FACULDADE · DE · CIÊNCIAS UNIVERSIDADE · DE · LISBOA

DEPARTAMENTO DE BIOLOGIA VEGETAL

MESTRADO EM BIOLOGIA CELULAR E BIOTECNOLOGIA

PHOTOPROTECTION AND PHOTOINHIBITION IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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THE DIATOMS (BACCILARIOPHYCEAE)

Phytoplankton, ≈ 200,000 species, all aquatic environments

Fucoxanthin, Chl *c*

Major carbon fixators

40% of total CO_2 in oceans 25% global

Phaeodactylum tricornutum (Pennales)

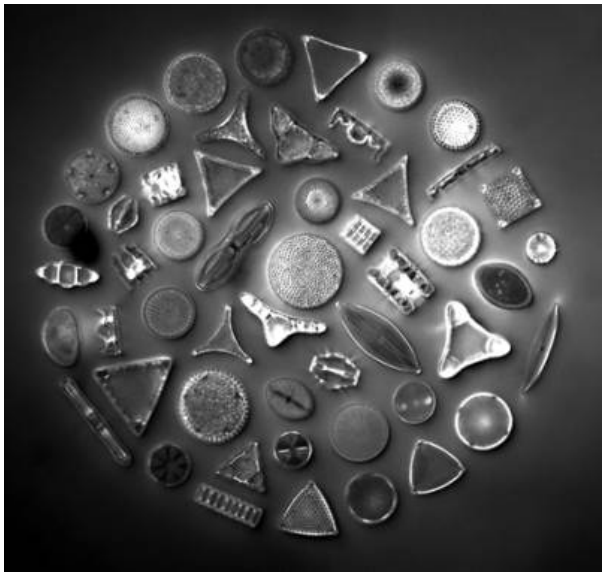


Fig. 1 – Diatoms intricate silica frustules (Photo by Randolph Femmer).

PHOTOSYNTHESIS IN WATER

Different pigments, bigger diversity | **Light transmission differs** | **iC may be limiting for some species**

PHOTOSYNTHETIC MACHINERY: PHOTOSYSTEM II

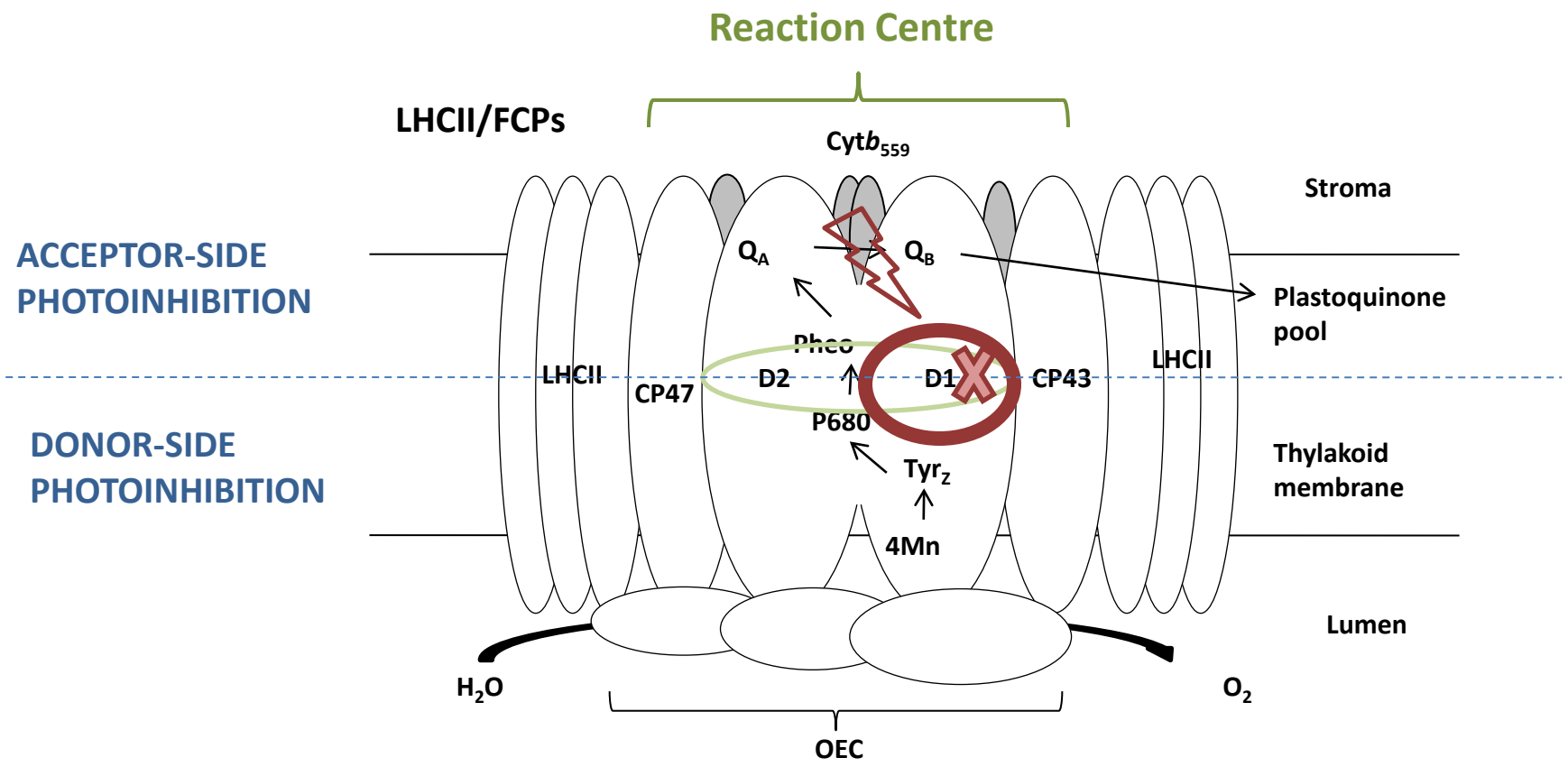


Fig. 2 – Assembled photosystem II, OEC and LHCII in thylakoid membranes. Adapted from Yamamoto et al. (2008).

Open Reaction Centres can start a new electron transfer reaction

PHOTOPROTECTION AND PHOTOINHIBITION

PHOTOPROTECTION

Negative phototaxis, Accessory pigments, ROS scavengers, FCPs, Sinalization of translation of specific enzymes…

NPQ – non-photochemical quenching

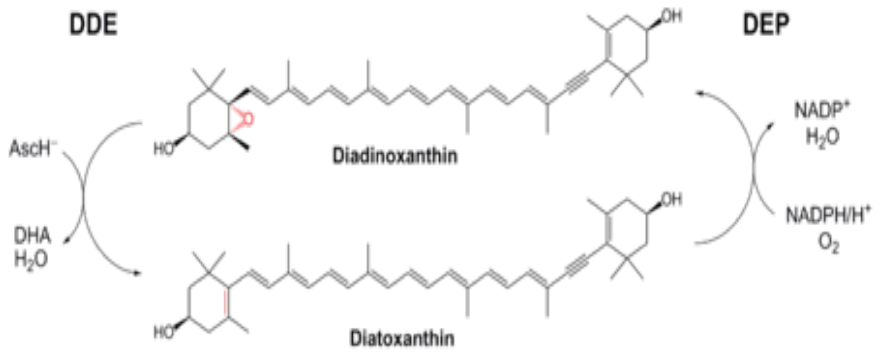


Fig. 3 – Epoxidation of DT to DD and de-epoxidation of DD to DT.

If repair can't keep up with the damage inflicted

PHOTOINHIBITION

Insufficient photoprotection mechanisms

Damaged PSII, mainly the D1 protein

PSII Repair

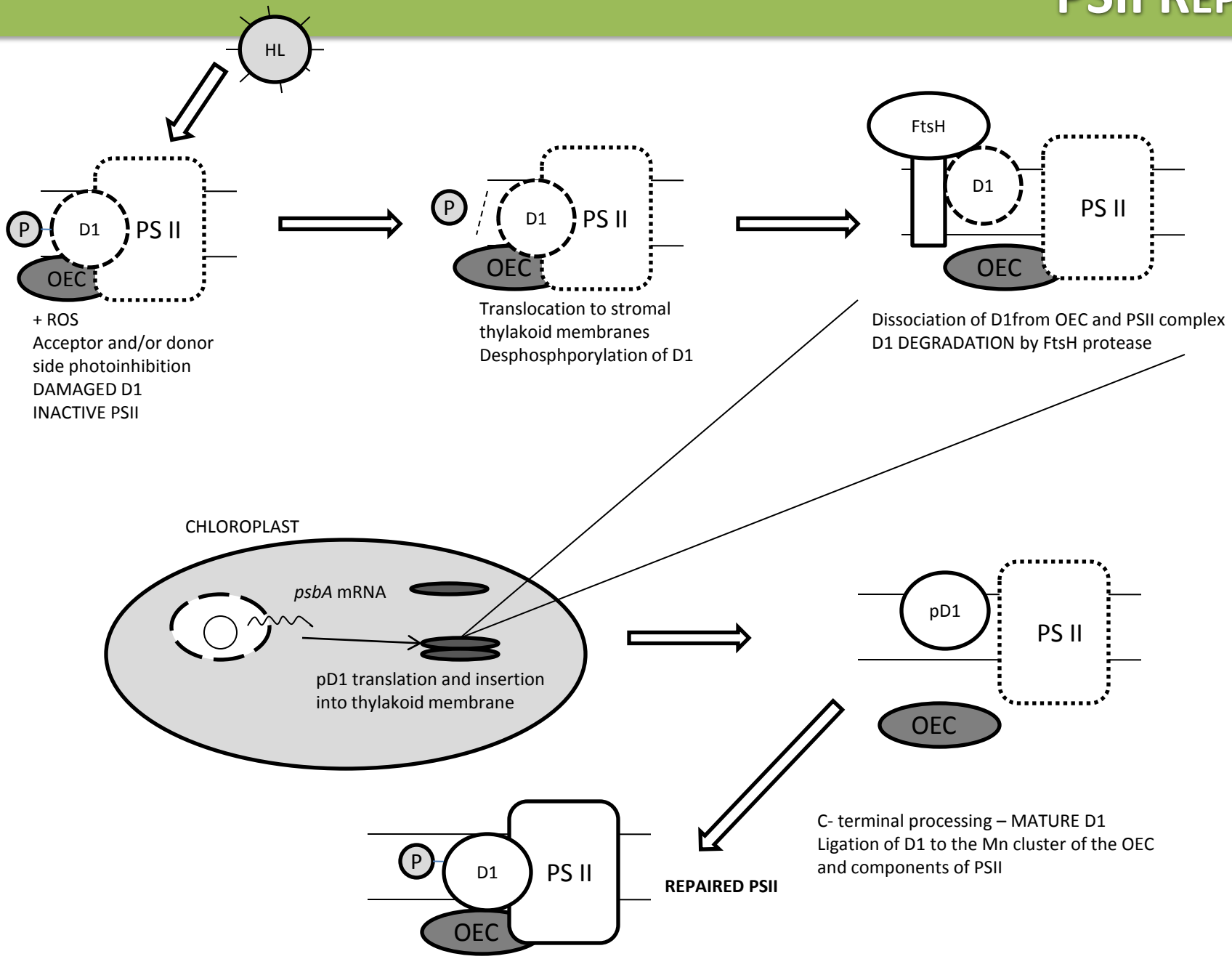


Fig. 4 – PSII repair. Adapted from Nixon et al. (2010).

METHODS – CULTURES AND PROTEIN EXTRACTION

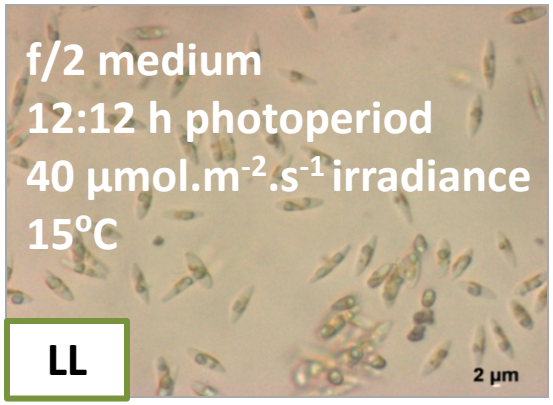
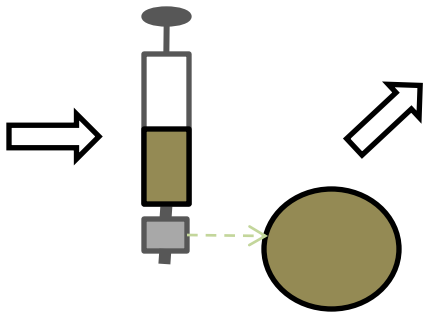


Fig. 5 – *Phaeodactylum tricornutum*.



EXTRACTION BUFFER:

Na_2HPO_4 / NaH_2PO_4 pH7.4
EDTA
Water
Tween 20
DTT
PMSF

HIGH LIGHT STRESS:
1h, 15ºC
1,250 µmol.m^{-2}.s^{-1} irradiance

HL

\+ **Lincomycin**
Chloroplast protein synthesis inhibitor

LLi
HLi

Sonication 1 min 220V

4x freeze-thaw cycles

QUANTIFICATION:

Bradford microassay
BSA standard < 25µg.mL^{-1}

Methods – Western immunoblotting

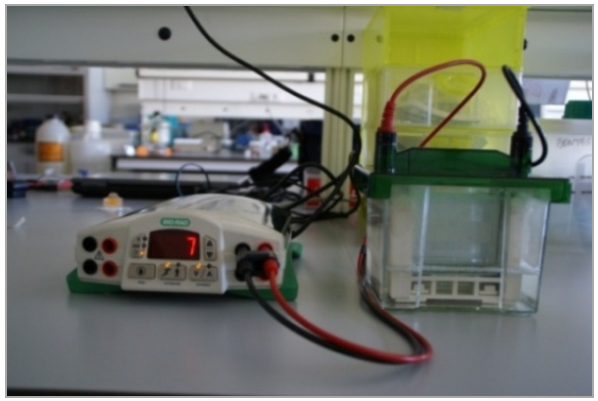


Fig. 6 – SDS-PAGE (Bio-Rad).

Fig. 7 – Transfer to membrane (Bio-Rad).

2 µg total protein

Anti-psbA antibody (1 : 20,000)

HSP anti-rabbit IgG (1 : 40,000)

Fig. 8 – Blocking reagent and antibodies' incubations.

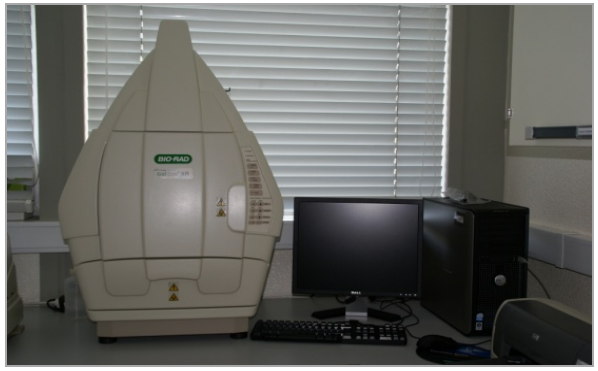


Fig. 9 – Imager (Bio-Rad).

METHODS – PAM FLUOROMETRY

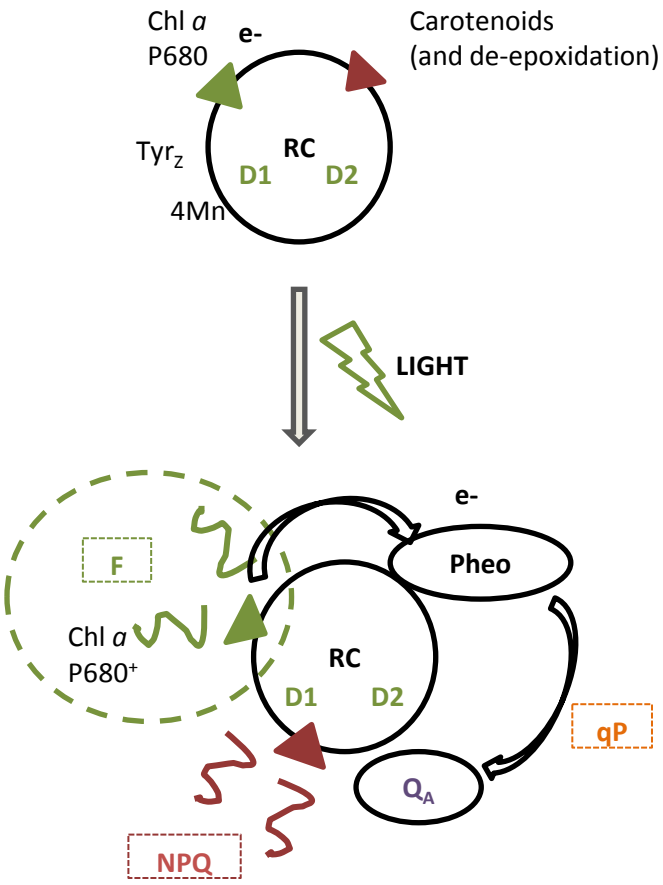


Fig. 10 – Open and closed reaction centres.

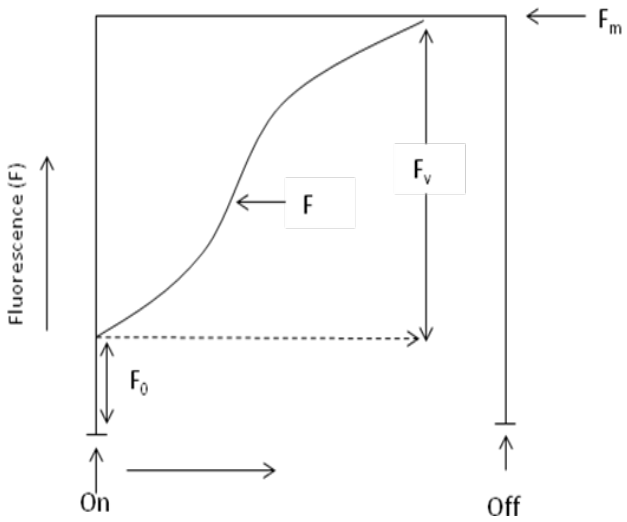


Fig. 11 – Kautsky curve. Adapted from Falkowsky e Raven (1997).

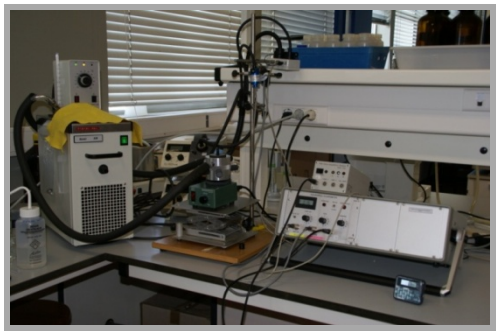


Fig. 12 – PAM101 fluorometer (Walz).

$$\Phi_{PSII} = F_v'/F_m' \times qP$$ (Genty et al., 1989)

$$NPQ = (F_m - F_m')/F_m'$$ (Bilger and Bjorkman, 1994)

Yield and **NPQ** changes before and after HL stress and recovery

METHODS – PIGMENT ANALYSIS BY HPLC

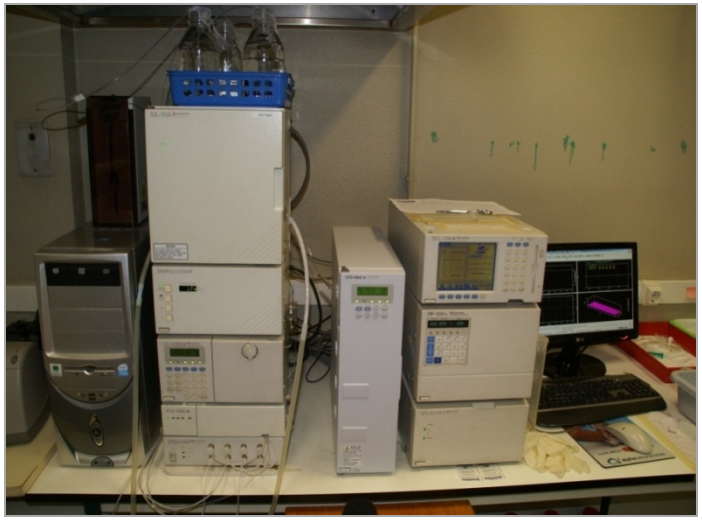


Fig. 13 – Shimadzu HPLC system with diode array detector (DAD).

HPLC-DAD

Flow rate 0.6 mL.min^{-1}
440 nm

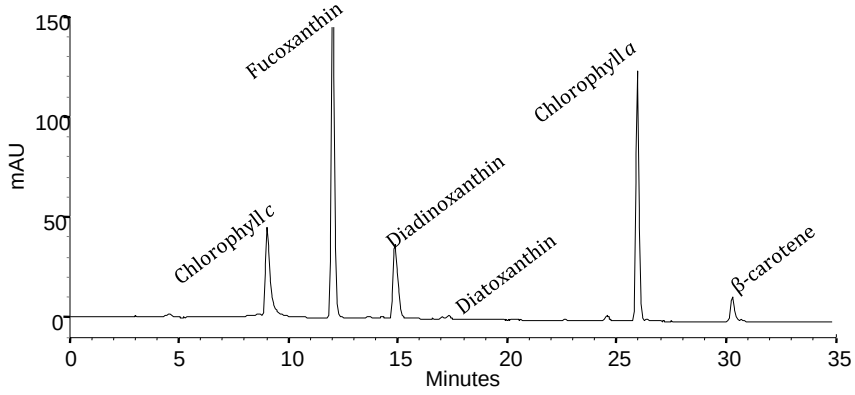


Fig. 14 – Chromatogram with several pigment areas.

Standards used for calibration

Peak areas for each pigment are determined

RESULTS AND DISCUSSION: PROTEIN EXTRACTION

50 µL extract

98.6% effciciency in extraction

Table I - Bradford microassay quantification

	Amount of total protein extracted				
	Abs. 595 nm				Total protein
	a)	b)	c)	Average	extracted (µg/mL)
LL1	*	0.637	0.639	0.638	**273**
LL2	0.773	0.761	0.74	0.758	**332**
LL3	0.758	*	0.753	0.756	**331**
LL4	0.774	0.765	0.782	0.774	**340**
HL1	0.667	*	0.68	0.674	**290**
HL2	0.704	*	0.702	0.703	**305**
HL3	0.707	*	0.71	0.709	**307**
HL4	0.581	0.594	*	0.588	**248**
LLi1	0.704	*	0.693	0.699	**303**
LLi2	0.615	*	0.616	0.616	**262**
LLi3	0.730	0.710	0.712	0.717	**312**
LLi4	0.849	0.847	0.851	0.849	**377**
HLi1	0.678	0.680	*	0.679	**293**
HLi2	0.663	0.662	*	0.663	**285**
HLi3	0.653	0.662	0.642	0.652	**280**
HLi4	0.527	*	0.523	0.525	**217**

$y = 0{,}0405x + 0{,}0859$; $r^2 = 0{,}998$

2 Experiments

1

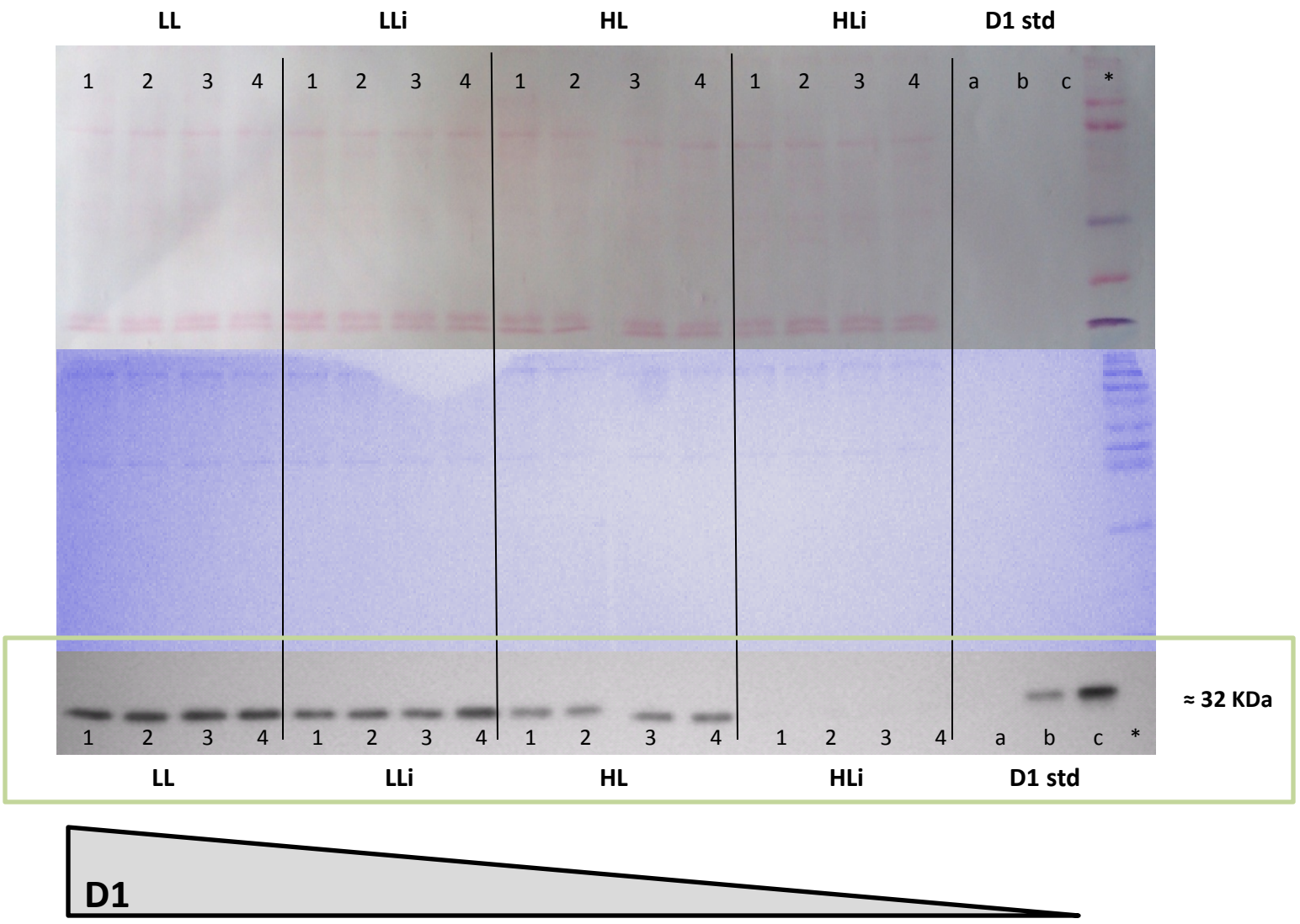


Fig. 12 – Immunoblotting steps and results.

D1 DYNAMICS

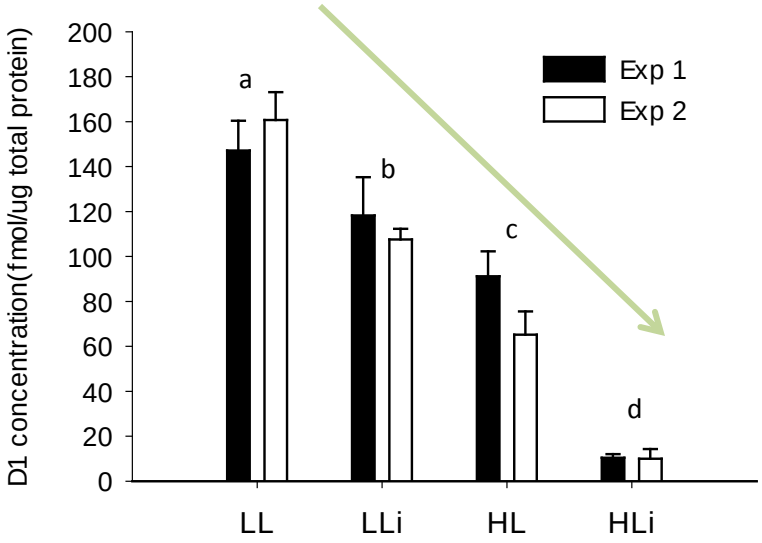


Fig. 13 – D1 concentrations in fmol/µg total protein.

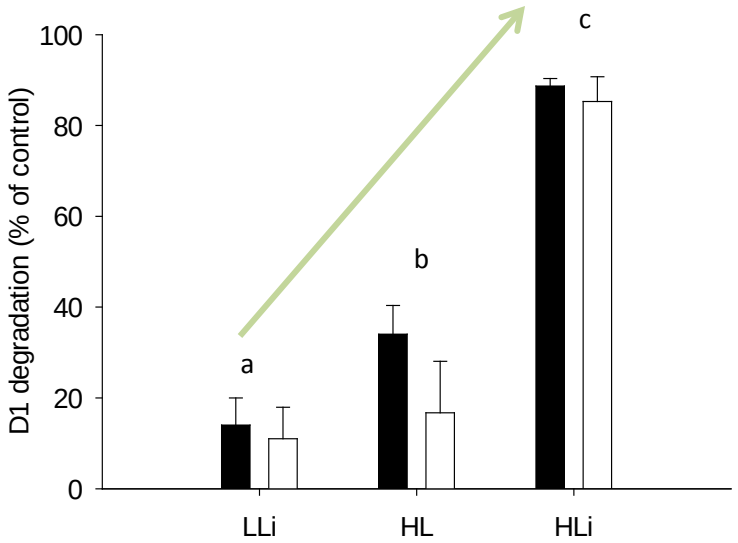


Fig. 14 – D1 degradation in all treatment (based on control LL levels).

PIGMENT VARIATIONS

Table II – Mean pigment concentration in pg/cell:

Pigment concentration (pg/cell)				
	LL		HL	
	Control	Inhibited	Control	Inhibited
Chl *a*	0.37 [a]	0.35 [a]	0.35 [a]	0.34 [a]
Fucoxanthin	0.21 [a]	0.20 [a]	0.20 [a]	0.18 [a]
Chl *c*2	0.07 [a]	0.07 [a]	0.07 [a]	0.06 [a]
β-carotene	0.016 [a]	0.013 [a]	0.014 [a]	0.014 [a]
DD	0.041 [a]	0.041 [a]	0.017 [b]	0.015 [b]
DT	0.0013 [a]	0.0014 [a]	0.029 [b]	0.031 [b]
DD+DT	0.043 [a]	0.043 [a]	0.046 [b]	0.046 [b]

De-epoxidation of DD

Synthesis *de novo* of DT

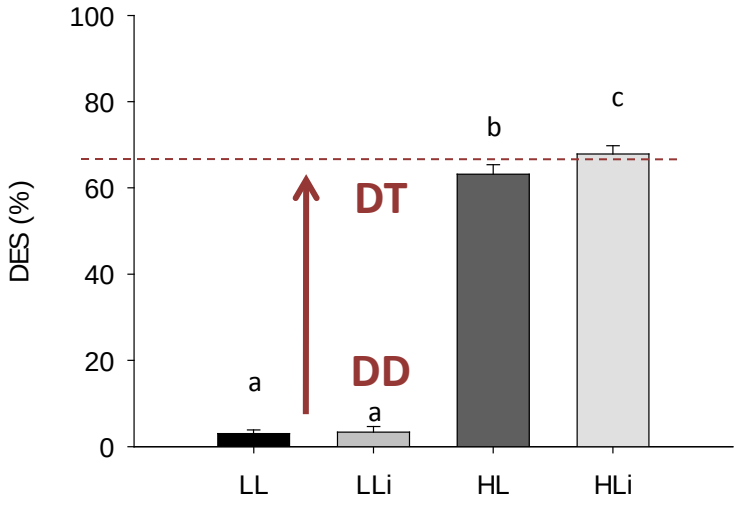
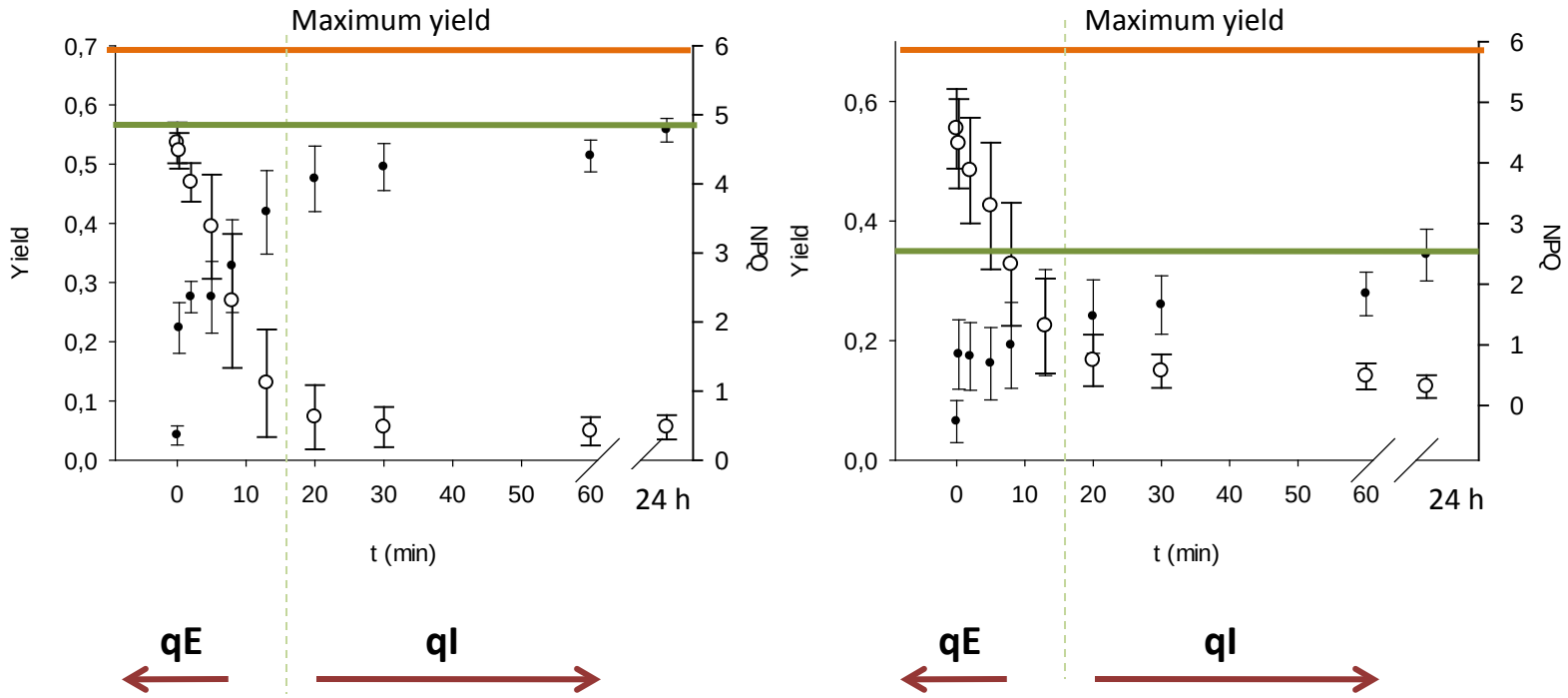


Fig. 14 – DES or [DT/(DD+DT)].

RECOVERY KYNETICS

NPQ = qE + qI



qE – energy dependent quenching
qI - photoinhibitory quenching

Fig. 15 – Yield and NPQ during recovery.

RECOVERY IN QUANTUM YIELD OF PSII

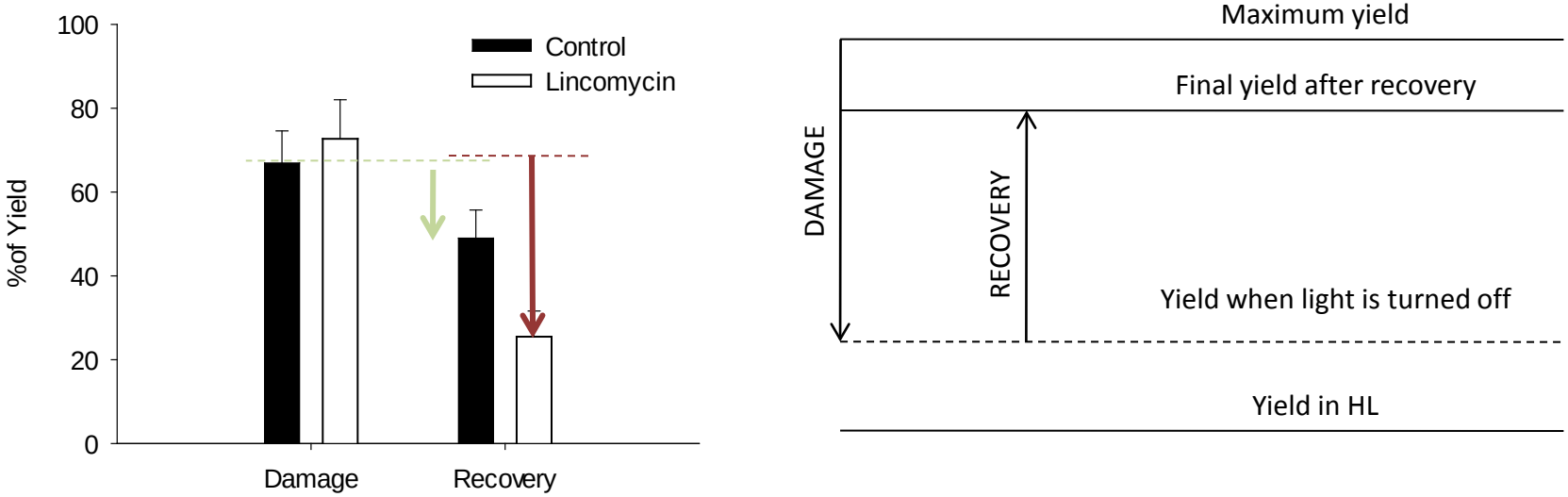


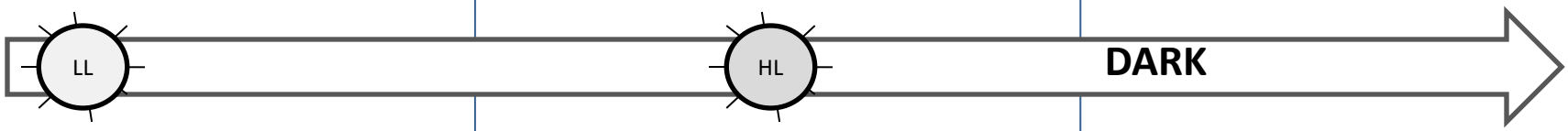
Fig. 16 – Damage effects on yield and recovery of photosynthetic capacity based on Yield.

$\Phi_{PSII} = F_v'/F_m'$ x qP when light is turned off

≈ 69 % damage

RECOVERY: ↑ HL 49% ↑ HLi 25.5%

CONCLUSIONS



Low Light (LL)

D1 degradation and resynthesis

Very low NPQ
(Almost no DT)

High yield

EFFICIENT REPAIR

High Light (HL)

Extreme D1 degradation
Insufficient D1 resynthesis

High NPQ
Photoprotective mechanisms insufficient

Very low yield

PHOTOINHIBITION

RECOVERY

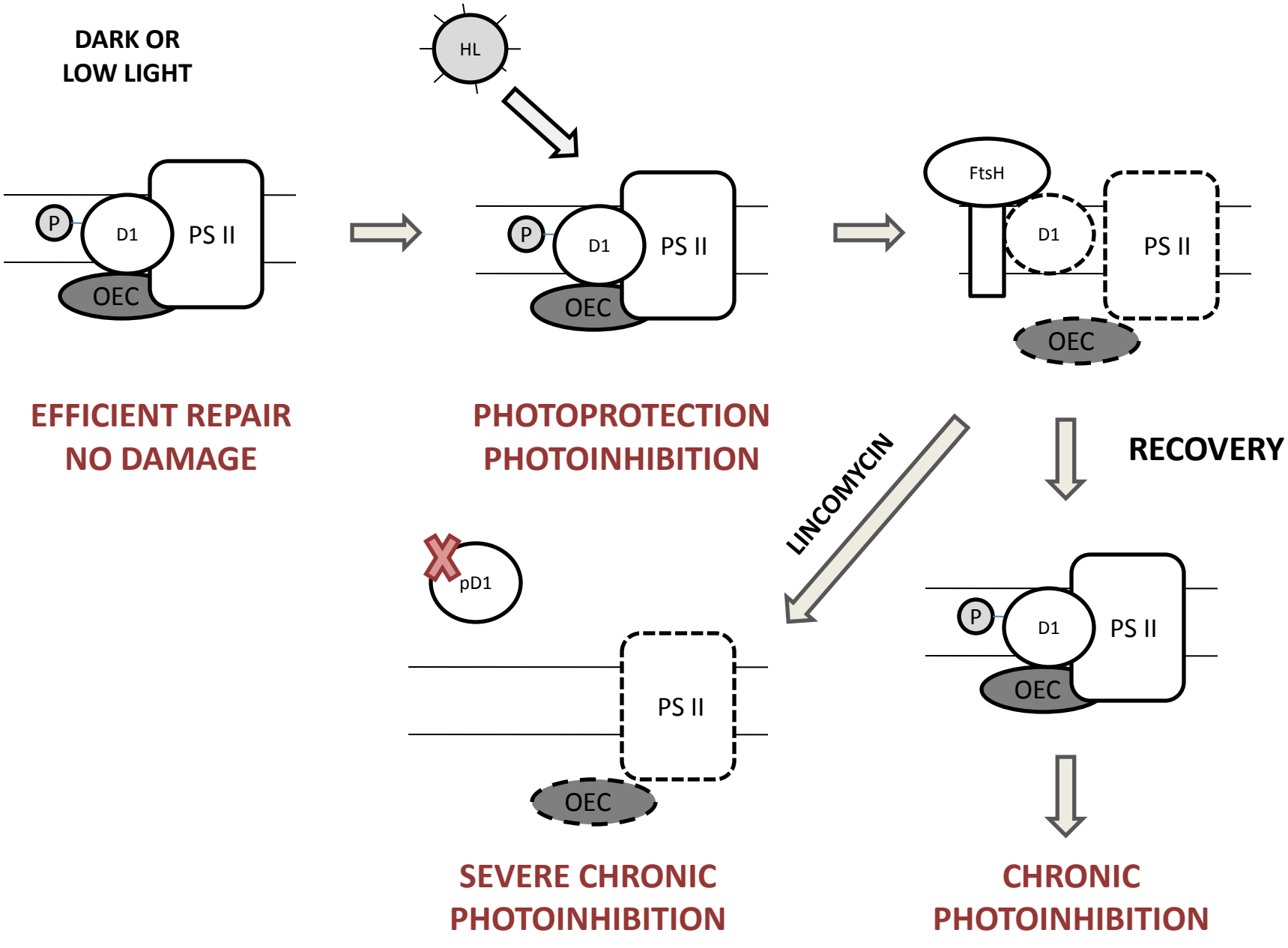
85% resynthesized D1

Epoxidation of DT to DD
Low NPQ

50% recovery in yield

CHRONIC PHOTOINHIBITION

CONCLUSIONS



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