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

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- 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.

<table>
<thead>
<tr>
<th>Darkness 1 hour</th>
<th>KCN + SHAM or Glucose oxidase in darkness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxydation of plastoquinones</td>
<td>Impairment of mitochondrial respiration → reduction of plastoquinones</td>
</tr>
<tr>
<td>Transition to state I</td>
<td>Transition to state II</td>
</tr>
</tbody>
</table>

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**Graph**

- **Fluorescence (a.u.)**
  - darkness 1h: state I
  - GO 20min: state II
  - KCN + Sham 20min: state II

- **F715/F685**
  - F715/F685 values for different conditions:
    - Green: darkness 1h: state I
    - Purple: GO 20min: state II
    - Red: KCN + Sham 20min: state II
OK but what else?

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

2 populations of PSII: PSII\(\alpha\) and PSII\(\beta\)
PSII heterogeneity

- Lavergne et al. (2004):

<table>
<thead>
<tr>
<th></th>
<th>PSIIα</th>
<th>PSIIβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion</td>
<td>80-90%</td>
<td>10-20%</td>
</tr>
<tr>
<td>Antenna</td>
<td></td>
<td>+- 1/3 of PSIIα (no LHCII)</td>
</tr>
<tr>
<td>Region of the thylakoïd membrane</td>
<td>appressed</td>
<td>non appressed</td>
</tr>
<tr>
<td>Multimer?</td>
<td>dimer</td>
<td>monomer</td>
</tr>
<tr>
<td>Connectivity (p)</td>
<td>0.5 – 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Fluorescence rise</td>
<td>sigmoidal</td>
<td>exponential</td>
</tr>
</tbody>
</table>

- 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).

\[
F(t) = \frac{\alpha \times (1 - p) \times PSII\ \alpha\ open \times (1 - e^{-K\alpha\times t})}{1 - p \times PSII\ \alpha\ open \times (1 - e^{-K\alpha\times t})} + \beta \times PSII\ \beta\ open \times (1 - e^{-K\beta\times t})
\]

sigmoid  

exponential
PSII heterogeneity: non-linear regression

```r
library(qplots)
library(mime)

### formule
formule <- f ~ ((propA*(1-pA))*1*(1-exp(-KlA*x)))+
### contraintes
debut <- c(propA=0.5, pA=0.5, KlA=0.001)
limiteinf <- c(propA=0.1, pA=0.01, KlA=0.0001)
limitesup <- c(propA=1, pA=1, KlA=0.01)

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

### determination 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.factor(tempnomexper))
    nomexper <- tempnomexper
  }
}
```

![Graph showing fluorescence over time](image)

- **propA**: 0.64086
- **pA**: 0.0037
- **KlA**: 100.42
- **KIB**: 1.12

- **Estimate**: 2.010
- **Std. Error**: 0.028
- **t value**: 50.8
- **P(>|t|)**: 2.6

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PSII heterogeneity: non linear regression

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<thead>
<tr>
<th></th>
<th>% α</th>
<th>% β</th>
<th>P α</th>
<th>K α</th>
<th>K β</th>
</tr>
</thead>
<tbody>
<tr>
<td>darkness 1h: state I</td>
<td>84 ± 0.7</td>
<td>16</td>
<td>0.78 ± 0.01</td>
<td>2.55.10^{-3} ± 4.10^{-5}</td>
<td>2.8.10^{-4} ± 1.10^{-5}</td>
</tr>
<tr>
<td>KCN + SHAM 20min: state II</td>
<td>73 ± 2</td>
<td>27</td>
<td>0.76 ± 0.01</td>
<td>2.4.10^{-3} ± 7.10^{-5}</td>
<td>4.8.10^{-4} ± 2.10^{-5}</td>
</tr>
<tr>
<td>GO 20min: state II</td>
<td>74 ± 4</td>
<td>26</td>
<td>0.77 ± 0.01</td>
<td>3.2.10^{-3} ± 1.10^{-4}</td>
<td>9.1.10^{-4} ± 6.10^{-5}</td>
</tr>
</tbody>
</table>
I. State II: rate of fluorescence rising increase → opposite effect expected

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