The Dynamics of Thylakoid Membranes from Higher Plants

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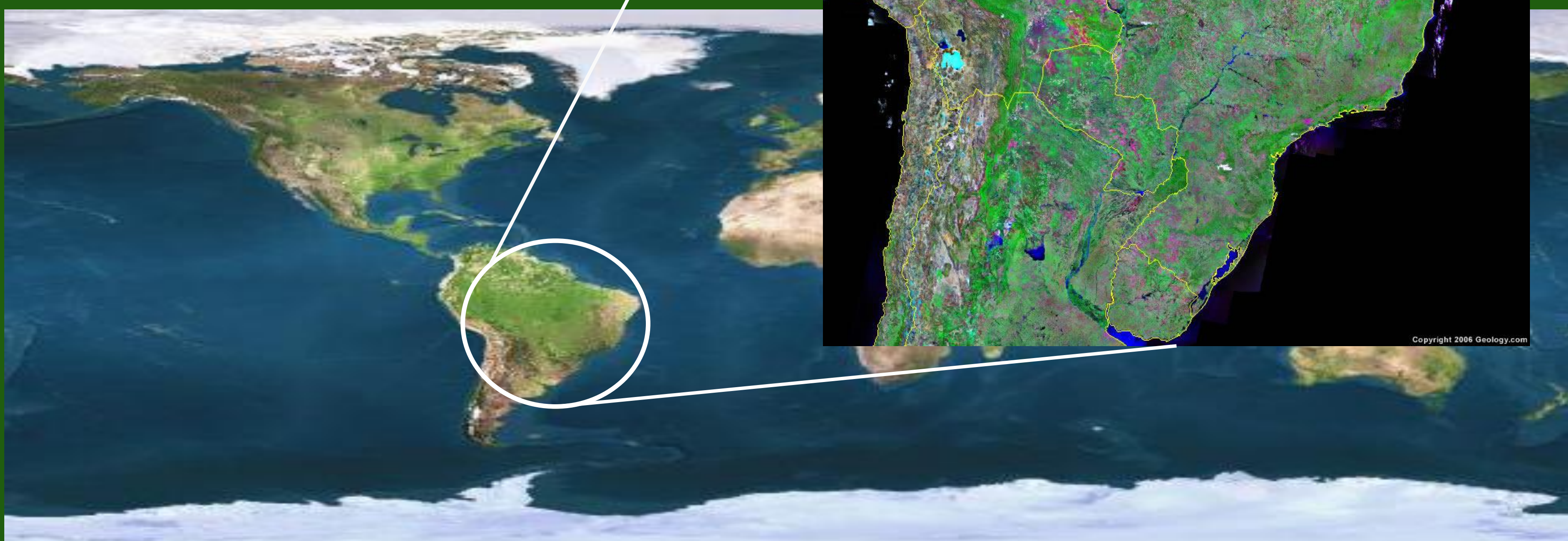
Photosynthesis – a global perspective

Takes place almost every
green plants, algae and p
can be found



Photosynthesis – a global perspective

- Energy for photosynthesis comes from sun light
- Two sets of reactions – light dependent and light independnt
- Affected by temperature, light intensity/quality and CO_2 level

Photosynthesis – a global perspective

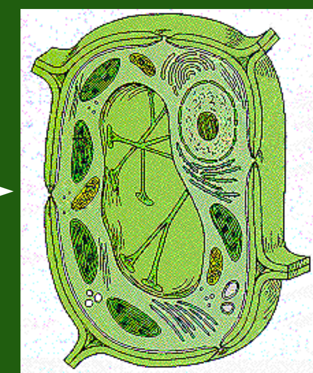
- Ultimate energy sourse for living organisms –all food and oxygen in Earth's biosphere arrive from photosynthesis
- Sourse for all fossil fuel reserves – products of photosynthesis were converted into fuels over millions of years
- One tree makes **12 kg** of biomass and outputs **9400 L** of oxygen in **24 h** – enough for **family of FIVE**!

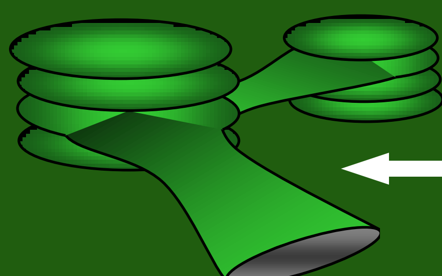
Photosynthesis – where it all takes place

Tree

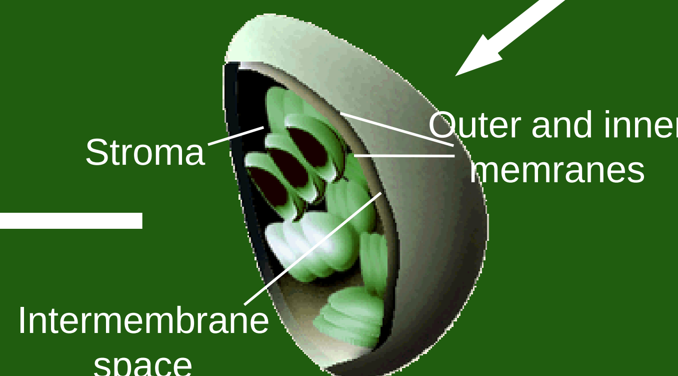
Leaf

Plant cell



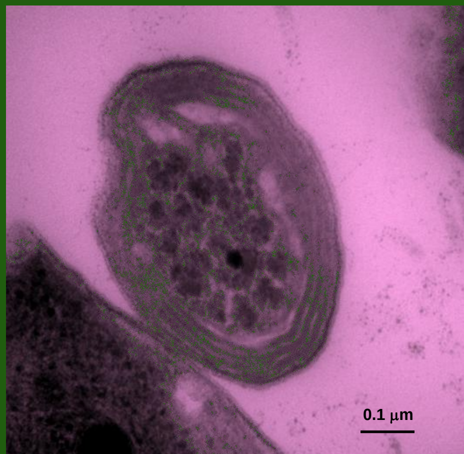


Thylakoid membrane

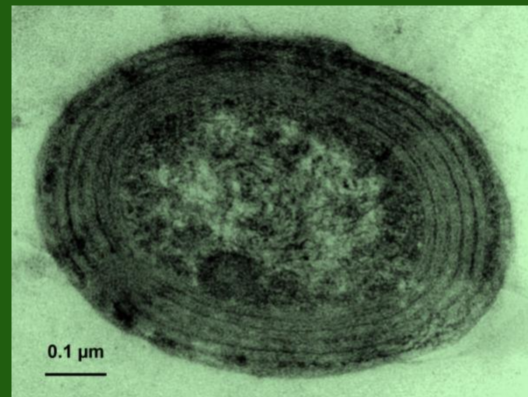


Chloroplasts

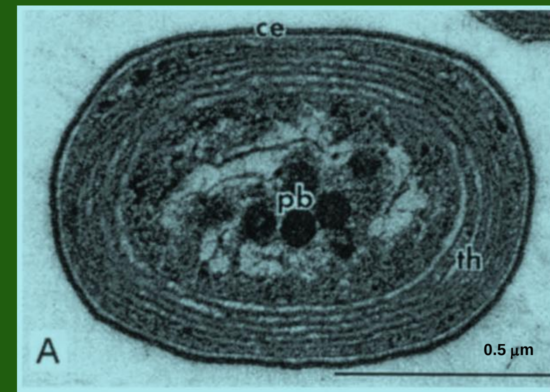
Photosynthetic membranes



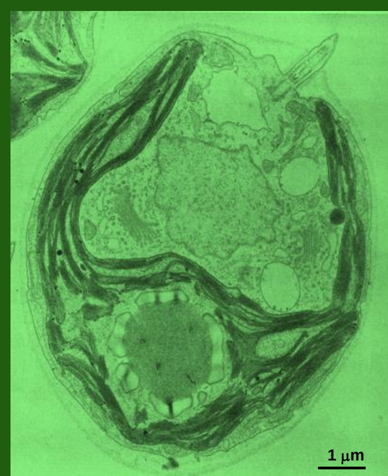
Photosynthetic bacteria
Rhodopseudomonas viridis



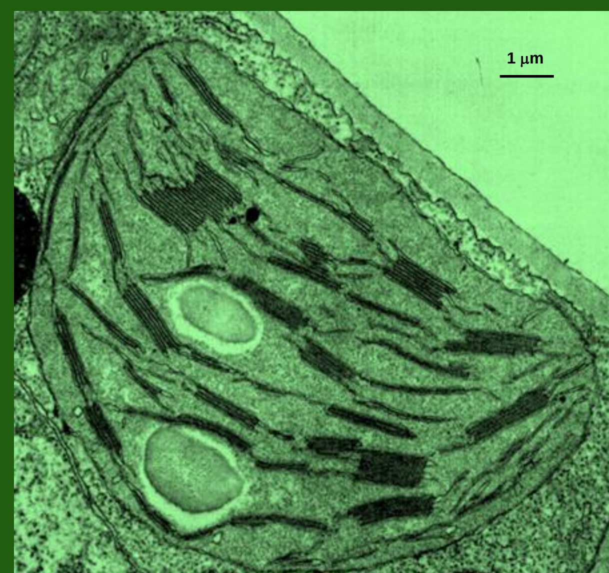
Cyanobacteria
Synechocystis sp. PCC 6803



Marine cyanobacteria
Prochlorococcus marinum

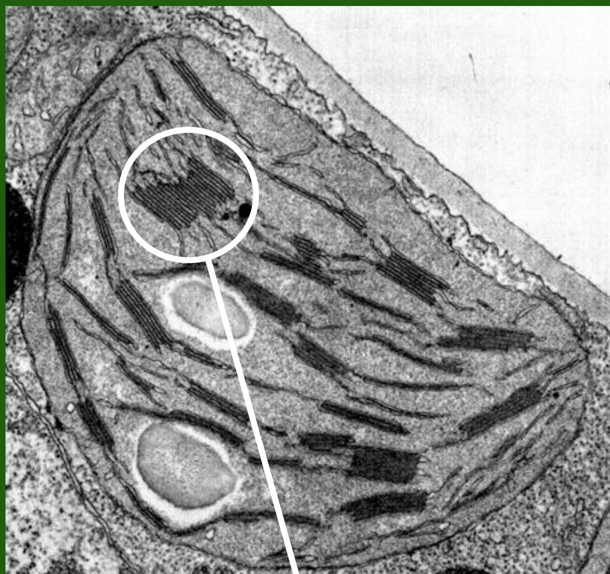


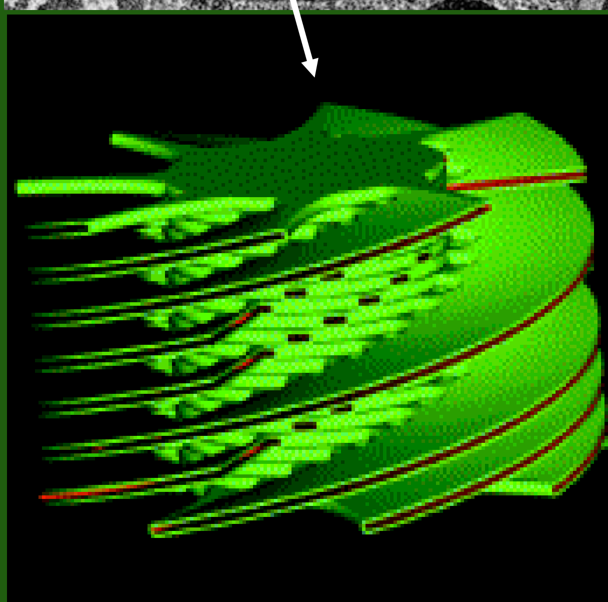
Green alga
Chlamydomonas reinhardtii

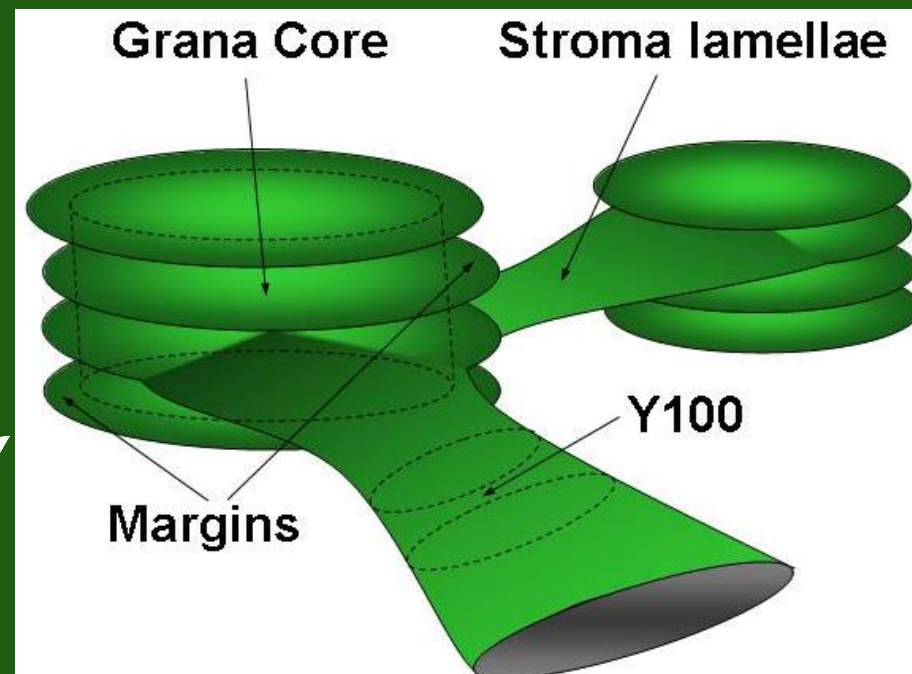


Chloroplast
Spinacia oleracia L.

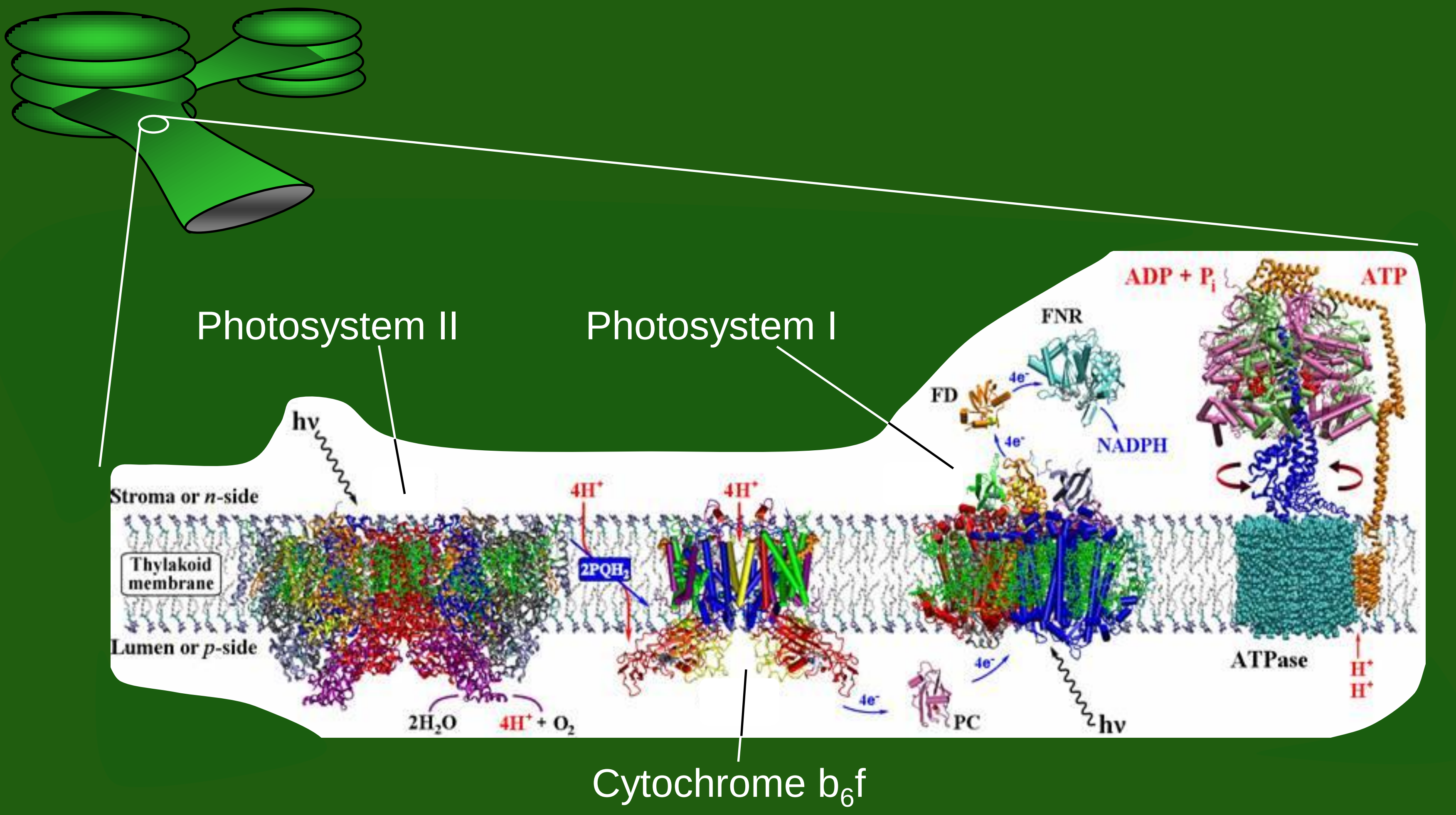
Domains of the thylakoid membrane from higher plants





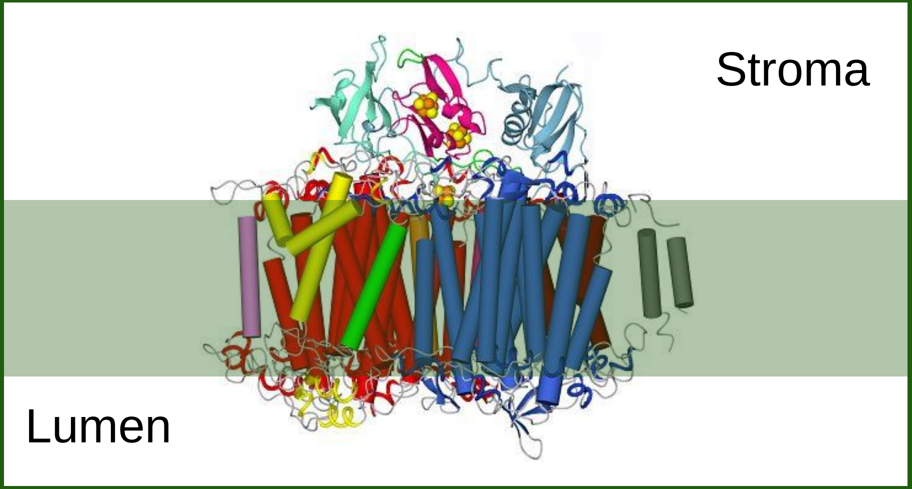


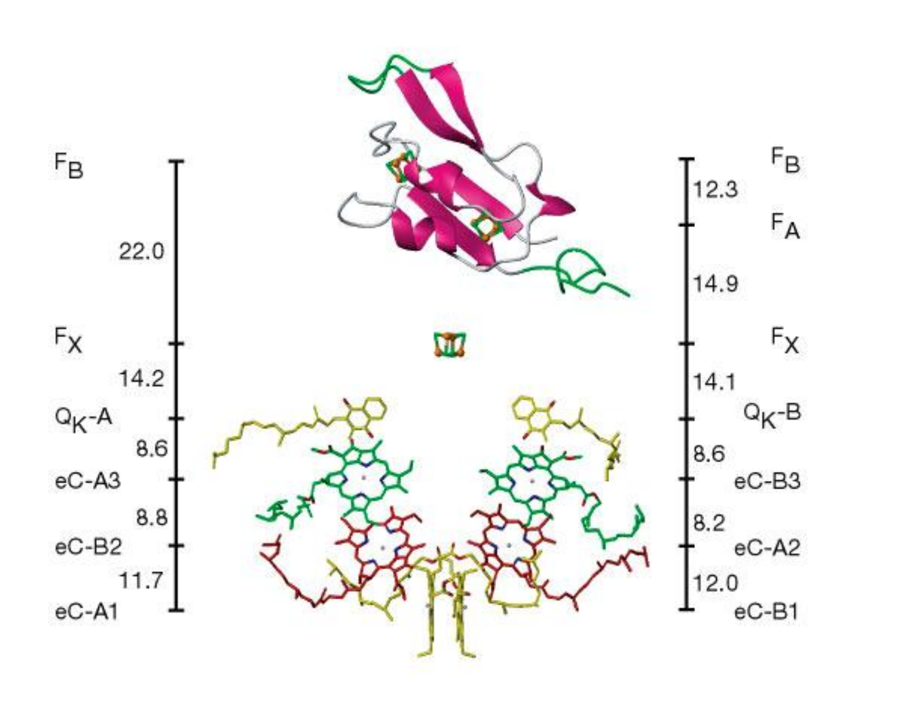
Thylakoid membrane complexes and electron/proton transfer reactions



Photosystem I

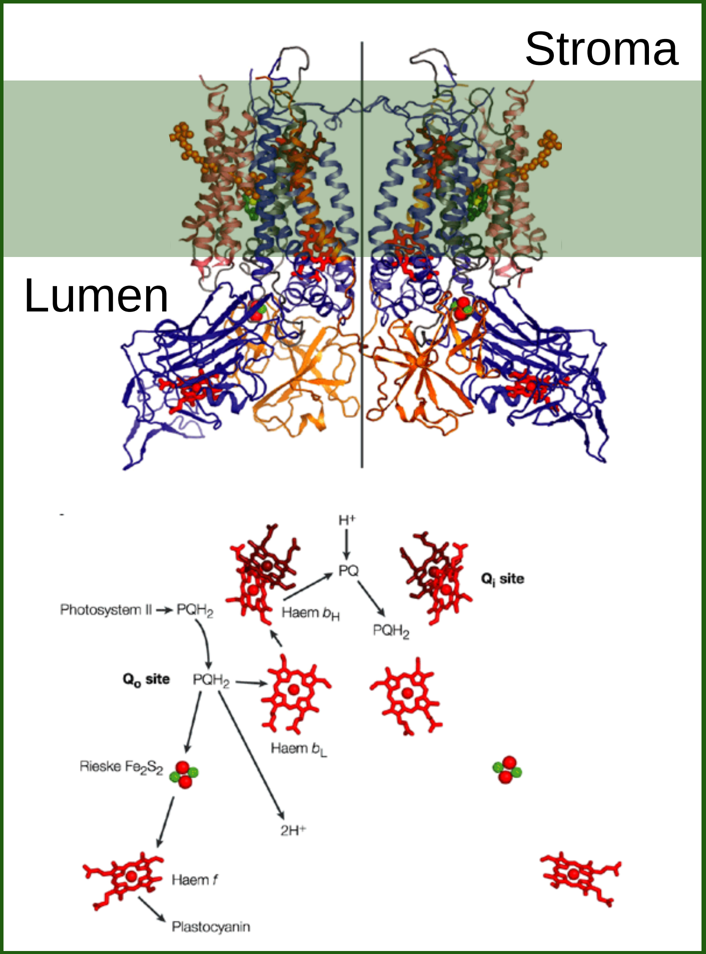
- **Light driven plastocianine feredoxine oxidoreductase**
- **Electron transfer reactions**
- **Analogous to green sulphur and hellobacteria (iron sulfur type reaction center)**
- **~ 300 kDa, about 15 protein subunits**
- **Trimer in cyanobacteria, monomer in higher plants**
- **Crystal structure is solved to 2.0 Å resolution**
- **Branched electron transfer is debated**





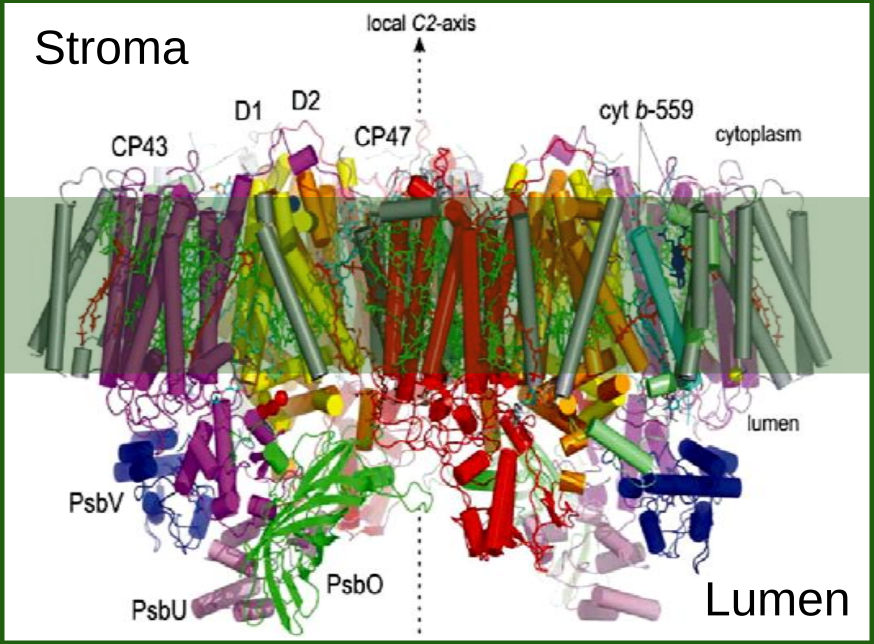
Cytochrome b_6f complex

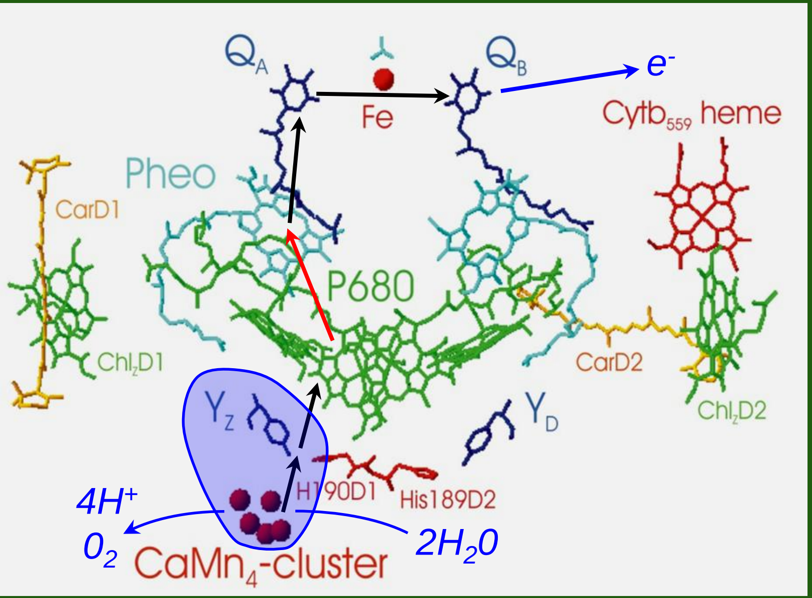
- Plastoquinone plastocyanine oxidoreductase
- Electron and proton transfer reactions
- Q cycle to translocate proton through the membrane
- Found in the dimeric form
- Analogous to Cytochrome bc_1 complex in photosynthetic bacteria and mitochondria
- Crystal structure is solved to the 3.0 Å resolution
- A single Chl molecule is found; function is unknown



Photosystem II

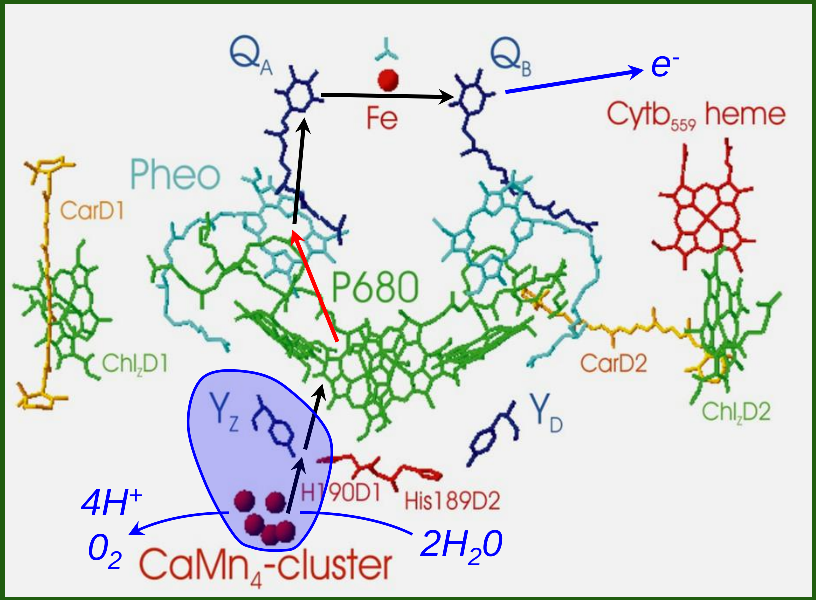
- Light driven water plastoquinone oxidoreductase. Can split water and O_2 is released as a byproduct, turnover rate is about 100 molecules per second
- Electron and proton transfer reactions
- Analogous to purple bacteria (quinone type reaction center)
- ~ 900 kDa, more than 25 protein subunits, structurally highly heterogenic
- Operates at highly oxidizing potentials
- Crystal structure is solved to the medium 3.0 Å resolution
- Water oxidation mechanism is unknown

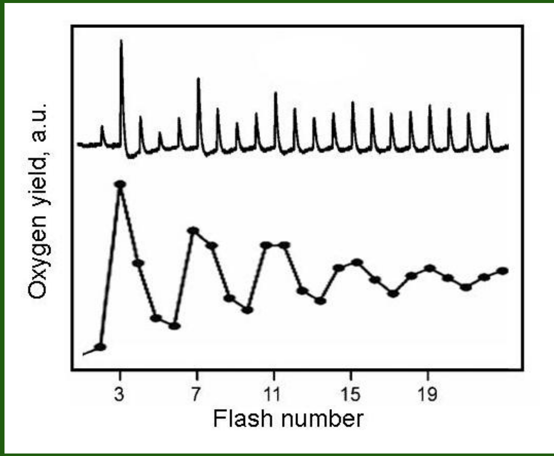


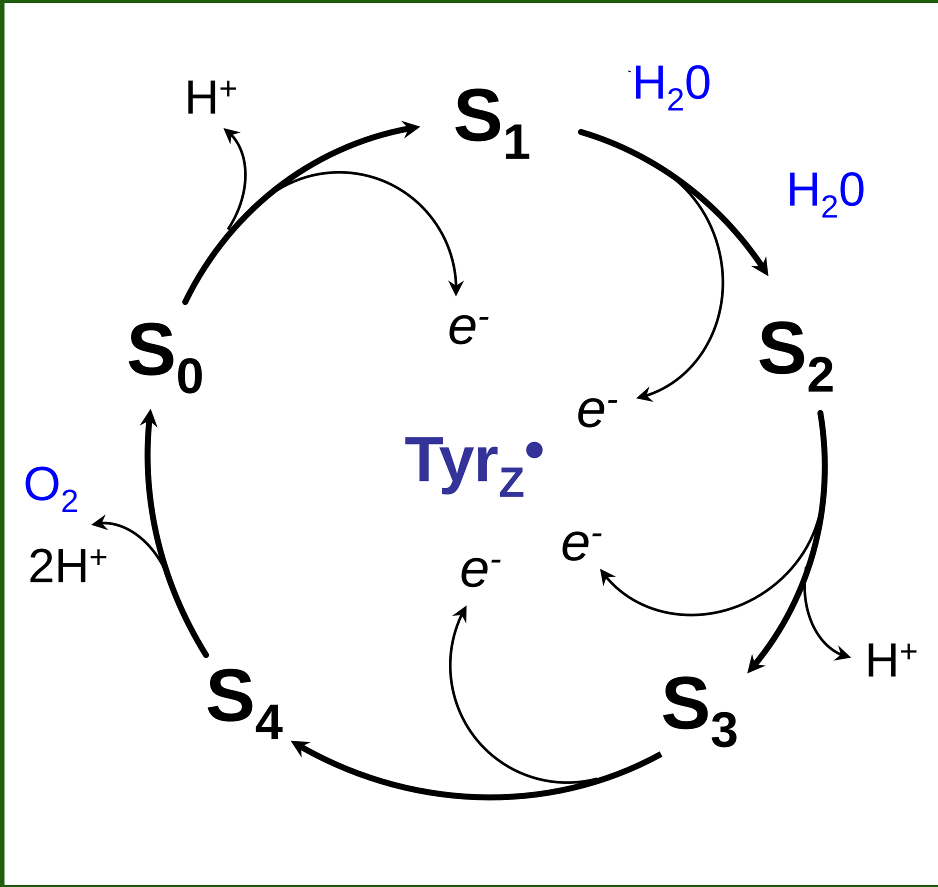


The catalytic site of Photosystem II

$CaMn_4$ cluster and the S-state cycle

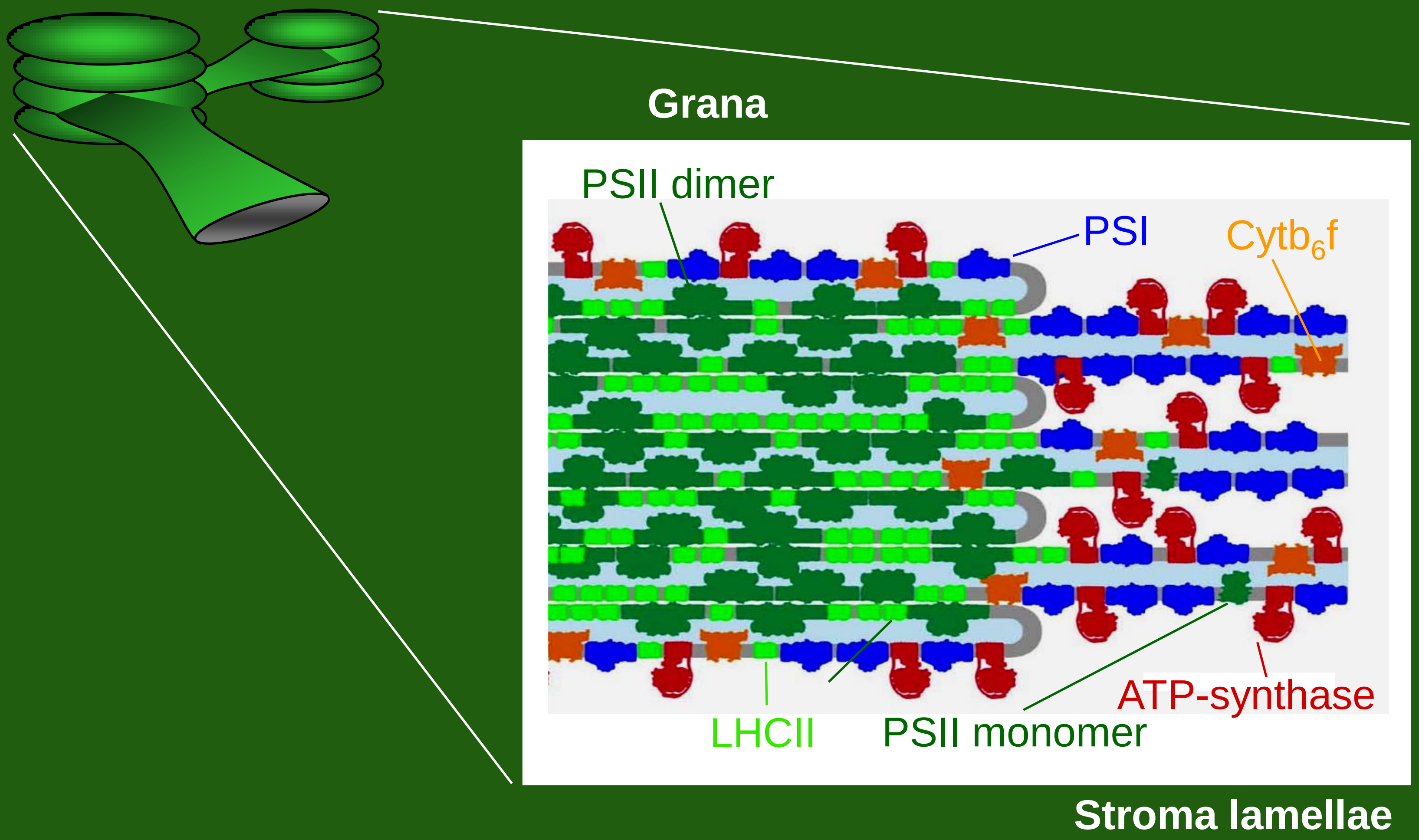






Oxygen release pattern: the S state cycle

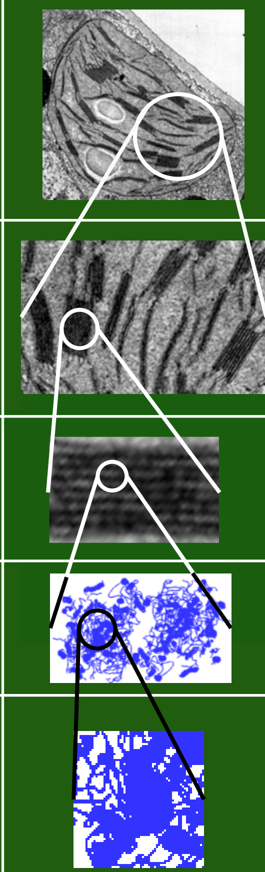
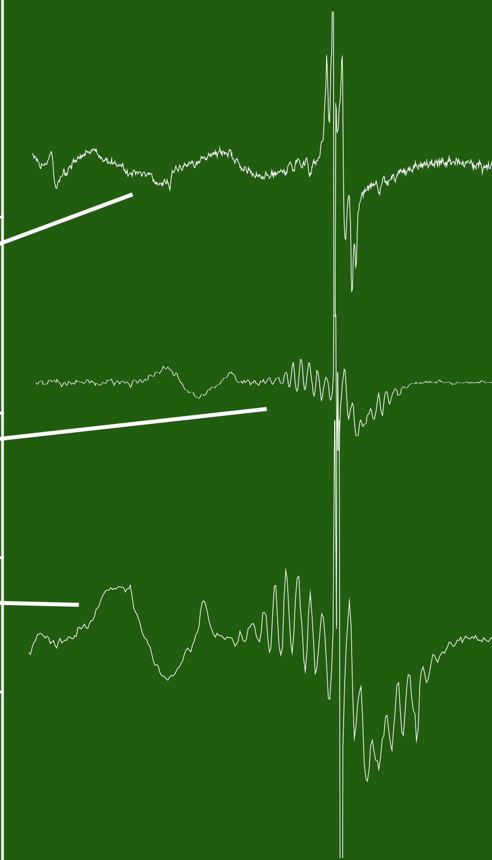
Distribution of Photosystems in the thylakoid membrane from higher plants



Methods to study photosynthetic complexes : Biochemistry

- Separation of different parts of the thylakoid membrane (different domains) without disturbing their native composition
- Isolation and purification of the photosynthetic membranes and complexes on the different levels – chloroplasts, thylakoid membranes, PSI or PSII membranes, PSI and PSII core complexes, reaction centers etc. from plants, green algae and cyanobacteria
- Supramolecular and protein composition analysis of different complexes in the thylakoid membrane

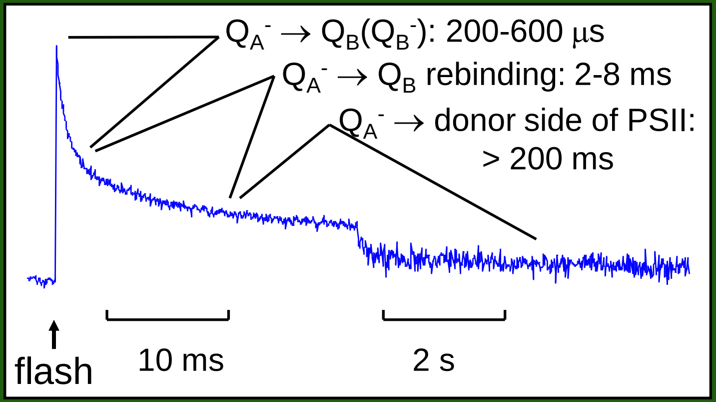
Methods to study photosynthetic complexes : Biochemistry

Preparation		Activity (i.e. PSII oxygen evolution, $\mu mol\ O_2$ / mg Chl h)	EPR signal
Cells, chloroplasts		~ 80	
Thylakoid embranes		120	
PSII membranes		500 - 700	
PSII core reparations		~ 5000	
Reaction Center preparations		0	

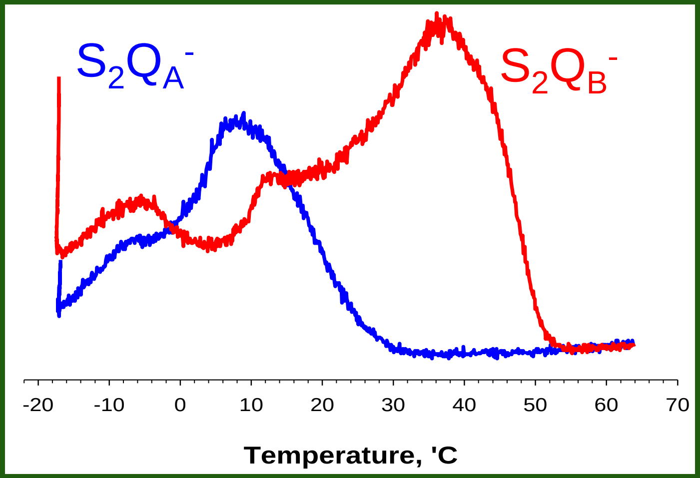
Methods to study photosynthetic complexes: Biophysics and Spectroscopy

- **Electron and proton transport measurements**
- **Optical and fluorescence spectroscopy; time resolved measurements**
- **EPR spectroscopy – conventional and advansed (pulse) methods**
- **Application of the short (ns) laser flashes to study different intermediates of the catalytic meachanisms (i.e. S states)**

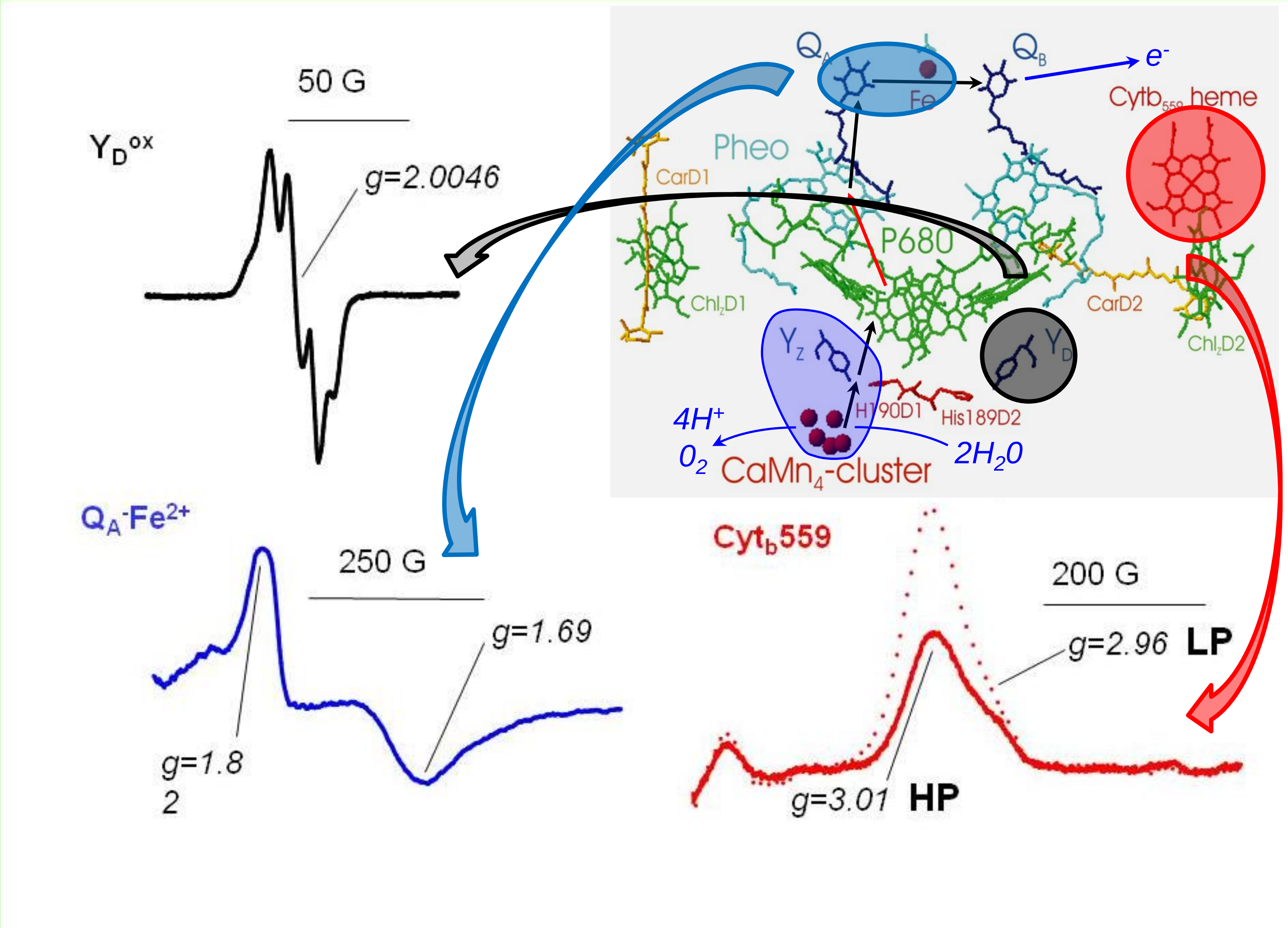
Variable fluorescence



Thermoluminescence

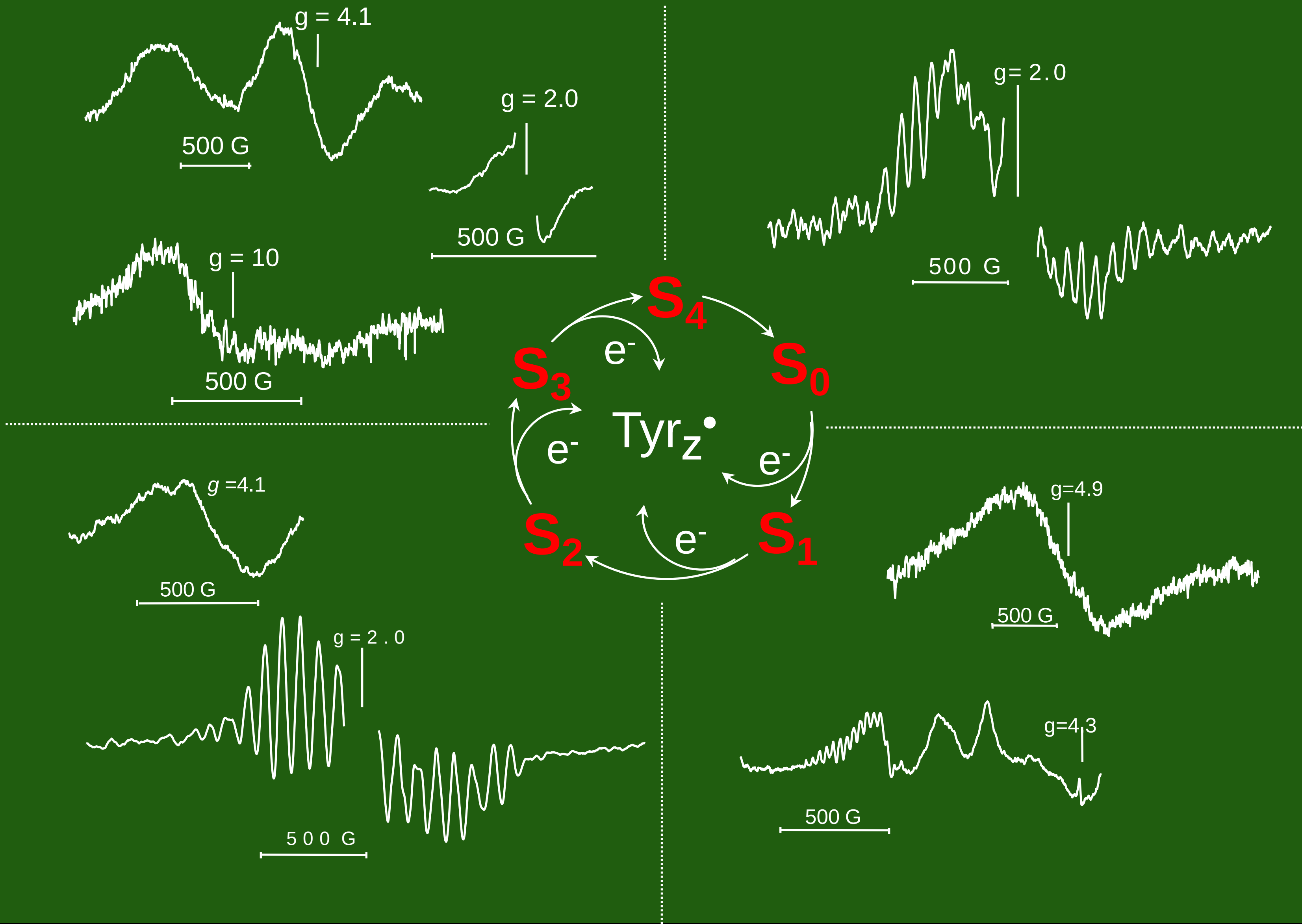


Electron Paramagnetic Resonance (EPR) spectroscopy from PSII



Electron Paramagnetic Resonance

Spectroscopy on the S-states

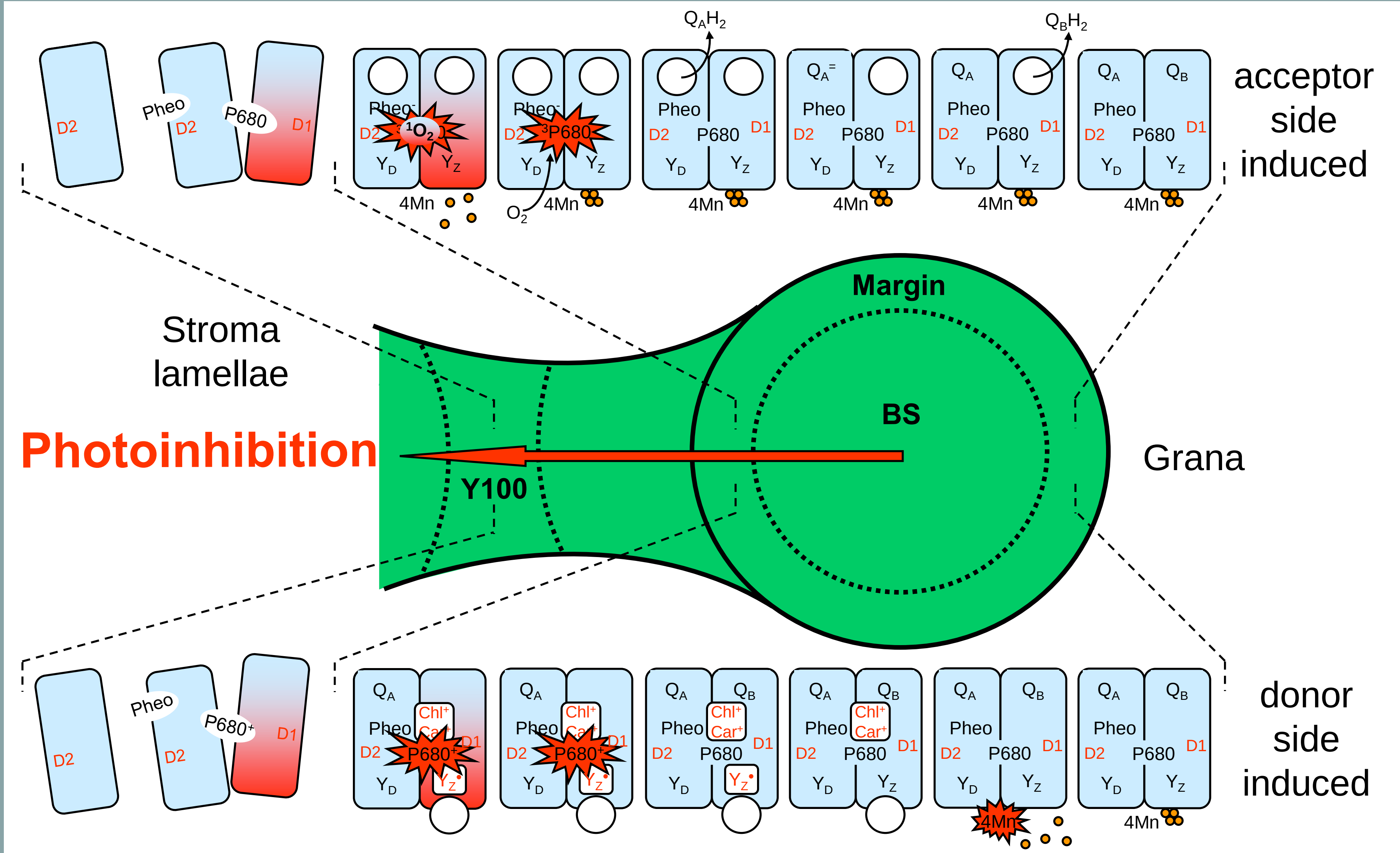


Photosystem II life cycle
photoinhibition / repair cycle

- Photosystem II is highly vulnerable to environmental stress
- Exhibit functional and structural heterogeneity and unevenly distributed in the thylakoid membrane
- Pocess several protective meachanisns such as energy dissipation in antenna, xanthophyll cycle, protein phosphorylation, state transition, etc
- Excess of light leads to inhibition of Photosystem II (photoinhibition). At the normal day light conditions every 30 min one Photosystem II is destroyed
- Reparation of Photosystem II is a complex process, which takes place in the different parts of the thylakoid membrane and requires the lateral movement of Photosystem II centers in the thylakoid membrane

Photosystem II life cycle

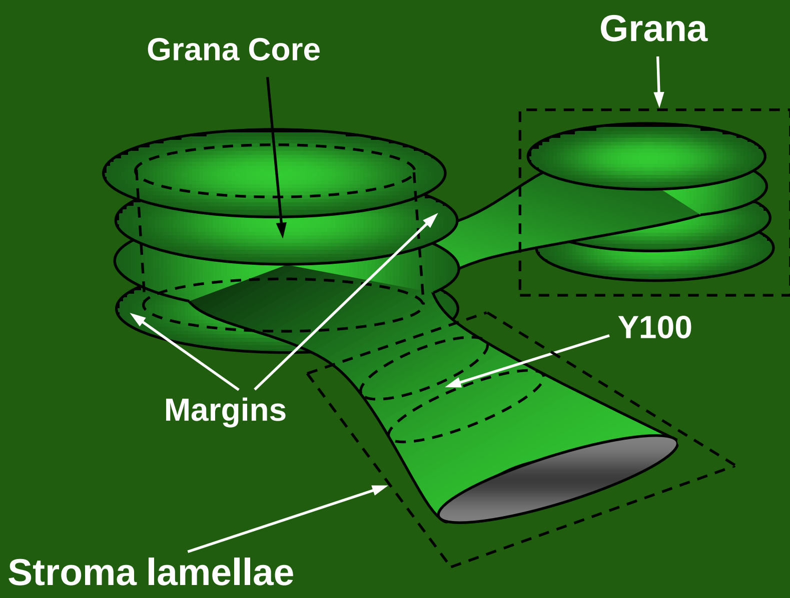
Photoinhibition



How to study Repair process?

- Separation of the thylakoid domains and study of their biochemical and biophysical properties
- Application of the imaging technology – confocal fluorescence miscroscopy, EPR imaging, etc.
- Biogenesis of the photosynthetic complexes. In this case, the assembly and activation of the PSI, PSII or cyt b_6f complexes can be studied during greening of the etiolated plants
- Photoactivation experiments (assembly of the $CaMn_4$ –cluster) (dark gron alga are an excellent model)

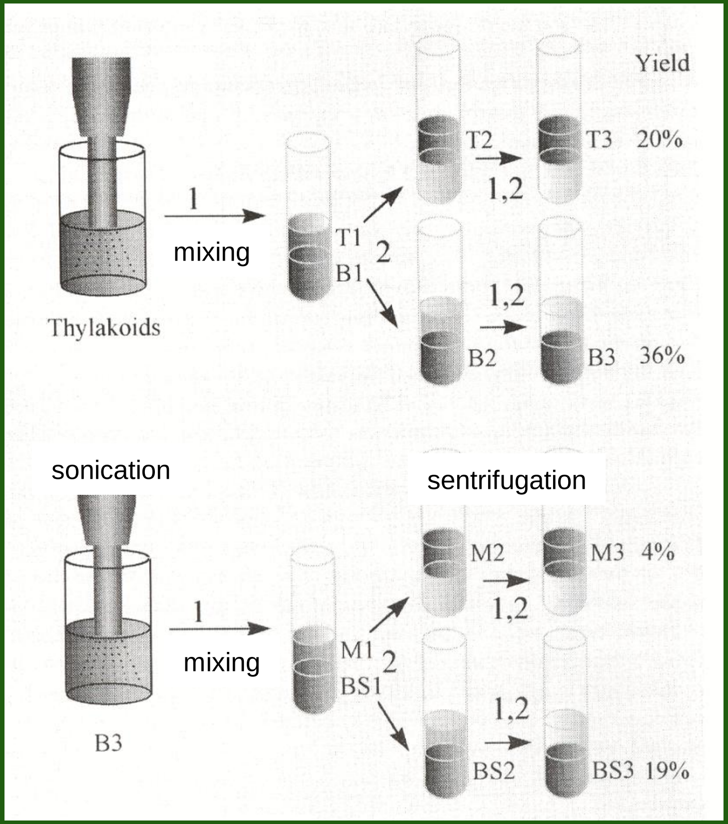
Understanding membrane dynamics – study of the thylakoid membrane domains



- Non-invasive, two phase separation of the different fractions of thylakoid membrane
- Biochemical and biophysical characterization of PSII, PSI and Cyt b_6f complex (antenna properties, protein composition, electron transfer reactions)

Isolation of the different membrane fractions

Two-phase separation technique



Stroma lamellae

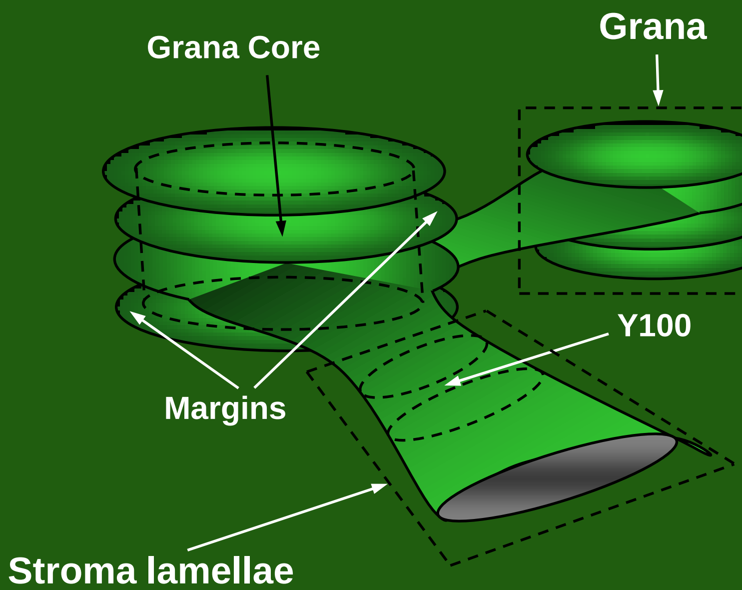
Grana

Grana Margins

Grana Core

The end membrane (End of Grana) and the purified stroma lamellae (Y100) also can be separated

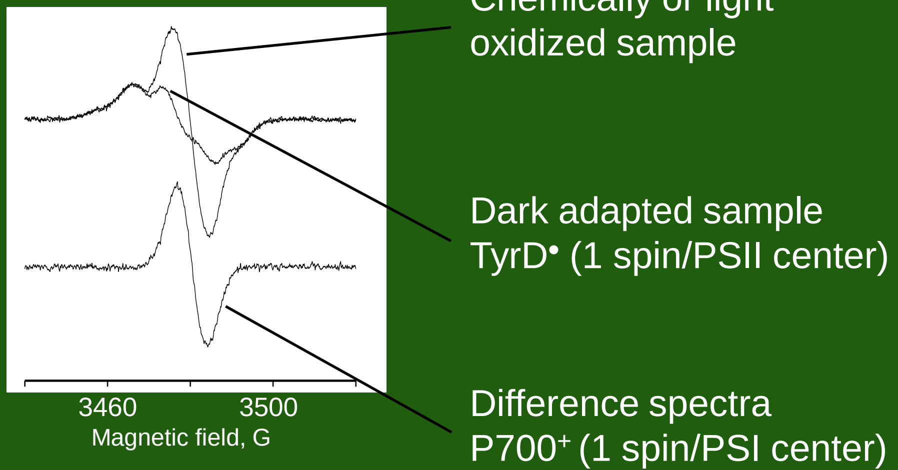
Characterization of Photosystem II in different fractions

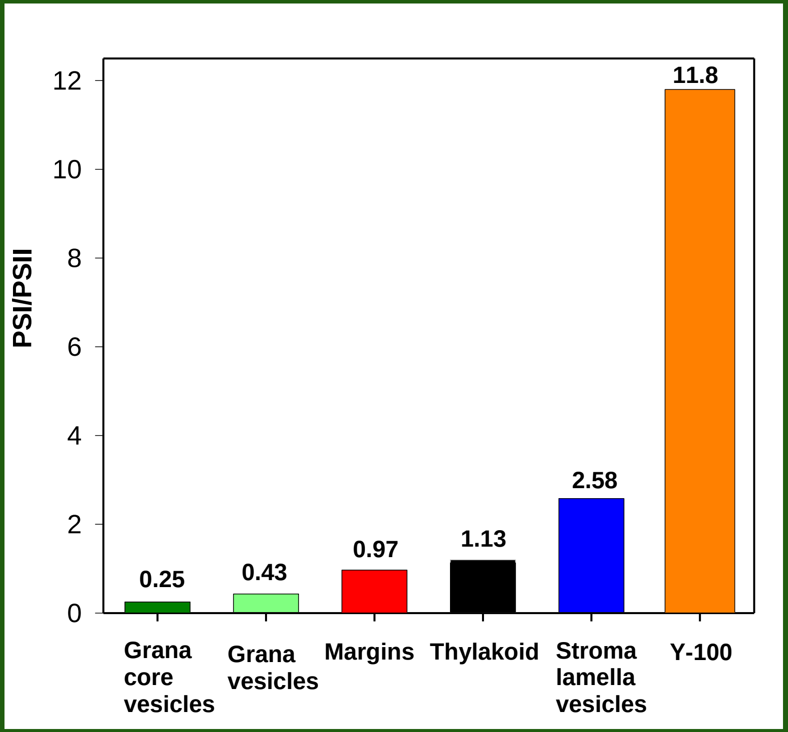


Domain	*O_2 evolution* *(μmol / mg of Chl × h)*	*O_2 evolving centers* *(% of total PSII centers)*	*Fv/Fo*	*Chl a/b* *(mol/mol)*
Grana Core	250-300	91	0.87-1.30	1.8-2.0
Grana	200-250	84	0.81-1.10	2.2-2.4
Margins	102	66	0.45-0.50	3.0-3.3
Stroma	80	43	0.27	4.5-5.0
Y100	0	0	0.20	6.0-6.7
Thylakoids	120	80	0.70	2.9

Thylakoid membrane domains
Quantification of PSI and PSII

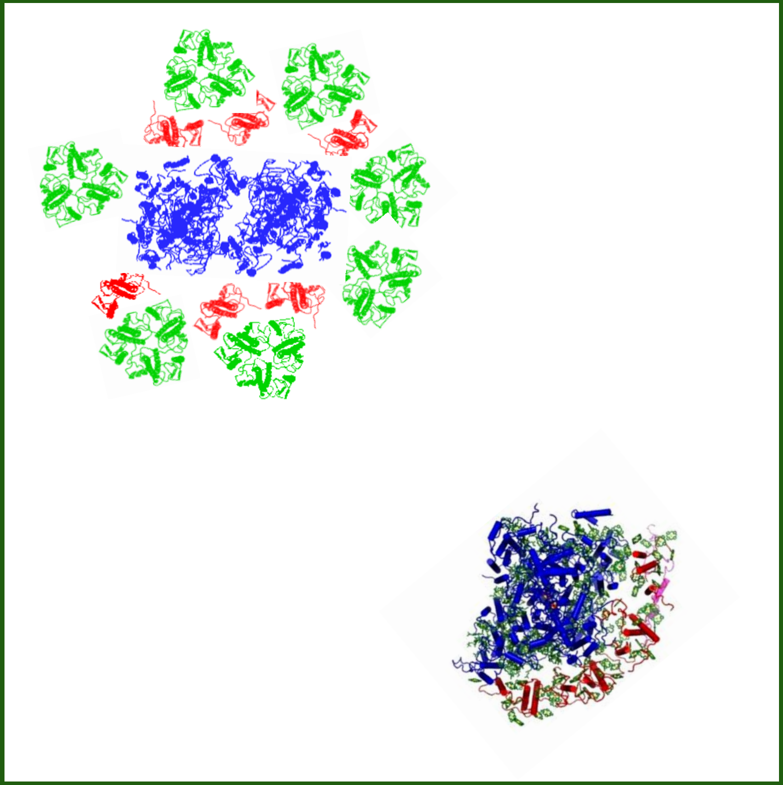
EPR spectroscopy

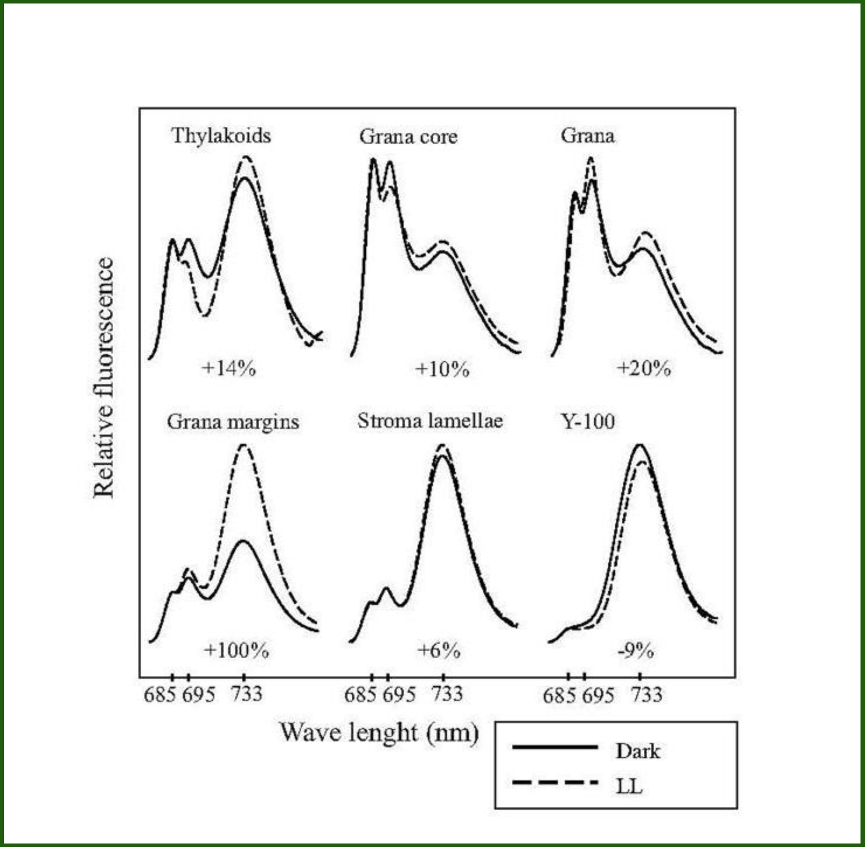




Thylakoid membrane domains
Antenna properties

State transition phenomenon

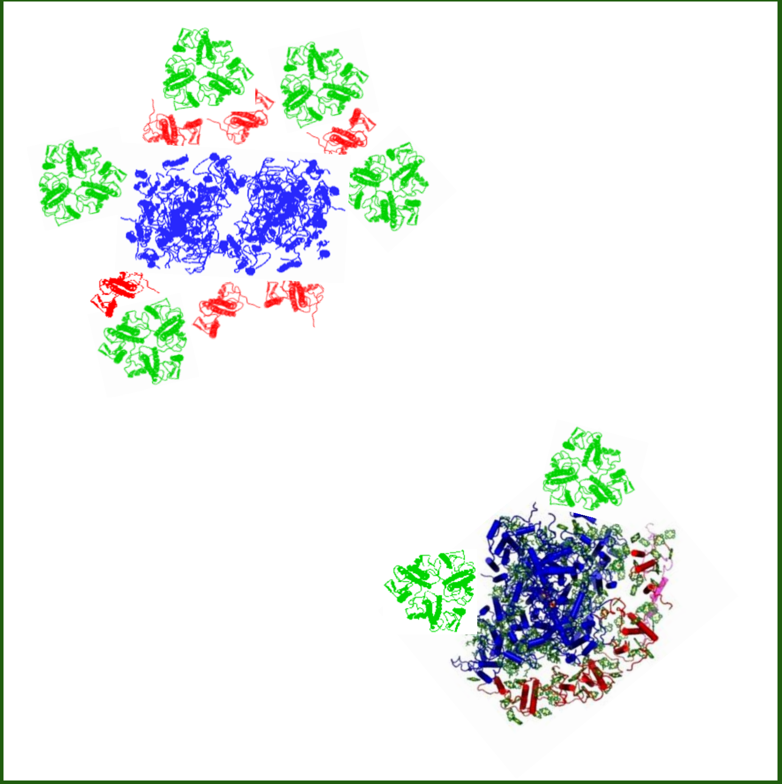


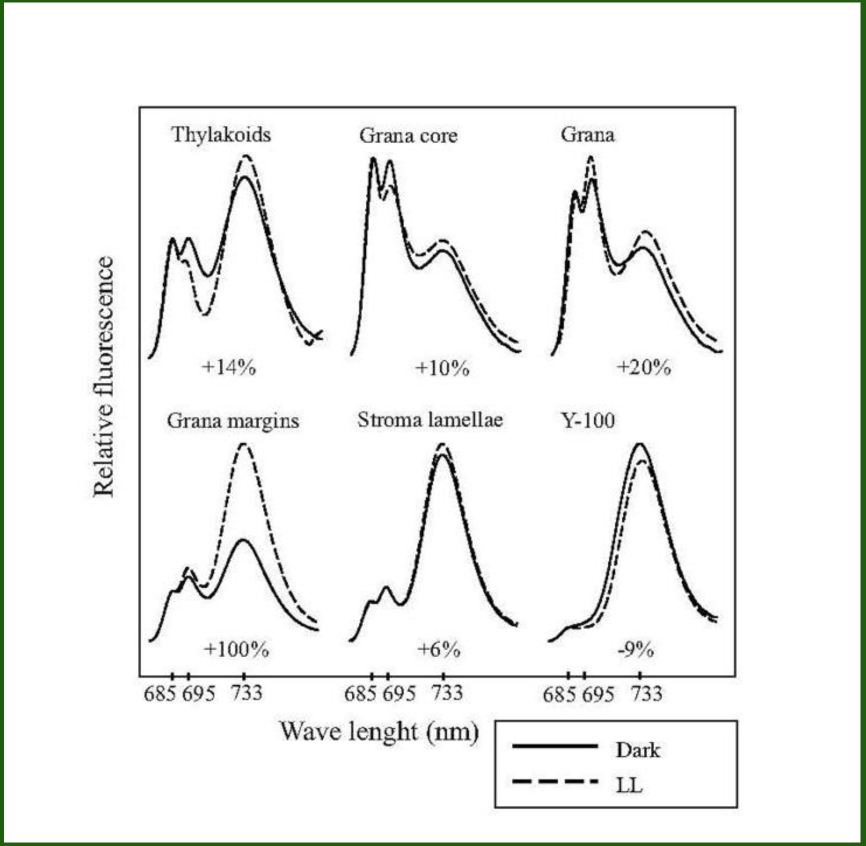


77 K fluorescence spectra

Thylakoid membrane domains
Antenna properties

State transition phenomenon

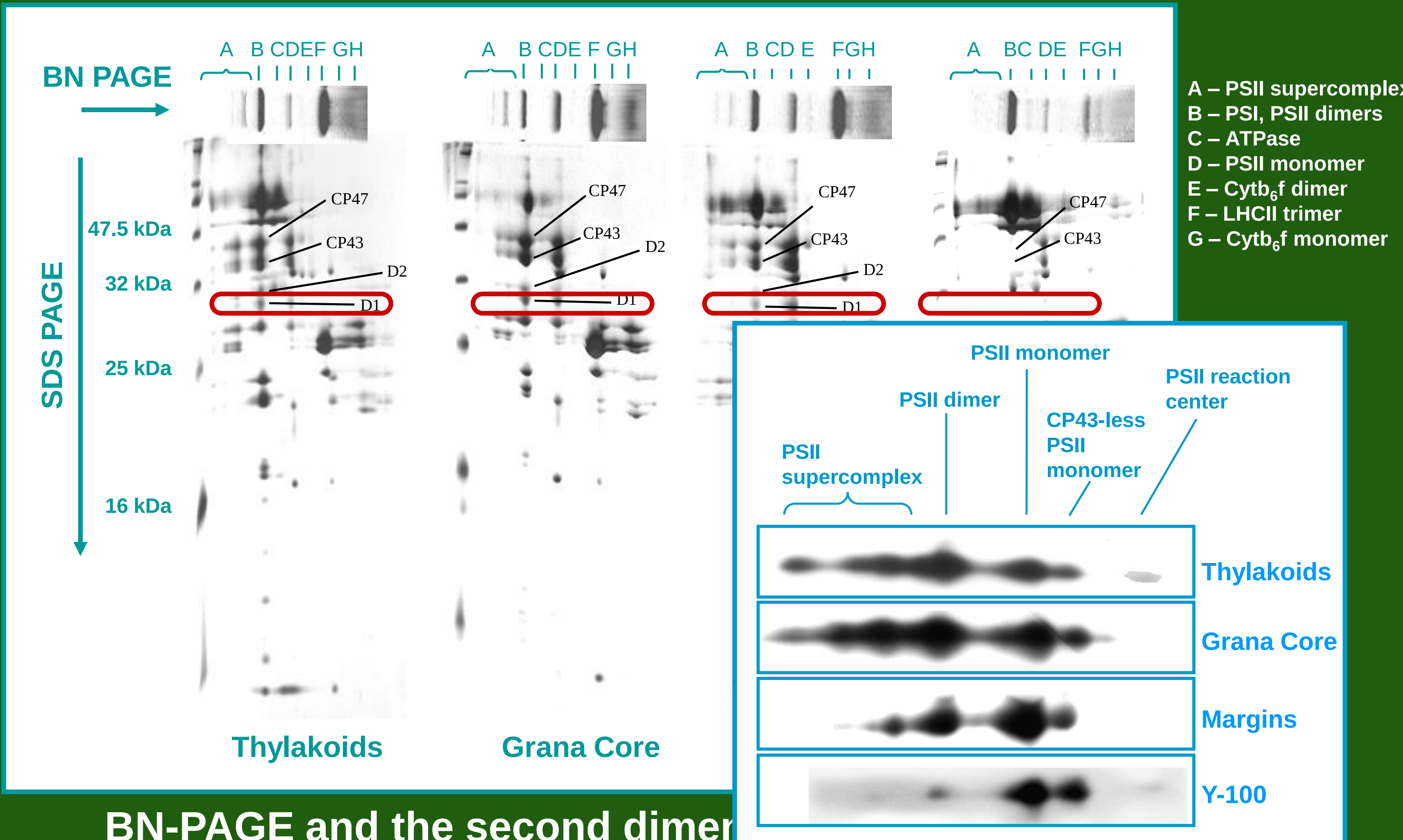




77 K fluorescence spectra

Thylakoid membrane domains

Supramolecular composition of Photosystem II

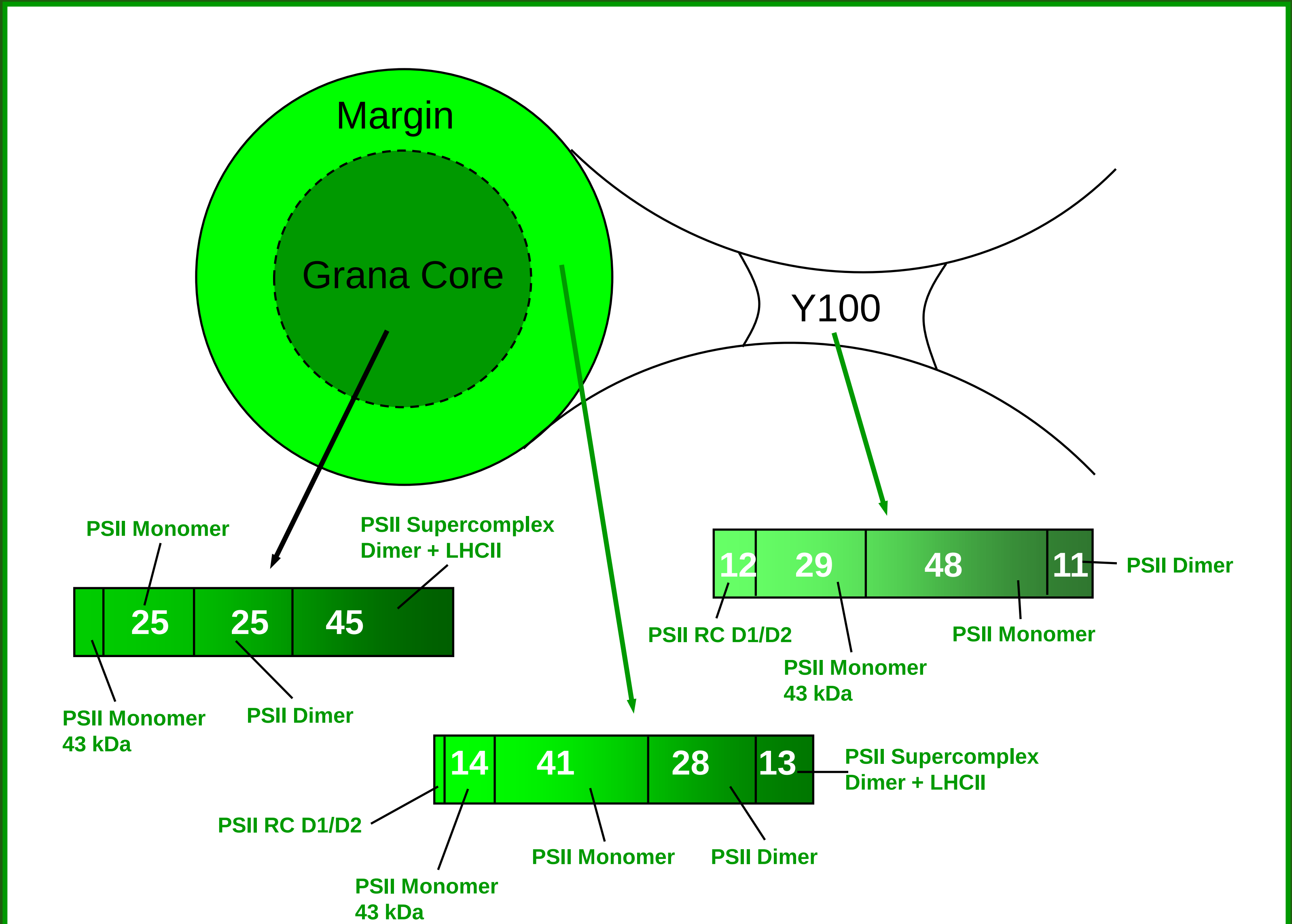


Immunoblot detection

BN-PAGE and the second dimer
of different fractions from the tl

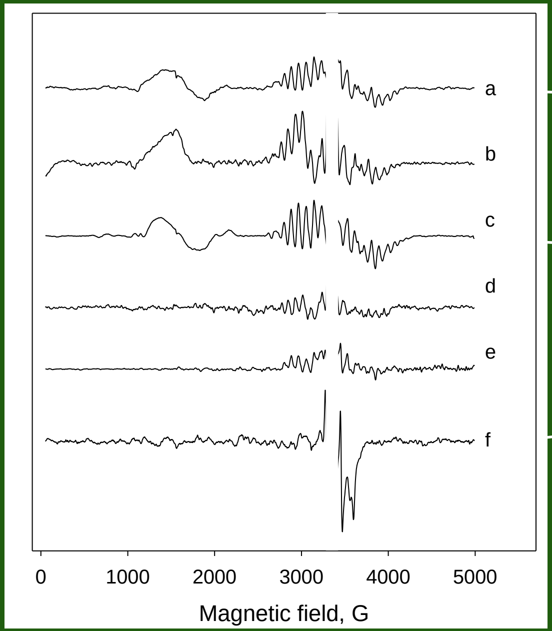
Thylakoid membrane domains

Supramolecular composition of Photosystem II



Thylakoid membrane domains

Electron transport properties – EPR spectroscopy

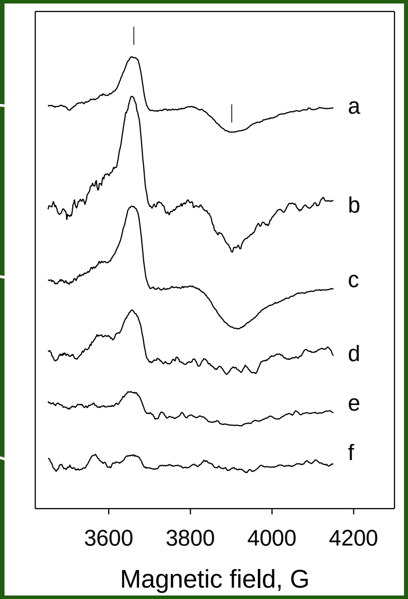


Grana Core

Margins

Y100

EPR measurements on different fractions



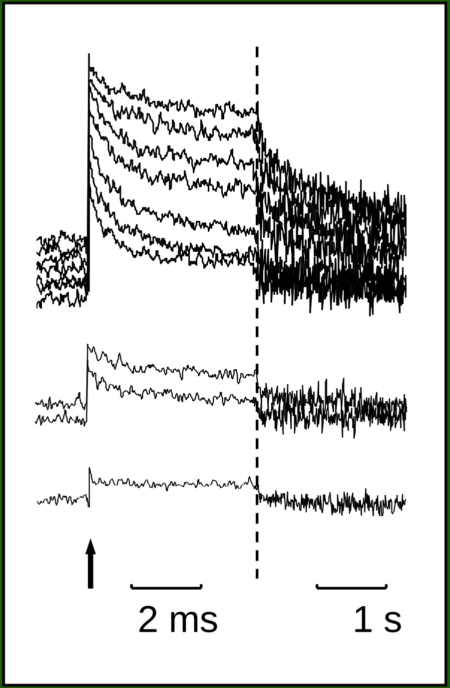
S_2 state multiline

$Q_A^- Fe^{2+}$ signal

Domain of the thylakoid	Tyr_D^{ox} %	Q_A^- Fe^{2+} %	S_2 State %	O_2 evolution %
Grana Core	**100**	**100**	**100**	**100**
Grana	82	94	92	81
Margin	59	39	40	37
Stroma	35	31	33	29
Y100	15	13	0	0
Thylakoid	66	70	81	43

Thylakoid membrane domains

Electron transport properties – Fluorescence



Grana Core

Margins

Y100

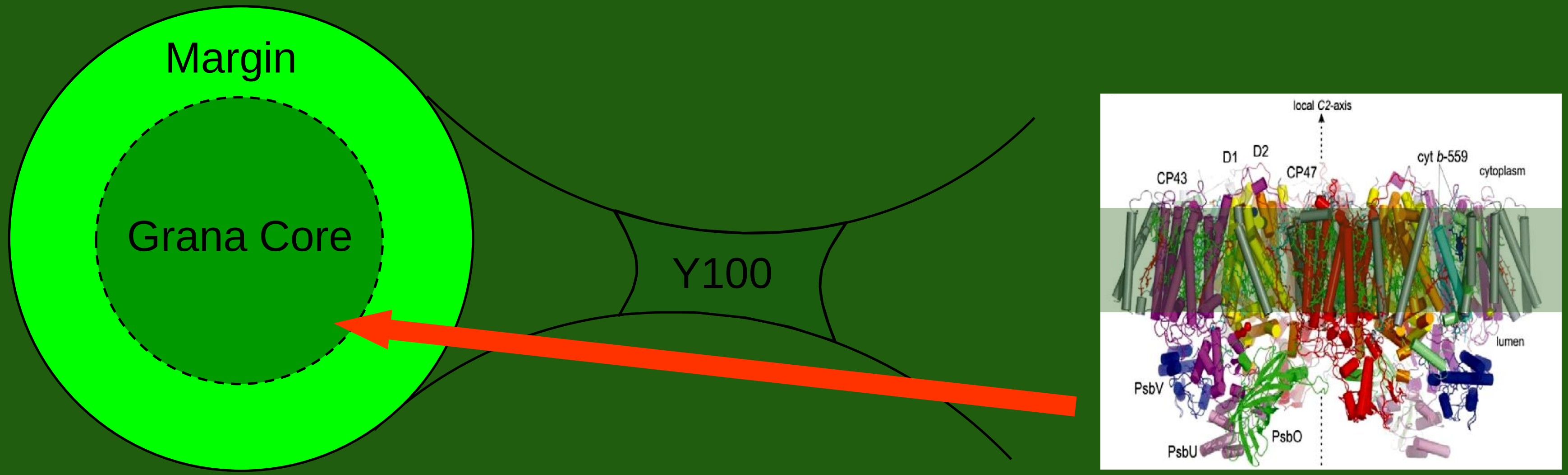
Flash-induced fluorescence decay in different fractions

Domain	Q_B binding, ms	Photoactivation, min	Q_B binding, ms
Y100	29	Dark grown	32
Stroma	29	2	27
Margin	46	5	18
---	---	10	14
---	---	30	12
Grana	6.5	60	10
Grana Core	5.9	Light grown	8

Domain	Recombination	Photoactivation, min	Recombination
Y100	39	Dark grown	90
Stroma	44	2	110
Margin	90	5	170
---	---	10	460
---	---	30	720
Grana	170	60	670
Grana Core	280	Light grown	930

+ DCMU

Thylakoid membrane domains
Conclusions on the repair process



- Photosystem II migrates from the stroma lamellae to the grana during reparation process. Concomitantly with this lateral migration:
- The number of the Photosystem II centers is gradually increases (from 5 to 60% of the total amount)
- Supramolecular and protein composition is changing from the minimal monomeric protein unit to the fully assembled PSII supercomplexes
- Electron transport on both acceptor and donor side is activated leading to the fully competent centers

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Botanical Garden
View from Uppsala Castle