Analysis of PSII antenna size and heterogeneity in state I and state II in *Chlamydomonas reinhardtii*

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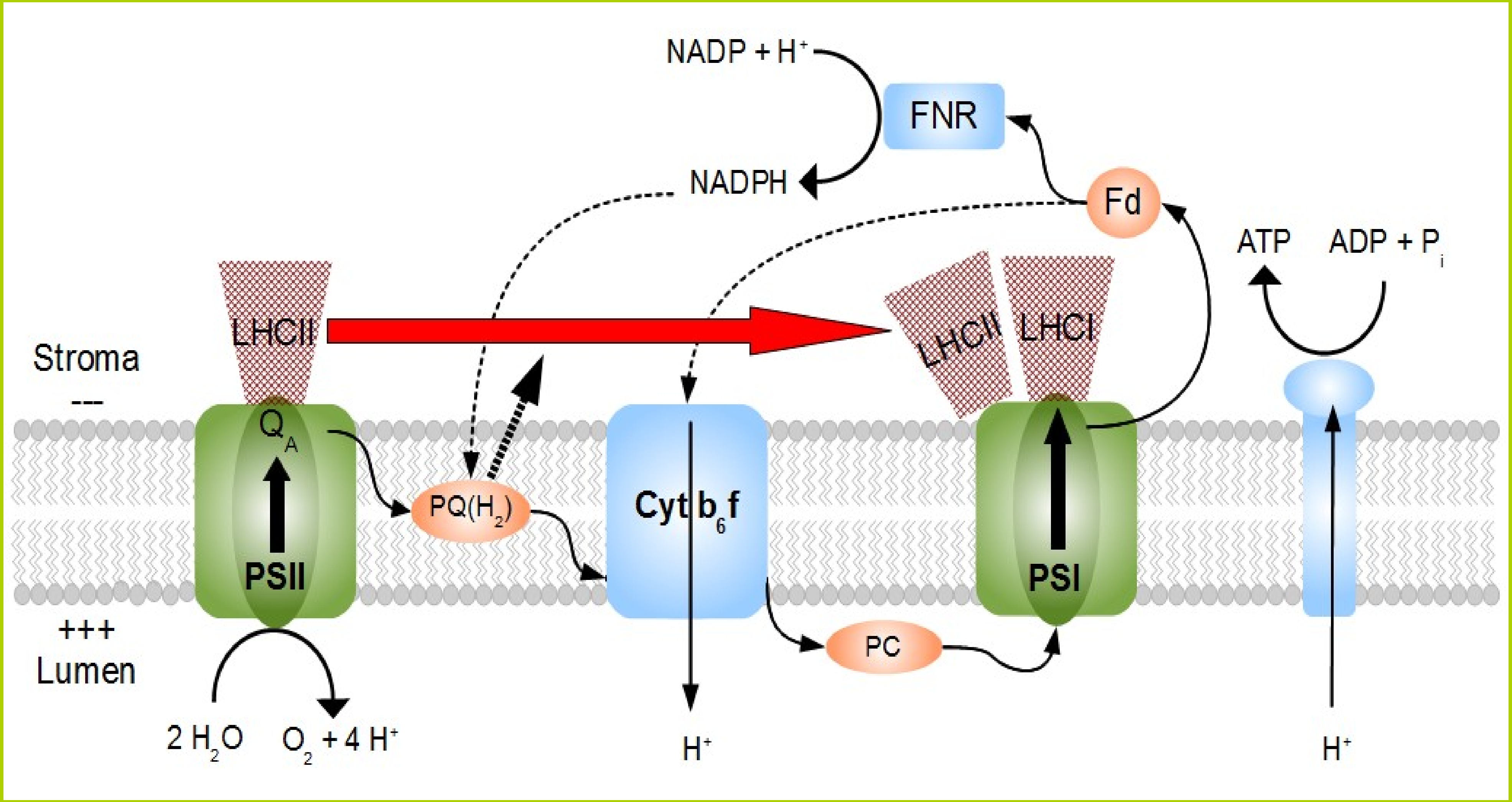
State transitions



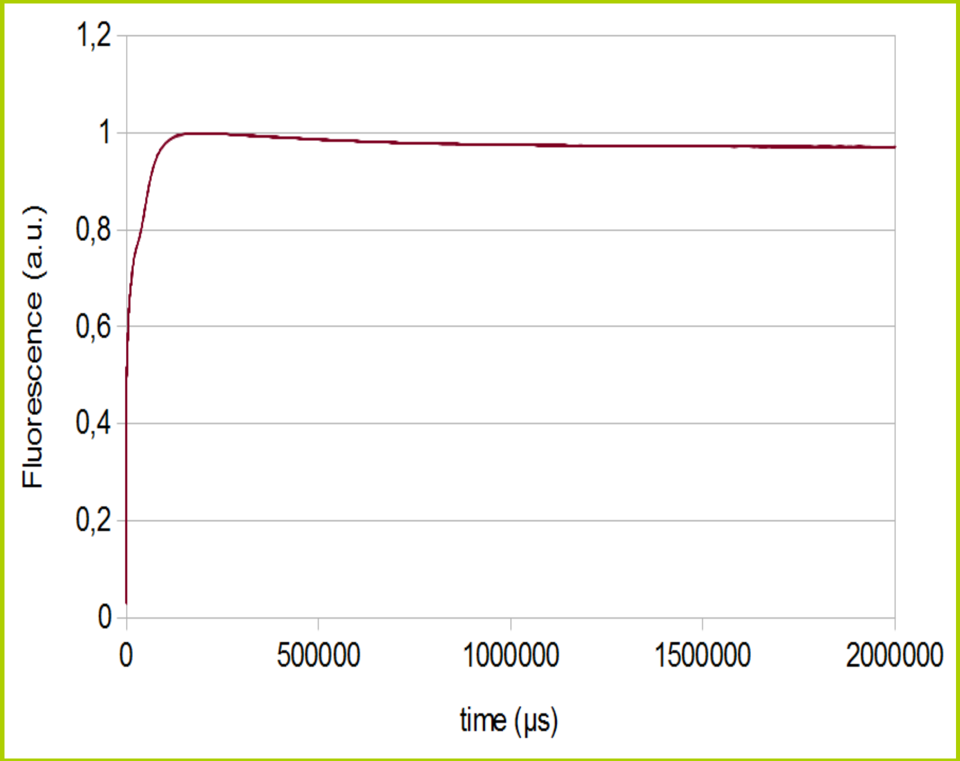
- State transition : migration (max 80%) of antenna from PSII to PSI
- Promotes cyclic electron transport

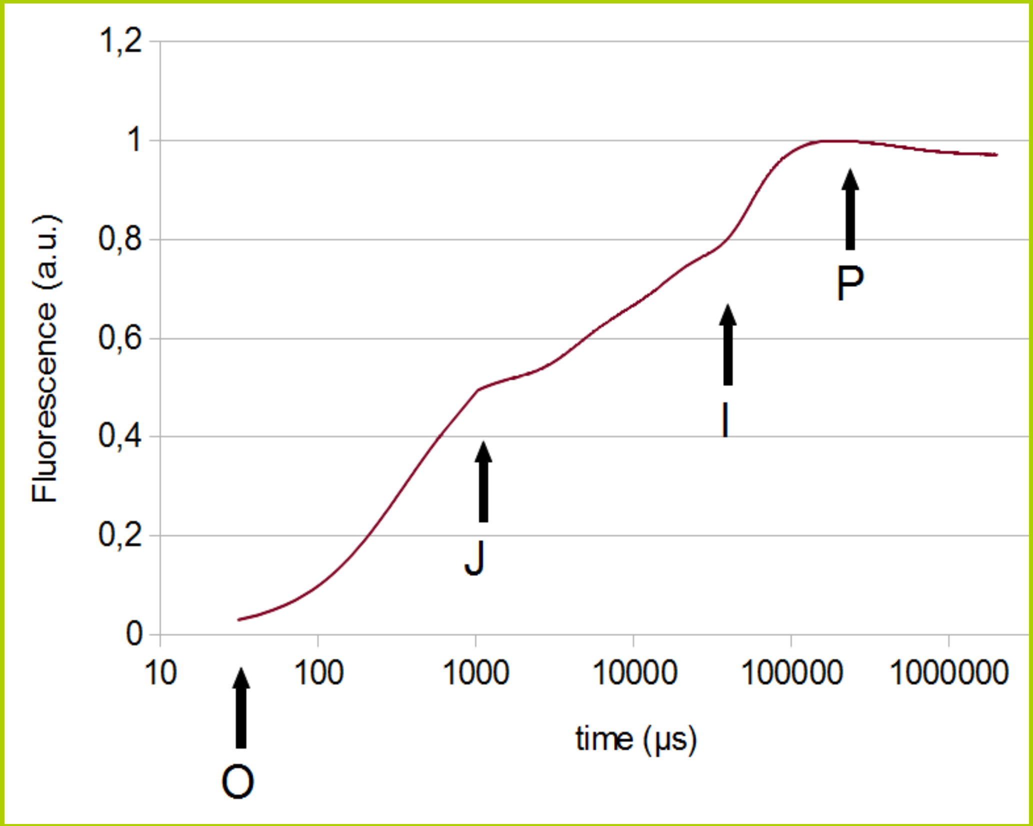
Fluorescence rise

- Purpose of this work : analyse PSII antenna size and heterogeneity in state I or in state II.
- Cardol et al.(2009) : Comparing antenna size of different strains by fluorescence rise experiments.

⬇

Rising of fluorescence from Fo to Fm corresponding to the reduction of Q_A in the reaction center of PSII.



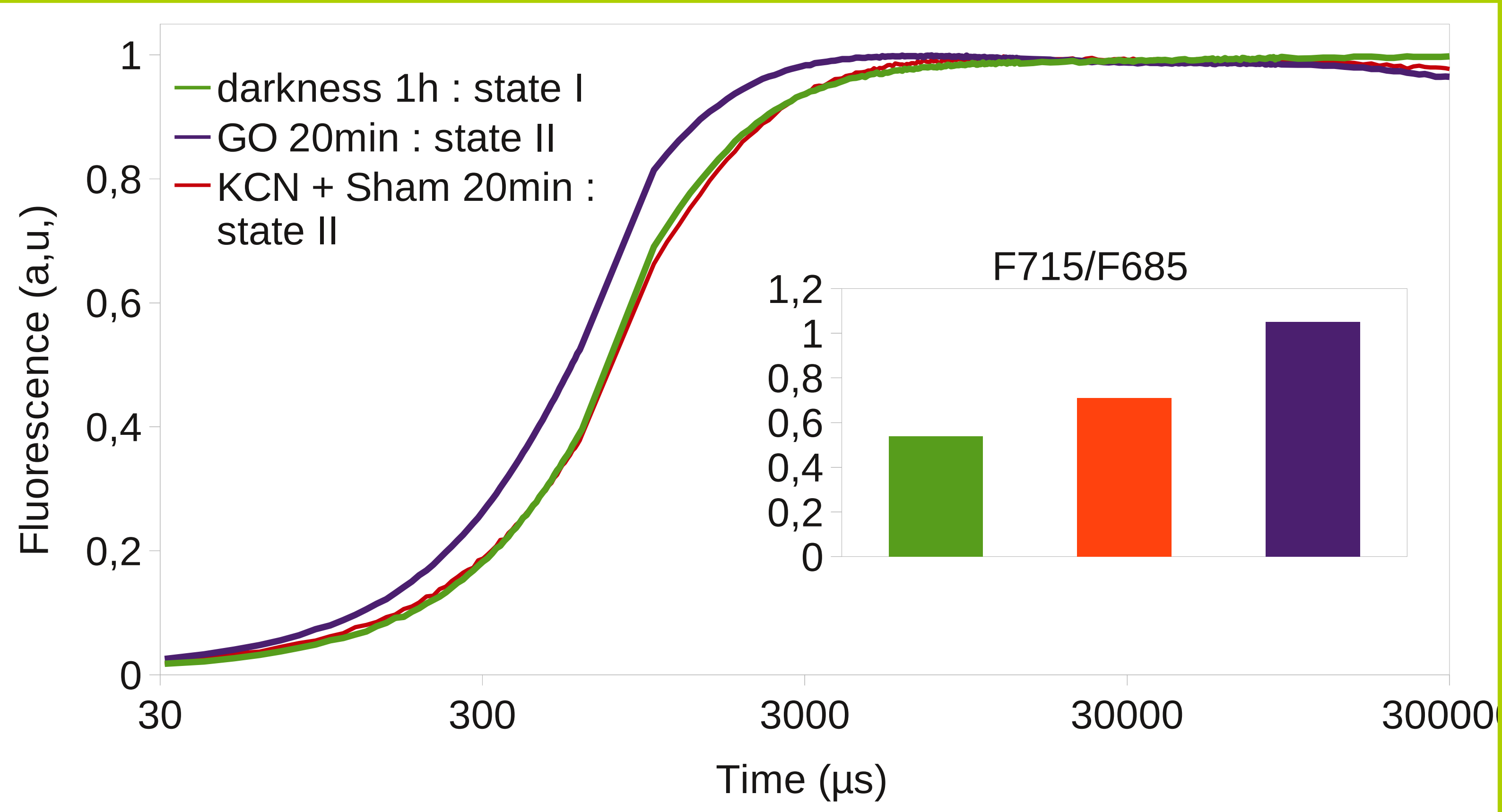


Principle of the experiment : Transition from state I to state II → the speed of rising should decrease because part of LHCII migrate from PSII to PSI.

Fluorescence rise : the experiment

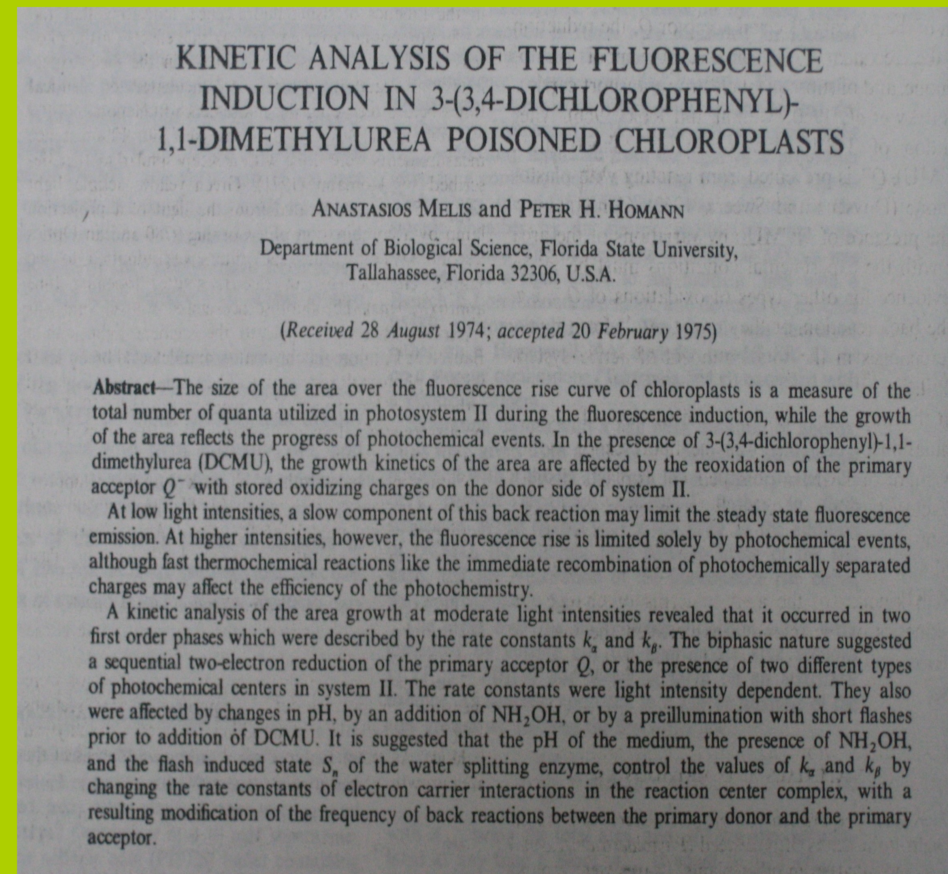
- Simplification : DCMU is added before the measure → only photochemical events.

Darkness 1 hour	KCN + SHAM or Glucose oxydase in darkness
Oxydation of plastoquinones ⬇ Transition to state I	Impairment of mitochondrial respiration → reduction of plastoquinones ⬇ Transition to state II



OK but what else?

- What sort of numeric informations can we calculate from such curves ?



KINETIC ANALYSIS OF THE FLUORESCENCE INDUCTION IN 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA POISONED CHLOROPLASTS

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(*Received* 28 *August* 1974; *accepted* 20 *February* 1975)

Abstract—The size of the area over the fluorescence rise curve of chloroplasts is a measure of the total number of quanta utilized in photosystem II during the fluorescence induction, while the growth of the area reflects the progress of photochemical events. In the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the growth kinetics of the area are affected by the reoxidation of the primary acceptor Q^- with stored oxidizing charges on the donor side of system II.

At low light intensities, a slow component of this back reaction may limit the steady state fluorescence emission. At higher intensities, however, the fluorescence rise is limited solely by photochemical events, although fast thermochemical reactions like the immediate recombination of photochemically separated charges may affect the efficiency of the photochemistry.

A kinetic analysis of the area growth at moderate light intensities revealed that it occurred in two first order phases which were described by the rate constants k_α and k_β. The biphasic nature suggested a sequential two-electron reduction of the primary acceptor Q, or the presence of two different types of photochemical centers in system II. The rate constants were light intensity dependent. They also were affected by changes in pH, by an addition of NH_2OH, or by a preillumination with short flashes prior to addition of DCMU. It is suggested that the pH of the medium, the presence of NH_2OH, and the flash induced state S_n of the water splitting enzyme, control the values of k_α and k_β by changing the rate constants of electron carrier interactions in the reaction center complex, with a resulting modification of the frequency of back reactions between the primary donor and the primary acceptor.

2 populations of PSII : PSIIα and PSIIβ

PSII heterogeneity

- Lavergne et al.(2004) :

	PSIIα	PSIIβ
Proportion	80-90%	10-20%
Antenna		+- 1/3 of PSIIα (no LHCII)
Region of the thylakoïd membrane	appressed	non appressed
Multimer?	dimer	monomer
Connectivity (p)	0,5 – 0,7	0
Fluorescence rise	sigmoidal	exponential

- Connectivity : quantify the probability of energy transfer between closed PSII to an open PSII

- Several authors noticed a third component γ... Is it a real component or a pure mathematical object ?

PSII heterogeneity and state transition

- In this case : determination of PSII heterogeneity in state I or in state II
- However, the method of Melis and Homann is approximative :

  an error of 0,6% in Fm → 53% of error in amplitude and rate of different photosystems

- non linear regression algorithm with equations from Lazár et al.(2001).

$$F(t)=\frac{\alpha\times(1-p)\times PSII\,\alpha\,open\times\left(1-e^{-K\alpha\times t}\right)}{1-p\times PSII\,\alpha\,open\times\left(1-e^{-K\alpha\times t}\right)}+\beta\times PSII\,\beta\,open\times\left(1-e^{-K\beta\times t}\right)$$

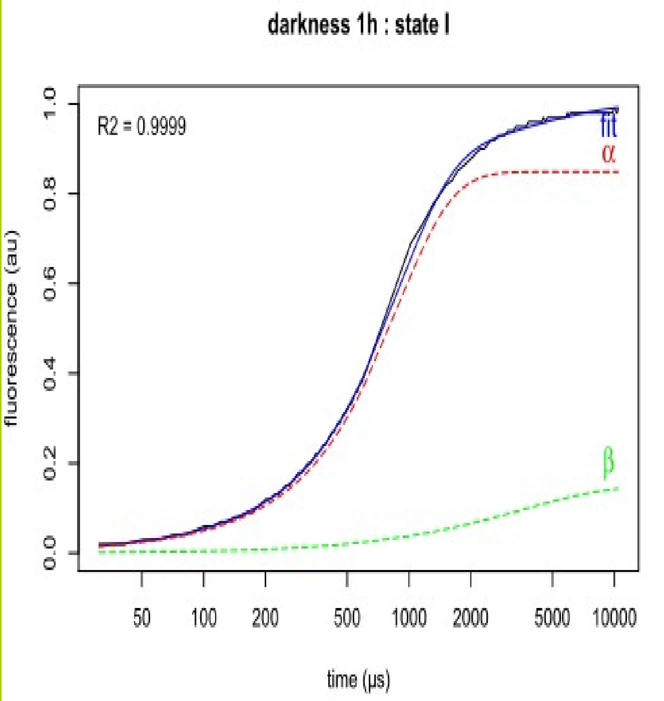
⬇ sigmoid

⬇ exponential

PSII heterogeneity : non linear regression

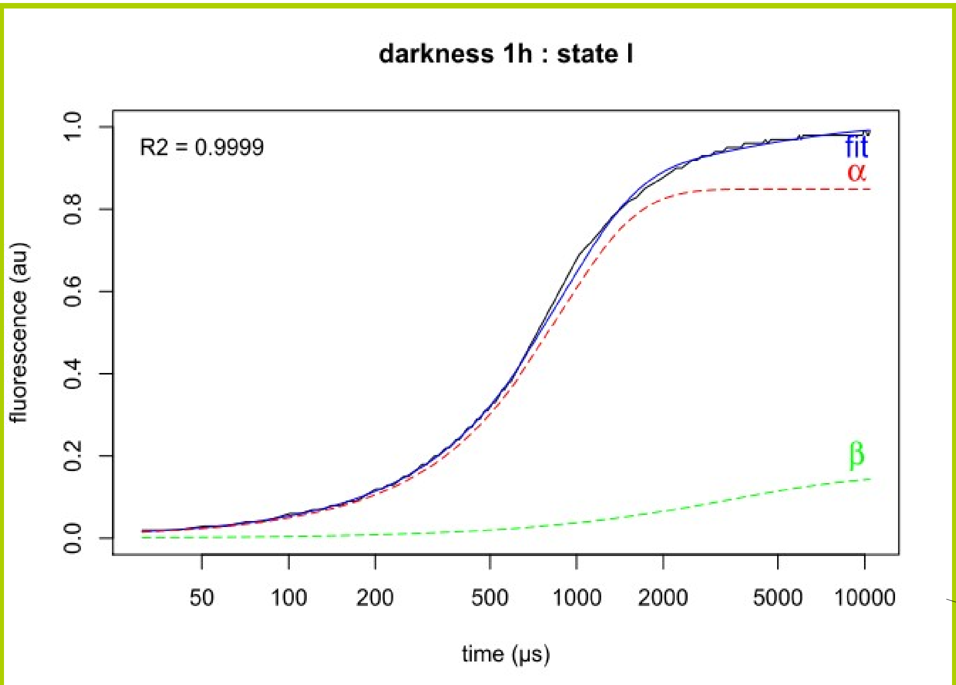
```
15-12-10
darkness 1h : state I    31   0,02
darkness 1h : state I    41   0,02
darkness 1h : state I    51   0,03
darkness 1h : state I    61   0,03
darkness 1h : state I    71   0,04
darkness 1h : state I    81   0,04
darkness 1h : state I    91   0,05
darkness 1h : state I    101  0,06
darkness 1h : state I    111  0,06
darkness 1h : state I    121  0,07
darkness 1h : state I    131  0,07
darkness 1h : state I    141  0,08
darkness 1h : state I    151  0,08
darkness 1h : state I    161  0,09
darkness 1h : state I    171  0,09
darkness 1h : state I    181  0,1
darkness 1h : state I    191  0,11
darkness 1h : state I    201  0,12
darkness 1h : state I    211  0,12
darkness 1h : state I    221  0,13
darkness 1h : state I    231  0,13
darkness 1h : state I    241  0,14
darkness 1h : state I    251  0,15
darkness 1h : state I    261  0,15
darkness 1h : state I    271  0,16
darkness 1h : state I    281  0,17
darkness 1h : state I    291  0,18
darkness 1h : state I    301  0,18
darkness 1h : state I    311  0,19
darkness 1h : state I    321  0,2
darkness 1h : state I    331  0,2
darkness 1h : state I    341  0,21
darkness 1h : state I    351  0,22
darkness 1h : state I    361  0,22
```

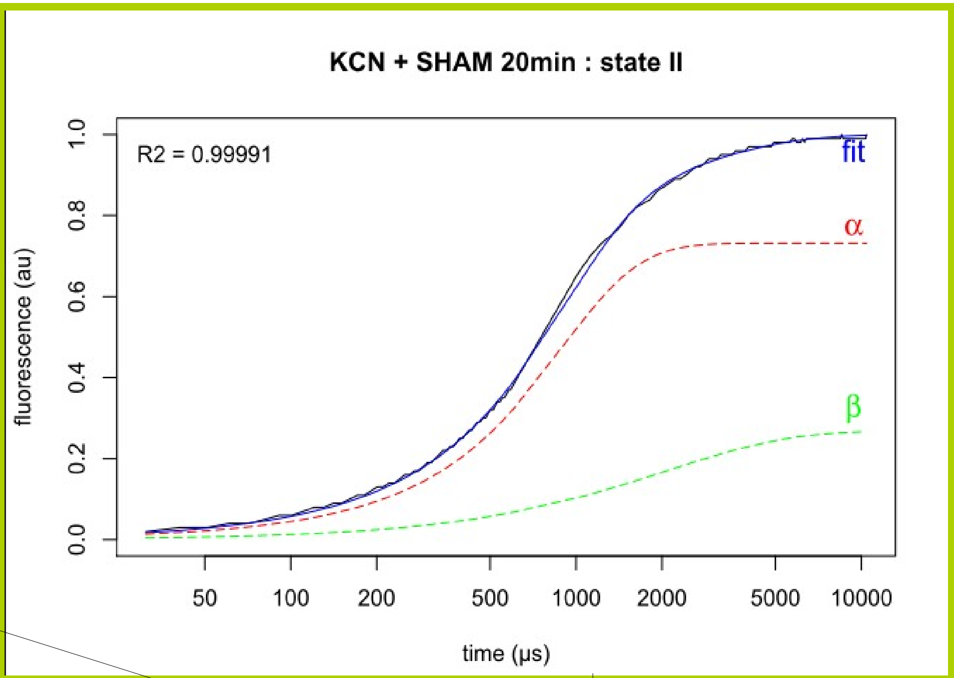
```r
library(gplots)
library(nlme)

### formule

formule <- f ~ ((propA*((1-pA)*1*(1-exp

### contraintes

debut <- c(propA=0.5, pA=0.5, KliA=0.00
limiteinf <- c(propA=0.1, pA=0, KliA=0.
limitesup <- c(propA=1, pA=1, KliA=1, K

### import des données

titre <- na.omit(scan("data.txt", sep="
import <- read.table("data.txt", sep="\
names(import) <- c("exper","t","f")

### détermination du nombre de manip

nomexper <- c("a")
conditions <- c(NULL)

for(i in 1:nrow(import)) {

    tempnomexper <- import[i,1]

    if(tempnomexper != nomexper){
        conditions <- c(conditions,as.c
        nomexper <- tempnomexper
        }

    }
```

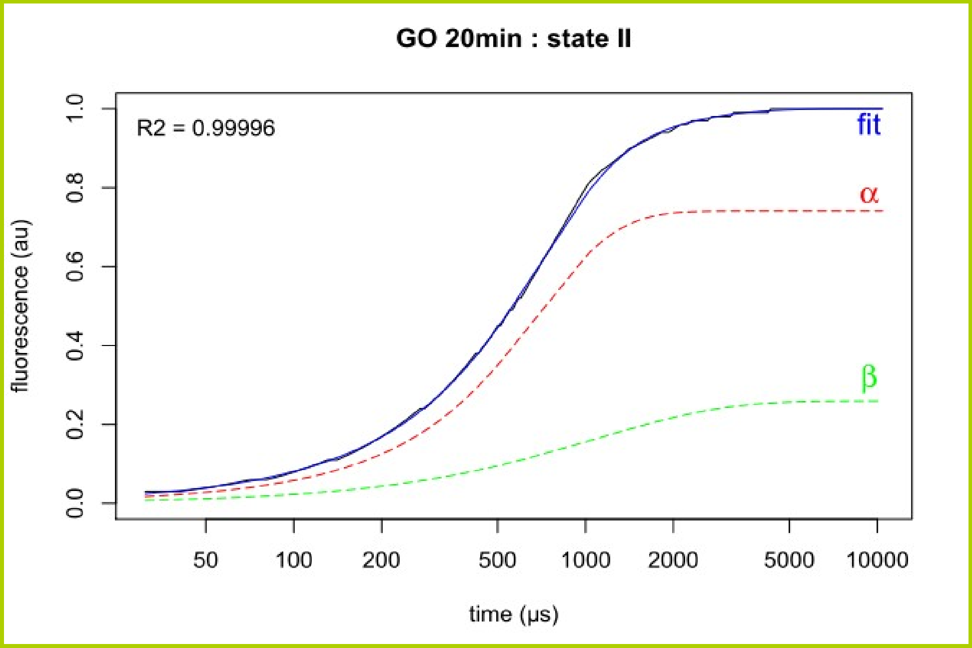


	Estimate	Std. Error	t value	Pr(>|t|)
propA	0.848864995842245	0.00796136082811174	106.623103030864	2.61919928521793e-143
pA	0.786065279017894	0.00605382447252561	129.846063853575	5.23707635253785e-156
KliA	0.00255161217353433	4.61378863149596e-05	55.3040543755253	1.14084927813656e-101
KliB	0.000285070316471041	1.25063472786475e-05	22.7940509022768	1.37921361277857e-50

PSII heterogeneity : non linear regression







% α	84 ± 0,7	73 ± 2	74 ± 4
% β	16	27	26
P α	0,78 ± 0,01	0,76 ± 0,01	0,77 ± 0,01
K α	$2{,}55.10^{-3} \pm 4.10^{-5}$	$2{,}4.10^{-3} \pm 7.10^{-5}$	$3{,}2.10^{-3} \pm 1.10^{-4}$
K β	$2{,}8.10^{-4} \pm 1.10^{-5}$	$4{,}8.10^{-4} \pm 2.10^{-5}$	$9{,}1.10^{-4} \pm 6.10^{-5}$

Conclusion

I. State II : rate of fluorescence rising increase → opposite effect expected

II. Fit : - state I → connectivity of PSIIα higher than reported in older litterature

- state II → ± 10% of PSIIα converted in PSIIβ

Preliminary conclusion : PSIIβ more abundant and more rapid in state II

In the future : - add γ photosystems to fitting procedure

- follow directly the reduction of Q_A with 320nm signal

Thanks for your attention