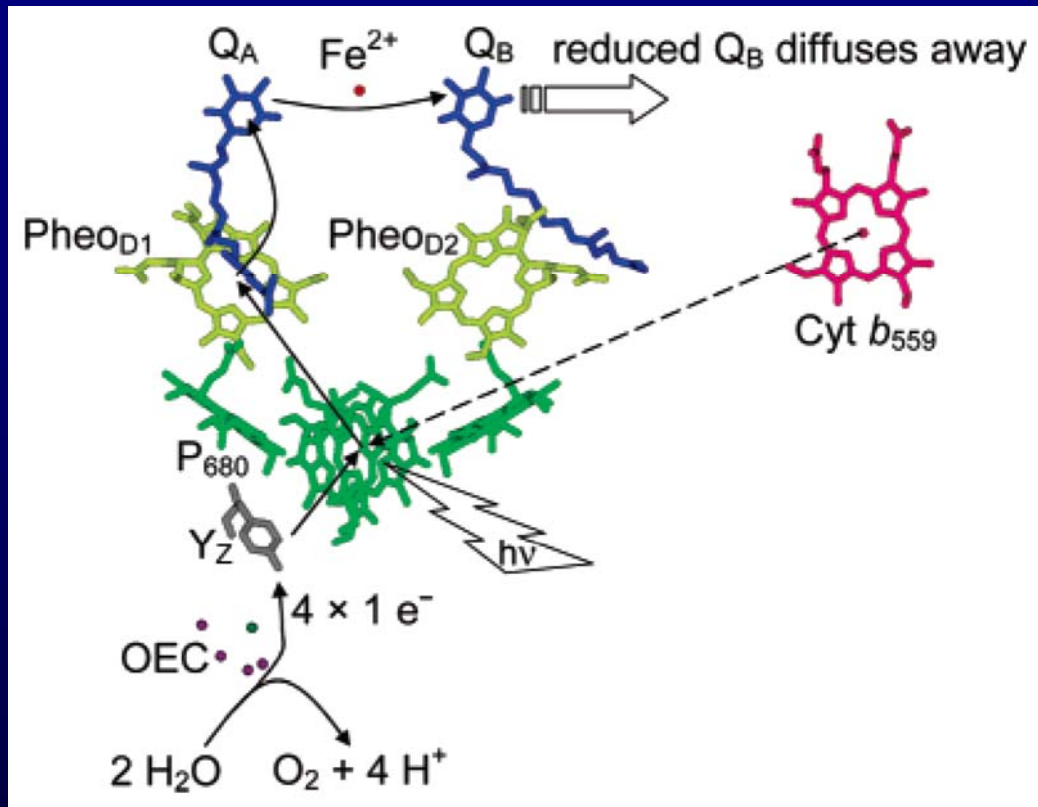
(b) proposed mechanism



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
- Ca²⁺ helps in binding the water

Non-heme iron in the photosystem II reaction centre



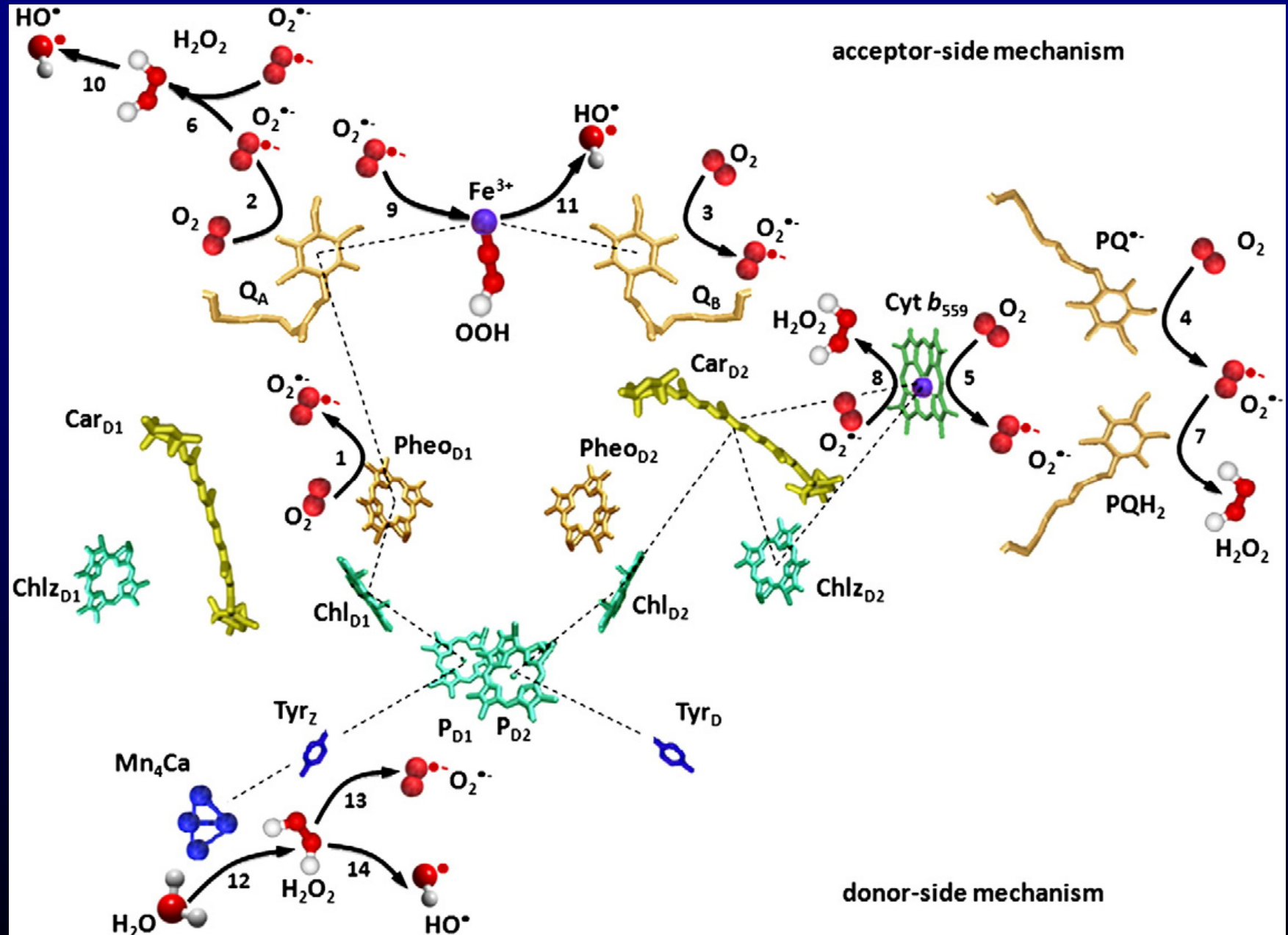
From: McEvoy JP, Brudvig GW, 2006, Chemical Reviews 106, 4455-83

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

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

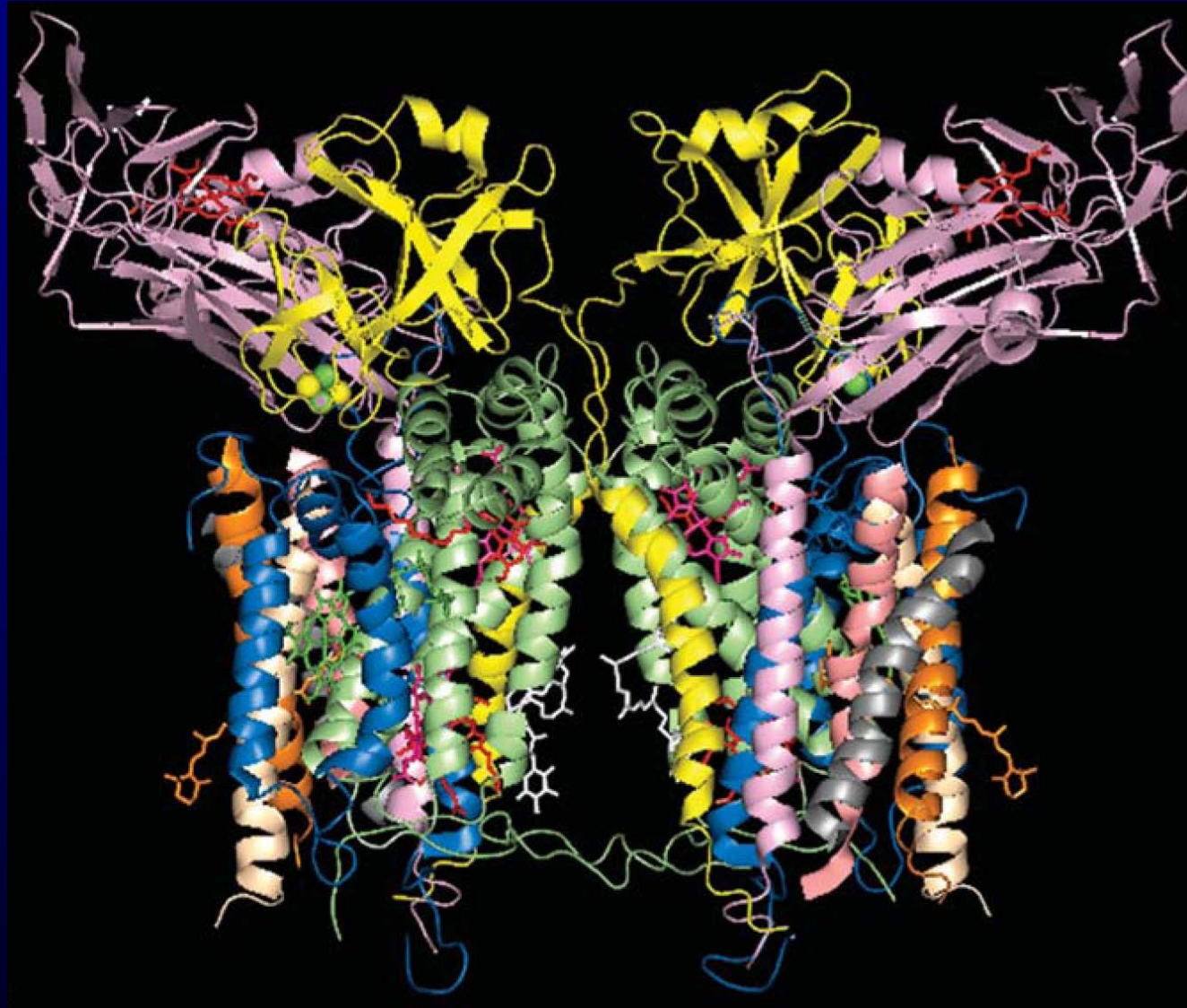
Photosynthesis related proteins with metal centres

PSIIRC: generation of ROS



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

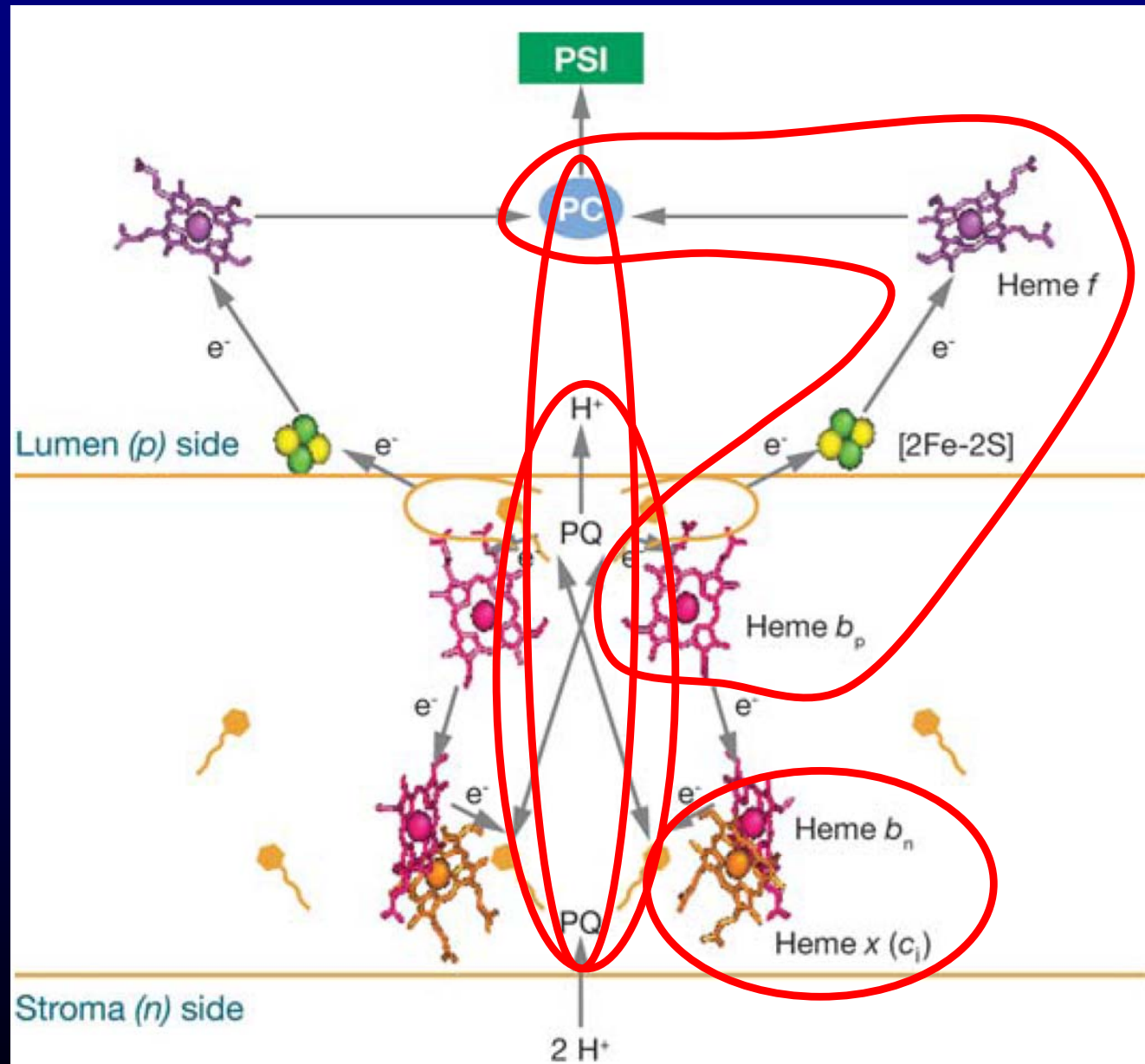
- Homodimer, each monomer consisting of 8 subunits totalling about 109 kDa
- Each monomer contains 13 transmembrane helices, and beta sheets in the Rieske subunit

Photosynthesis related proteins with metal centres

3. Cytb₆f complex: Mechanism

Functional characteristics

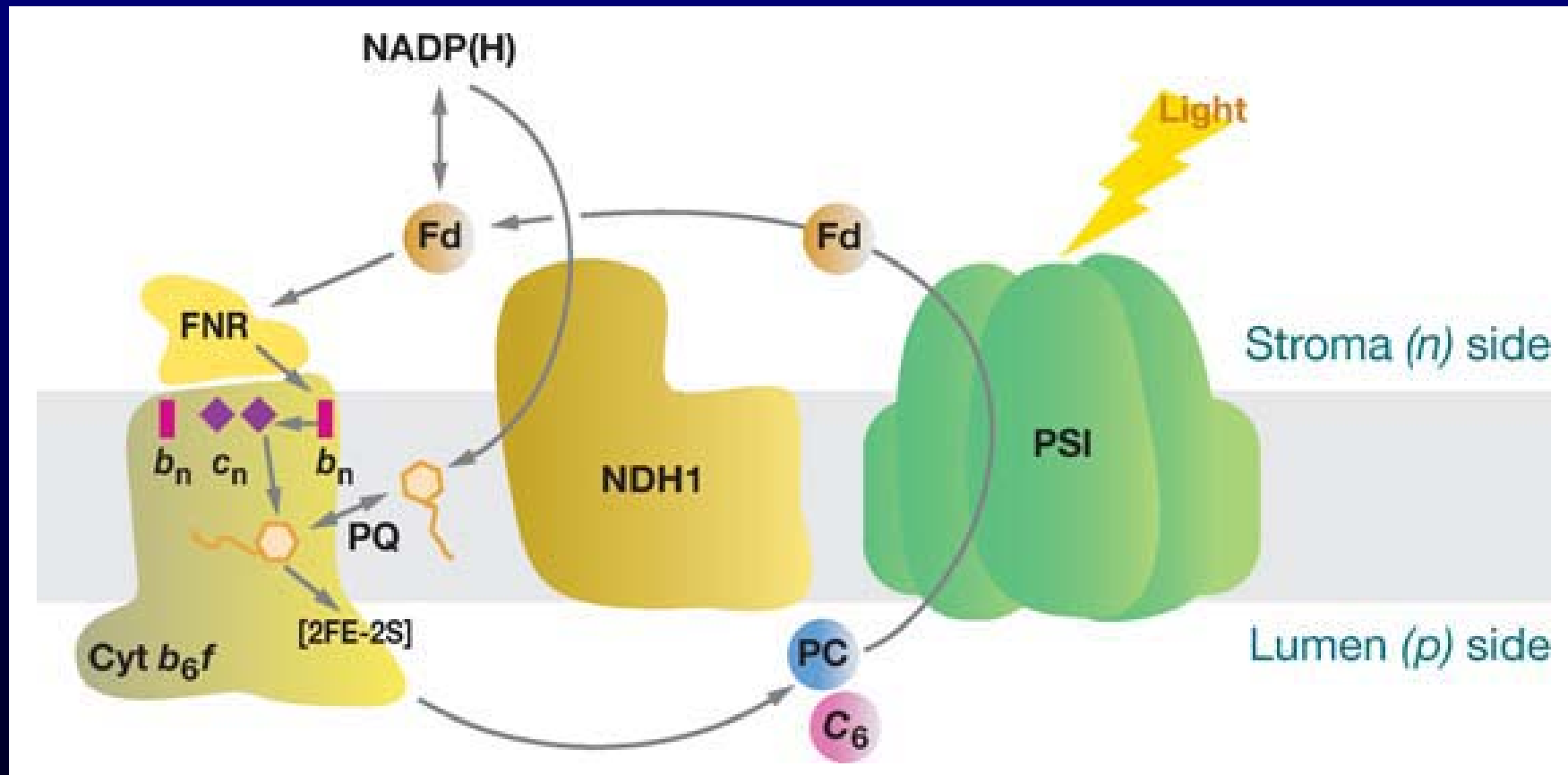
- transfers e⁻ from PQ to plastocyanin (PC),
- It uses the difference in potential between Q_B and PC for translocating a proton via 2x2 heme *b* groups and 2x1 heme *x* group
- Electrons are transferred from the heme *b* groups to PC via a “Rieske” [2Fe2S]-cluster and a heme *f* group
- Cyclic electron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



Photosynthesis related proteins with metal centres

3. Cyt_b₆f complex: Mechanism

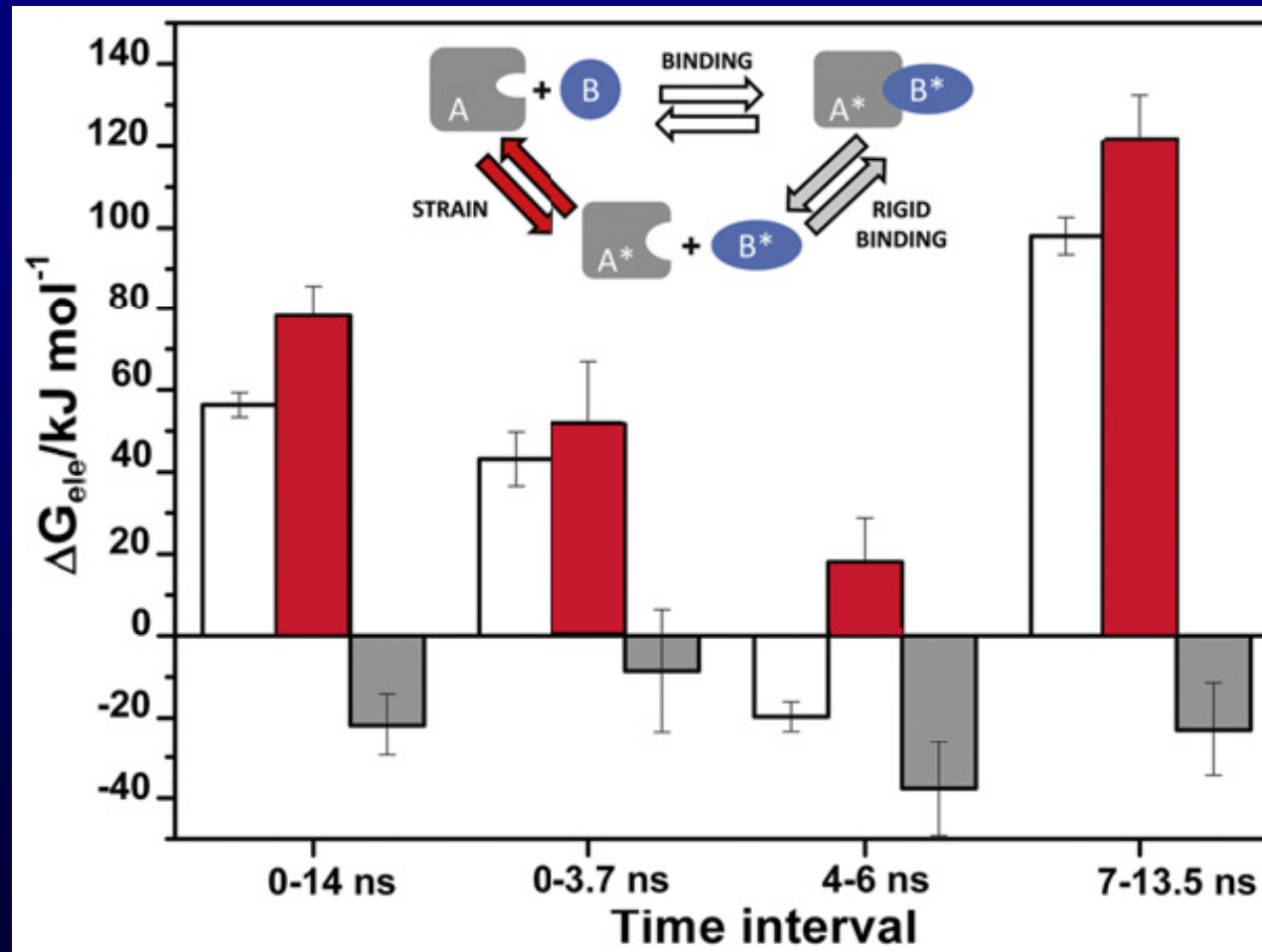
Cyclic electron transport occurs via coupling of ferredoxin to heme x



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

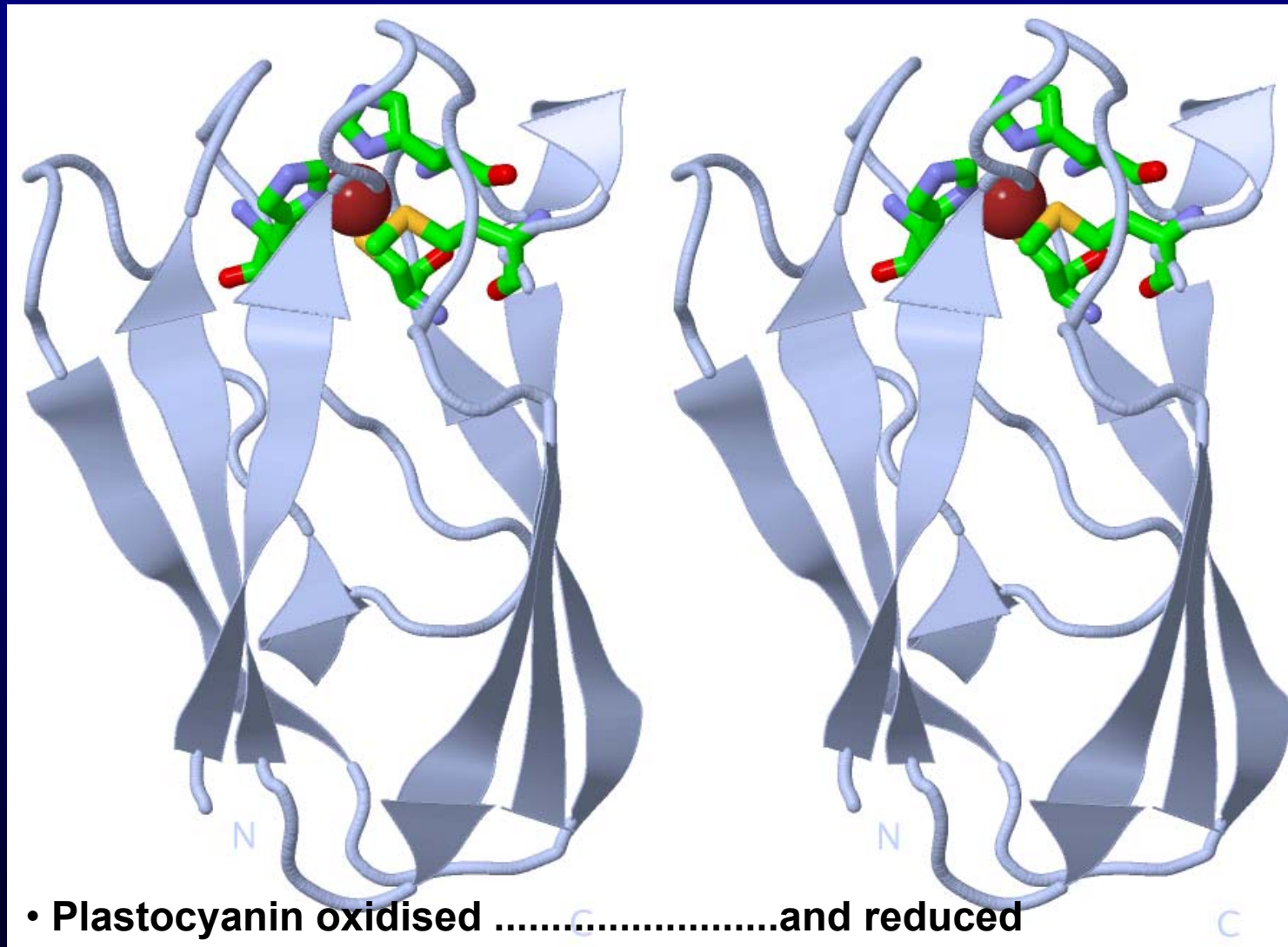


From: Cruz-Gallardo I, et al. (2012) FEBS Lett 586, 646-52

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

Photosynthesis related proteins with metal centres

4. Plastocyanin



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

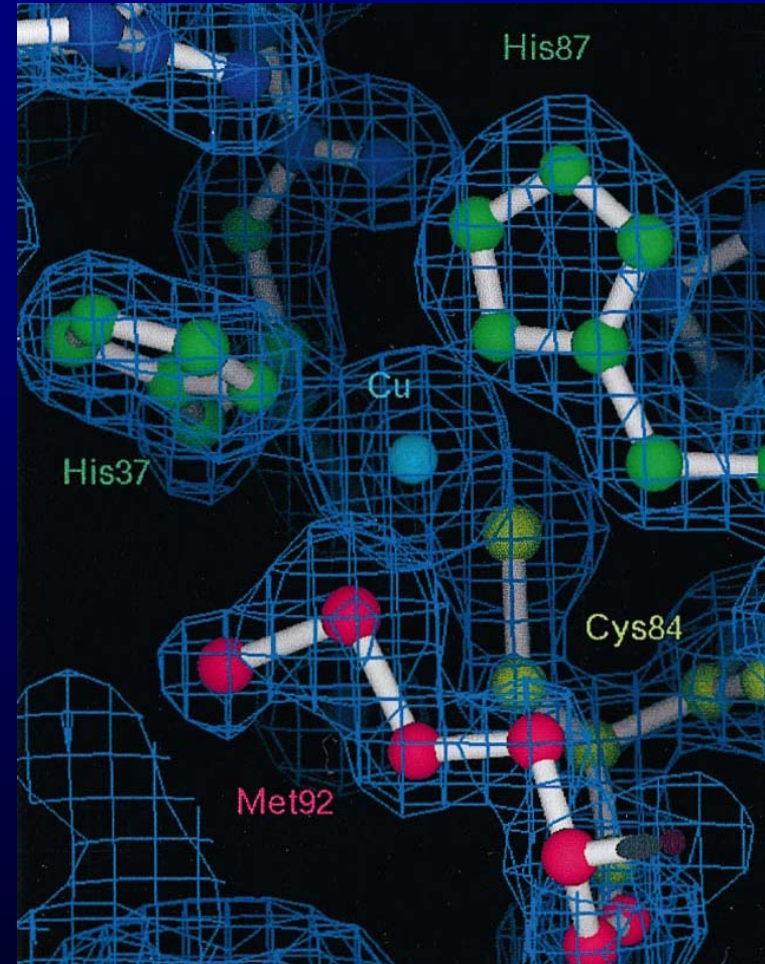
- about 100 amino acids, soluble protein
- type 1 (“blue”) copper protein
- copper bound by 2 His, 1 Cys, and 1 Met residue in distorted tetrahedral geometry

Photosynthesis related proteins with metal centres

4. Plastocyanin

Functional characteristics

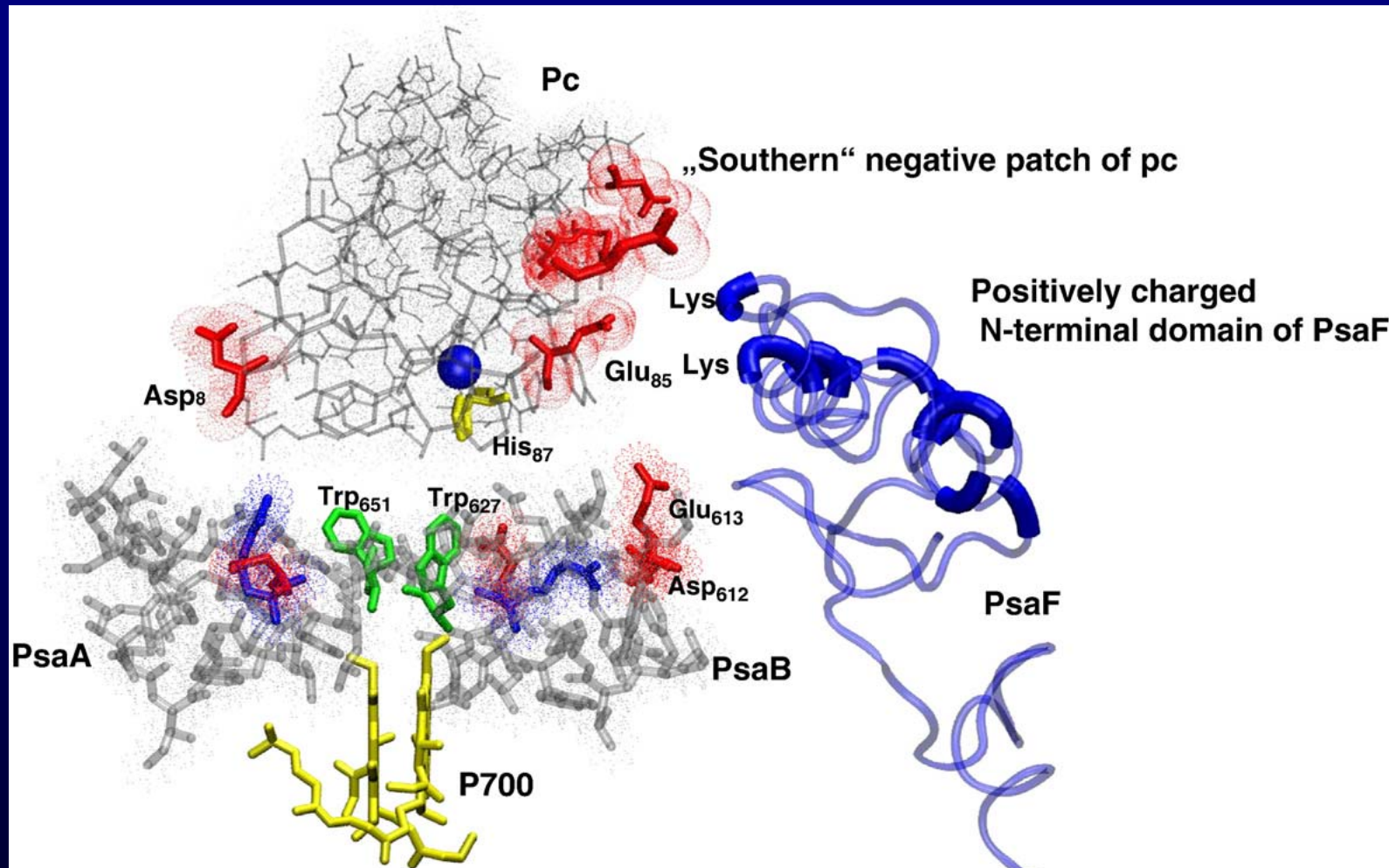
- Oxidised (Cu^{2+}) plastocyanin accepts electron from Cyt_{b6f} complex,
- Reduced ($\rightarrow \text{Cu}^+$) plastocyanin diffuses to the PSIRC
- Plastocyanin releases the electron ($\text{Cu}^+ \rightarrow \text{Cu}^{2+}$)
- Rigid protein structure facilitates fast red/ox-changes, but recent data 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



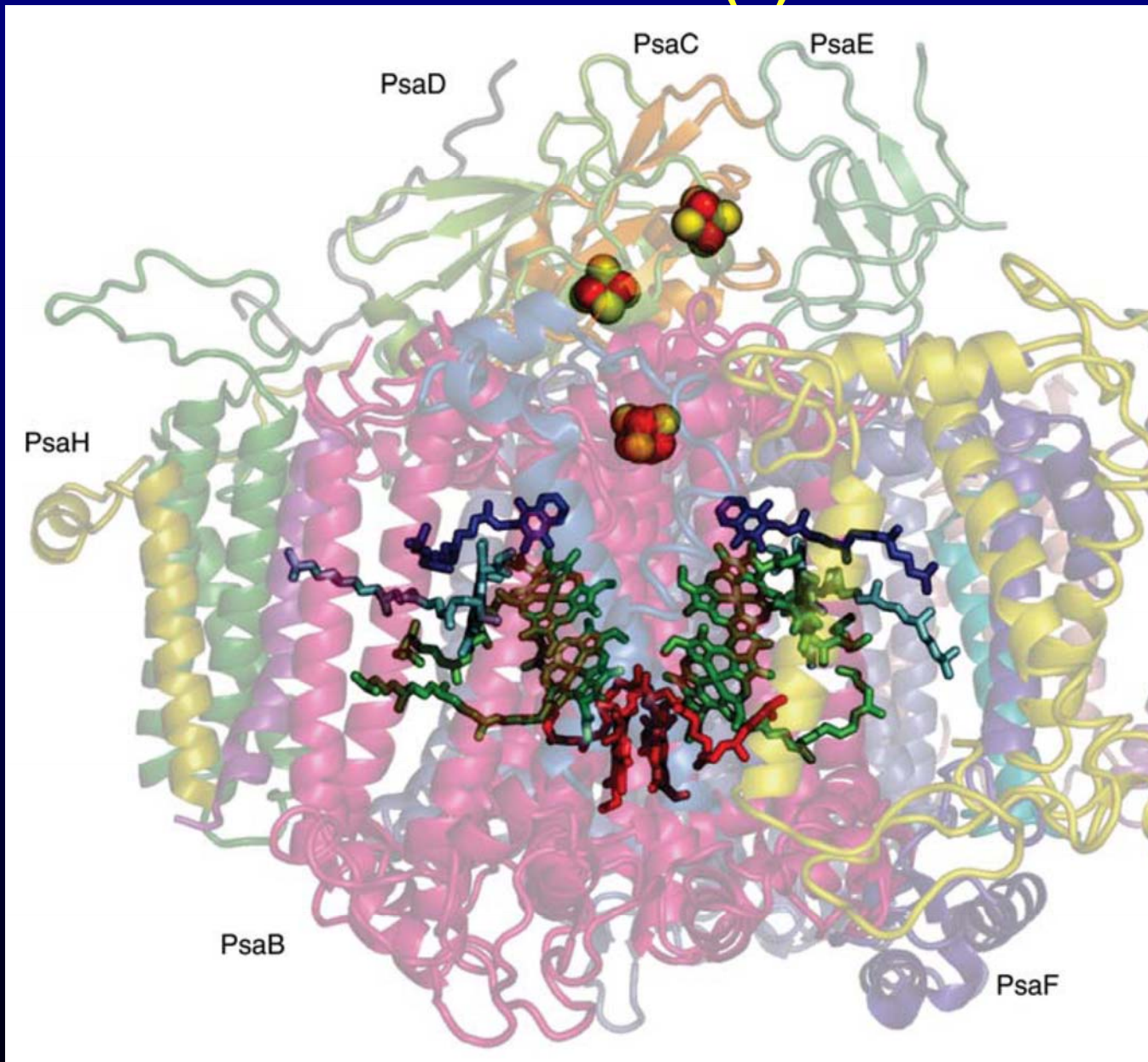
From: Busch A, Hippler M (2011) BBA1807, 864-77

- 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

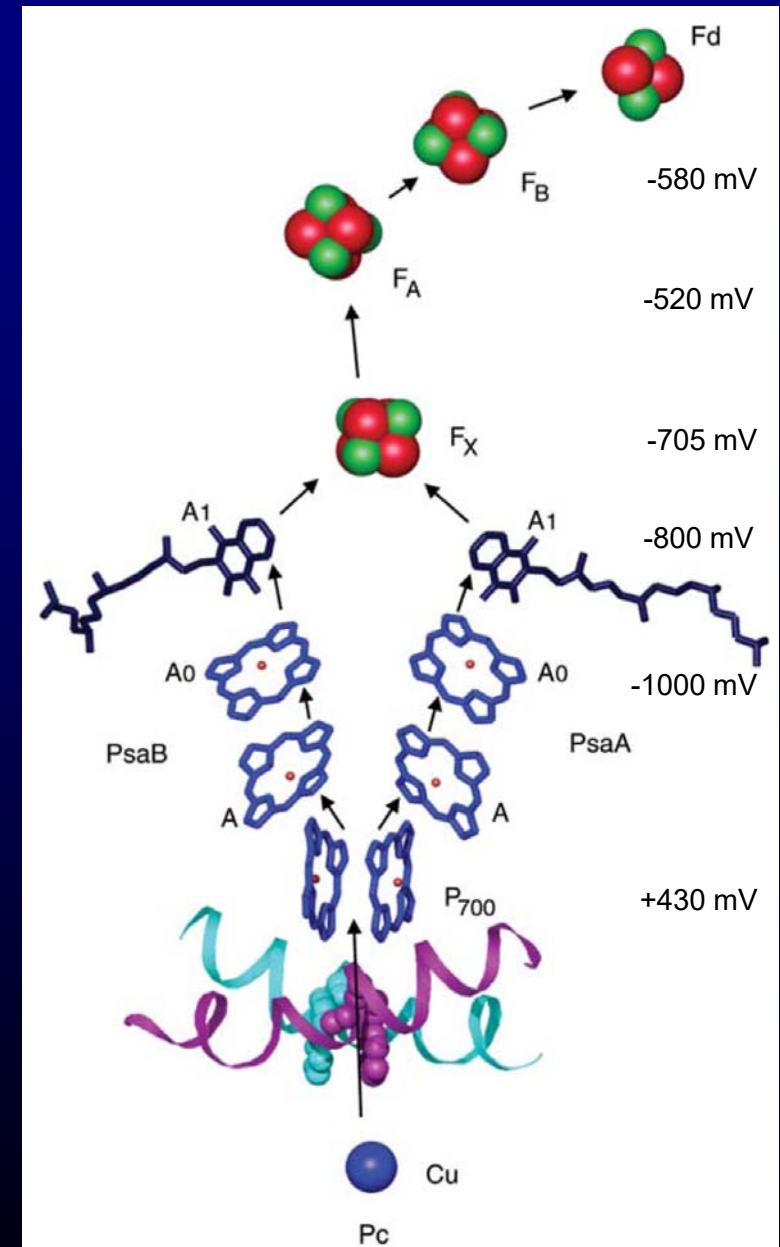
Photosynthesis related proteins with metal centres

5. Photosystem I reaction centre

(a) Overview

Functional characteristics:

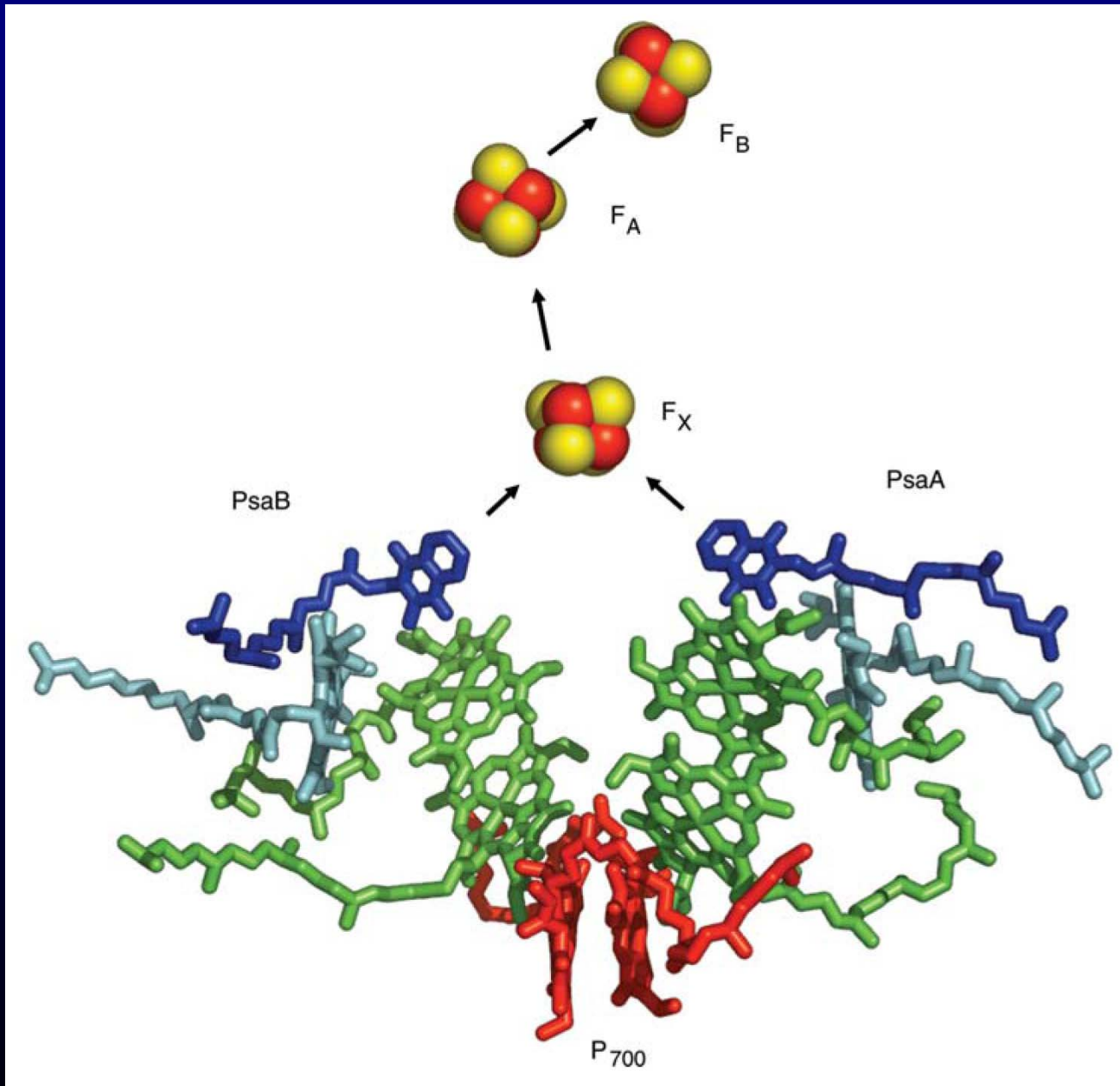
- primary charge separation:
special pair (=P700, Chl a / Chl a' heterodimer),
releases e^- to A_0 via A (both Chl a)
- e^- transport via A1 (phylloquinone) and the
[4Fe4S]-clusters F_x , F_A and F_B to the [4Fe4S]-
cluster of ferredoxin
- P700 is re-reduced by plastocyanin



Photosynthesis related proteins with metal centres

5. Photosystem I reaction centre

(b) iron-sulphur clusters

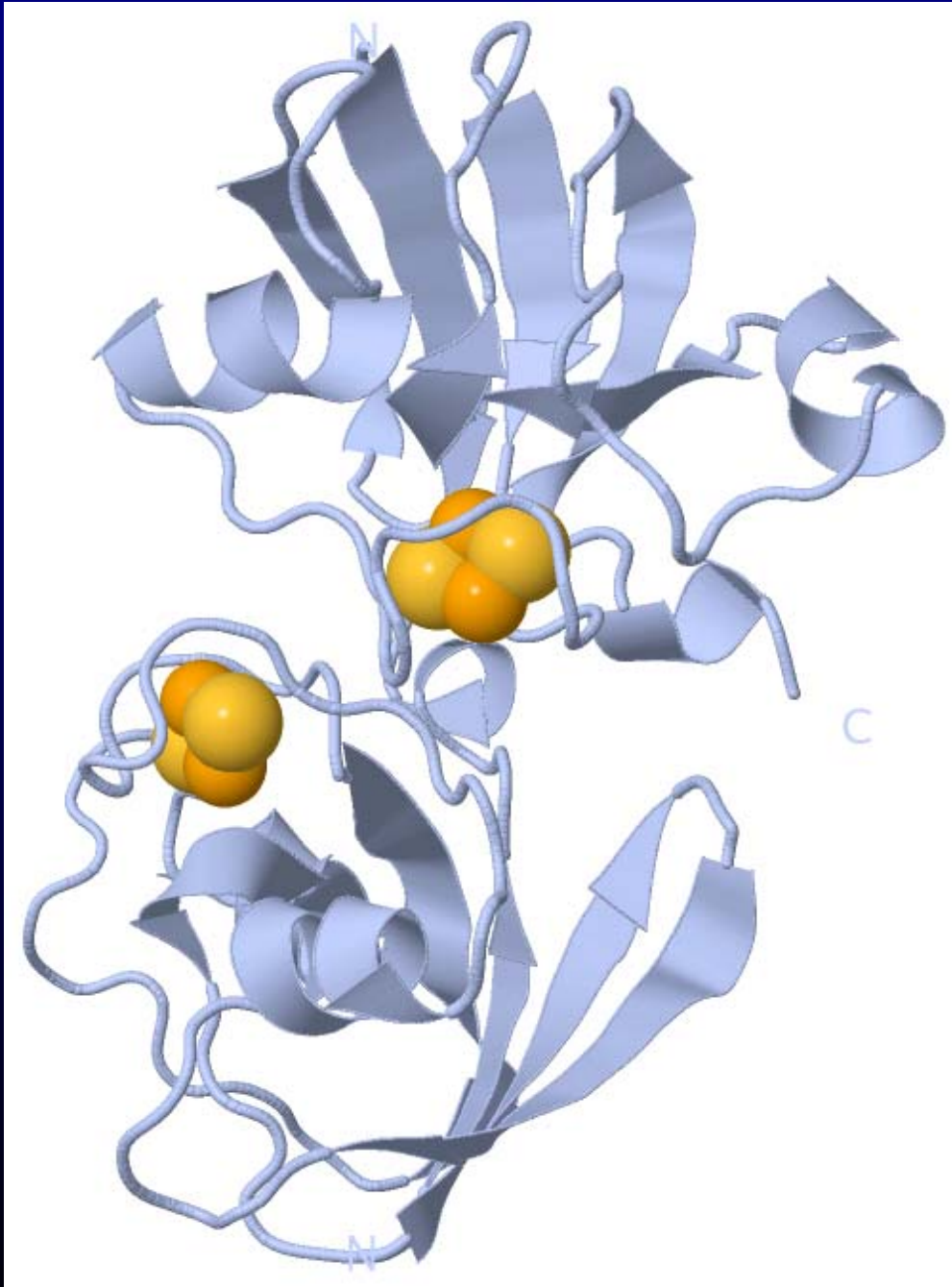


Function of the 4Fe4S-clusters in PSIRC

- accept electrons from the phylloquinones (“A₁”)
- transfer the electrons to ferredoxin

Photosynthesis related proteins with iron centres

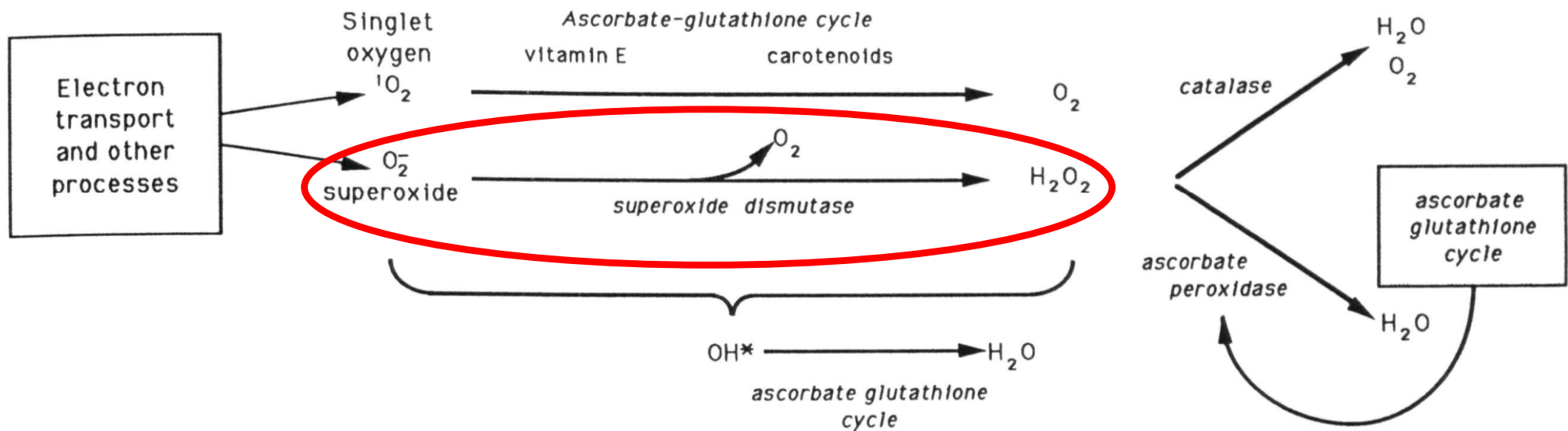
Ferredoxin



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)

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



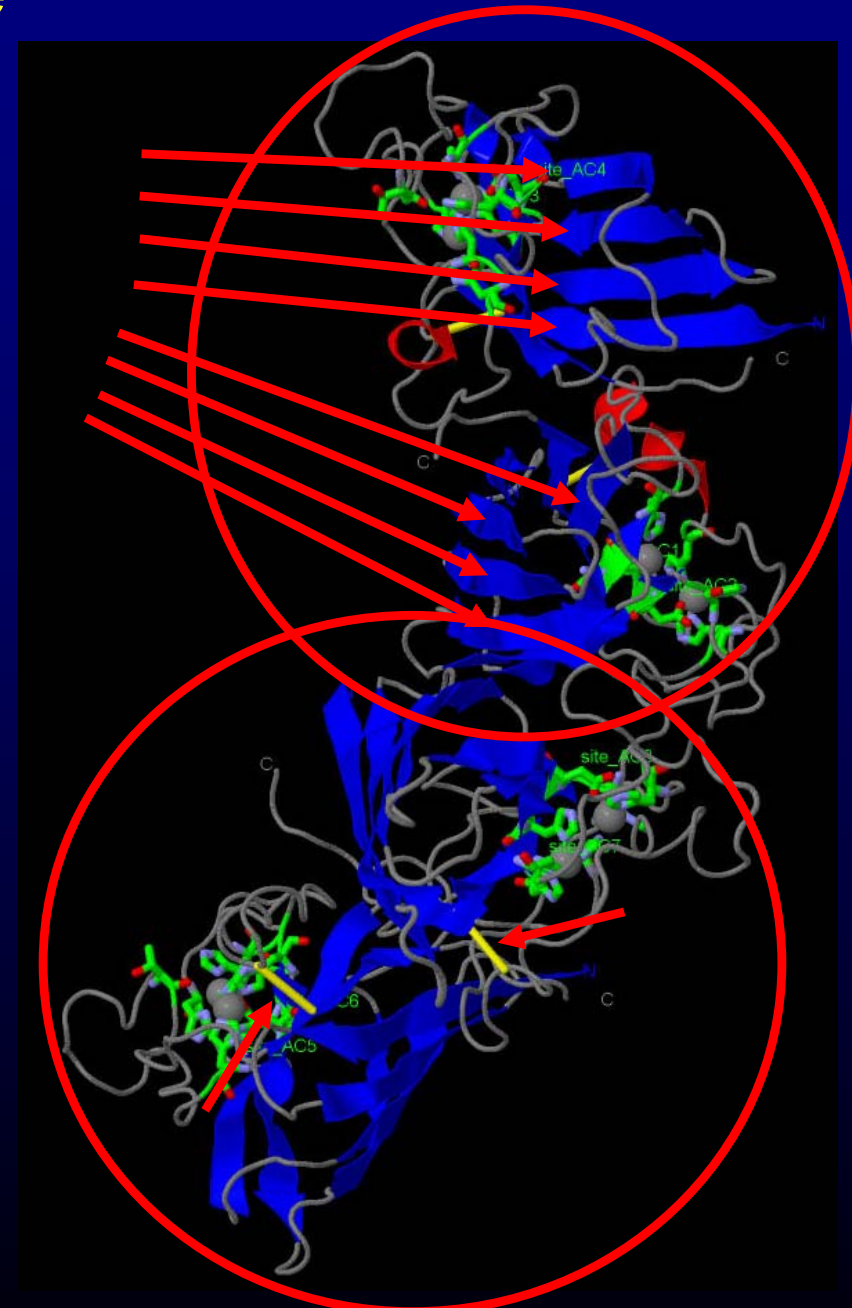
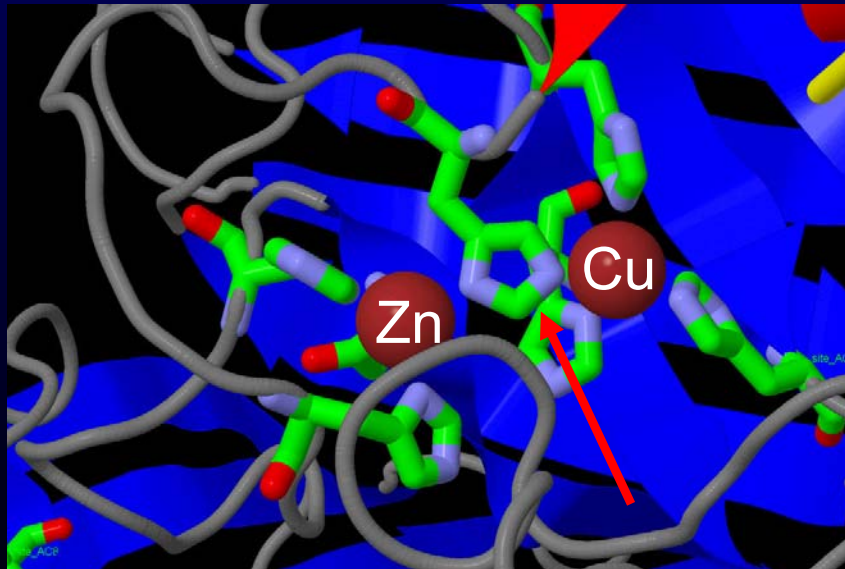
From: Foyer CH et al., 1994, PlantCellEnvi17_507-23

- 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

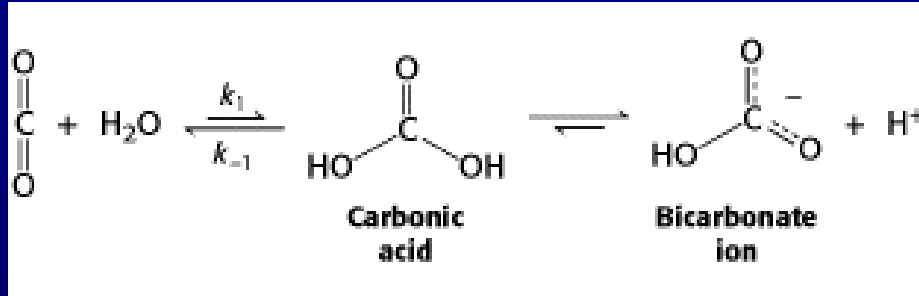
- Dimer of two identical subunits, in crystals 2 dimers together
- Each subunit consists of:
 - 8 anti-parallel β -strands forming a flattened cylinder,
 - 3 external loops
- 1 Cys-Cys disulfide bond stabilises loops
- 1 Cu^{2+} and 1 Zn^{2+} per subunit
- Cu^{2+} bound by 4 His, Zn^{2+} by 3 His + 1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



Photosynthesis related Enzymes with metal centres

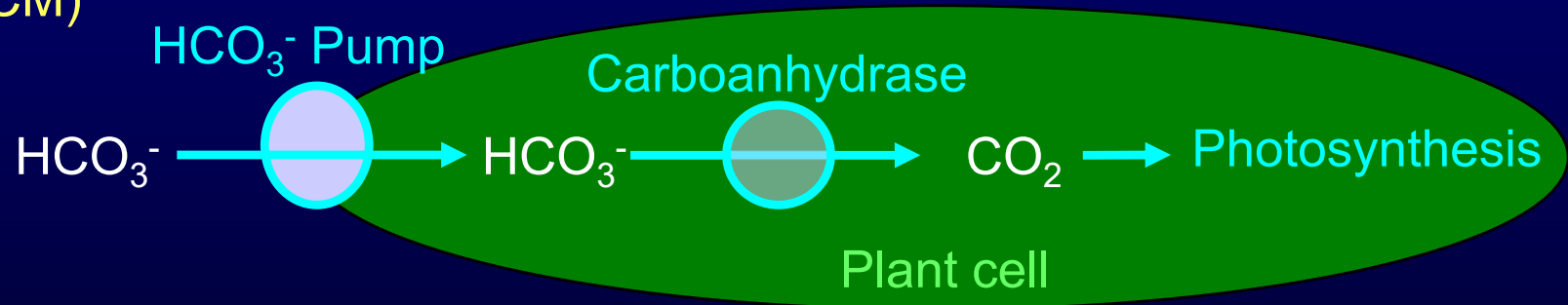
CO₂ delivery: Cd- and Zn- carboanhydrases

(a1) function

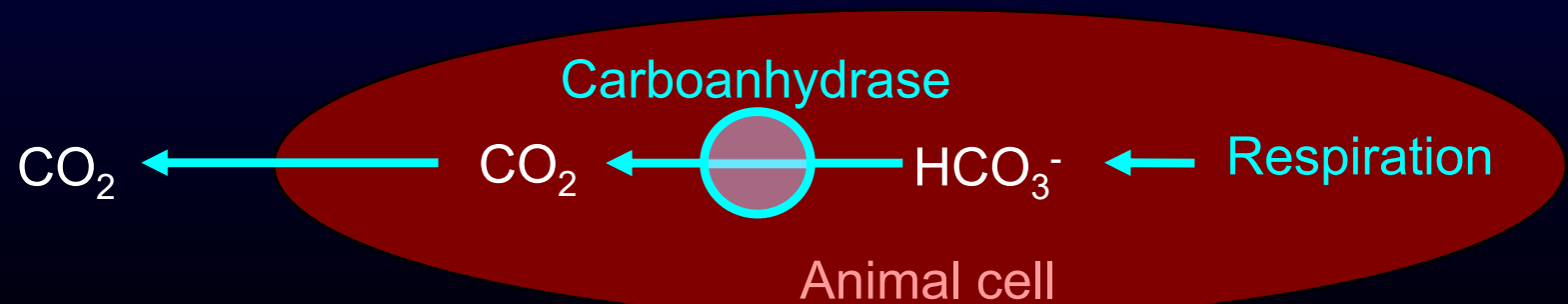


function of carboanhydrases (from: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer&part=A1199)

- 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 CO₂ from the body by exhalation

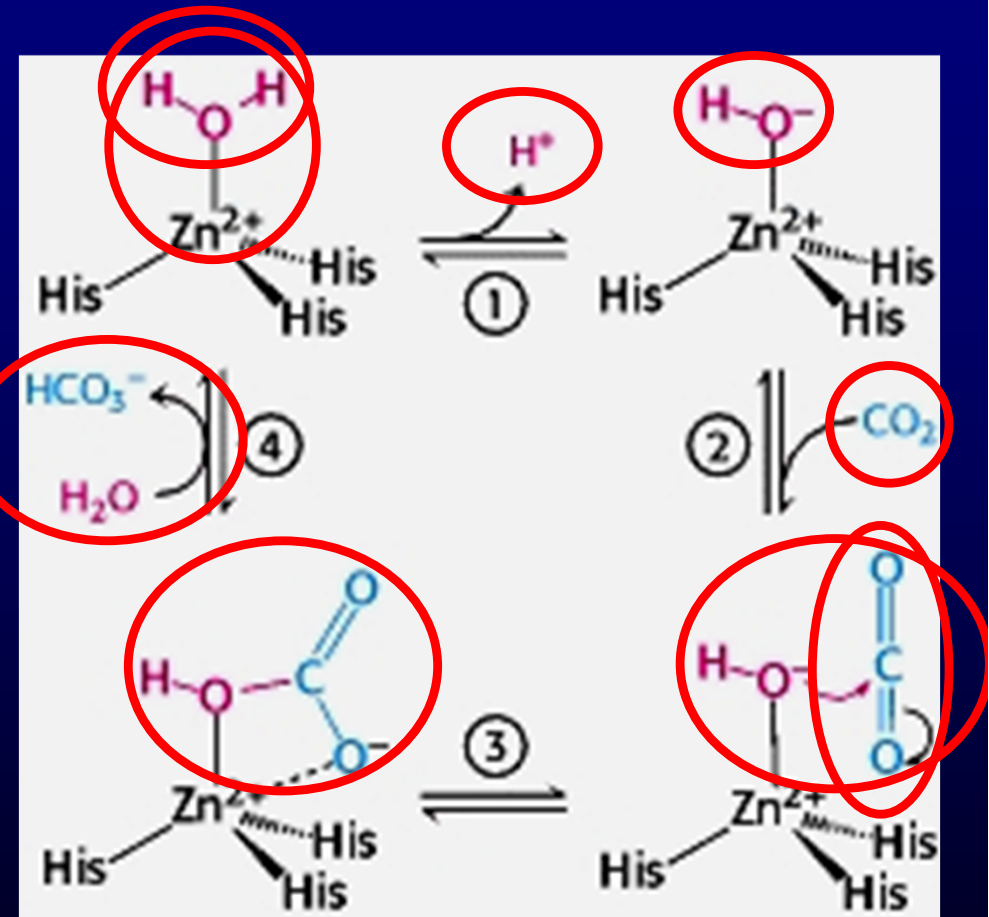


Photosynthesis related Enzymes with metal centres

CO₂ delivery: Cd- and Zn- carboanhydrases

(a2) reaction mechanism from CO₂ 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.



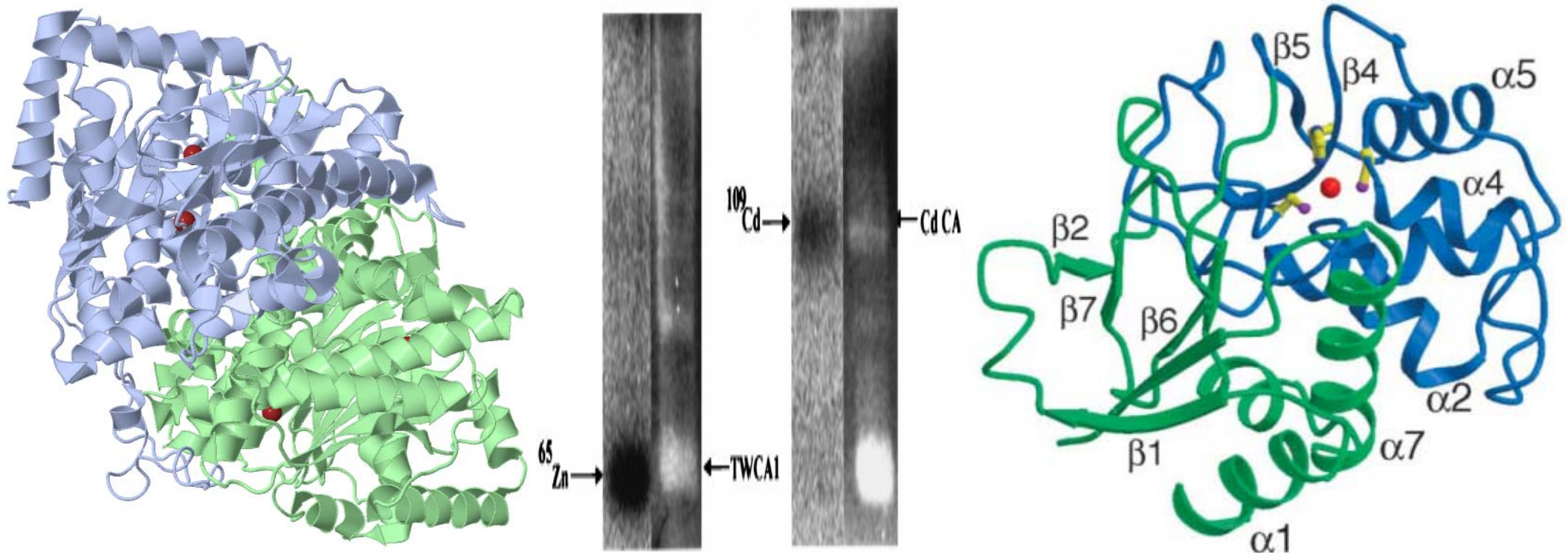
Reaction mechanism of Zn-carboanhydrases (from: www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=stryer&part=A1199)

Photosynthesis related Enzymes with metal centres

CO₂ 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.

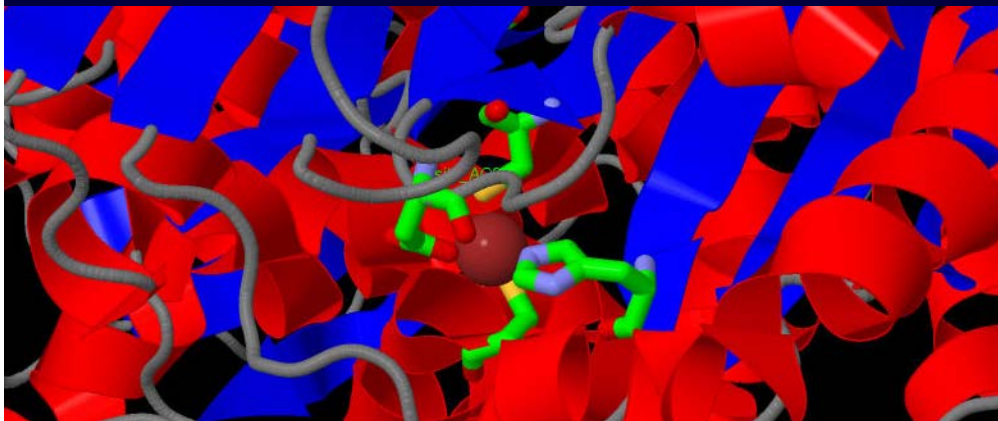
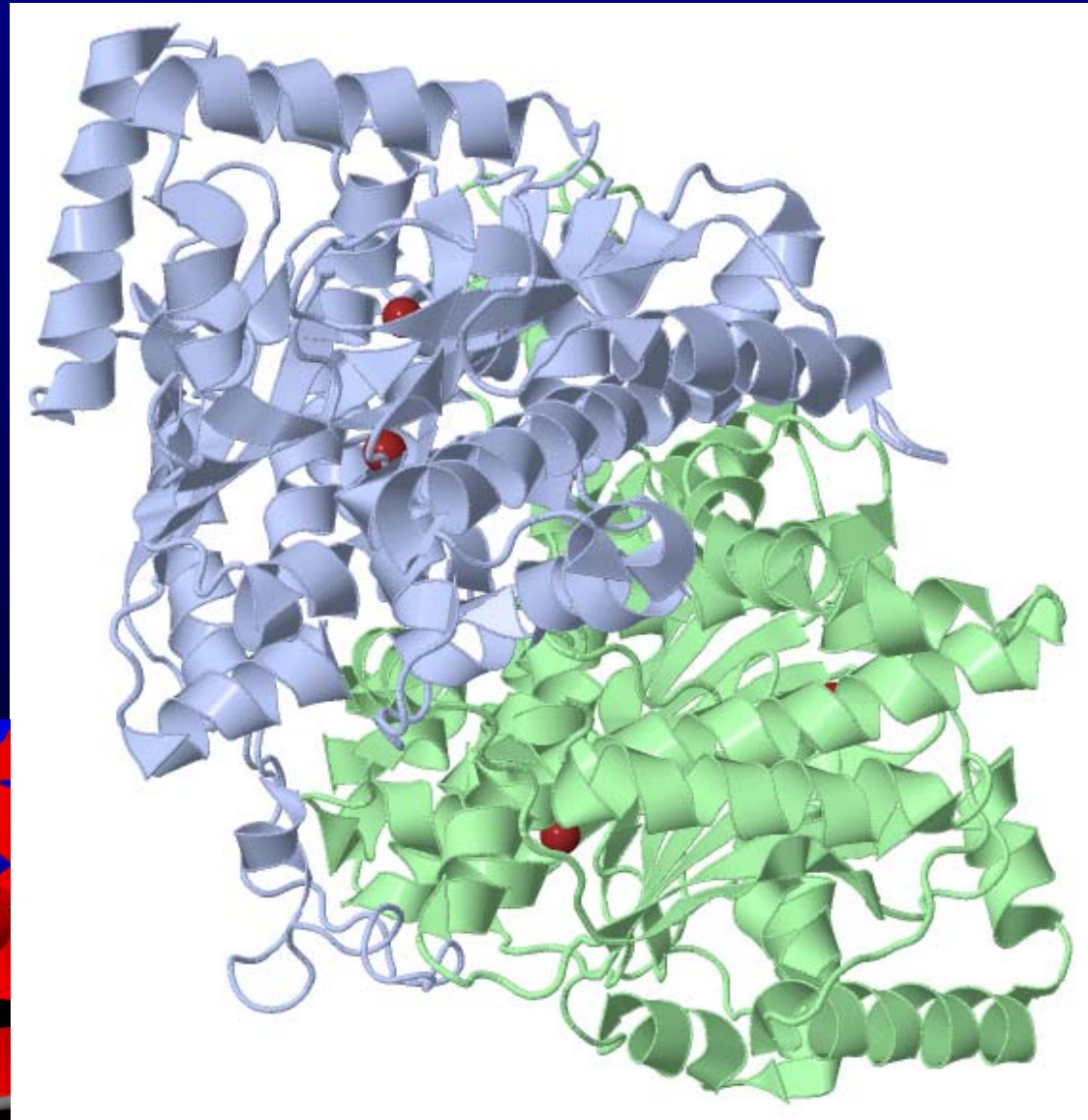


Photosynthesis related Enzymes with metal centres

CO₂ delivery: Cd- and Zn- carboanhydrases

(b1) structure and properties of a typical Zn-CA

- Zn-CA is a homodimer
- Each monomer consists of an α/β -domain and 3 α -helices
- Zn²⁺ is coordinated by 2 Cys, 1 Asp and 1 His

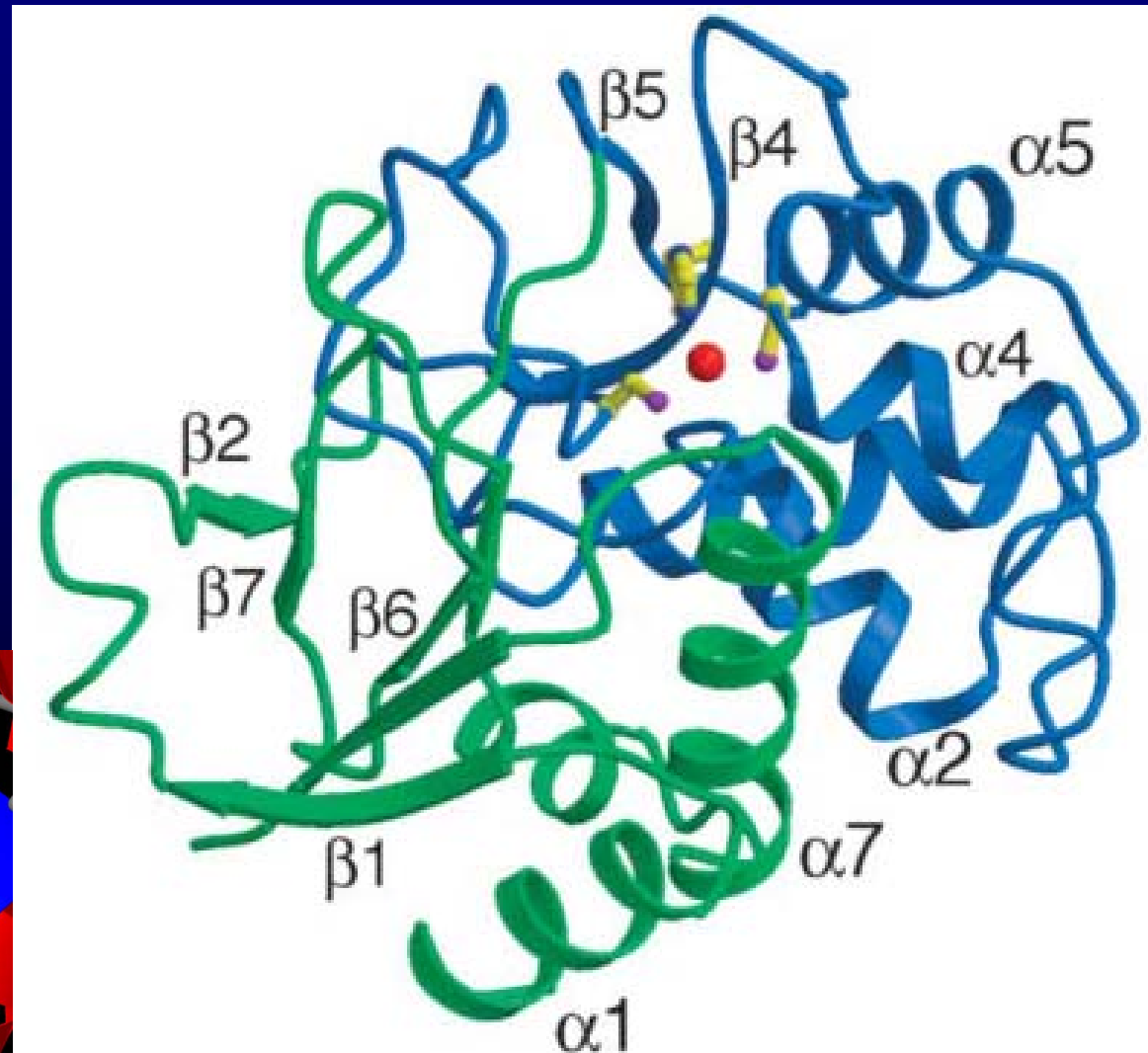
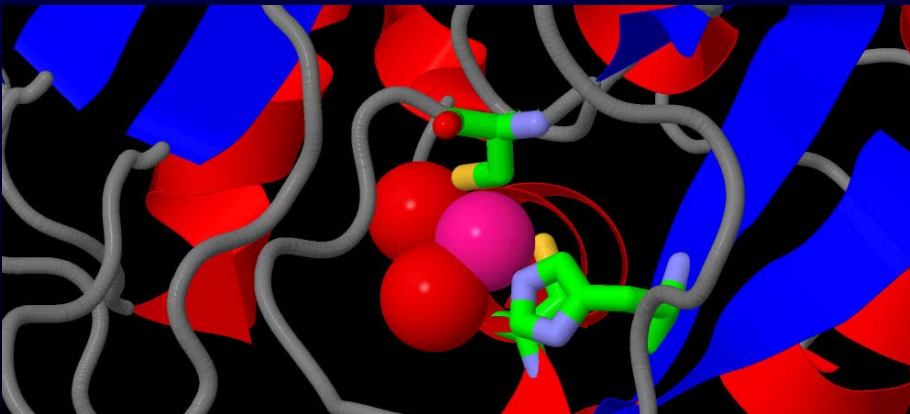


Photosynthesis related Enzymes with metal centres

CO₂ delivery: Cd- and Zn- carboanhydrases

(b2) Properties and Structure of the Cd-Carboanhydrase

- Cd-CA has 7 α -helices and 9 β -sheets,
- Cd is at the lower End of a funnel-like substrate binding pocket
- Cd²⁺ is bound by 2x Cys and 1x His, plus 1x Water (\rightarrow tetrahedral coordination).



**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