Analysis of PSII antenna size heterogeneity of *Chlamydomonas reinhardtii* during state transitions

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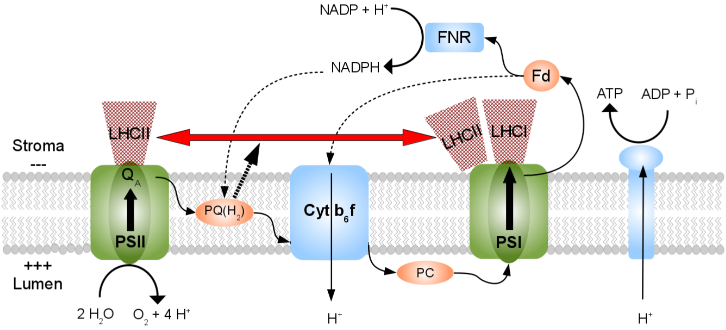
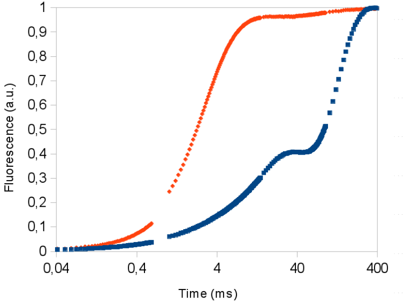
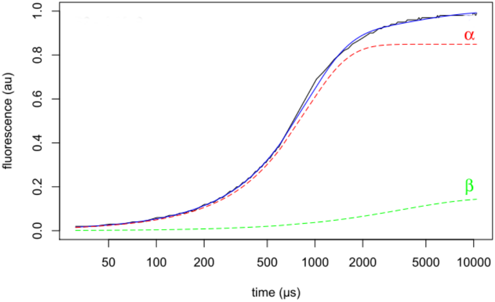


- State transition :
  - migration of LHCII pigment-proteins between PSII and PSI
  - up to 80% in *Chlamydomonas reinhardtii* (Delosme et al. 1996)
- Different types of PSII with different antenna sizes : PSII antenna size heterogeneity (Melis and Homann (1975))

Fluorescence rise from F_O to F_M corresponding to the reduction of Q_A in the reaction center of PSII.





- DCMU addition → the photochemical phase
- DCMU fluorescence rise induction kinetic is not a first order kinetic → PSIIα and PSIIβ

- Lavergne et al.(2004) :

	PSIIα	PSIIβ
Proportion	$+$	-
Antenna	210-250 Chl	$\approx$ 100 Chl
Region of the thylakoïd membrane	appressed	non appressed
Multimer?	dimer	monomer
Connectivity (p)	$\approx$ 0,7	0
Shape of fluorescence rise	sigmoidal	exponential

- Connectivity (p) : quantifies the probability of energy transfer between closed PSII to an open PSII (Joliot and Joliot, 1964)

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- Connectivity (p) : quantifies the probability of energy transfer between closed PSII to an open PSII (Joliot and Joliot, 1964)
- Purpose of this work : determination of PSII antenna size heterogeneity in state I and in state II
- Method of Melis and Homann (complementary area over the DCMU-FR) is very approximative due to approximations in the F_M level
- Non linear regression algorithm with equations derived from Lazár et al.(2001) :
  - better F_M determination
  - p determination
  - simultaneous fitting of several curves

$$rF_V(t) = \sum_{i=1}^{3} \frac{(1-p_i)\mathrm{PSII}_i^{closed}(t)}{1-p_i\mathrm{PSII}_i^{closed}(t)} \qquad \mathrm{PSII}_i^{closed}(t) = \mathrm{PSII}_{i,0}^{open}(1-e^{(-k_i t)})$$

1. $rF_V(t) = \%\mathrm{PSII}_\alpha \frac{(1-p_\alpha)(1-e^{-k_\alpha t})}{1-p_\alpha(1-e^{-k_\alpha t})} + \%\mathrm{PSII}_\beta\ (1-e^{-k_\beta t}) + \%\mathrm{PSII}_\gamma\ (1-e^{-k_\gamma t})$
2. $rF_V(t) = \%\mathrm{PSII}_\alpha \frac{(1-p_\alpha)(1-e^{-k_\alpha t})}{1-p_\alpha(1-e^{-k_\alpha t})} + \%\mathrm{PSII}_\beta \frac{(1-p_\beta)(1-e^{-k_\beta t})}{1-p_\beta(1-e^{-k_\beta t})} + \%\mathrm{PSII}_\gamma\ (1-e^{-k_\gamma t})$
3. $rF_V(t) = \%\mathrm{PSII}_\alpha \frac{(1-p_\alpha)(1-e^{-k_\alpha t})}{1-p_\alpha(1-e^{-k_\alpha t})} + \%\mathrm{PSII}_\beta \frac{(1-p_\beta)(1-e^{-k_\beta t})}{1-p_\beta(1-e^{-k_\beta t})} + \%\mathrm{PSII}_\gamma \frac{(1-p_\gamma)(1-e^{-k_\gamma t})}{1-p_\gamma(1-e^{-k_\gamma t})}$

The *Akaike's information criterion* (AIC) : comparison of different models by introducing a penalty for the number of parameters used.

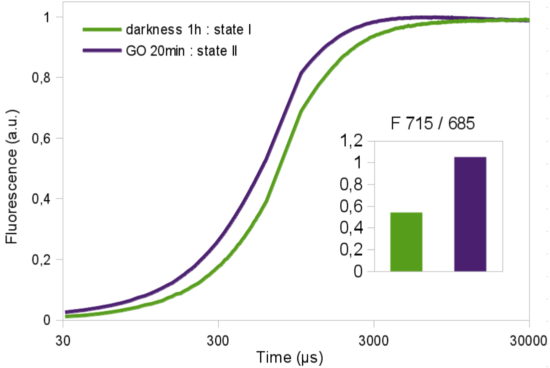
$$\mathrm{AIC} = 2k - 2log(L)$$

model	AIC
1: connectivity allowed only for PSIIα	-14599
2: connectivity allowed for PSIIα and PSIIβ	-17025
3: connectivity allowed for PSIIα, PSIIβ and PSIIγ	-17007

- $\mathrm{AIC}_{model2} < \mathrm{AIC}_{model3} < \mathrm{AIC}_{model1} \rightarrow$ model 2 describes the experimental data better than models 1 and 3
- connectivity for PSII$\beta \neq 0$, in contrast with a majority of studies (except the work of Lavergne and Trissl (1995) and Lazár et al.(2001))

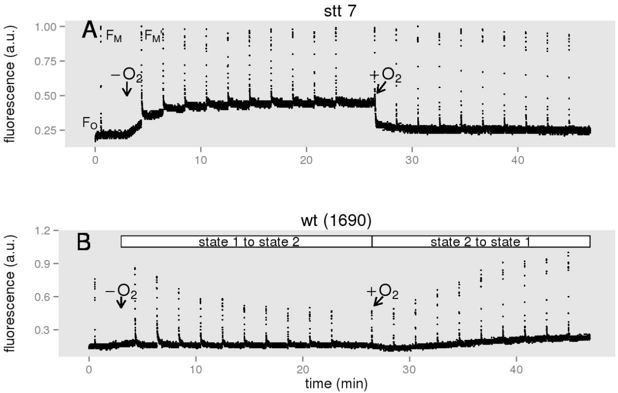
Experiment :

- Darkness 1 hour → Oxydation of plastoquinones → Algae close to state I
- Arrest of mitochondrial respiration → Reduction of plastoquinones → Transition to state II

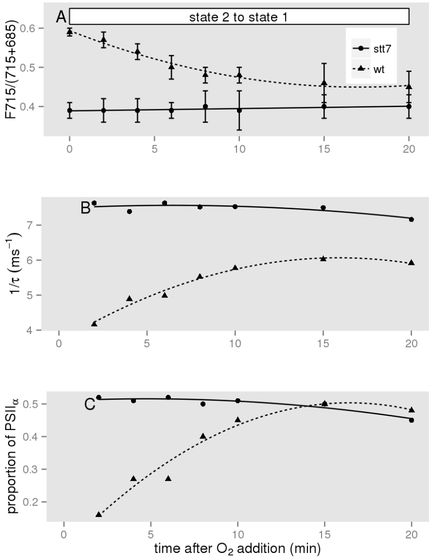


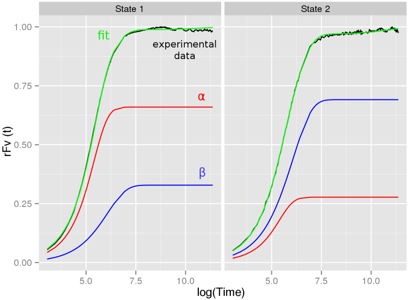
When PQ pool is highly reduced (state 2) → many PSII centers have a Q_B^- bound and the addition of DCMU leads to Q_A reduction before the illumination.

PQ pool had to be rapidly oxidized before the addition of DCMU → development of a method with N_2 bubbling



- to monitor the redox state of Q_A without the influence of state transitions → mutant *stt7*
- Complete reoxydation of PQH_2 by O_2 in 2 minutes
- In 2 minutes, back transition to state 1 is not significant in the *wt* → ideal delay

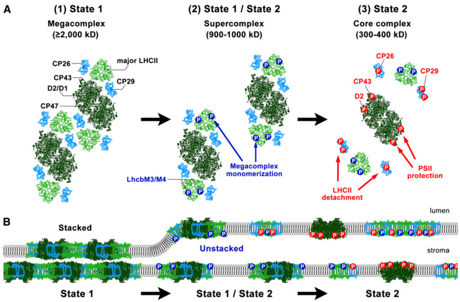




- the conversion of PSIIα to PSIIβ during transition from state 2 to state 1 parallels the decrease of the low T fluorescence ratio.
- state transitions can be described as changes in the proportions of two PSII populations with constant properties

	$\%PSII_\alpha$ S1	$\%PSII_\alpha$ S2	p_α	p_β	k_α (s^{-1})	k_β (s^{-1})	$\frac{k_\alpha}{k_\beta}$
wt	0.48	0.16	0.62	0.15	48.6	15.4	3.1

- In addition to this description of heterogeneity by functional analysis of the fluorescence rise of PSII *in vivo* : biochemical studies (mainly based on isolation of PSII complexes and subsequent analysis).
- Iwai et al., 2008



- We suggest that PSIIα phase refers to PSII mega- and super- complexes and that PSIIβ phase refers to PSII core complexes.

Summary :

- development of a protocol for PSII heterogeneity analysis during state transition and improvement of mathematical analysis
- connectivity for PSIIβ
- demonstration of an interconversion of PSIIα to PSIIβ during state transitions for the first time *in vivo*
- link between functional approach and biochemical and structural studies

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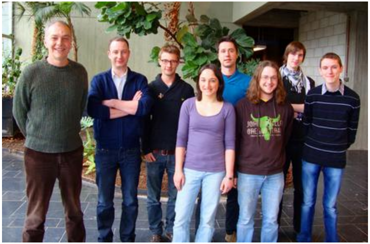
Thank you for your attention



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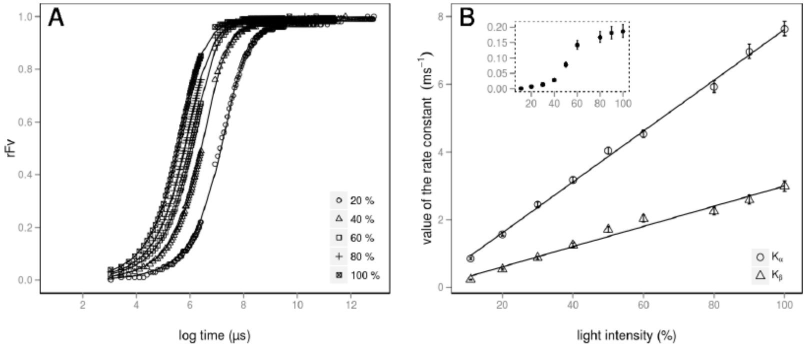
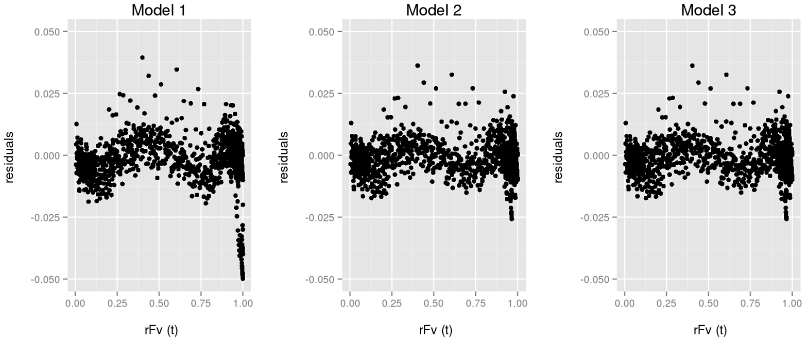

A
1.0
0.8
0.6
0.4
0.2
0.0
rFv
2
4
6
8
10
12
log time (µs)
20 %
40 %
60 %
80 %
100 %
B
value of the rate constant (ms⁻¹)
8
6
4
2
0
20
40
60
80
100
light intensity (%)
0.20
0.15
0.10
0.05
0.00
Kα
Kβ


Model 1
Model 2
Model 3
residuals
0.050
0.025
0.000
-0.025
-0.050
0.00
0.25
0.50
0.75
1.00
rFv (t)

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$$\mathrm{k}_i(t) = \frac{\mathrm{k}_i^0}{1-p_i\mathrm{PSII}_i^{closed}(t)}$$

$$\mathrm{k}_i^0 = \frac{1-p_i}{t(p_i rF_{V,i}(t)+1-p_i)}\Big(\ln\big(1-p_i(1-rF_{V,i}(t))\big) - \ln(1-rF_{V,i}(t)) - \ln(1-p_i)\Big)$$