WORKSHOP ON

METABOLOMICS IN THE CONTEXT OF SYSTEMS BIOLOGY: A RATIONAL APPROACH TO SEARCH FOR LEAD MOLECULES

25-26/02/10

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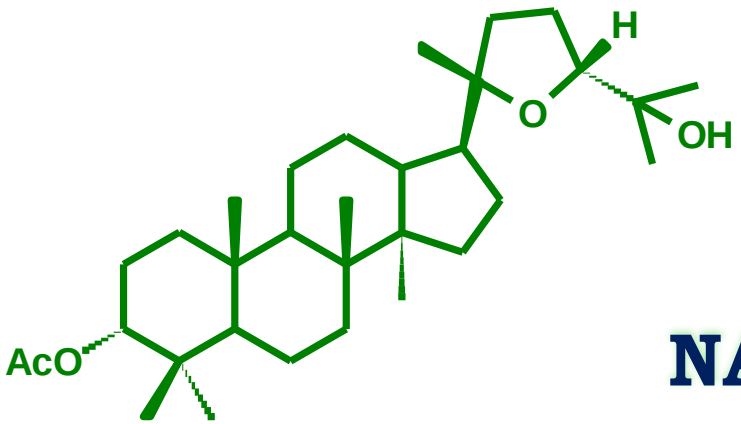
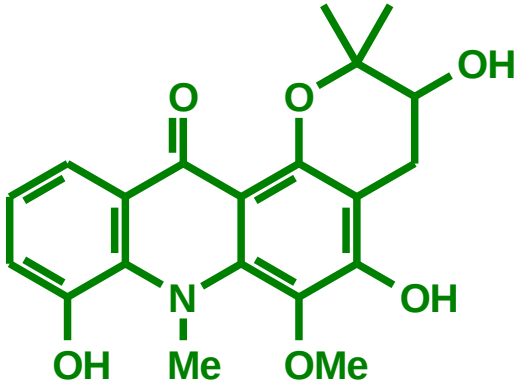
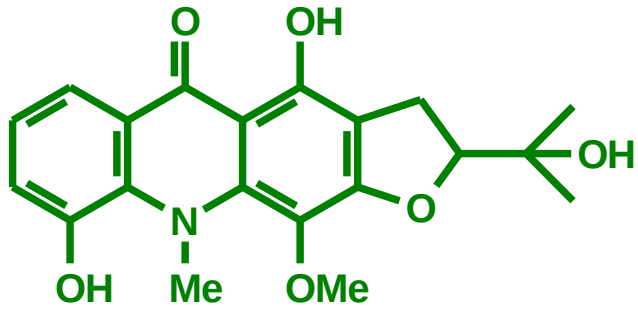


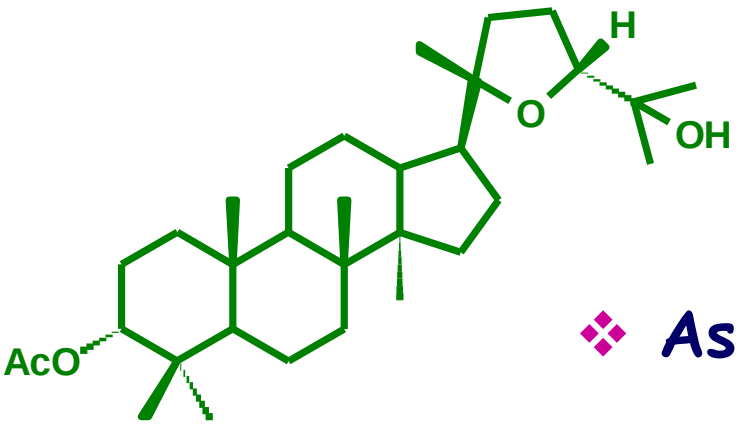
NATURAL PRODUCTS AND PHOTOSYNTHESIS INTERACTION



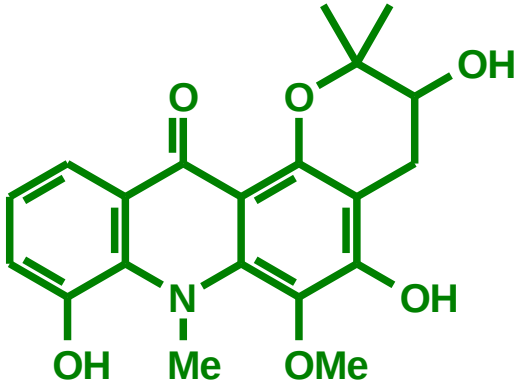


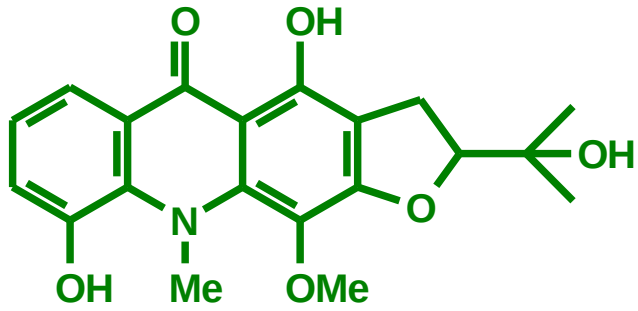
- Photosynthesis is the primary metabolic process disrupted by a diverse range of herbicides.

- However, other targets for herbicide action are cell division, cell elongation, plant growth regulation metabolism and biosynthesis of lipids, amino acids and pigments.

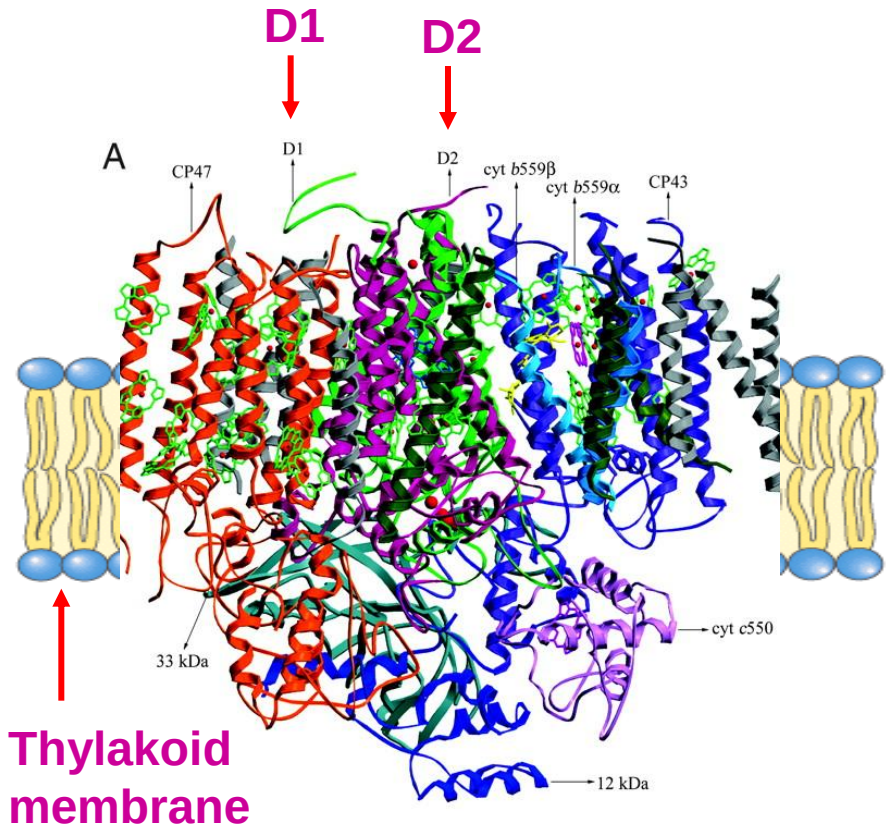


❖ As part of our efforts to find potential lead compounds as herbicides, we have been assayed natural products as inhibitors of photosynthesis.





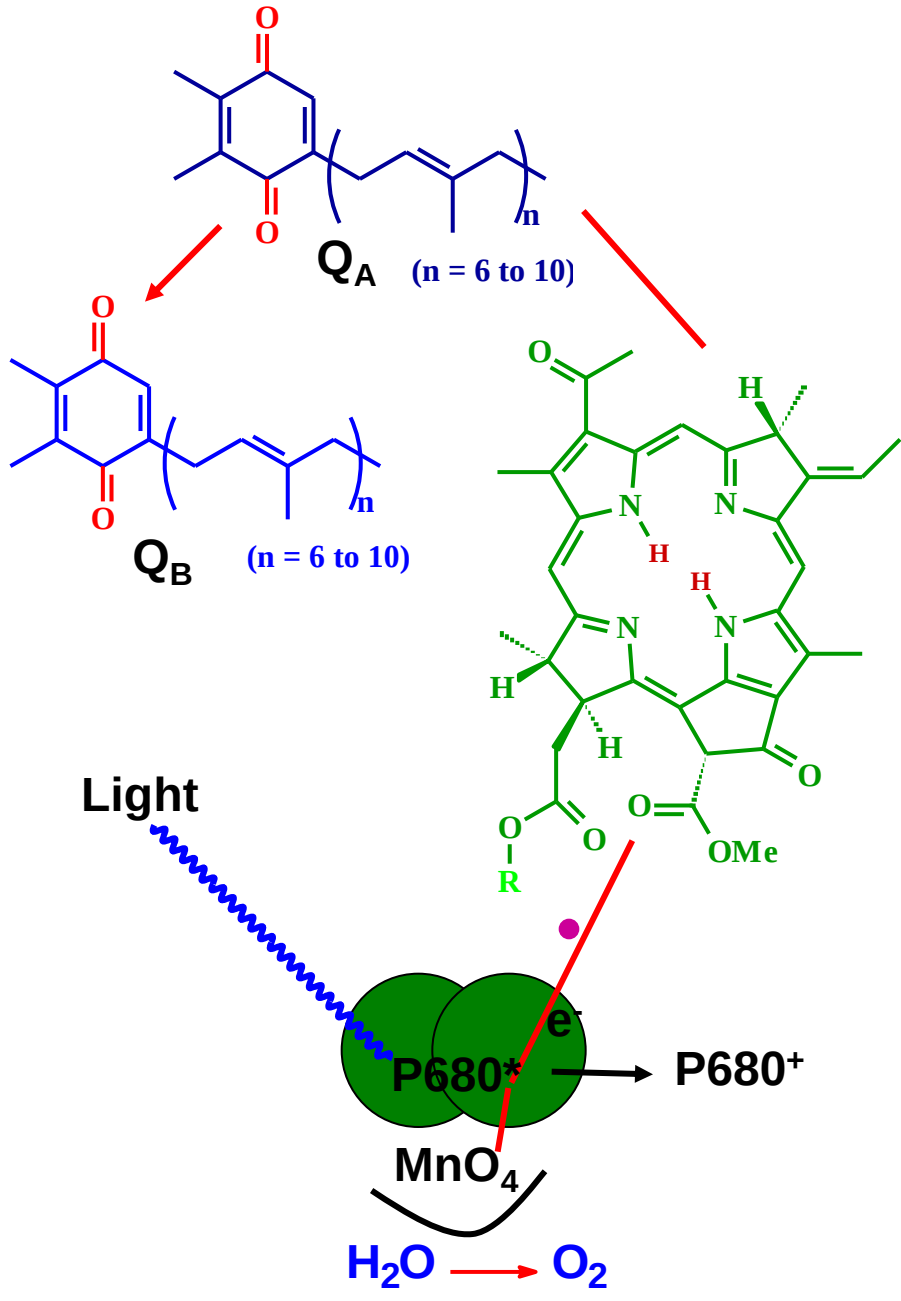
❖ Photosynthesis in green plants is mediated by two linked photosystems: Photosystem II and I



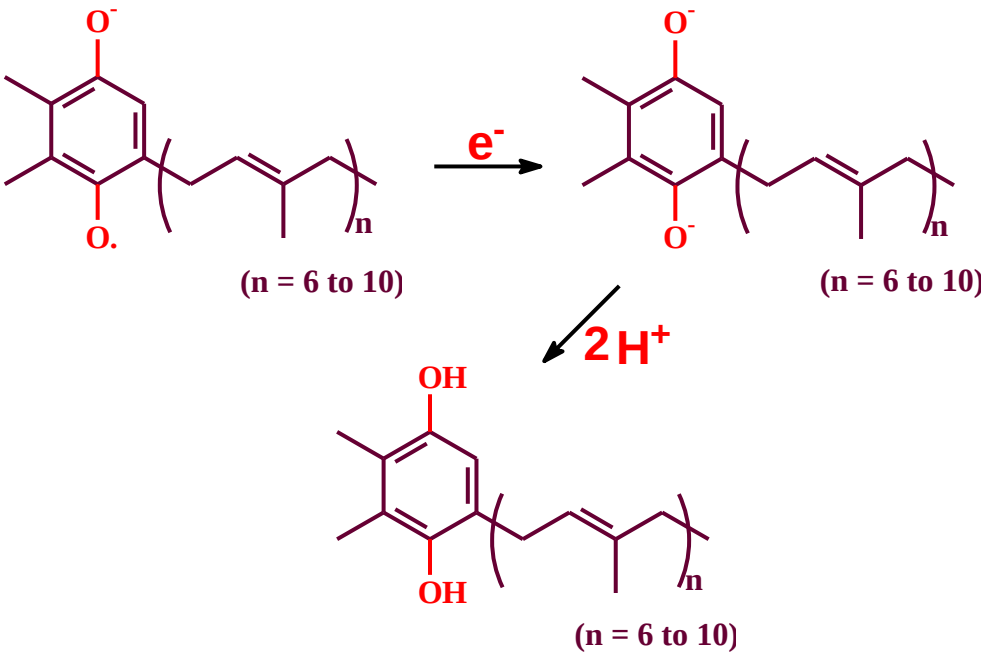
❖ Photosystem II, begins with excitation of a special pair of chlorophyll molecules that are bound by D1 and D2 subunits of proteins system.

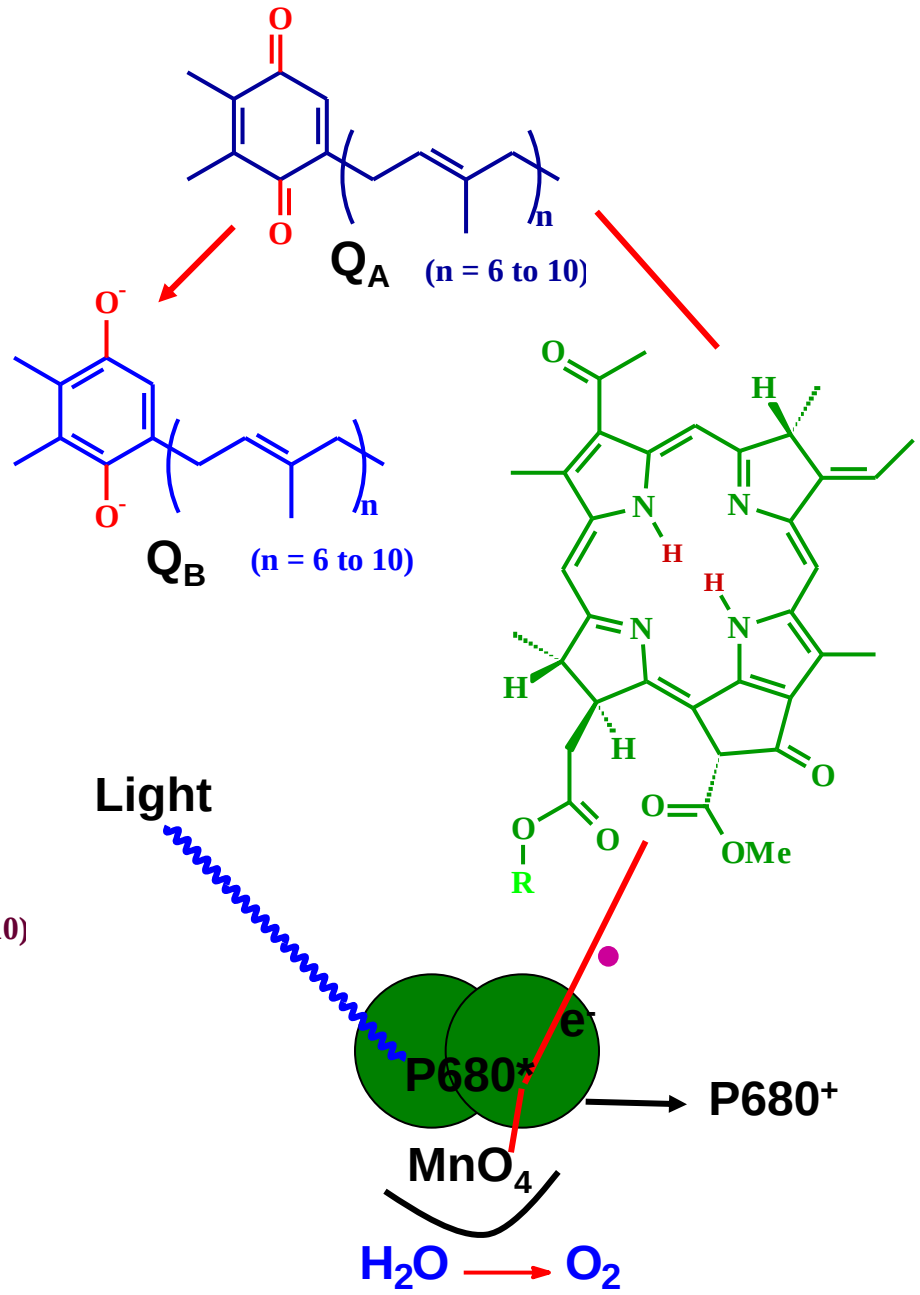
- The special pair of chlorophyll *a* molecules absorb light at 680 nm, thus it is often called P680.

- On excitation P680 rapidly transfers an electron to a pheophytin, which transfers it to a bound plastoquinone at site Q_A and then to a mobile plastoquinone at site Q_B.

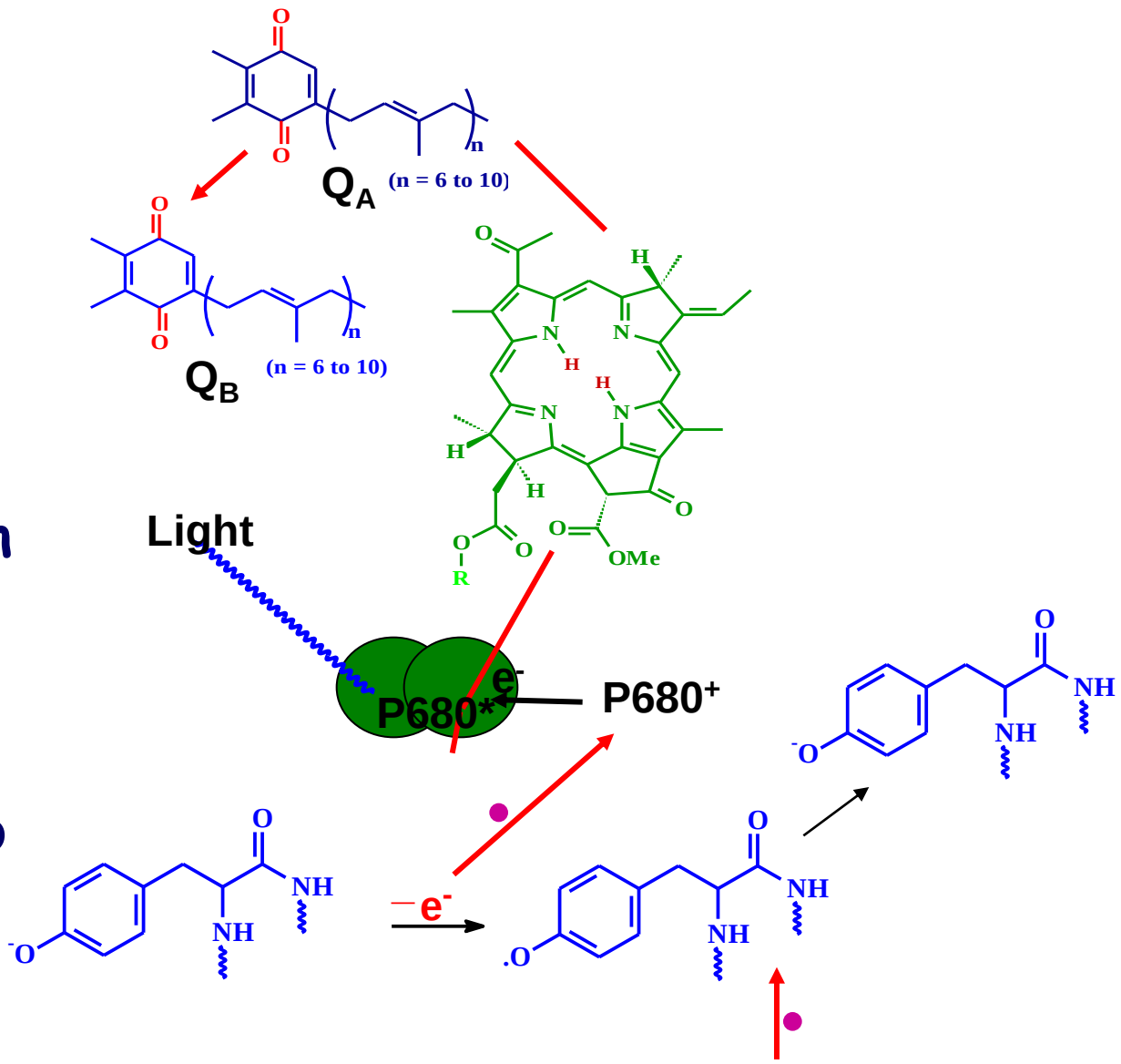


❖ With the arrival of a second electron and the uptake of two protons, the mobile plastoquinone is reduced to QH_2



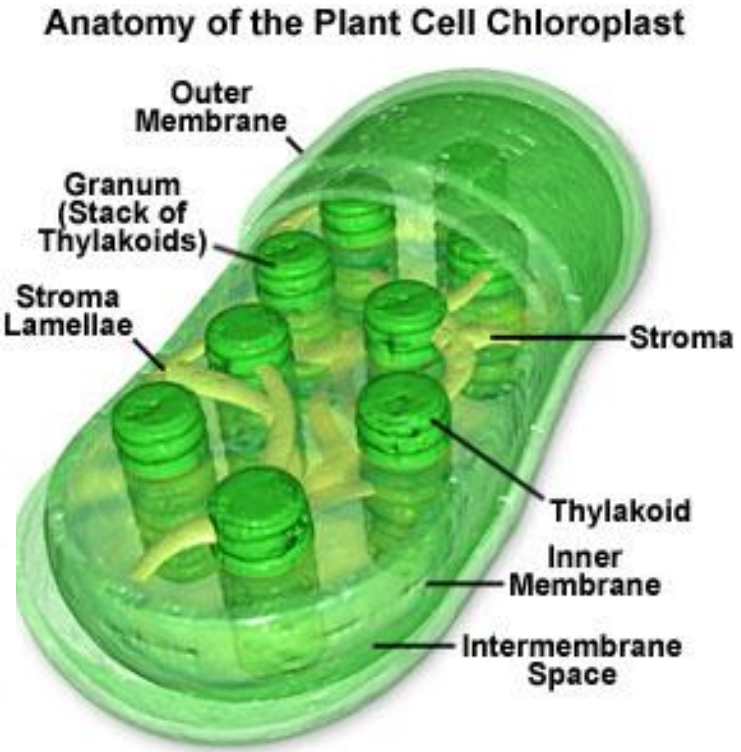


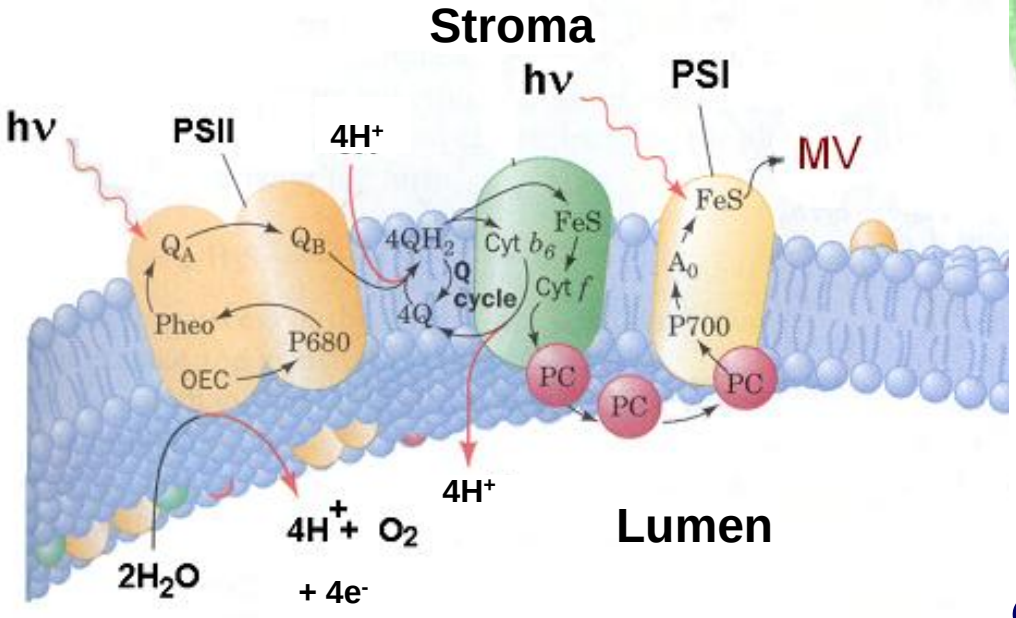
- The pair $P680^+$ extracts electrons from tyrosine residue of subunit D1 of PSII.
- Oxidized tyrosin removes electrons from water molecules bound to the manganese center.



$$2\ H_2O + (Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{5+}) \rightarrow O_2 + 4\ e^- + 4\ H^+$$

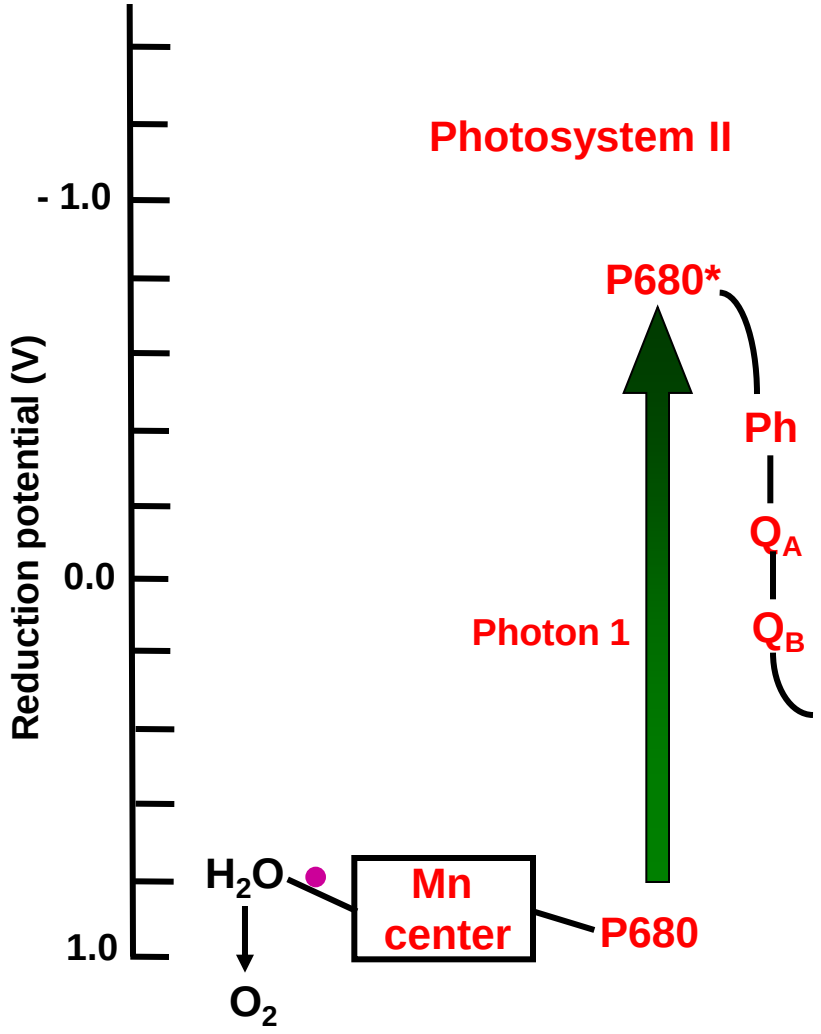
❖ Photosystem II occurs in the thylakoid membrane.

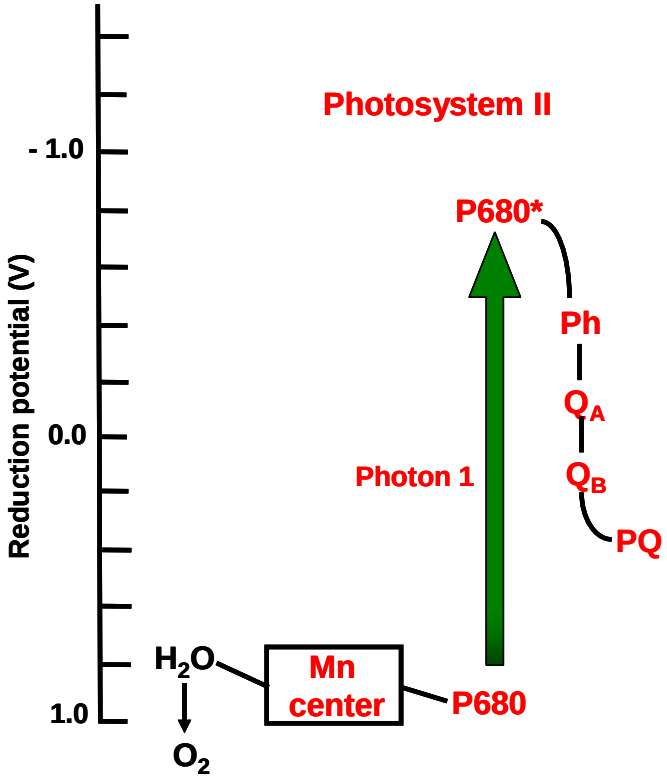




❖ The manganese center, or the water oxidation occurs in the thylacoide lumen

- In short
- Electrons flow from H_2O to Q_B (QH_2)

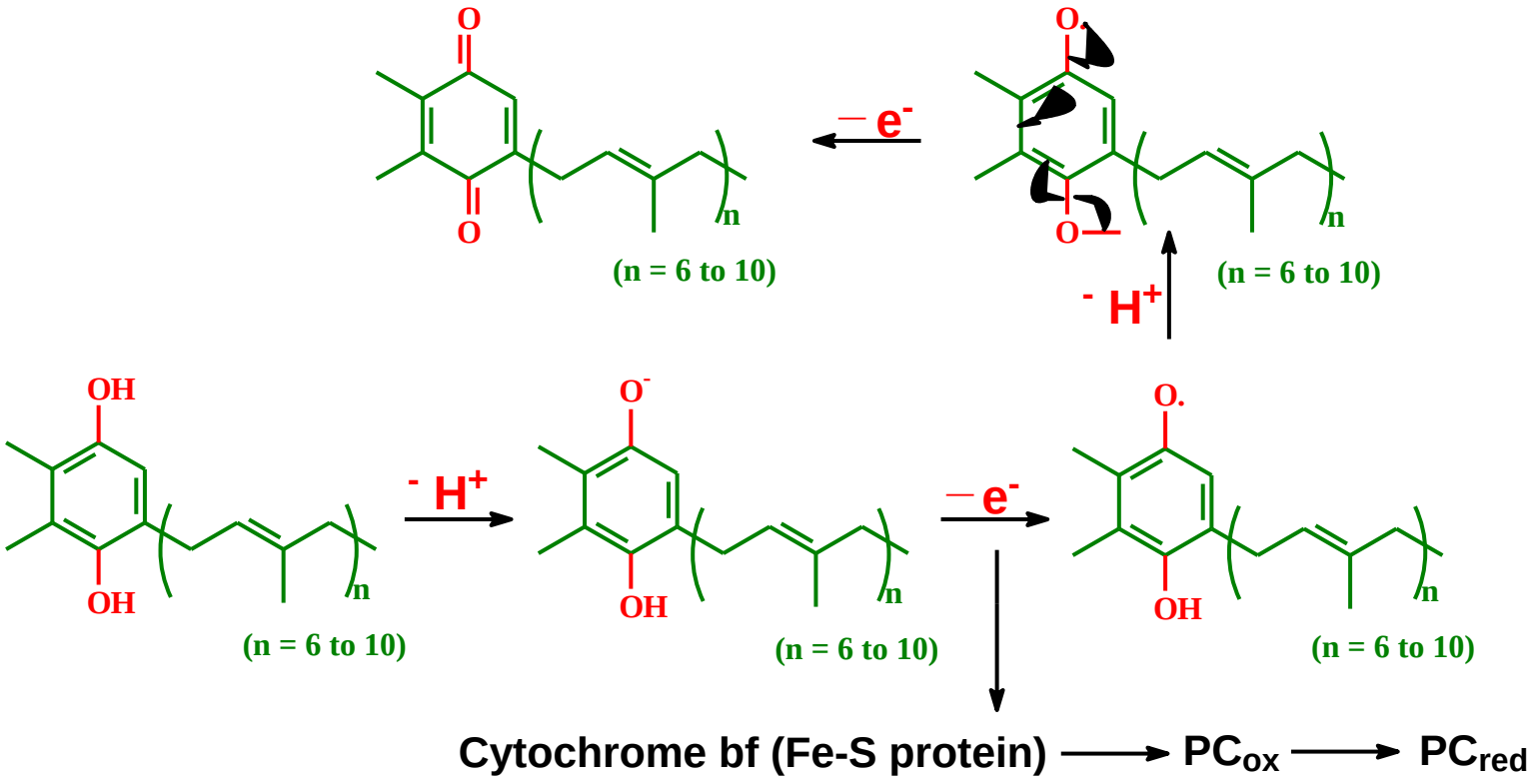


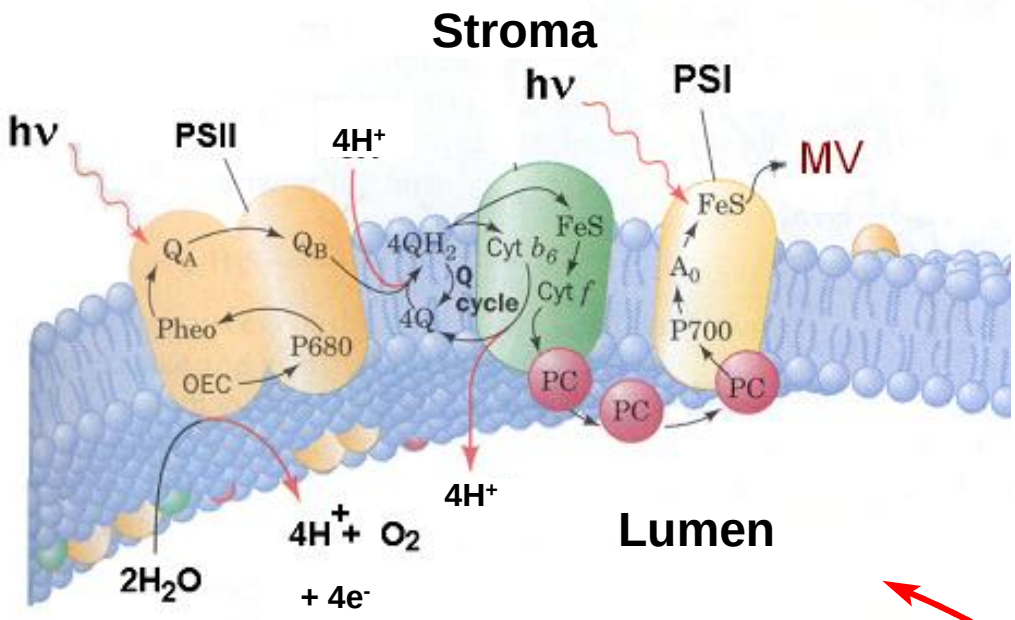


❖ Cytochrome bf links Photosystem II to Photosystem I

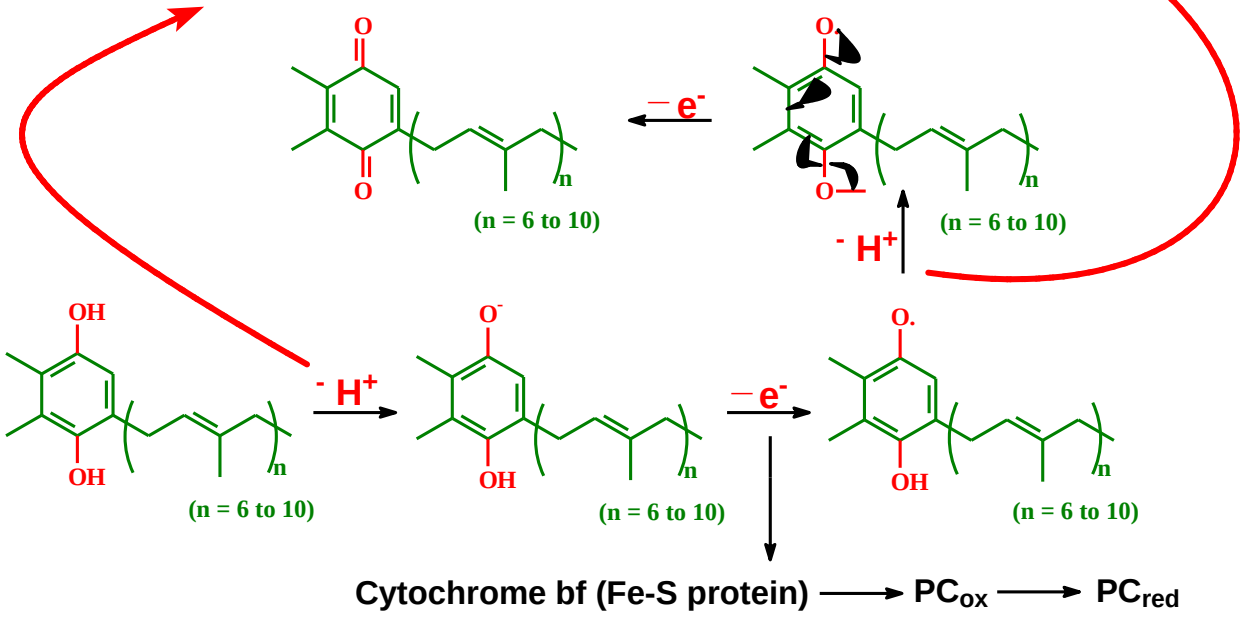
❖ This complex catalyzes the transfer of electrons from plastoquinol (QH_2) to plastocianin (Pc), a small, soluble copper protein in the thylakoid.

- Plastoquinol is oxidized to plastoquinone
- The electrons flow through the iron-sulfur protein of Cytochrome bf complex to convert oxidized plastocyanin into its reduced form.





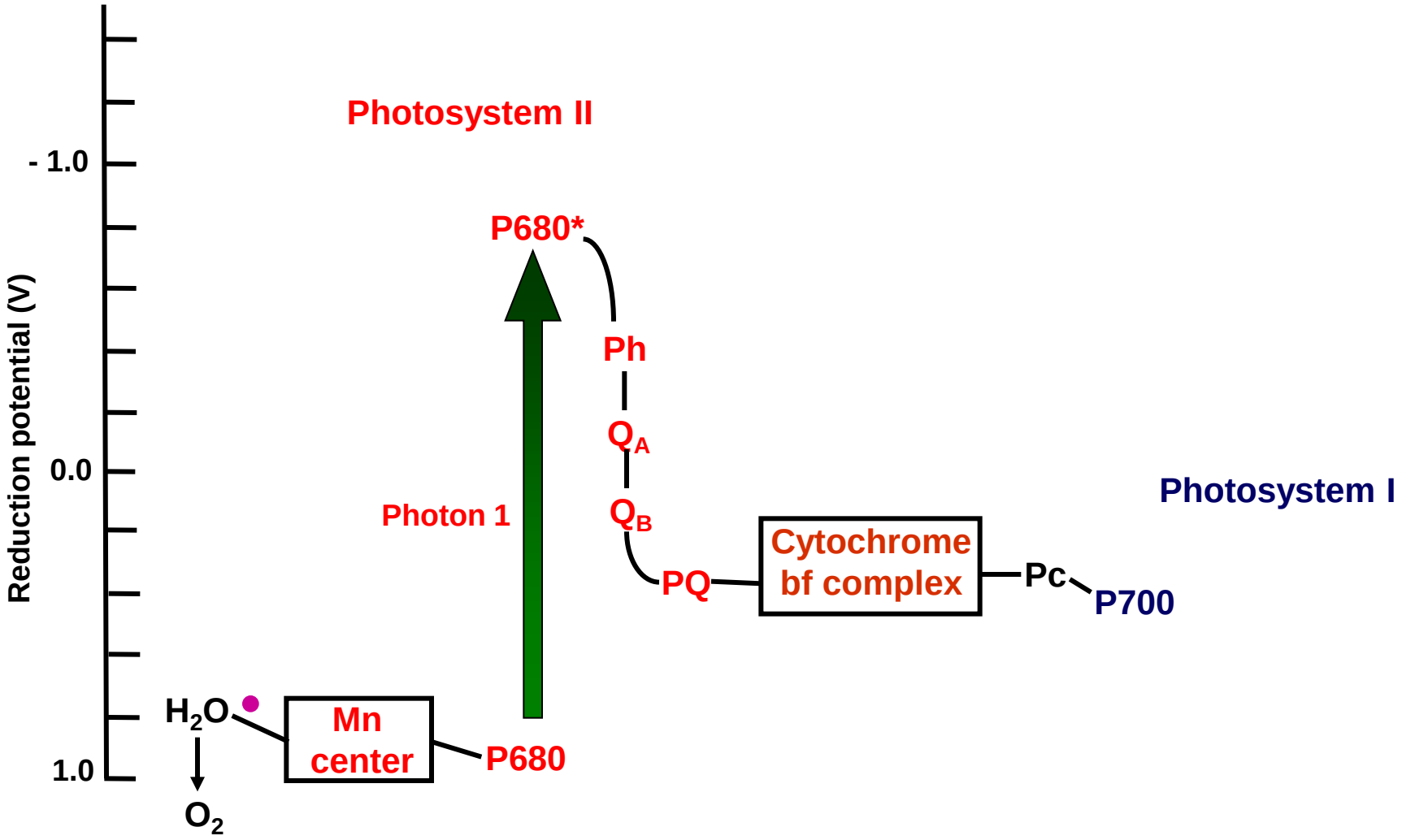
Thylakoide lumen low pH



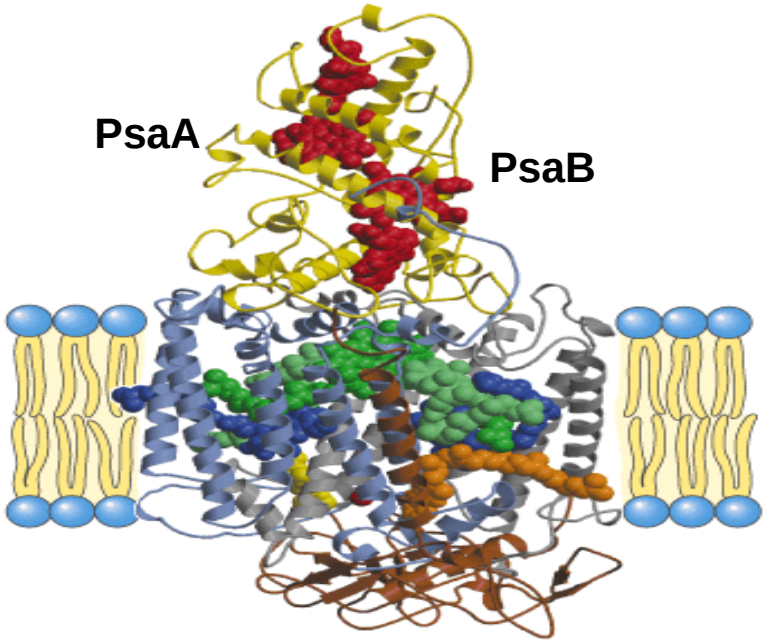
❖ These protons are released in the thylakoide lumen

❖ In short

❖ Electrons flow from H_2O to Plastocyanin, then to PSI



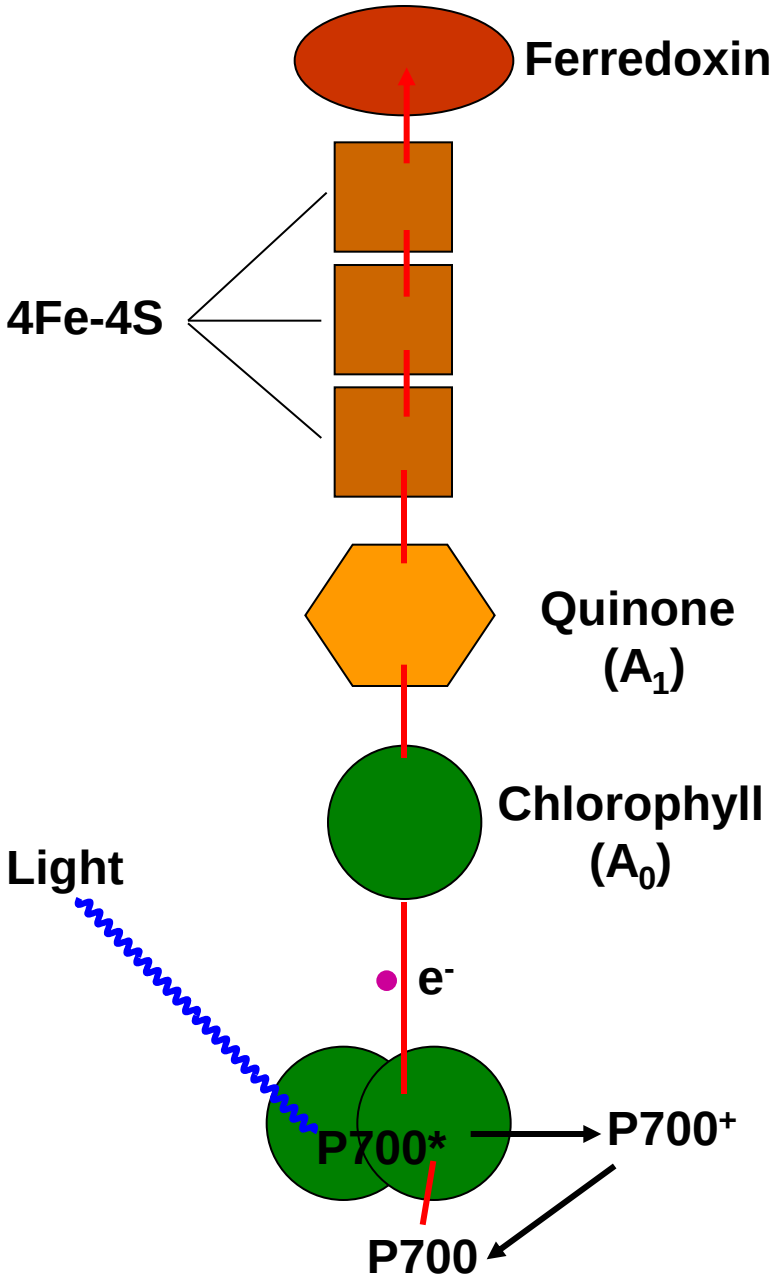
- **Photosystem I**, begins with excitation of a special pair of chlorophyll a molecules that are bound by psaA and psaB subunits of proteins system.



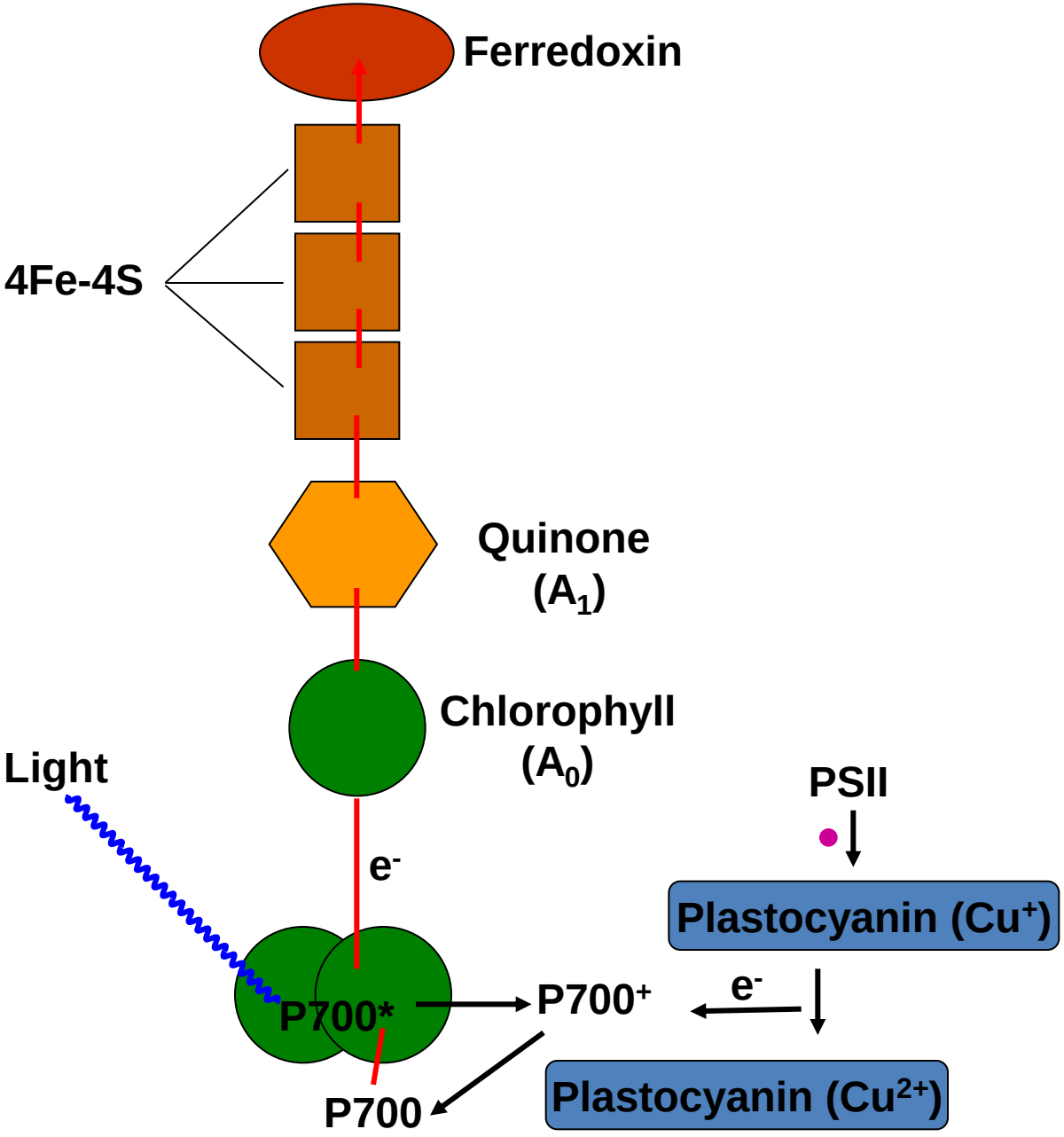
- The special pair of chlorophyll *a* molecules absorb light at 700 nm, thus it is often called P700.

❖ Photosystem I

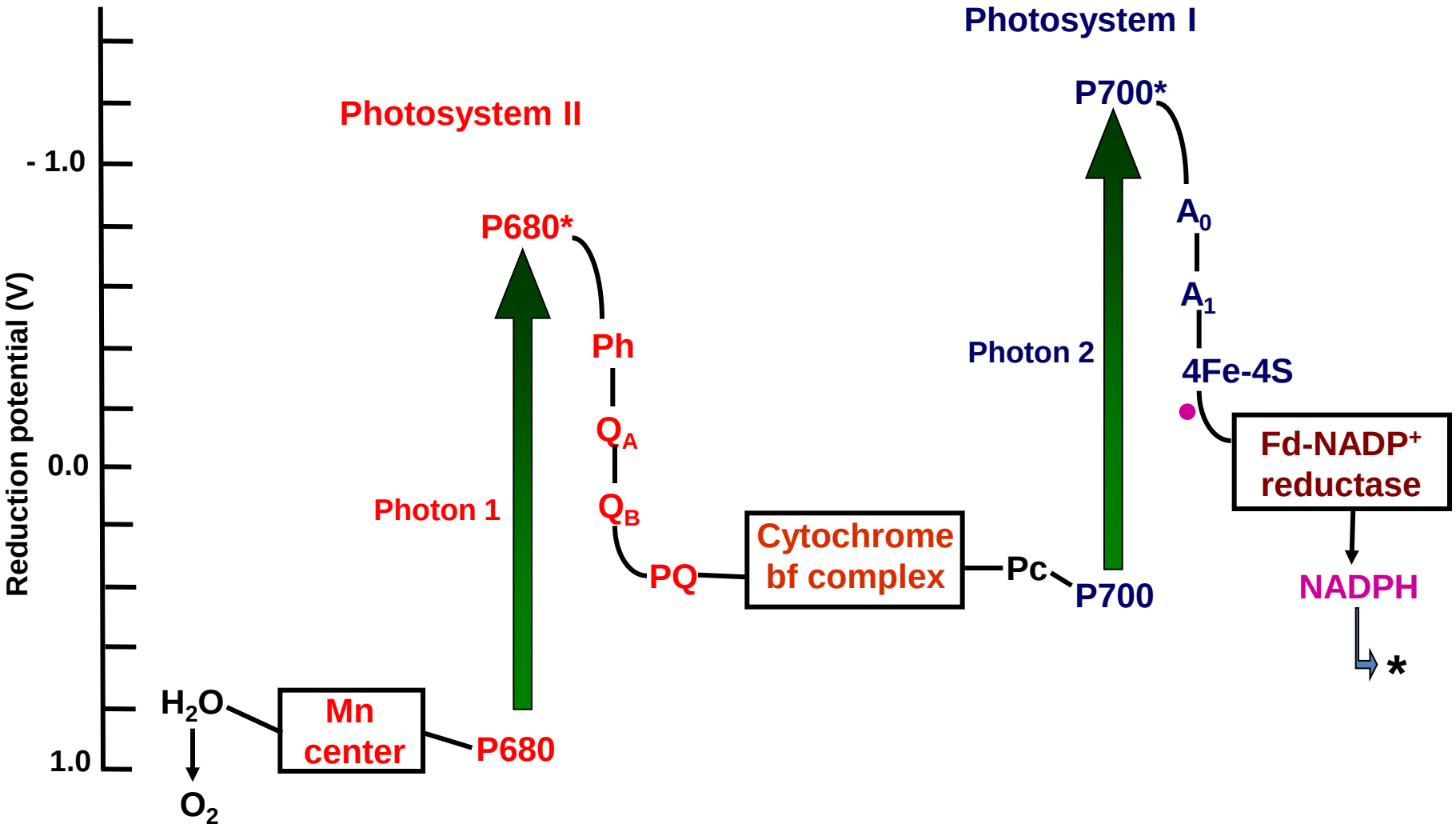
❖ On excitation P700 rapidly transfers an electron to chlorophyll at site A_0, which transfers it to quinone at site A_1 and then to a set of iron (Fe)-Sulfur clusters, and finally to ferredoxin (Fd) .



- $P700^+$ captures an electron from reduced plastocyanin to return to P700.

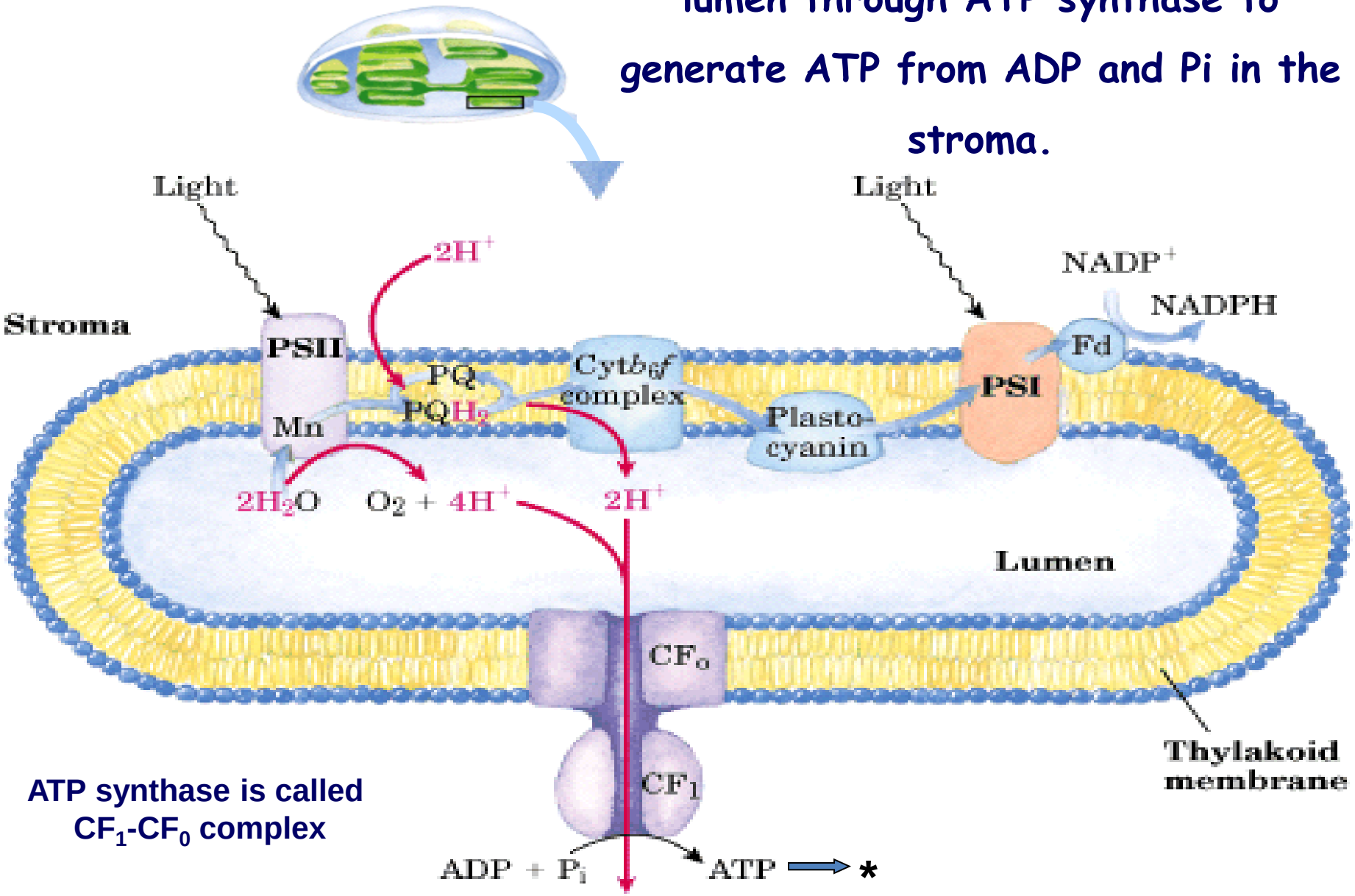


- Ferredoxin transfers electrons to $NADP^+$ and the reaction is catalyzed by ferredoxin-$NADP^+$ reductase to form NADPH



Photosynthesis

- The excess of protons flow out of lumen through ATP synthase to generate ATP from ADP and Pi in the stroma.

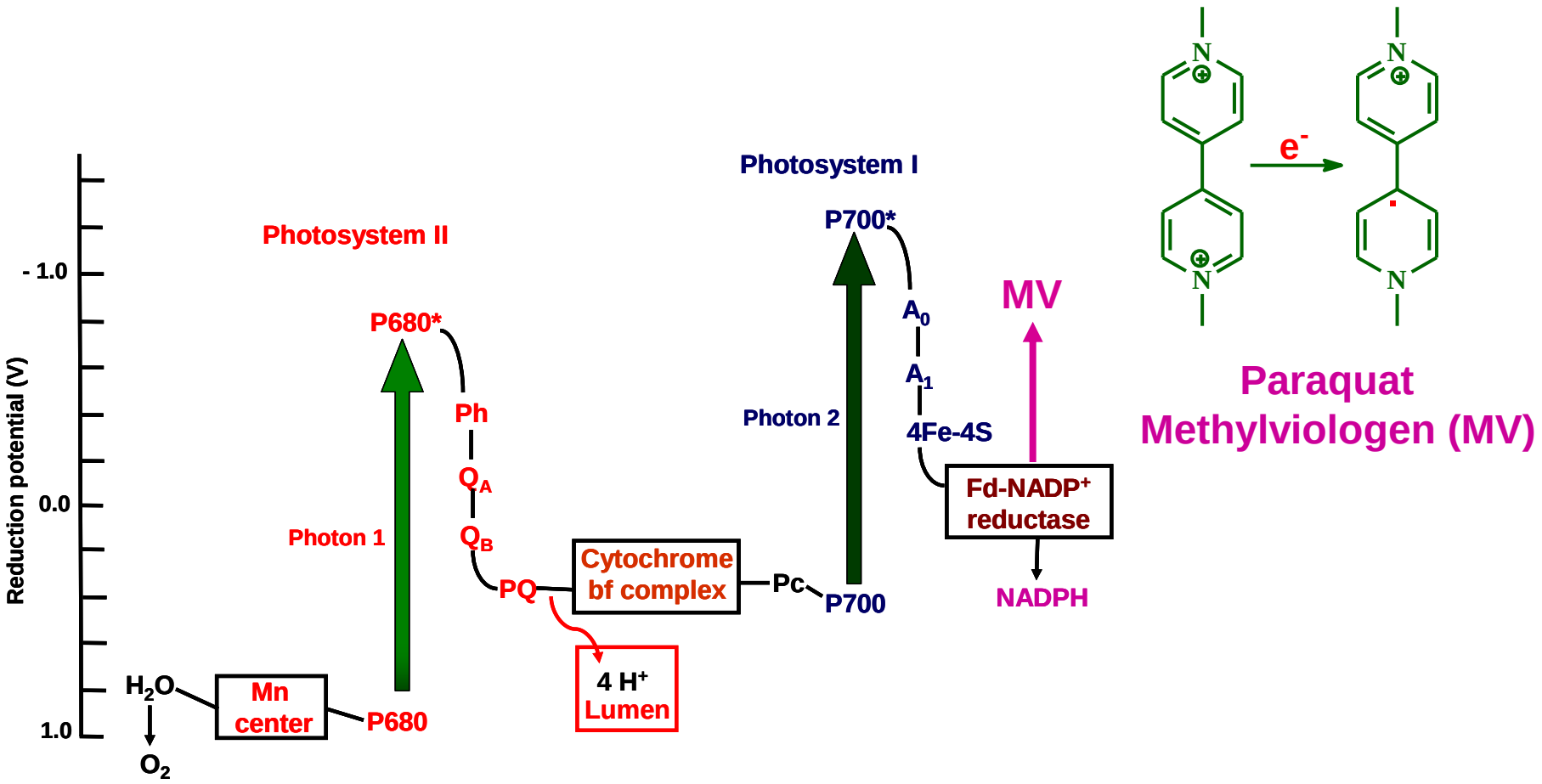


ATP synthase is called CF_1-CF_0 complex

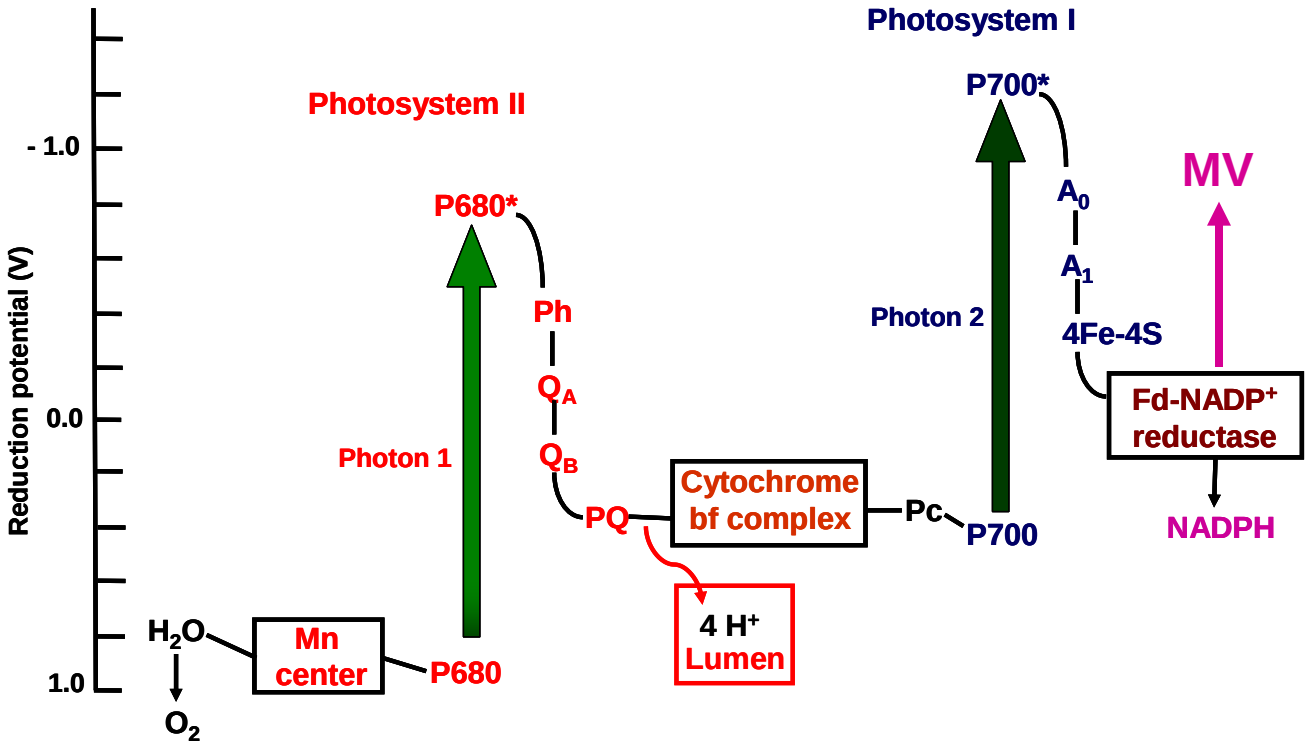
Inhibition of photophosphorylation and electron transport chain in thylakoids by Natural Products

- A number of compounds isolated in our own investigations of plants from Sapindales (Rutales) were assayed.

- We use artificial electron donors and acceptors, which bind in specific site of PSII or PSI

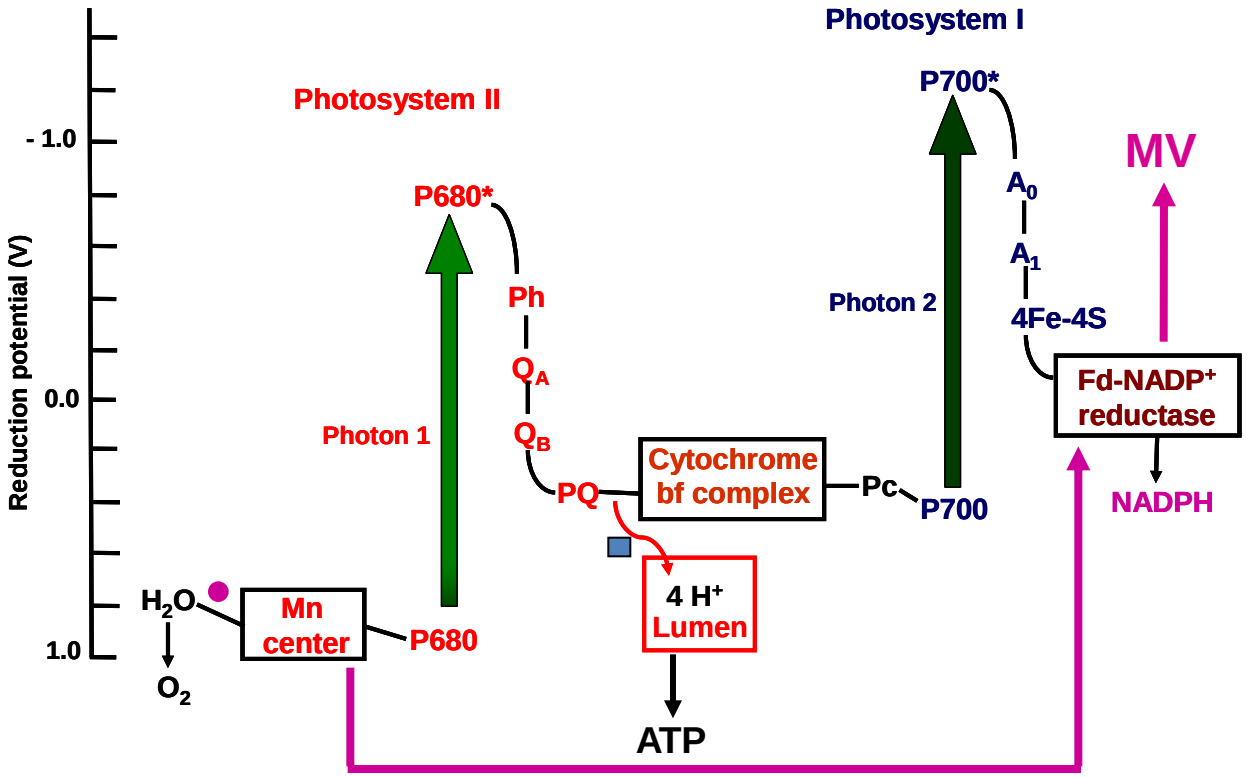


❖ Measurement of ATP Synthesis



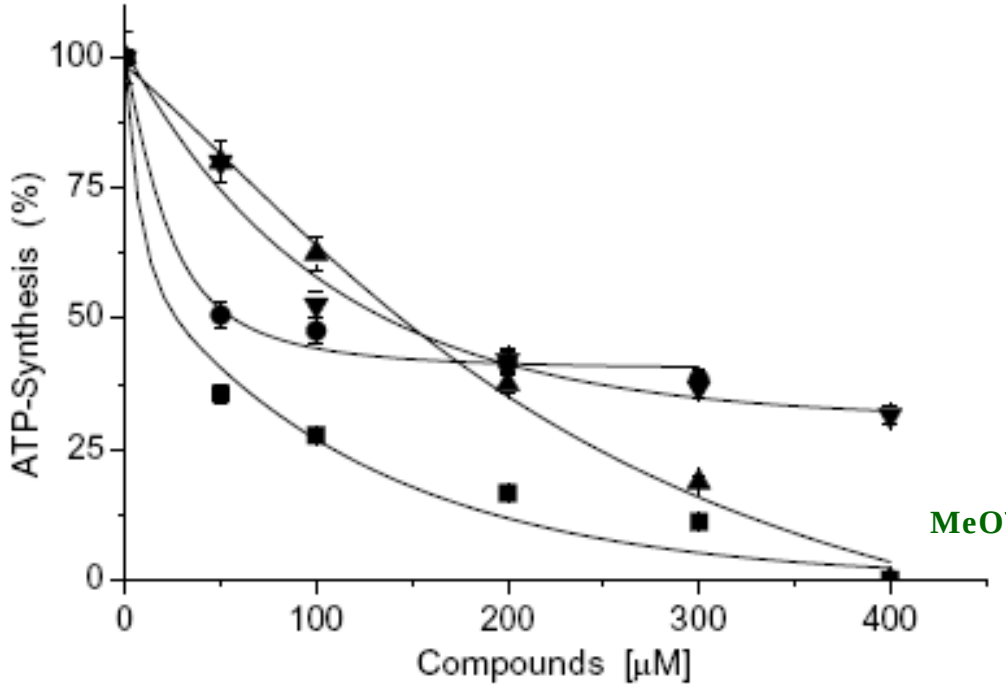
➢ We use methylviologen (MV) as electron acceptor, and chlorophyll from the intact chloroplasts freshly lysed.

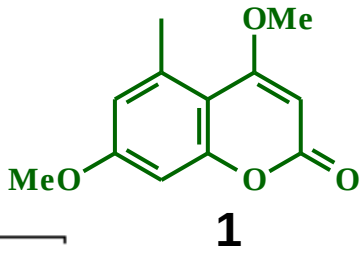
➢ Stock solution of NP is prepared using DMSO.



- We analyzed the effect of N P on electron transport from H_2O to MV.
- First of all, we measure the proton concentration, or ATP synthesis

- **Photosynthetic phosphorylation from water to MV as electron acceptor was inhibited by siderin in a concentration-dependent manner.**



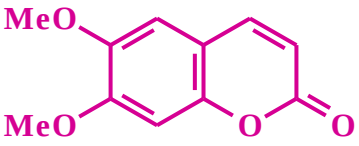


1

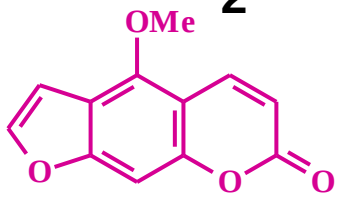
Effect of compounds 1 (■), 2 (●), 3 (▲), and 4 (▼) on ATP synthesis.

Veiga, da Silva et al, Arch. Biochem. Biophys. 465, 38-43, 2007

- The I_{50} value for siderin (1) was 27.0 μM.
- The other coumarins were almost inactive as inhibitor to ATP synthesis.



2



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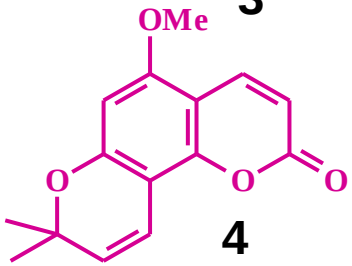

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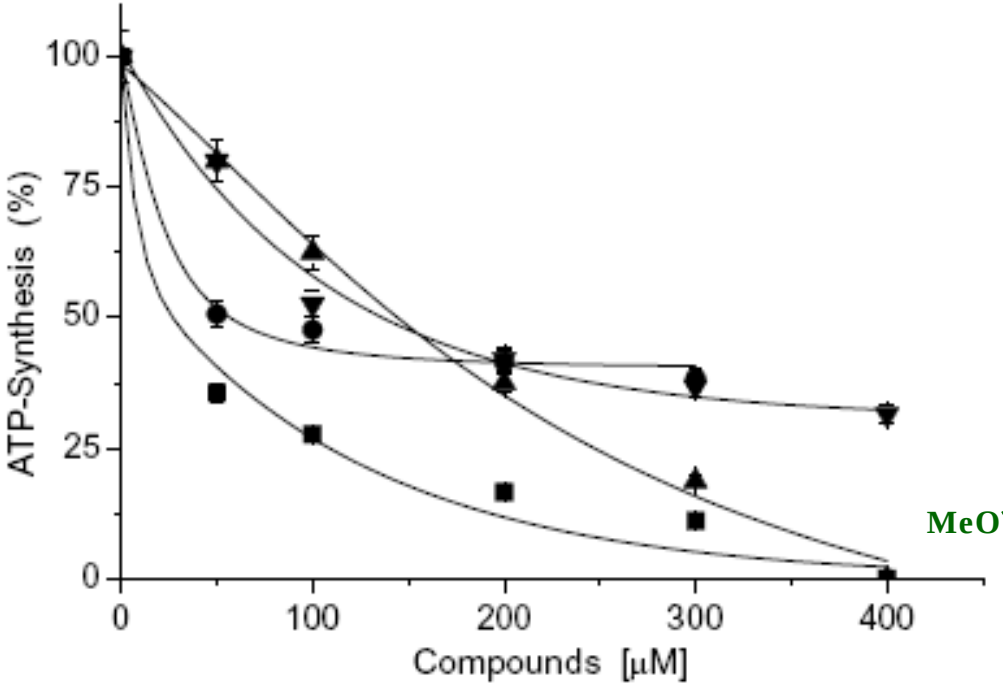
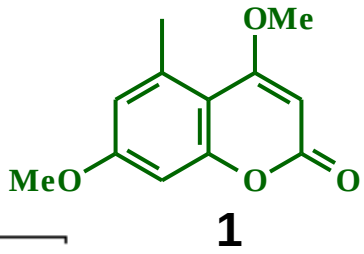
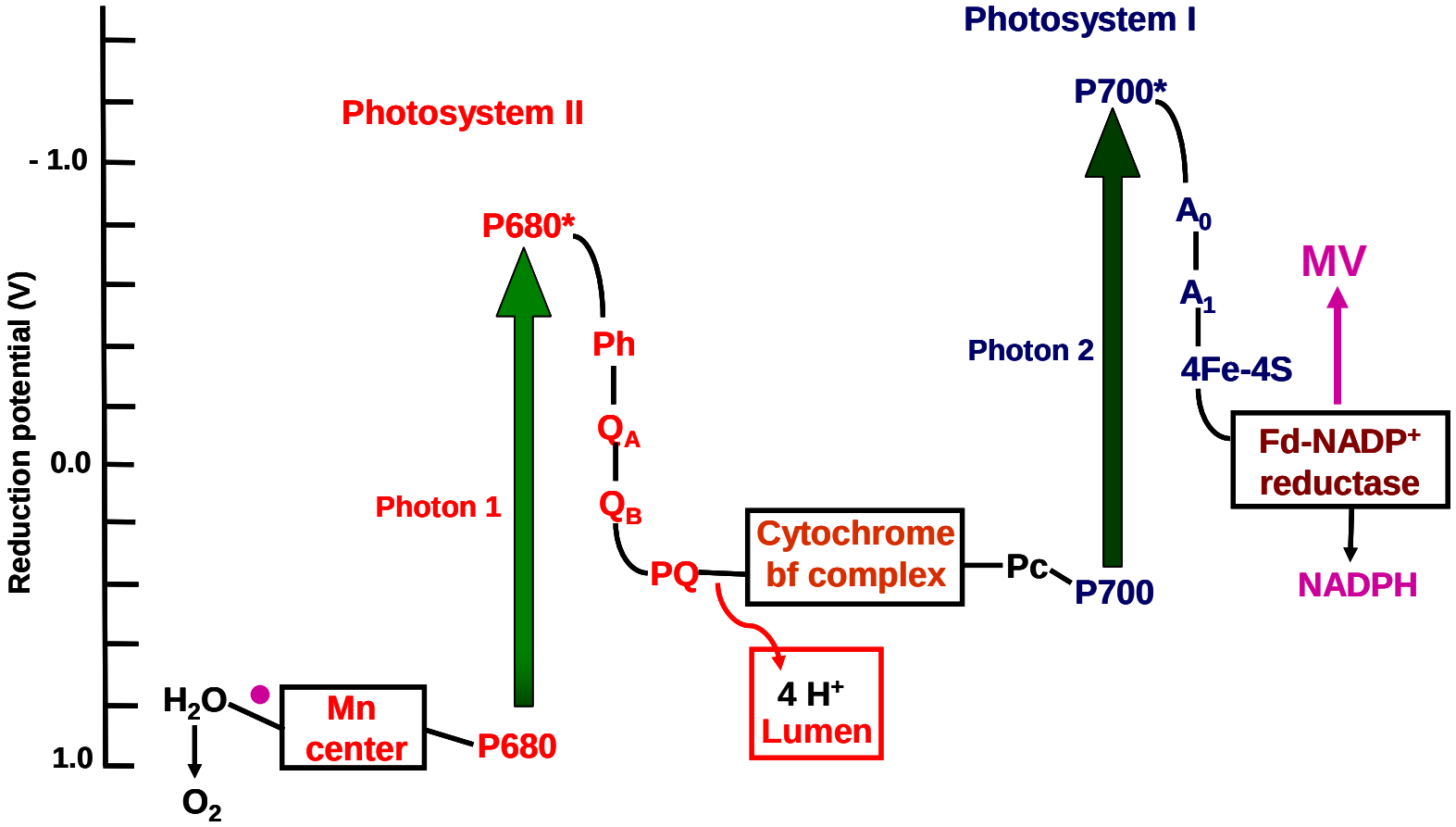




1

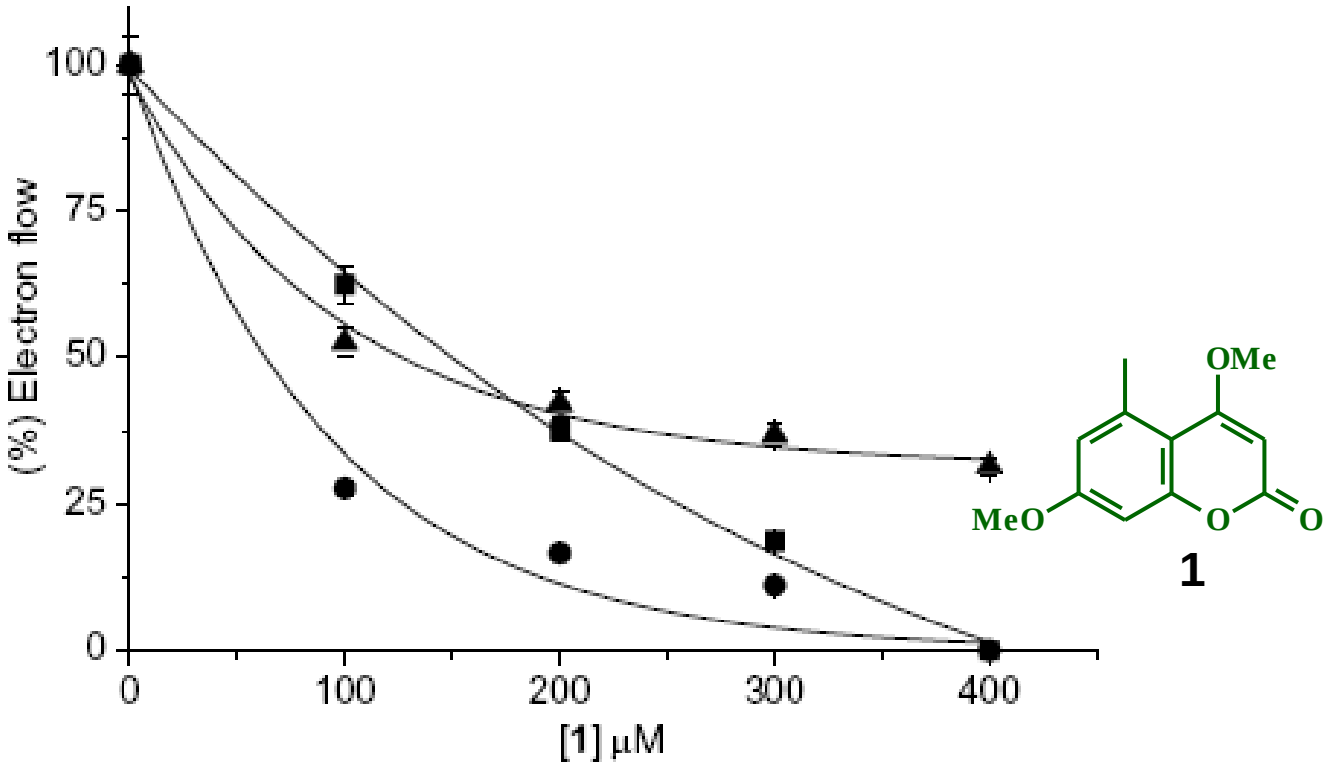
Effect of compounds 1 (■), 2 (●), 3 (▲), and 4 (▼) on ATP synthesis.

❖ Measurement of non-cyclic electron transport



➢ We measure the oxygen concentration with an oxygen electrode (Clark-type electrode)

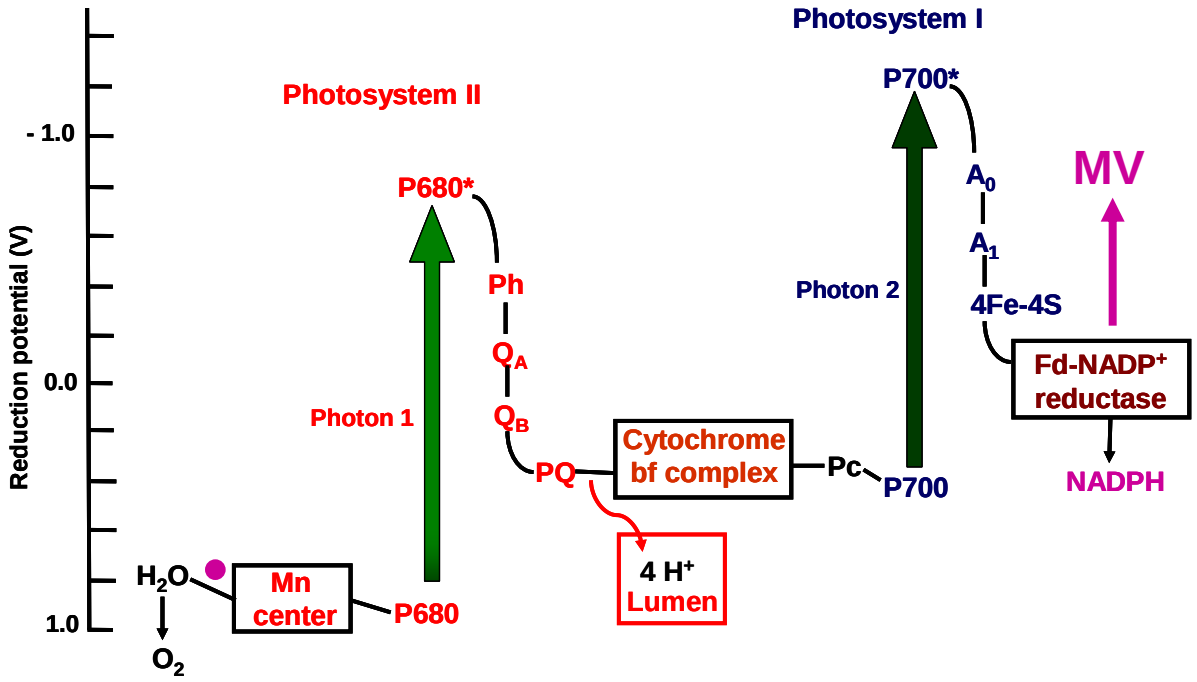
- **Siderin inhibited the phosphorylating electron transport rate 100% at 400 μM.**



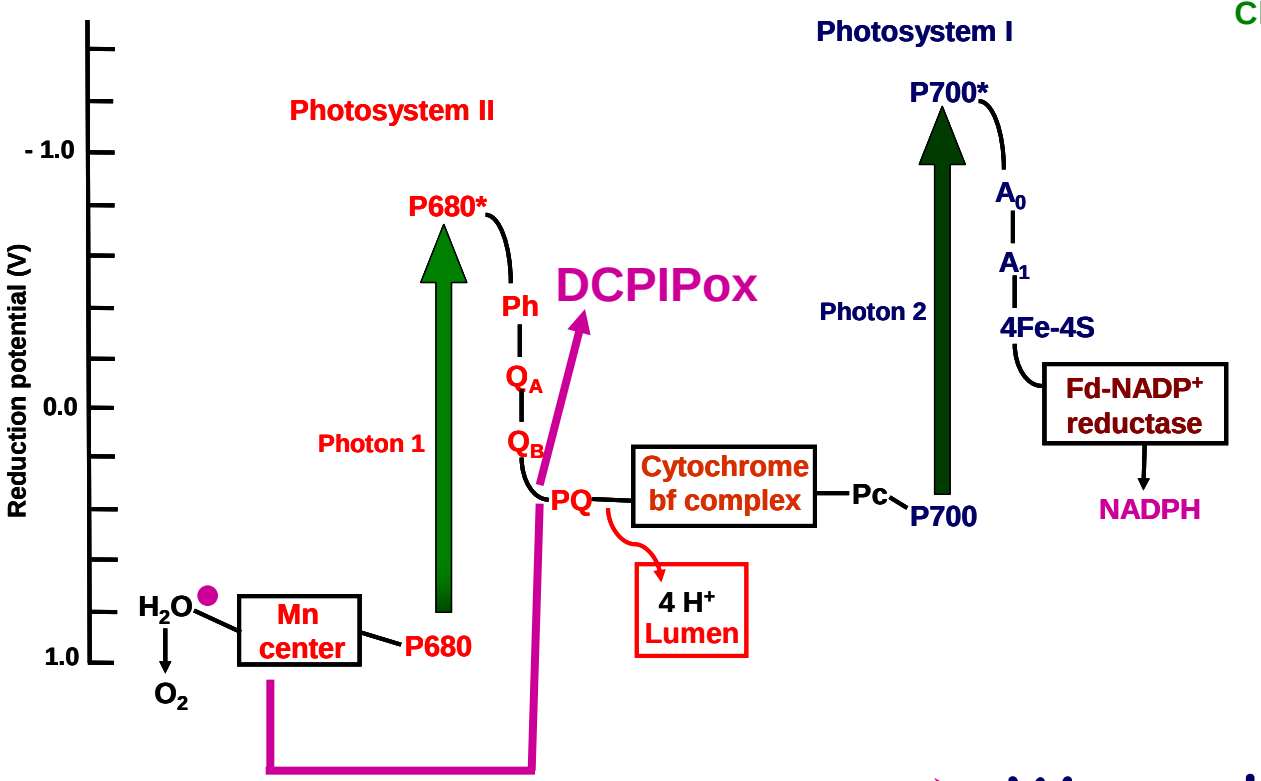
Effect of siderin on electron flow [basal (■), phosphorylating (●), and uncoupled (▲)] from water to MV in spinach chloroplasts

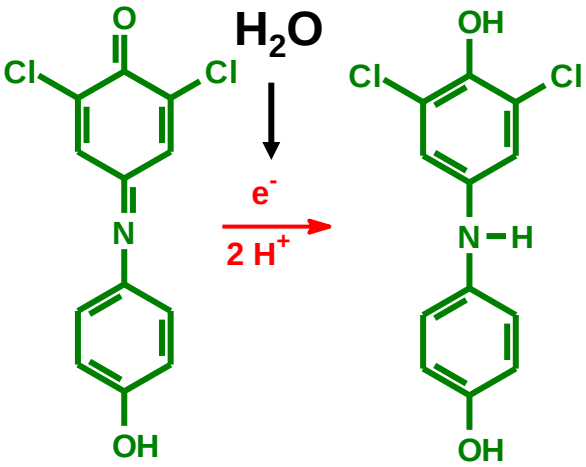
ELUCIDATION OF THE MECHANISM OF ACTION

LOCALIZATION OF SIDERIN SITES OF INTERACTION ON PSII OR PSI



- We use artificial electron donors and acceptors, which bind in specific site of PSII or PSI

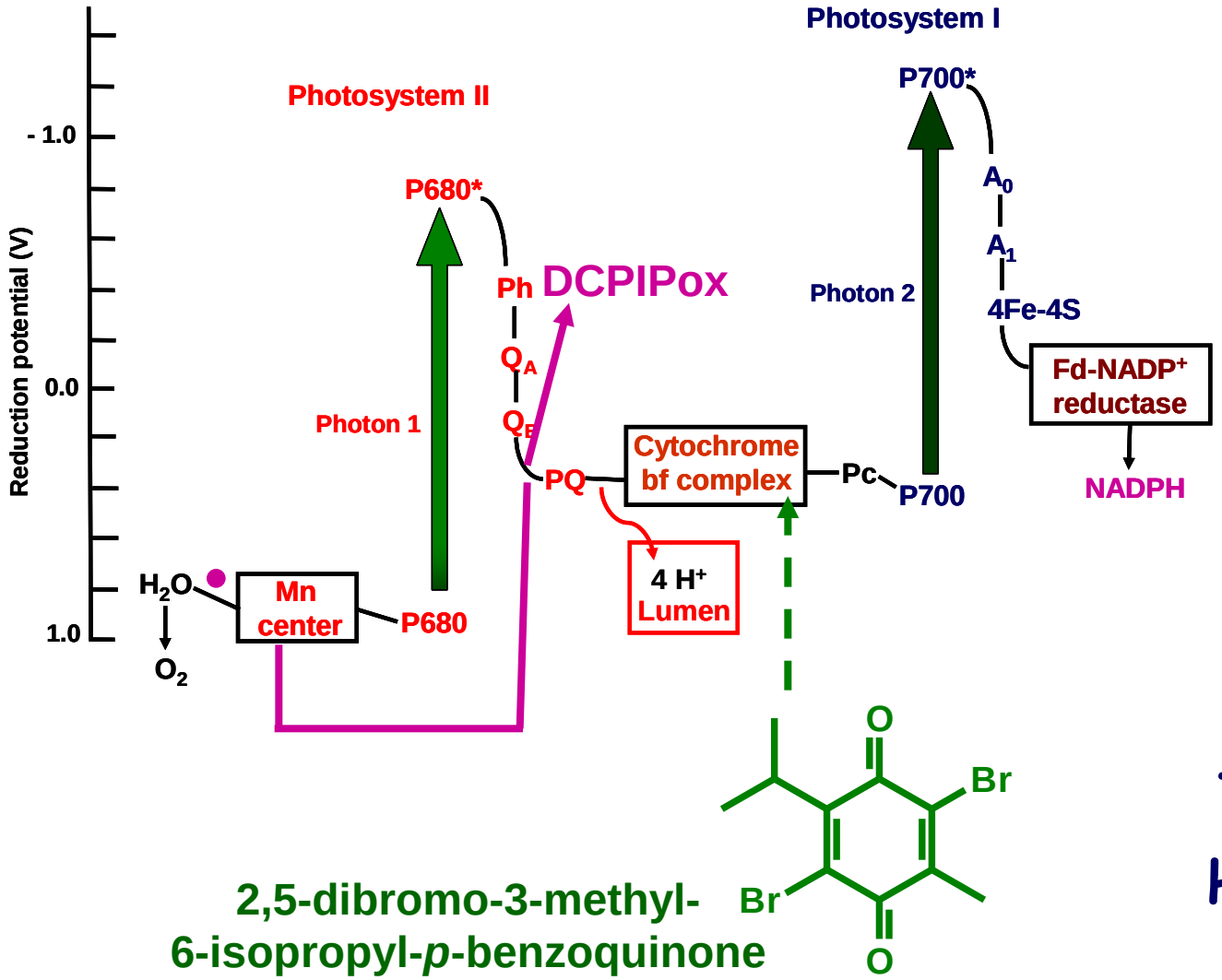




Dichlorophenol-indophenol

- We measure the oxygen concentration with an oxygen electrode (Clark-type electrode)

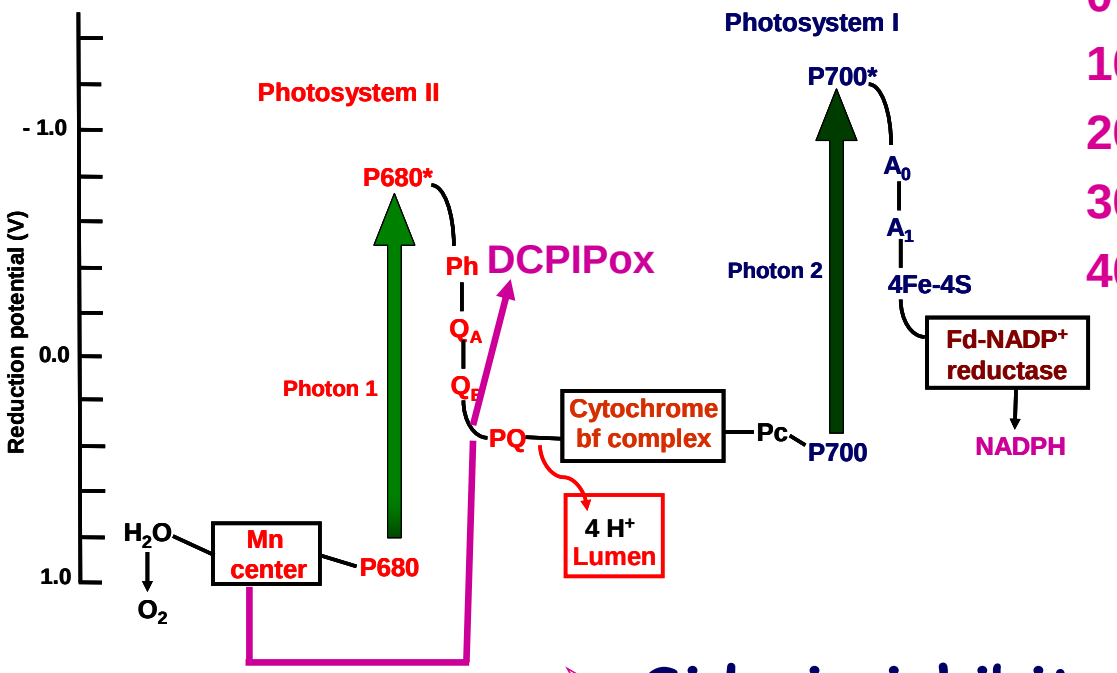
- We analyzed the effect of siderin on electron transport from H_2O to DCPIPox; then to Q_B.



2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone

- We also add DBMIB, which binding in Cytochrome bf complex, inhibiting the electron transport from H_2O to Quinone pool (Qp).

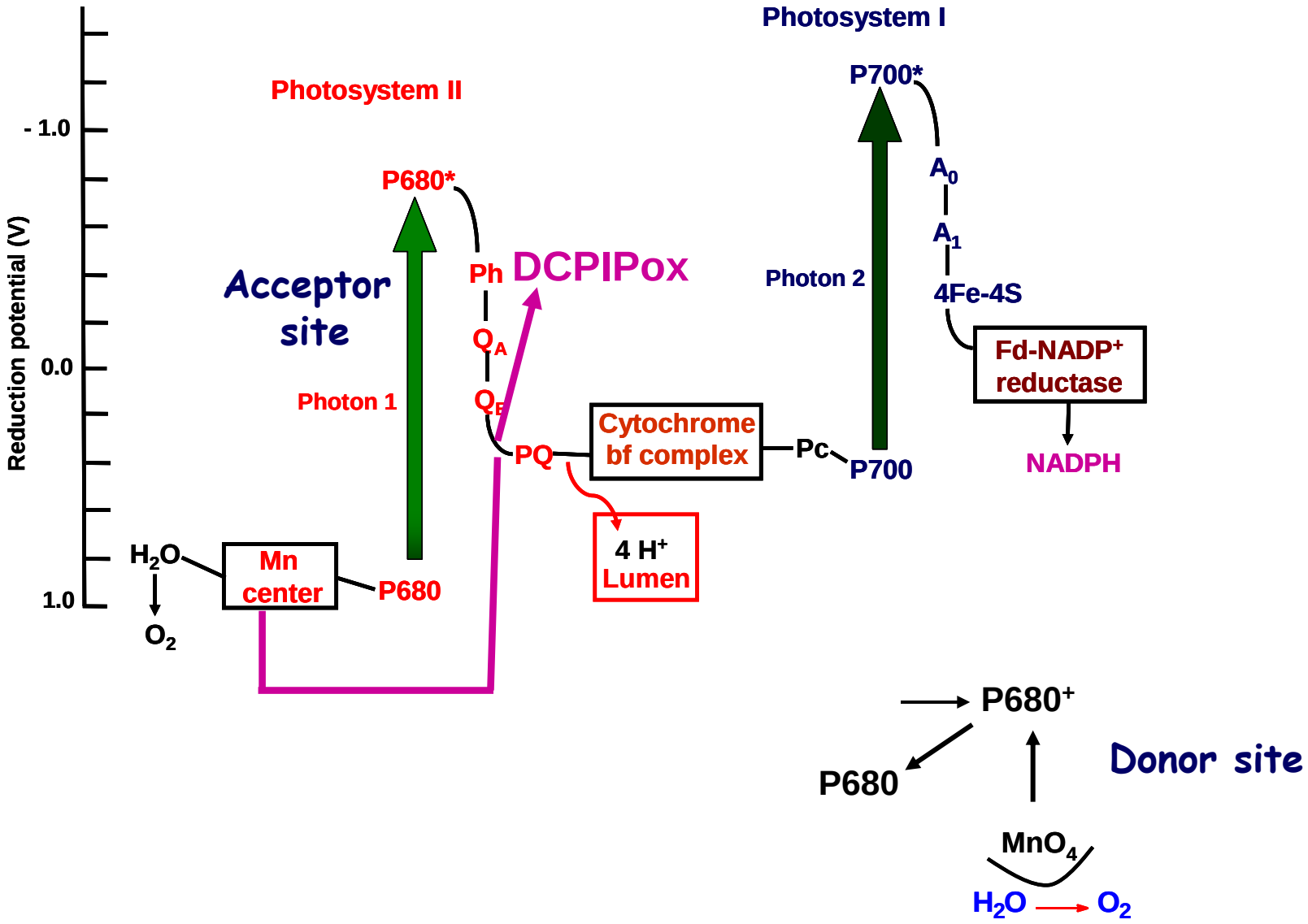
- Then, electrons flow to DCPIPox.

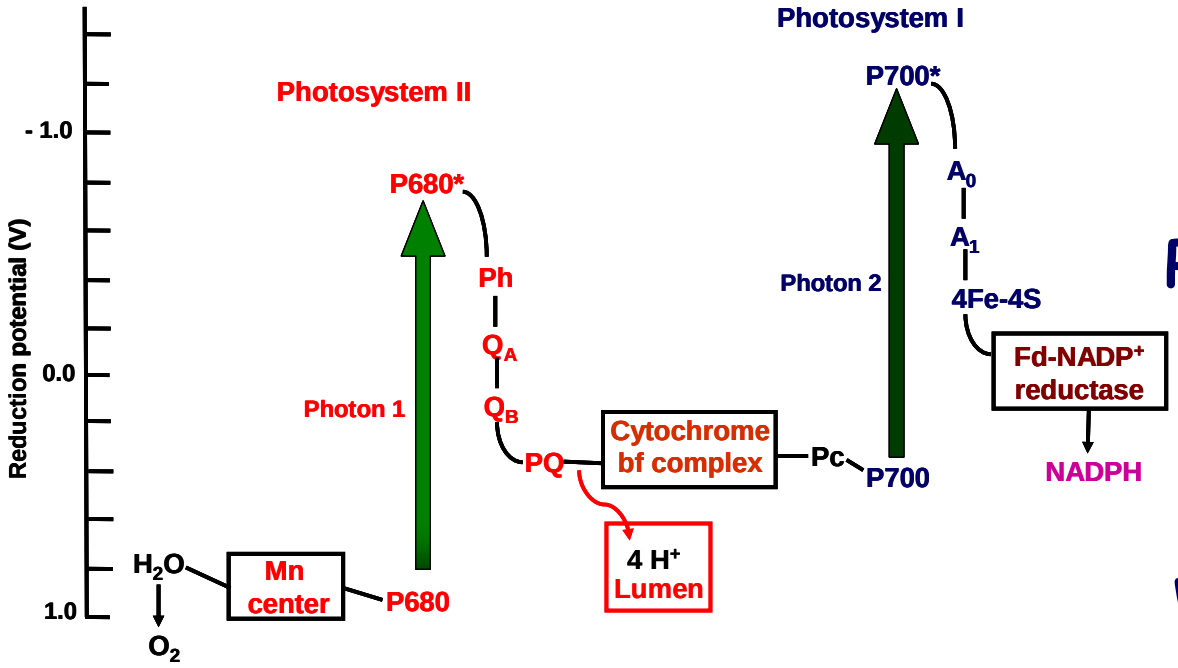


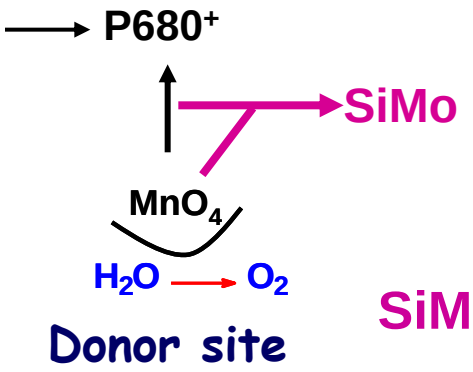
1 Conc. μM	PSII H_2O to DCPIPox (%)
0	100
100	78
200	70
300	52
400	0

- Siderin inhibits electron transport from H_2O to DCPIPox, then to Q_B, by 100% at 400 μM

Veiga, da Silva et al, Arch. Biochem. Biophys. 465, 38-43, 2007

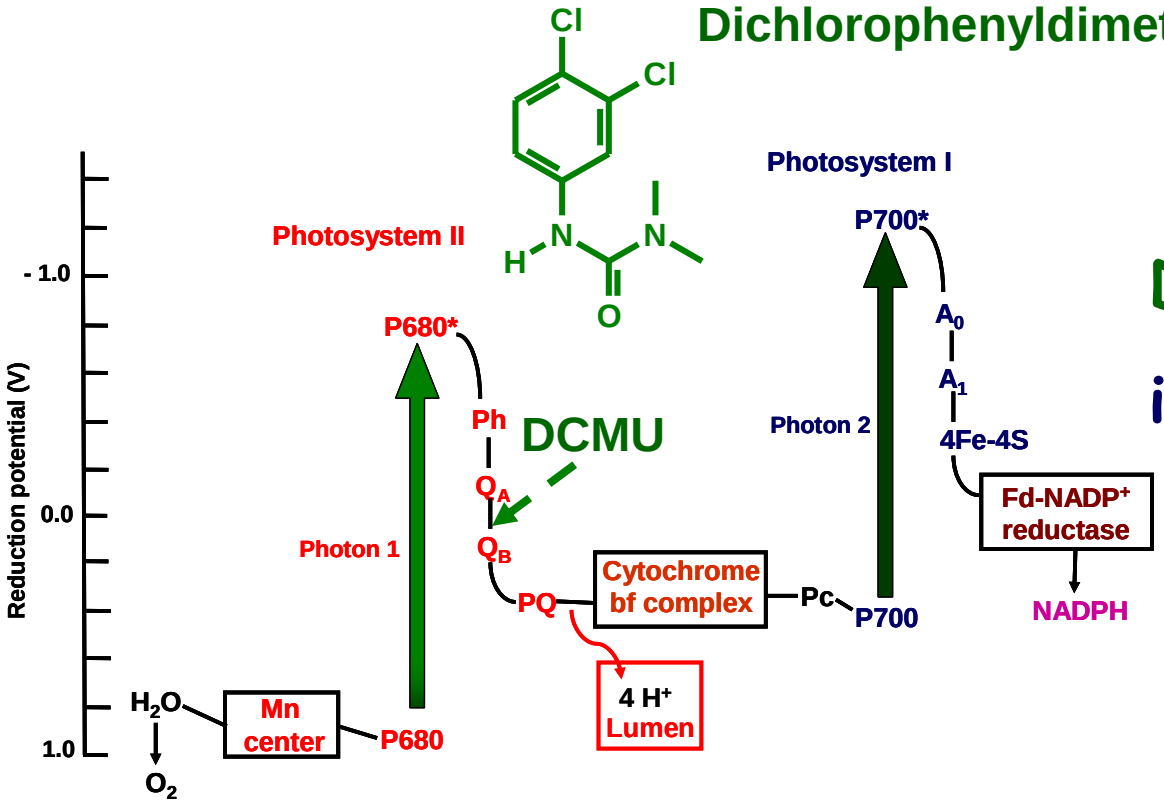

Photosystem I
Photosystem II
- 1.0
0.0
1.0
Reduction potential (V)
P700*
P680*
A0
A1
4Fe-4S
Ph
DCPIPox
Acceptor site
Photon 2
QA
QB
Photon 1
Fd-NADP+ reductase
Cytochrome bf complex
Pc
PQ
P700
NADPH
4 H+ Lumen
H2O
Mn center
P680
O2
P680+
P680
Donor site
MnO4
H2O
O2



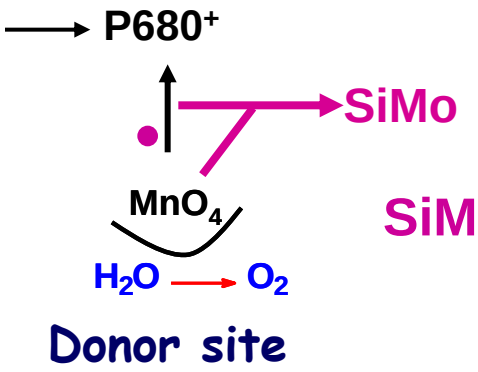


❖ To localize the siderin site of interaction on PSII between H_2O to Q_B, we use electron acceptor which binds in the donor site of PSII, as silicomolybdate.

$$SiMo_{12}O^{-4}_{40} + 2e^- + 2\,H^+ \rightarrow H_2SiMo_{12}O^{-4}_{40}$$

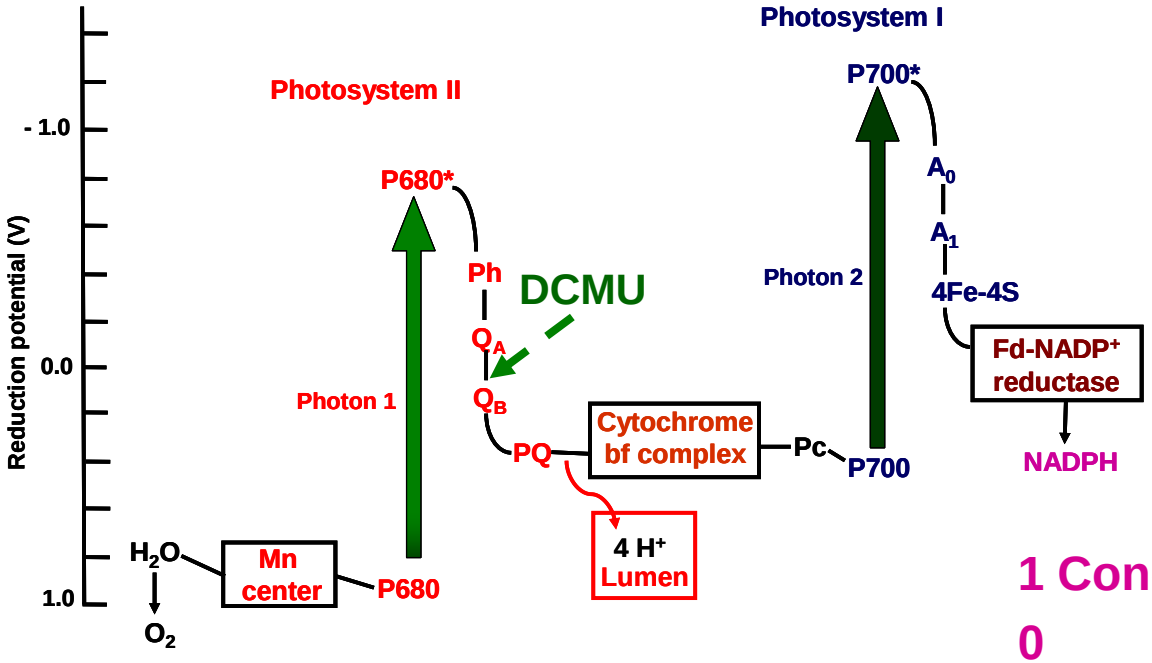


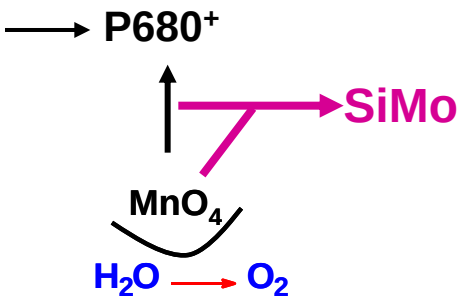
➢ We also add DCMU, which binding in plastoquinone site, inhibiting the electron transport from H_2O to Q_B.



➢ Then, electrons flow to SiMo.

$$SiMo_{12}O^{-4}_{40} + 2e^- + 2\,H^+ \rightarrow H_2SiMo_{12}O^{-4}_{40}$$



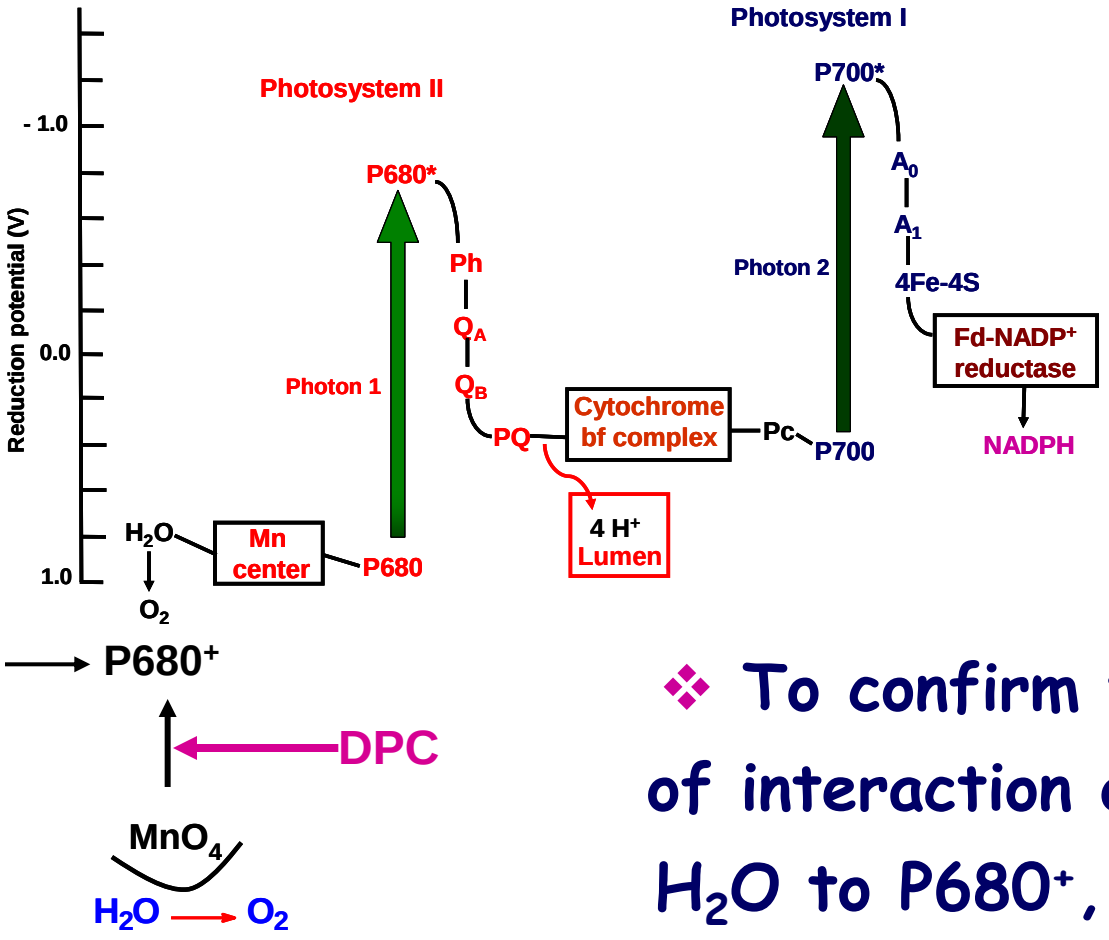


1 Conc. μM	PSII H_2O to SiMo (%)
0	100
100	75
200	50
300	12
400	0

Donor site

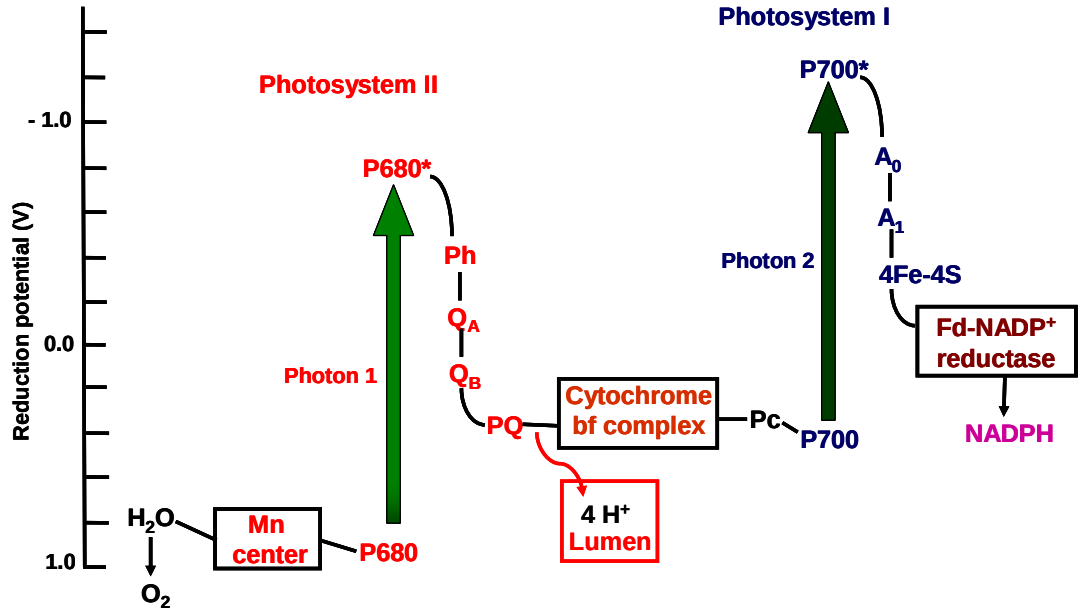
➢ Siderin inhibits electron transport from H_2O to SiMo by 100% at 400 μM

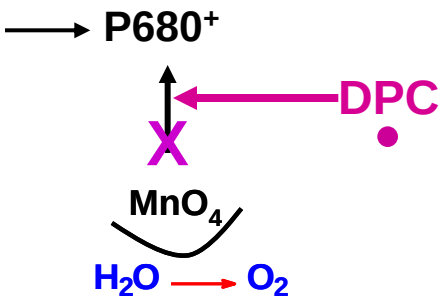
Veiga, da Silva et al, Arch. Biochem. Biophys. 465, 38-43, 2007



Donor site

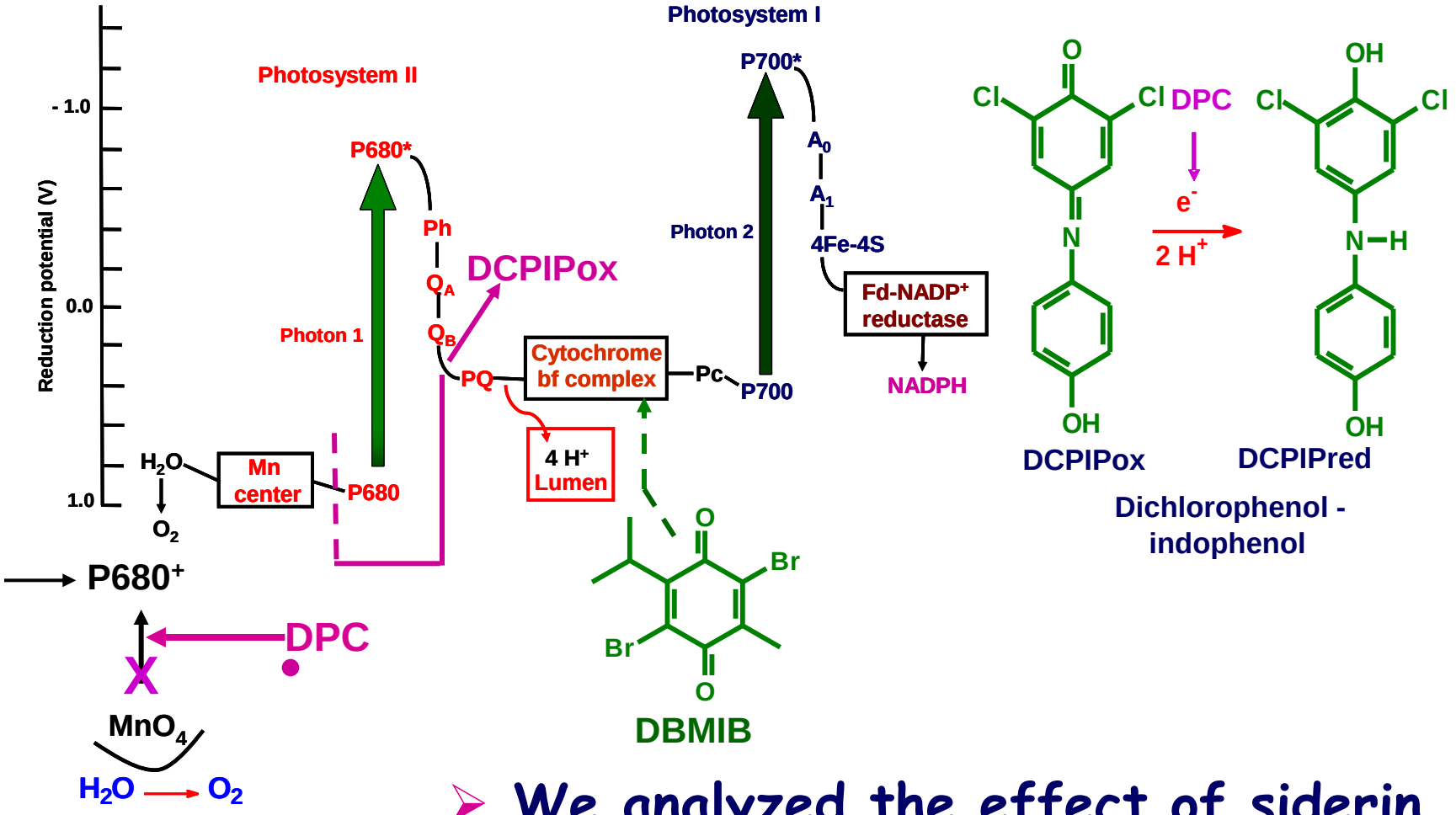
❖ To confirm the siderin site of interaction on PSII between H_2O to $P680^+$, we use electron donor which bind in the donor site of PSII, as DPC, diphenylcarbazide.





Donor site

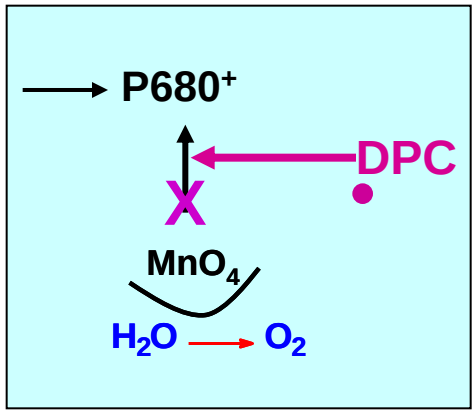
❖ With DPC, we use Tris-treated chloroplasts that inhibit the water-oxidation enzyme activity, thus we do not have the electron flow from water, but from DPC.

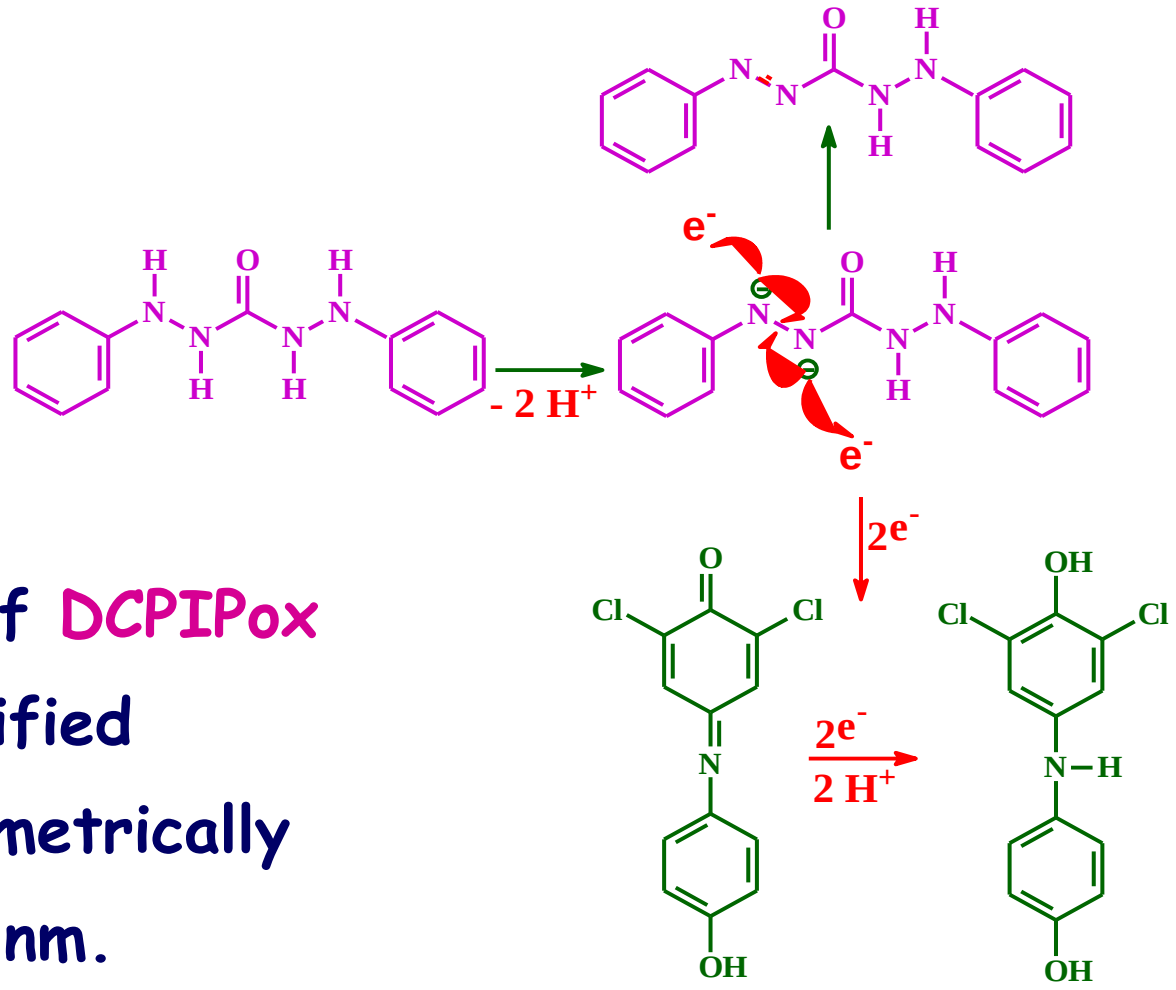


Donor site

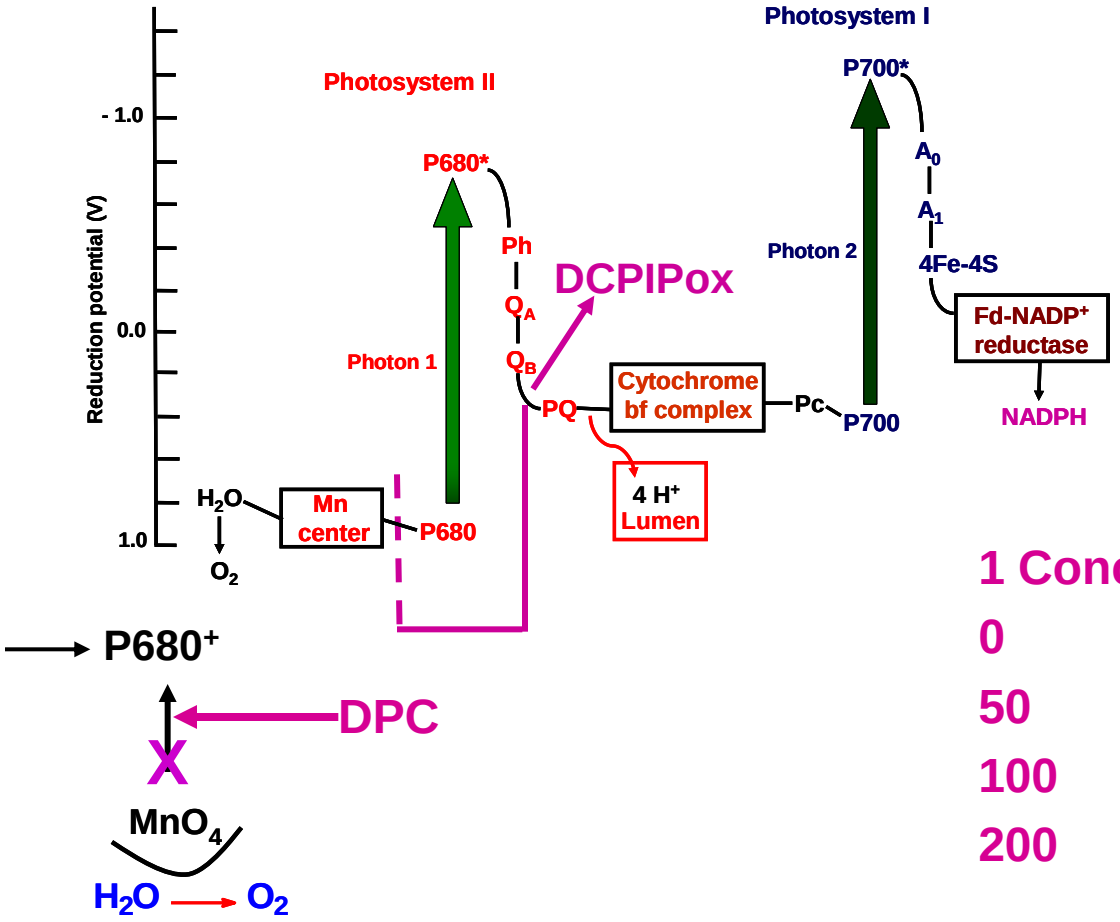
➢ We analyzed the effect of siderin on electron transport from DPC to DCPIPox; then to Q_B.

➢ Reaction of electron transport from DPC (diphenylcarbazide) to DCPIPox:





➢ Reduction of DCPIPox is quantified spectrophotometrically at 600 nm.



PSII	
1 Conc. μM	DPC to DCPIP (%)
0	100
50	83
100	58
200	57

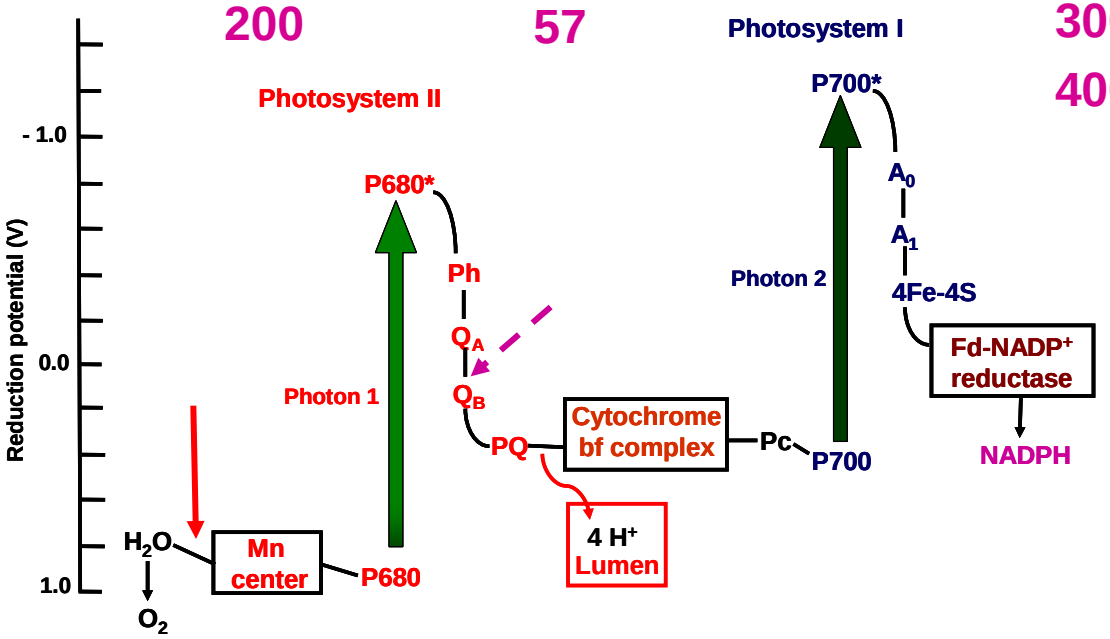
Donor site

- Electron transport from DPC to DCPIPox was partially inhibited at all concentrations tested.

Veiga, da Silva et al, Arch. Biochem. Biophys. 465, 38-43, 2007

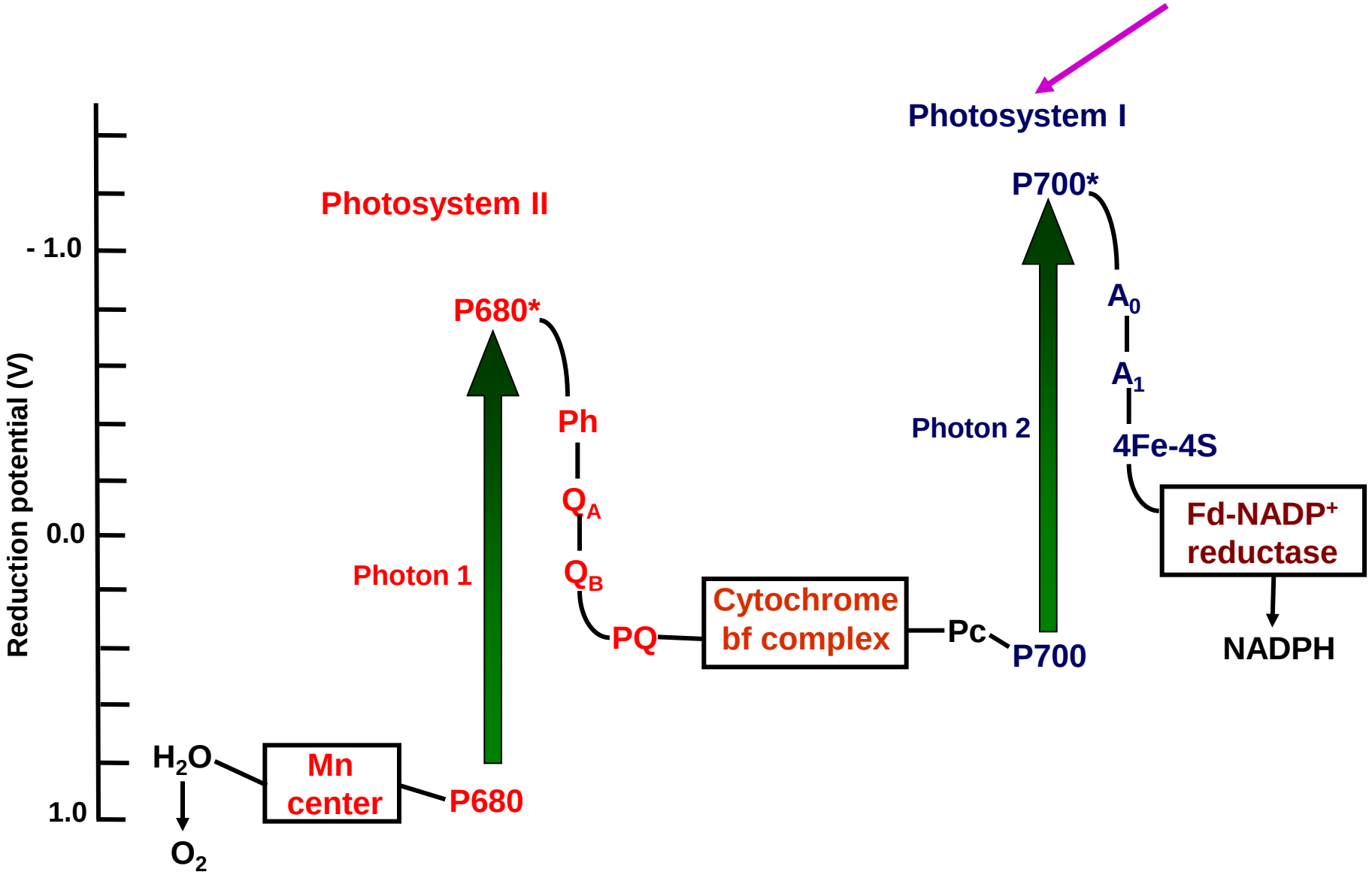
1 Conc. μM	PSII DPC to DCPIP (%)
0	100
50	83
100	58
200	57

1 Conc. μM	PSII H_2O to SiMo (%)
0	100
100	75
200	50
300	12
400	0

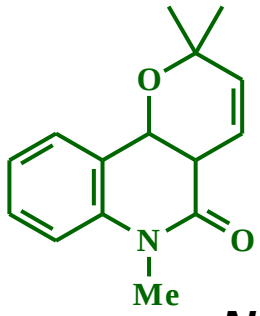


- The two results indicated that the target of siderin is located at the donor and acceptor sides of PSII, between P_{680} to Q_A.

➢ However, siderin did not affect PSI



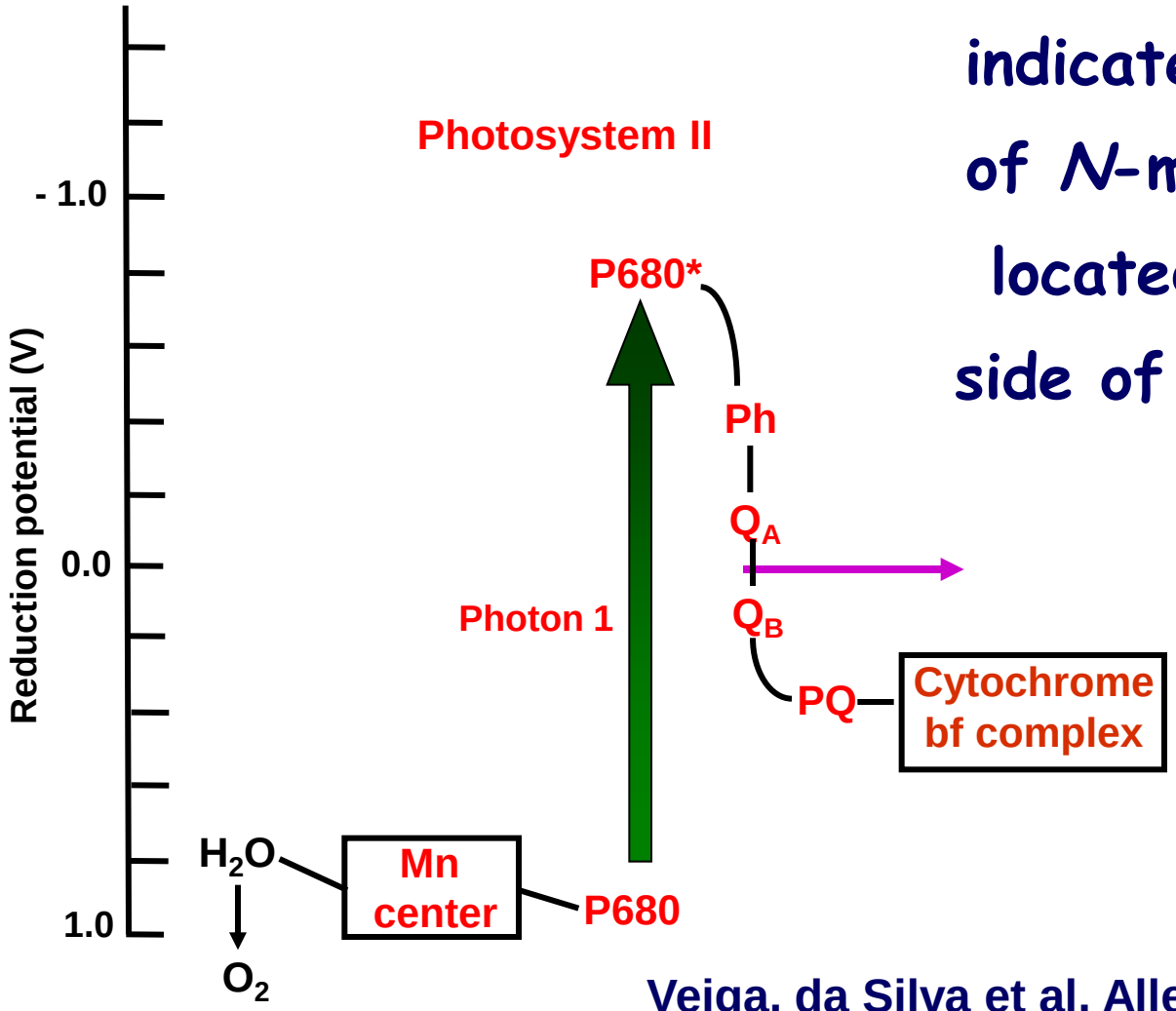
Veiga, da Silva et al, Arch. Biochem. Biophys. 465, 38-43, 2007



N-Me-Flindersine

- *N*-methylflindersine does not inhibit electron transport from H_2O to SiMo, but inhibited electron transport from DCP to DCPIP.

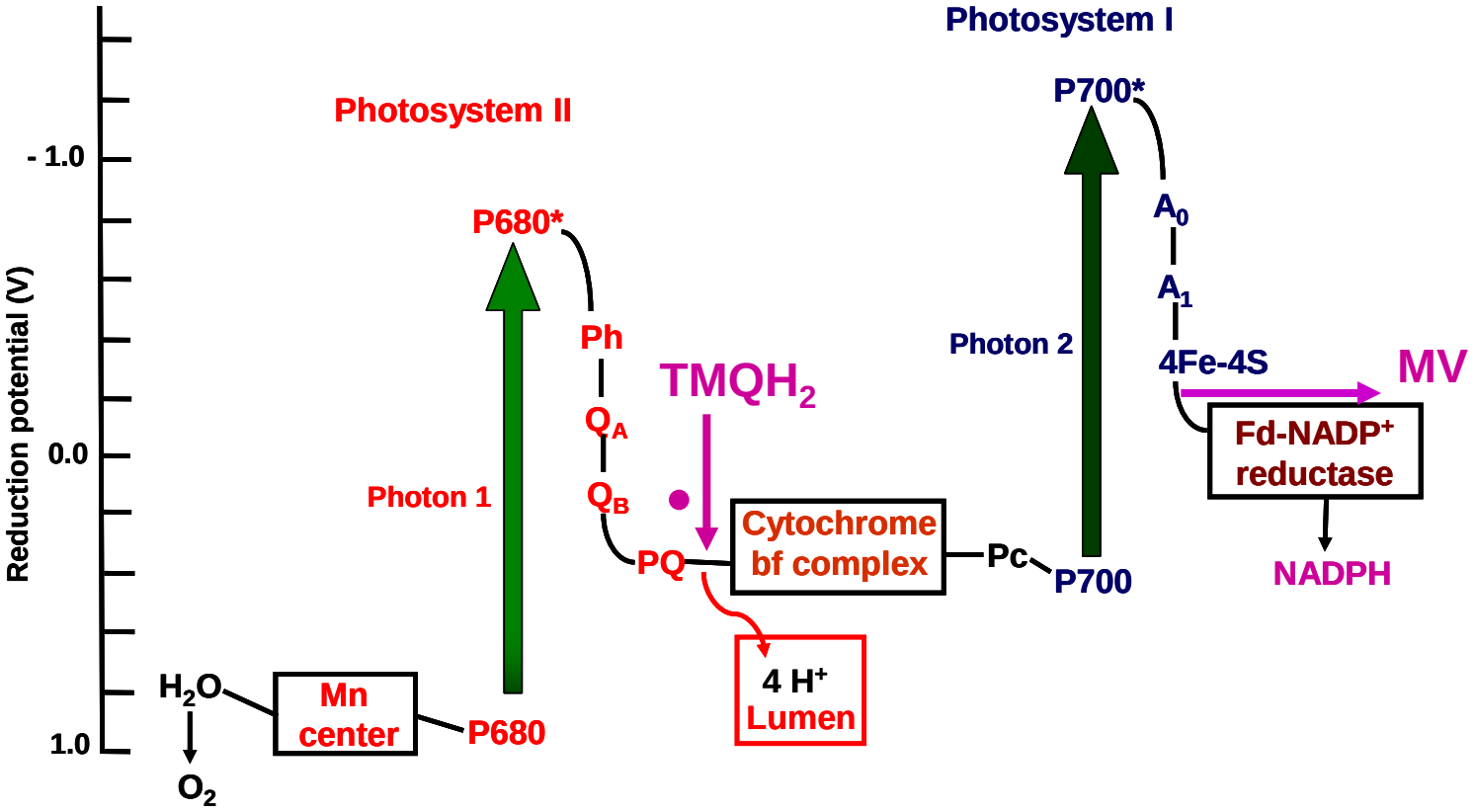
Veiga, da Silva et al, Allelopathy J. 21, 133-144, 2008



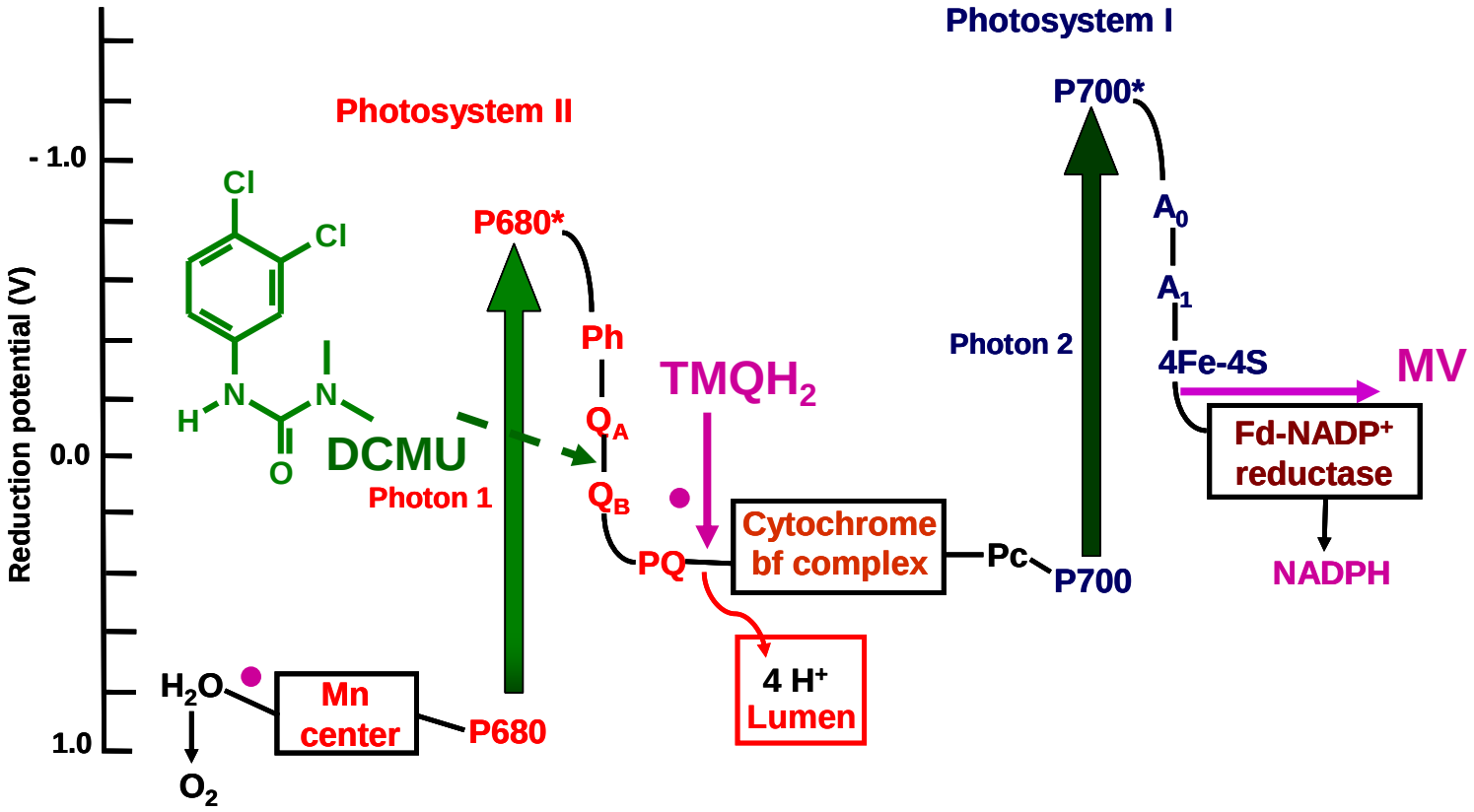
➢ Thus, the results indicated that the target of *N*-methylflindersine is located at the acceptor side of PSII, between Q_A and Q_B.

Veiga, da Silva et al, Allelopathy J. 21, 133-144, 2008

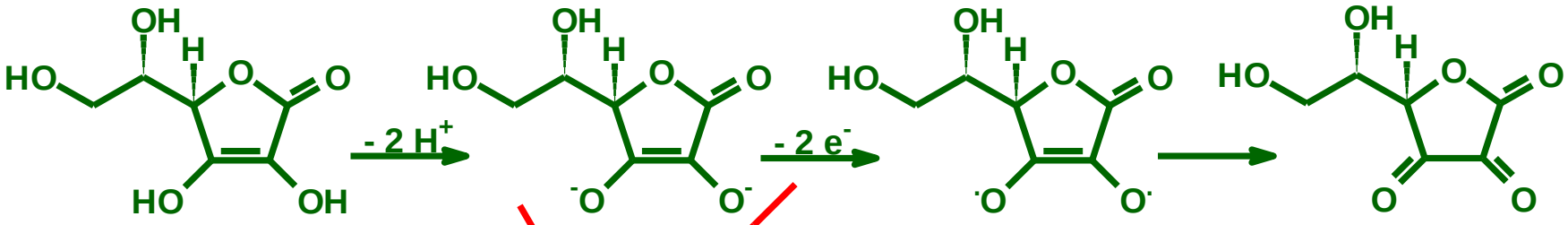
Localization of *N*-methylflindersine sites of interaction on PSI



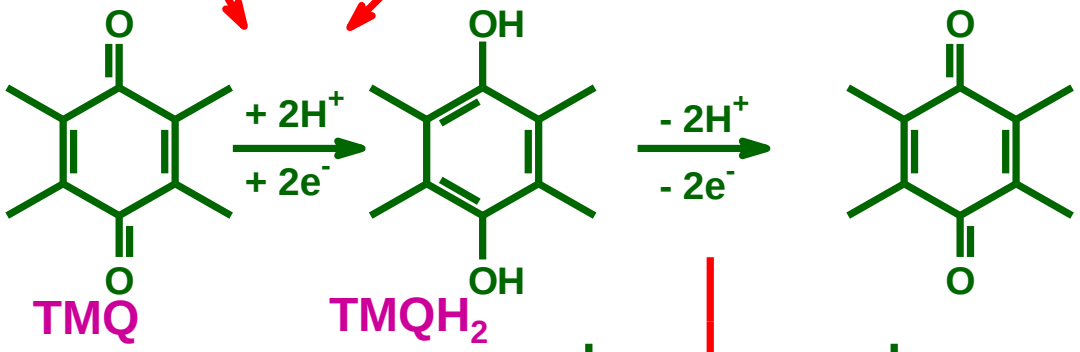
- We analyzed the effect of *N*-methylflindersine on electron transport from PSII using $TMQH_2$ to MV.



- We also add DCMU, which binding in plastoquinone site, inhibiting the electron transport from H_2O to Q_B, or PSI.
- Then, electrons flow from $TMQH_2$ to MV.

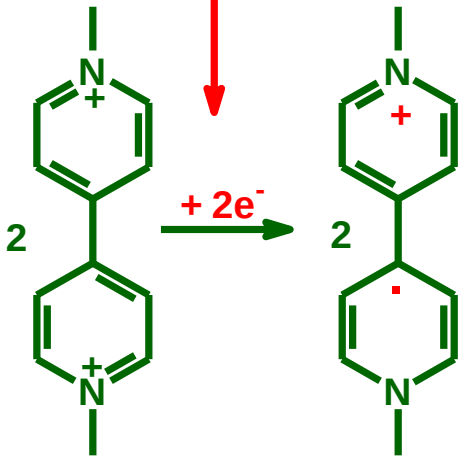


Ascorbic acid

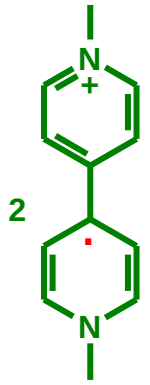


TMQ $TMQH_2$

Tetramethylhydroquinone ($TMQH_2$) is oxidized to tetramethylquinone and transfer electrons to MV.

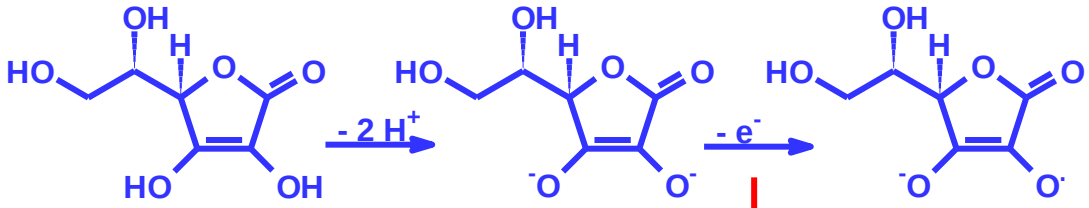


Methylviologen (MV)



$2MV^{\bullet+}$

$$2MV^{\bullet+} \xrightarrow{2O_2} 2MV^{2+} + 2O_2^{\bullet-}$$

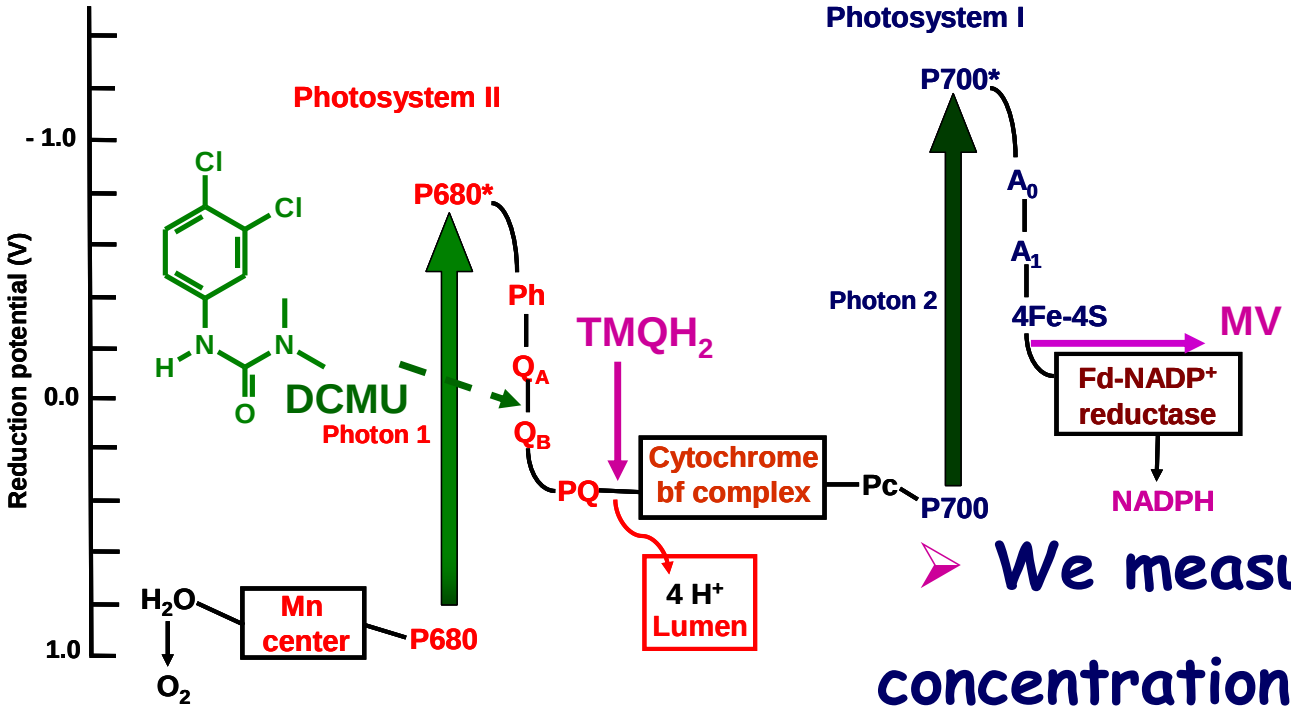


$$O_2^{\bullet-} \rightarrow O_2$$

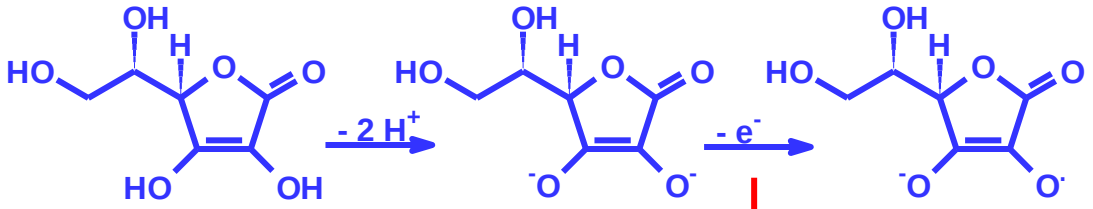
$$O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2$$

Hydrogen peroxide

The oxygen concentration decrease.



➢ We measure the oxygen concentration with an oxygen electrode (Clark-type electrode)



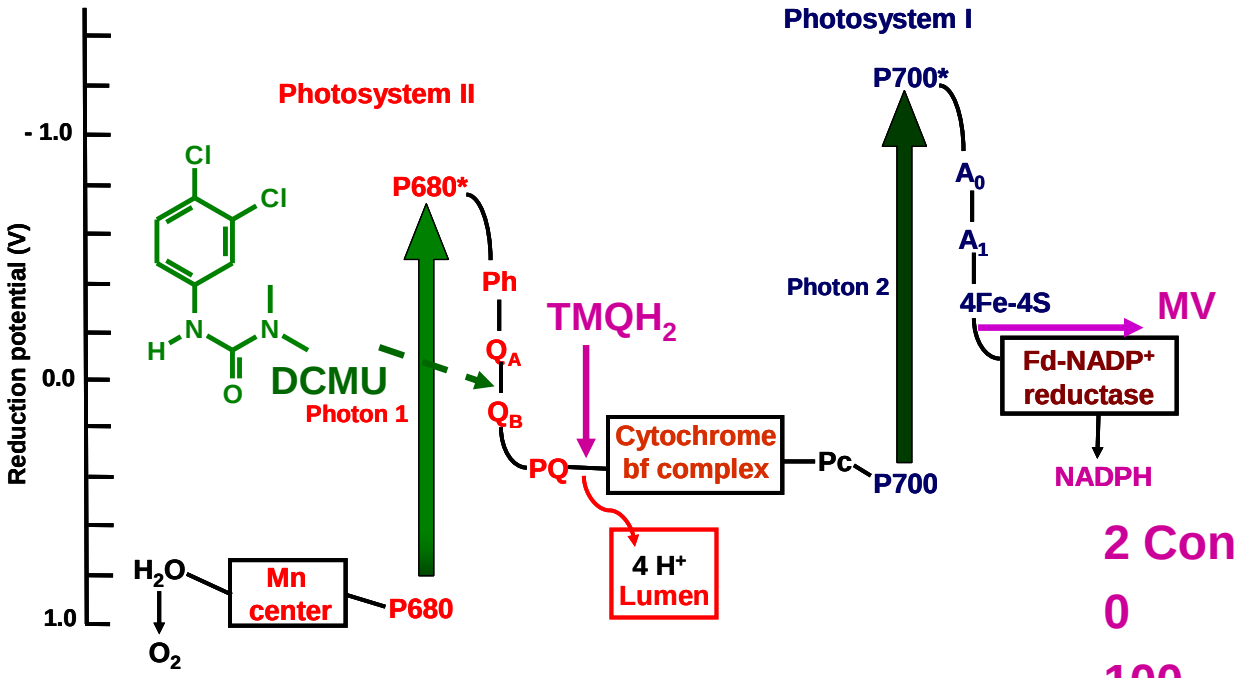
$O_2^{\cdot -}$ → O_2

$O_2^{\cdot -}$ | $2H^+$

H_2O_2

The oxygen concentration decrease.

Localization of *N*-methylflindersin sites of interaction on PSI

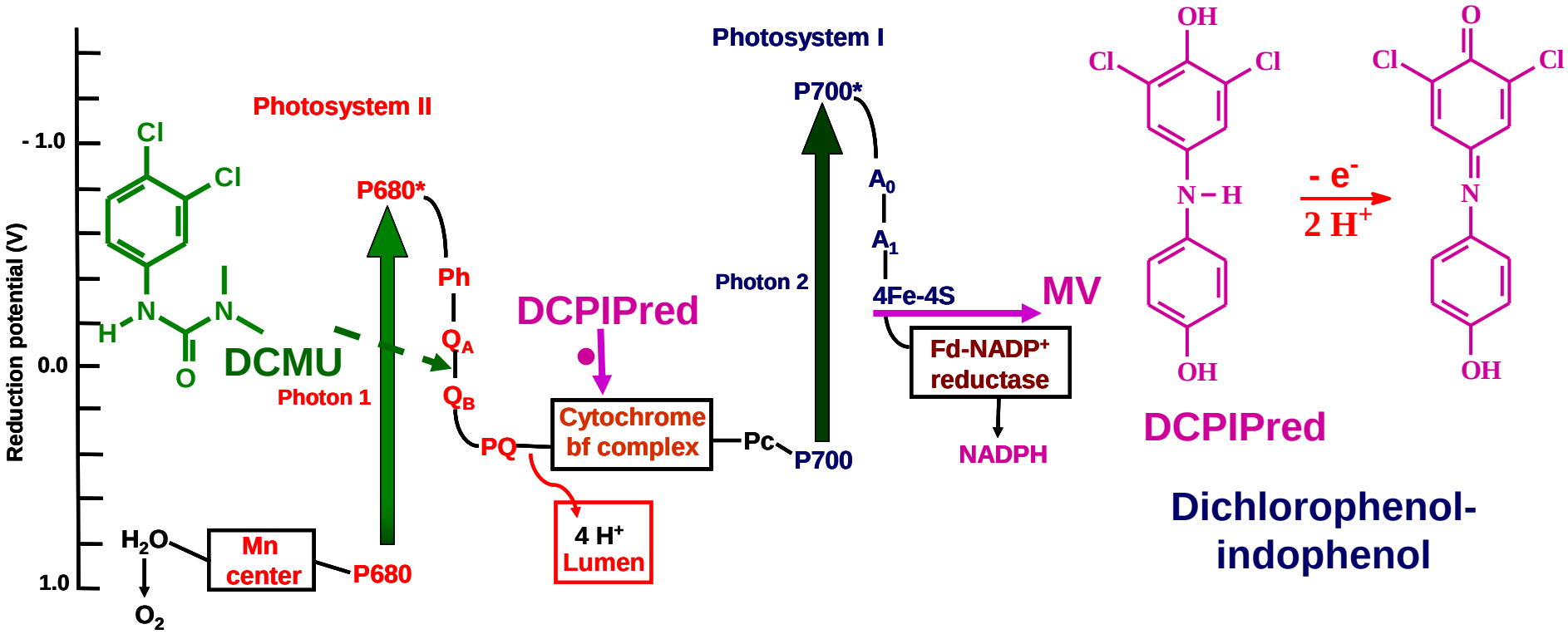


PSI	
2 Conc. μM	$TMQH_2$ to MV (%)
0	100
100	82
200	73
300	45
400	36
500	0

- At 500 μM *N*-methylflindersine completely inhibited electron transport from $TMQH_2$ to MV.

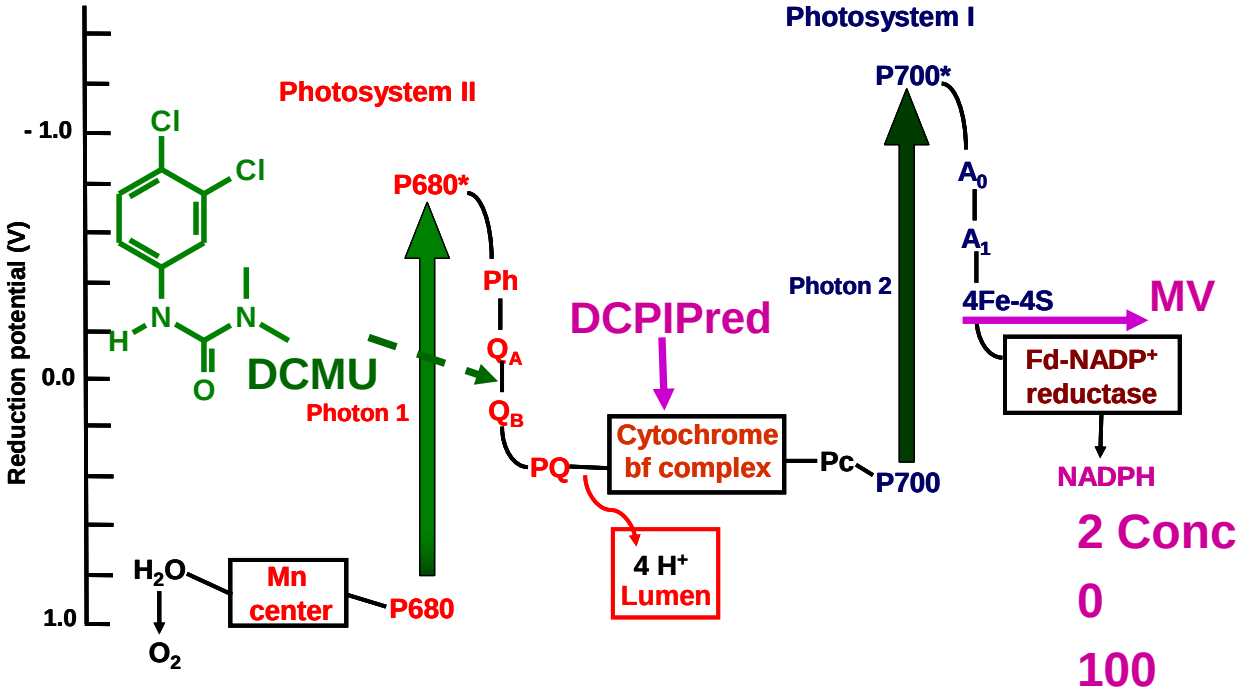
Veiga, da Silva et al, Allelopathy J. 21, 133-144, 2008

Localization of *N*-methylflindersin sites of interaction on PSI



- We analyzed the effect of *N*-methylflindersine on electron transport from Cy bf complex using DCPIPred to MV; then to 4Fe-4S.

Localization of *N*-methylflindersin sites of interaction on PSI

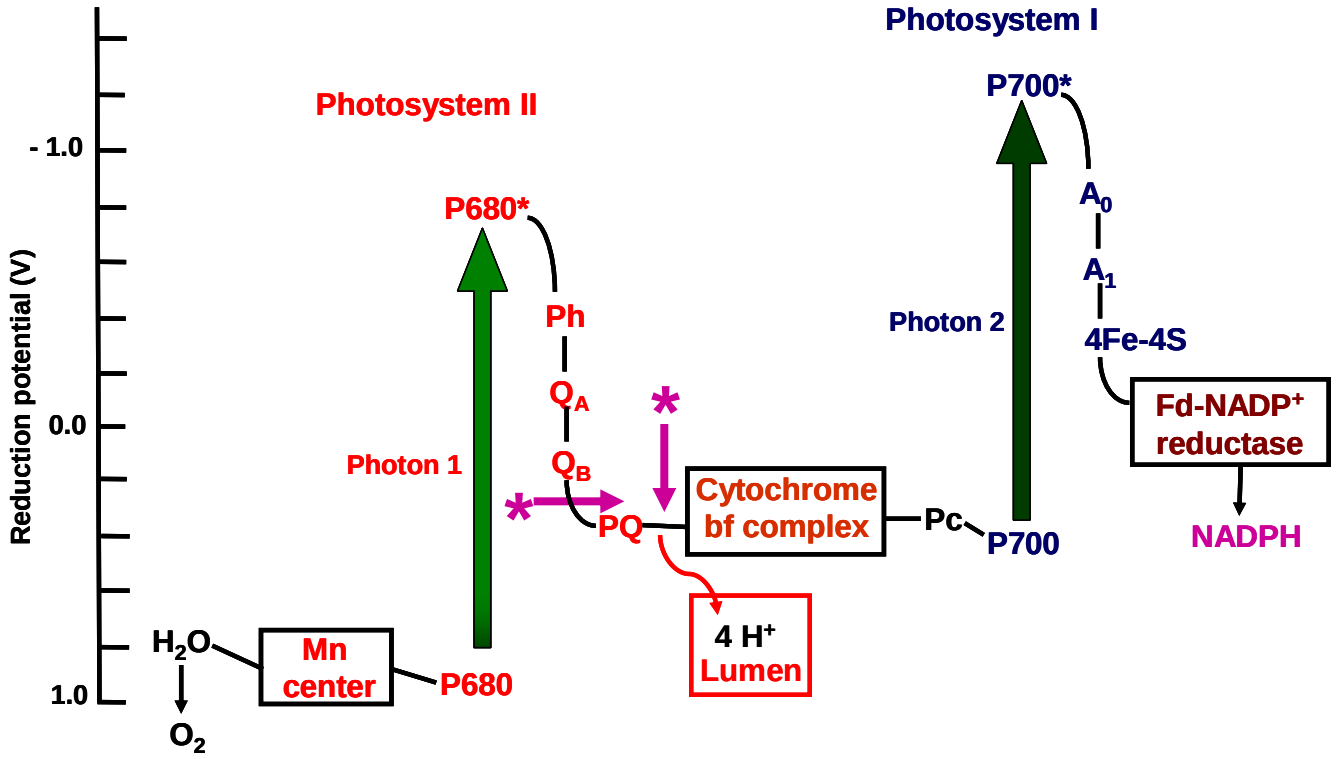


2 Conc. μM	PSII DCPIPred to MV (%)
0	100
100	-
200	100
300	100
400	-
500	-

- *N*-methylflindersine did not inhibit electron transport from DCPIPred to MV.

Veiga, da Silva et al, Allelopathy J. 21, 133-144, 2008

Localization of *N*-methylflindersin sites of interaction on PSI



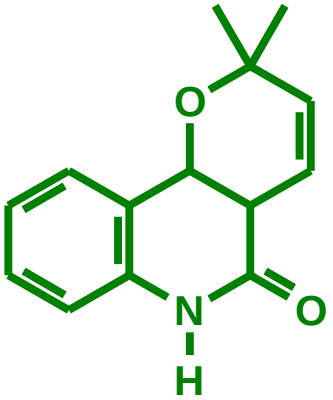
➢ Thus *N*-methylflindersine has two sites of interaction and inhibition, one where Q_B interacts and the second one is at PQH_2 oxidation site.

- Several natural products isolated for our group have been evaluated as inhibitor of photophosphorylation and electron transport chain in thylakoids.

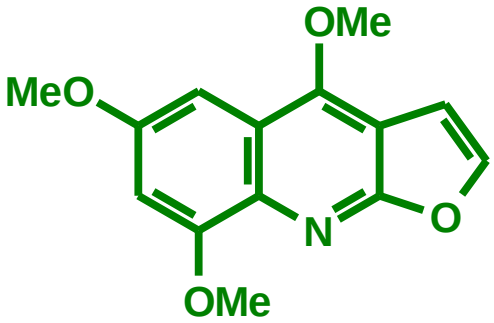
- Among the 200 compounds evaluated, only 16 were the most active:

Veiga, PhD Thesis, UFSCar, Brazil, 2008

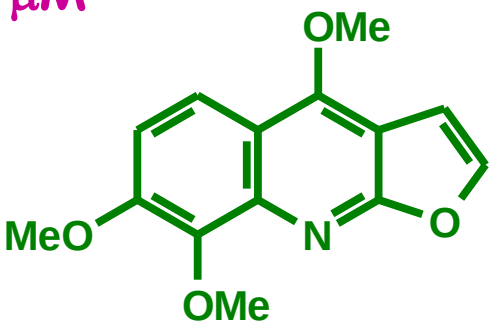
❖ ATP synthesis inhibition by:



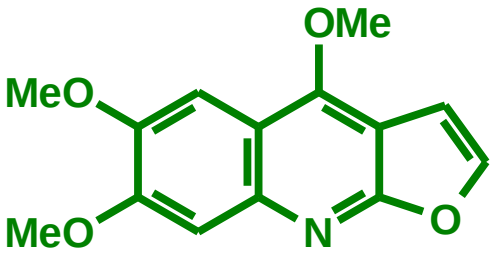
I_{50} 26.0 μM



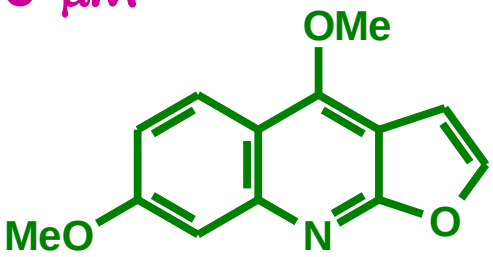
I_{50} 48.0 μM



I_{50} 46.0 μM

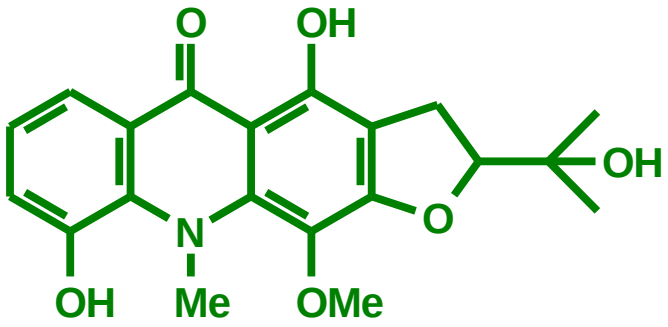


I_{50} 61.5 μM

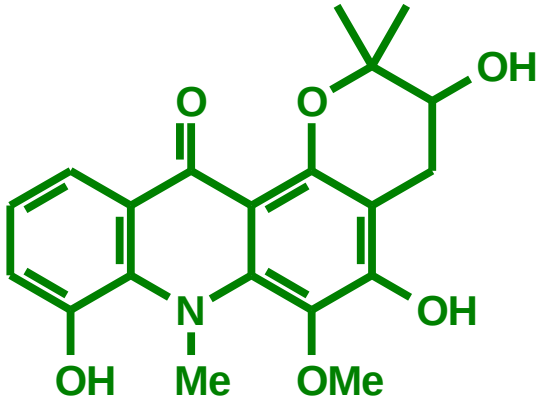


I_{50} 82.0 μM

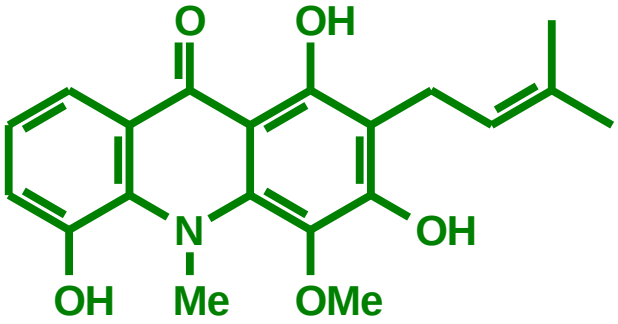
❖ ATP synthesis inhibition by:



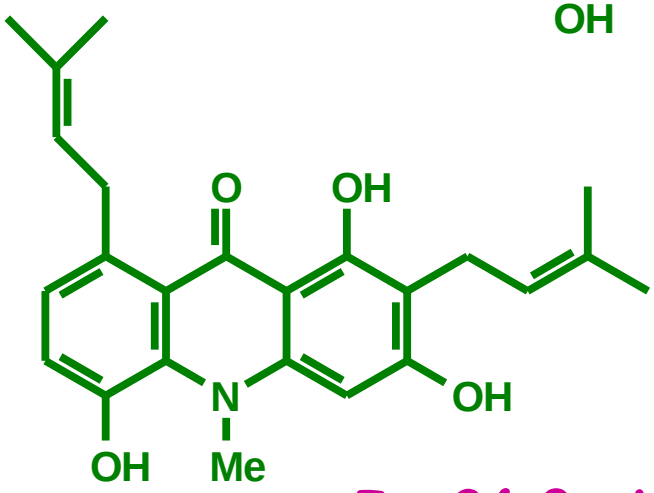
I_{50} 3.3 μM



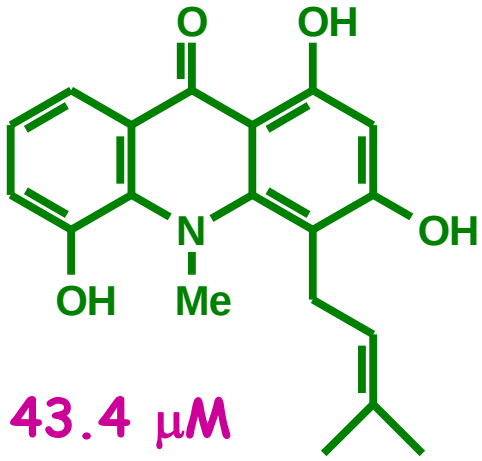
I_{50} 6.5 μM



I_{50} 33.0 μM

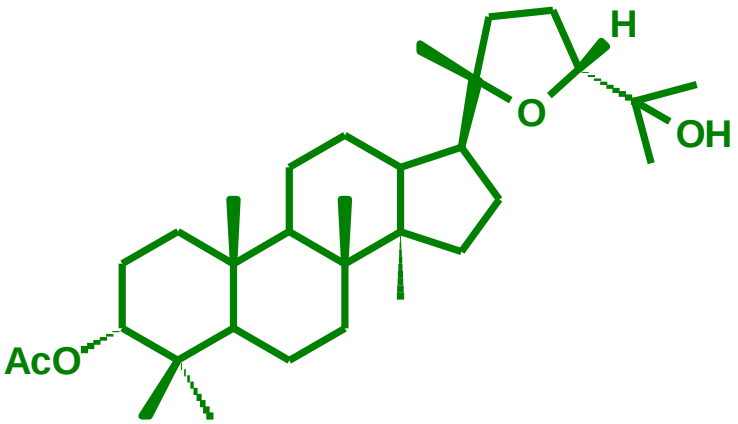


I_{50} 24.0 μM

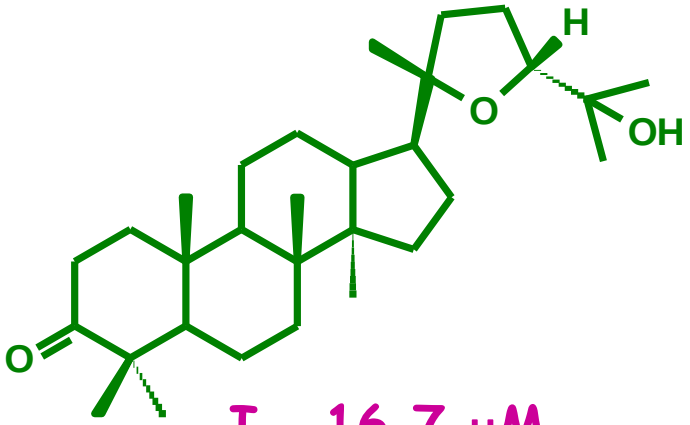


I_{50} 43.4 μM

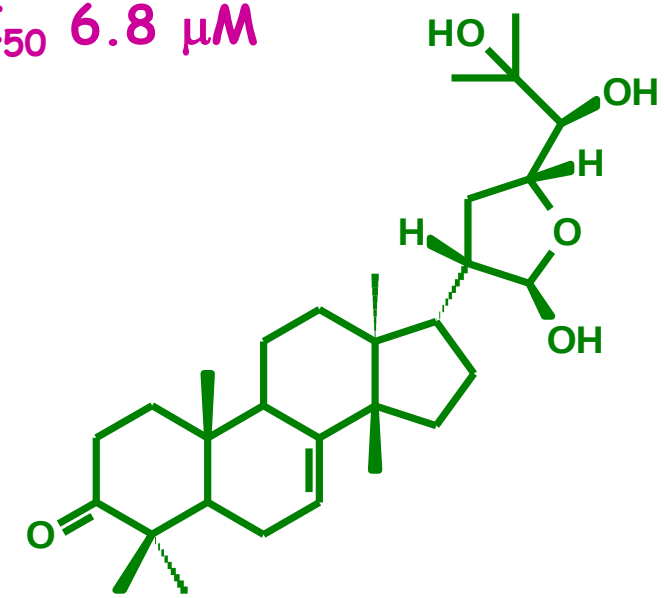
❖ ATP synthesis inhibition by:



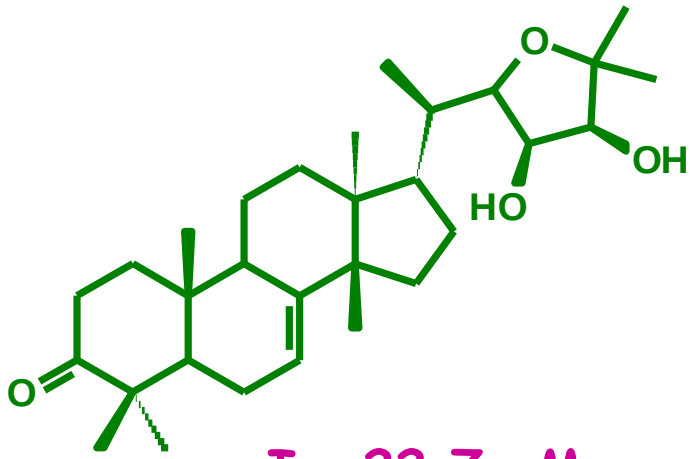
I_{50} 6.8 μM



I_{50} 16.7 μM



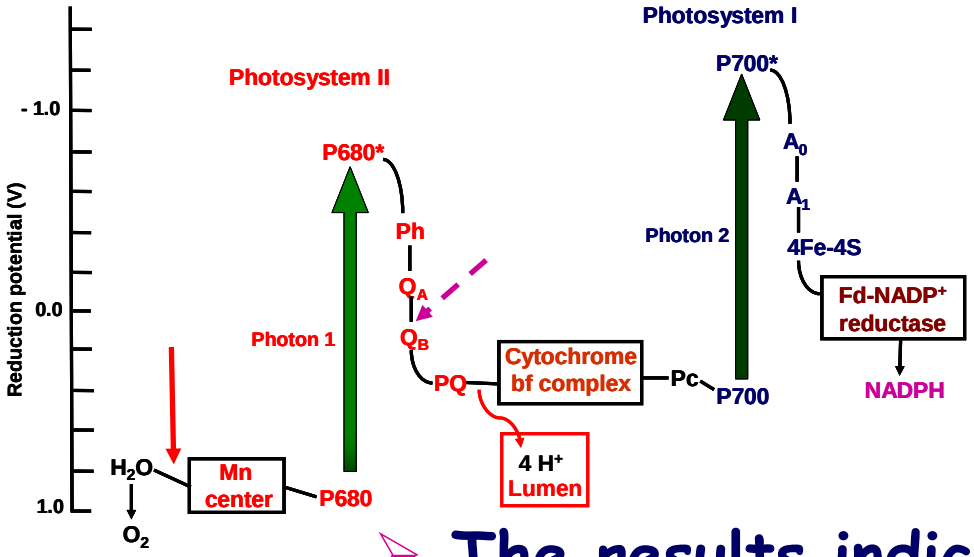
I_{50} 13.1 μM

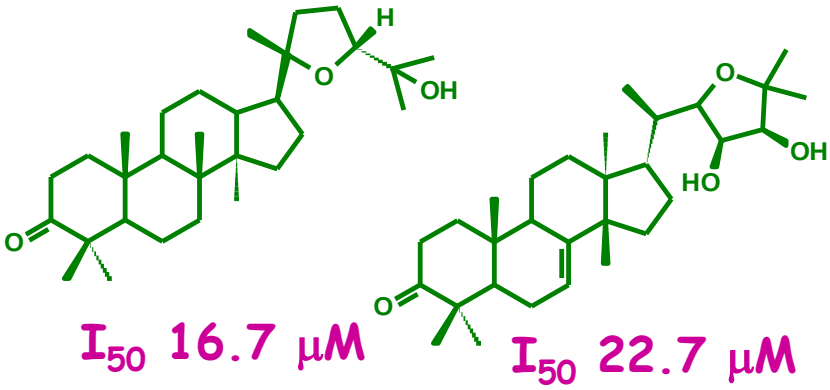


I_{50} 22.7 μM

Veiga, PhD Thesis, UFSCar, Brazil, 2008

❖ Localization of compounds sites of interaction on PSII

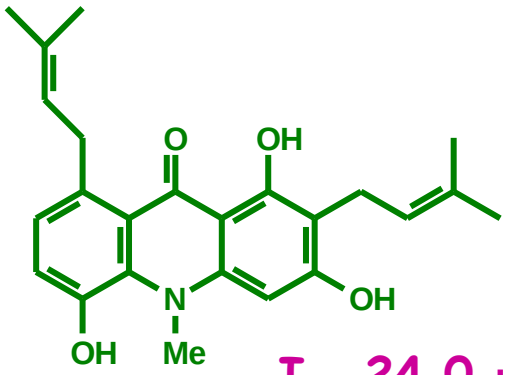




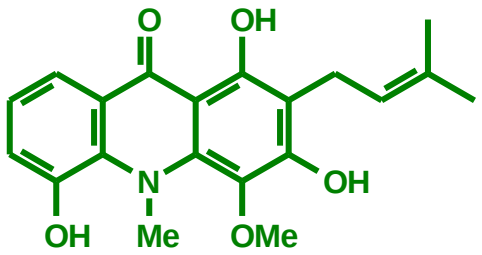
I_{50} 16.7 μM

I_{50} 22.7 μM

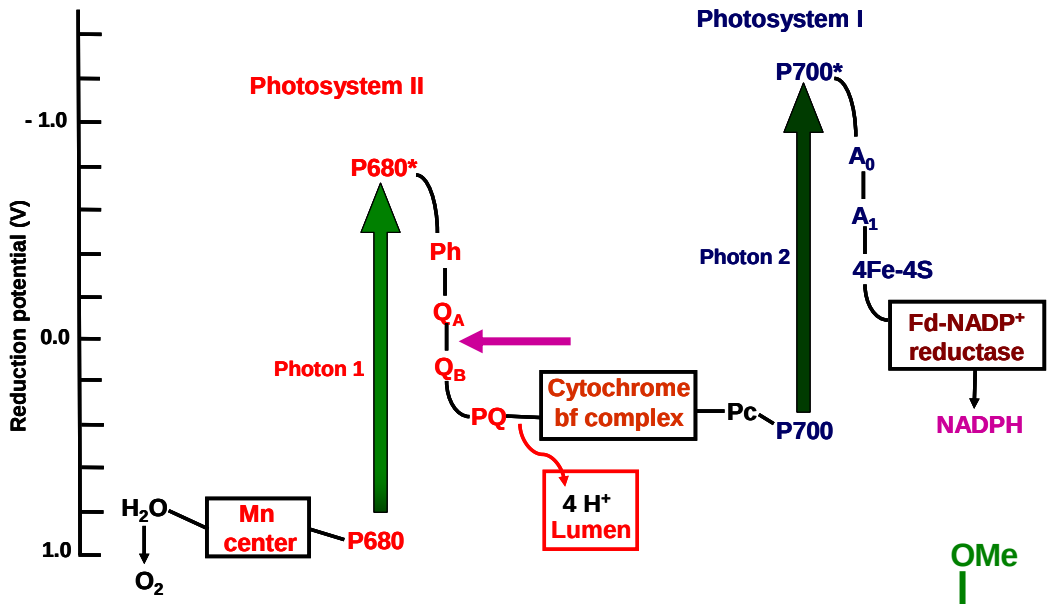
➢ The results indicated that the targets of these compounds are located at the donor and acceptor sides of PSII, between P_{680} to Q_A.

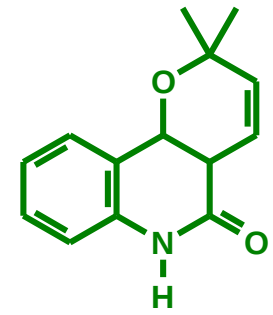


I_{50} 24.0 μM

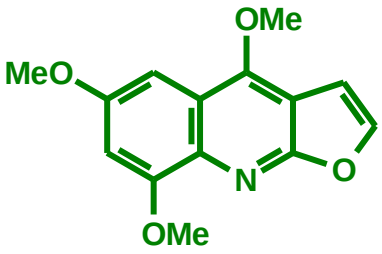


I_{50} 33.0 μM

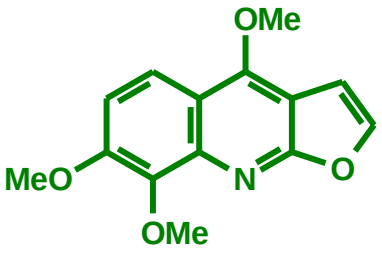




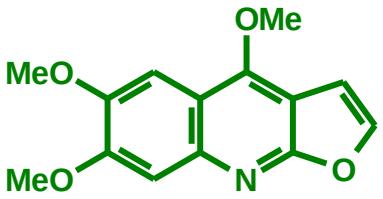
I_{50} 26.0 μM



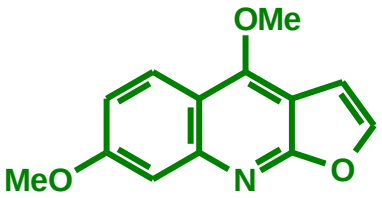
I_{50} 48.0 μM



I_{50} 46.0 μM



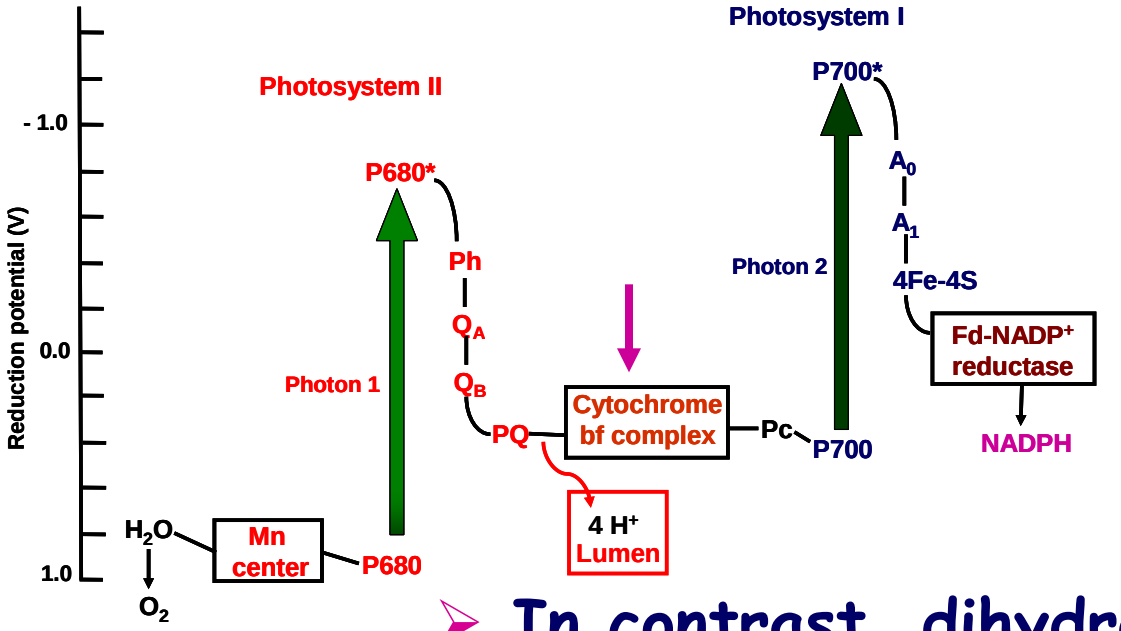
I_{50} 61.5 μM



I_{50} 82.0 μM

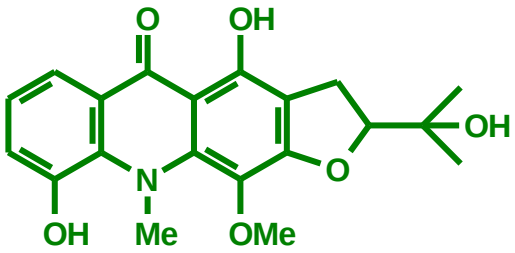
❖ Localization of compounds sites of interaction on PSII

➢ The results indicated that the actions of these compounds are located at the acceptor side of PSII, between Q_A and Q_B.

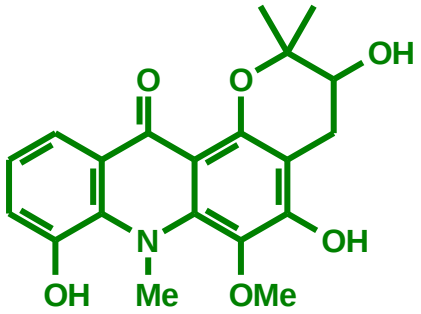


❖ Localization of compounds sites of interaction on PSI

➢ In contrast, dihydropyranoacridones and dihydrofuroacridones have the site of interaction and inhibition at Cy bf complex.



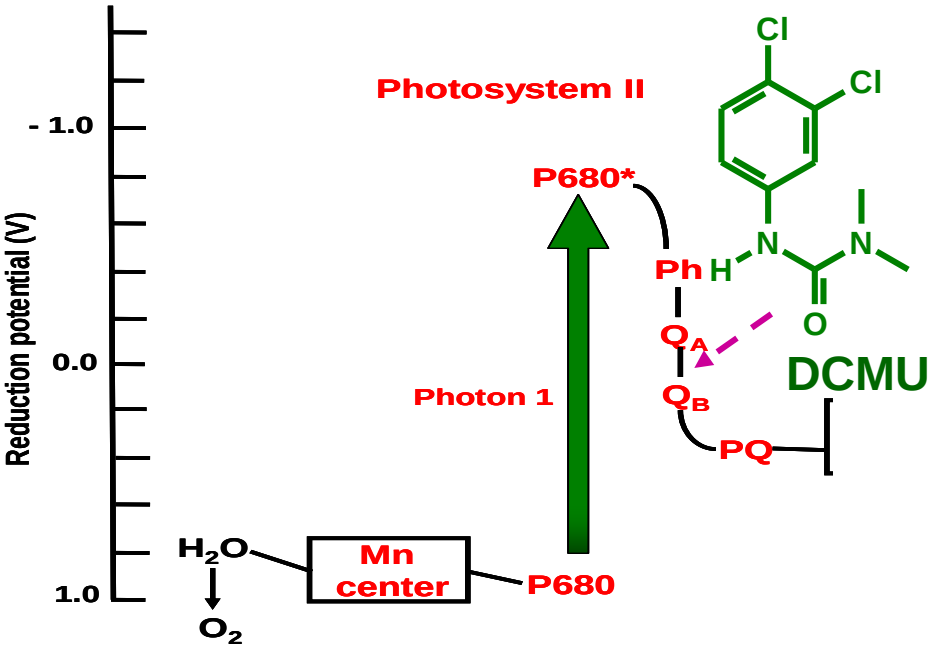
I_{50} 3.3 μM

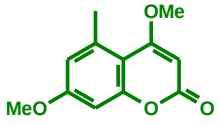


I_{50} 6.5 μM

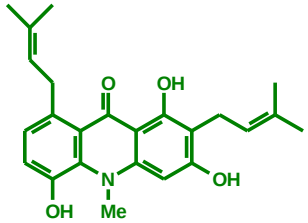
Veiga, PhD Thesis, UFSCar, Brazil, 2008

Concluding Remarks

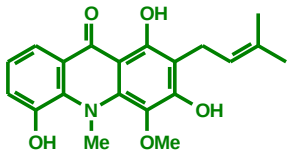




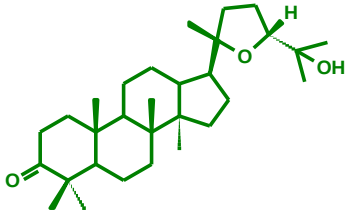
I_{50} 27.0 μM



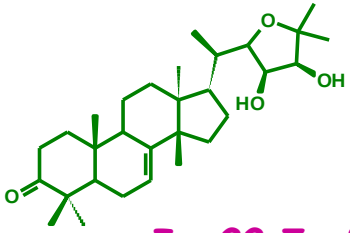
I_{50} 24.0 μM



I_{50} 33.0 μM



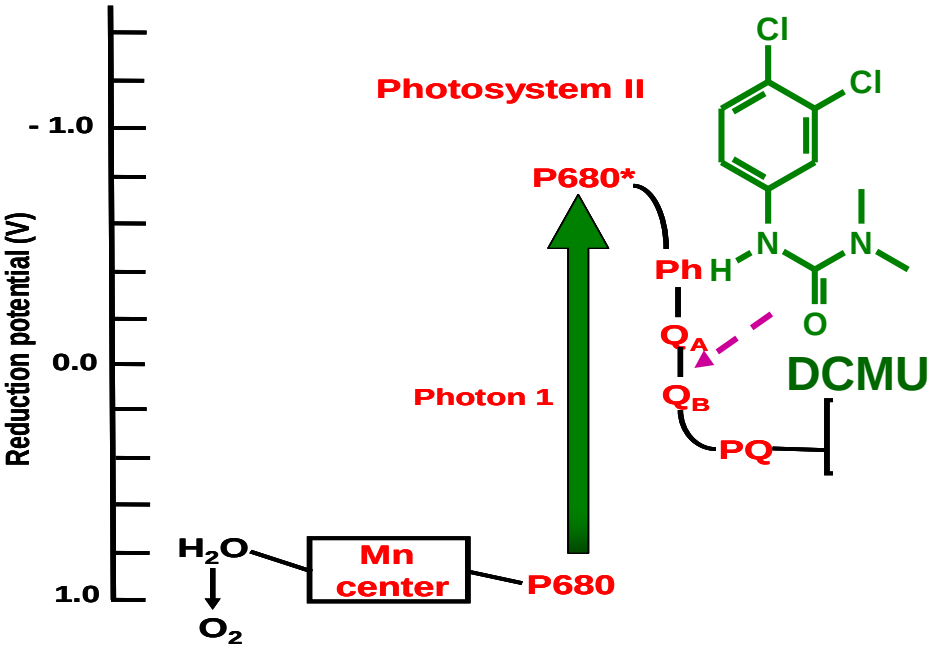
I_{50} 16.7 μM

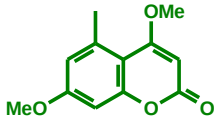


I_{50} 22.7 μM

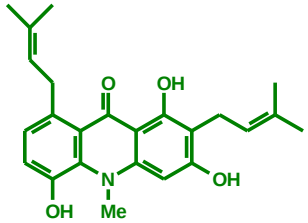
- Many commercial herbicides kill weeds by interfering with the action of PSII or PSI. PSII inhibitors include urea derivatives, such as *diuron* (DCMU), one of the most sold herbicides. The mechanism of action of these compounds were similar to DCMU.

Concluding Remarks

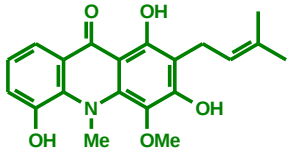




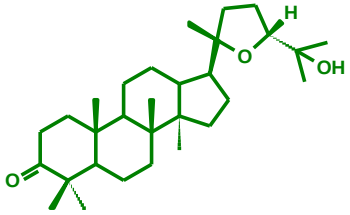
I_{50} 27.0 μM



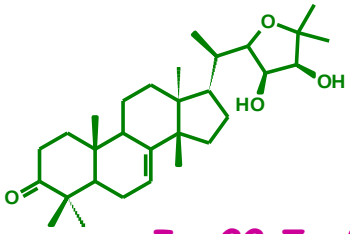
I_{50} 24.0 μM



I_{50} 33.0 μM



I_{50} 16.7 μM



I_{50} 22.7 μM

- If its assumed that it is possible to modify the chemical structure of compounds to improve activity, our results are so good.
- Experiments *in vivo* are in progress.

FINALLY WE ARE GRATEFUL TO OUR STUDENTS

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UNVERSIDAD NACIONAL AUTONOMA DE MÉXICO, MÉXICO

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