叶绿素荧光原理及使用技巧

郭峰

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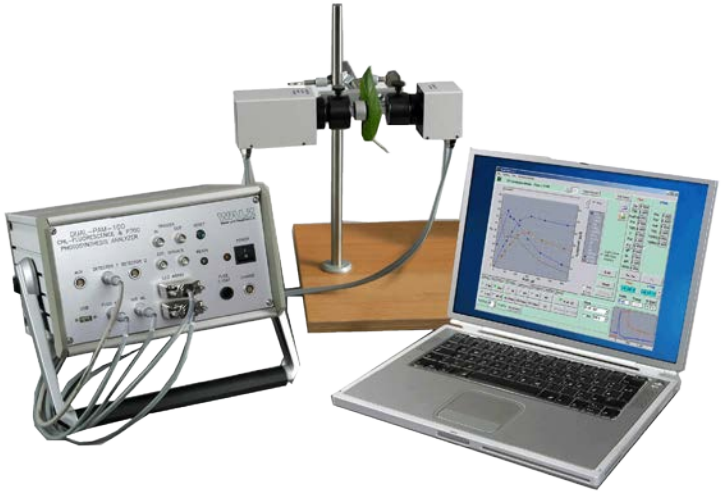
丰富多彩的PAM



德国

WALZ

泽泉 Zealquest®

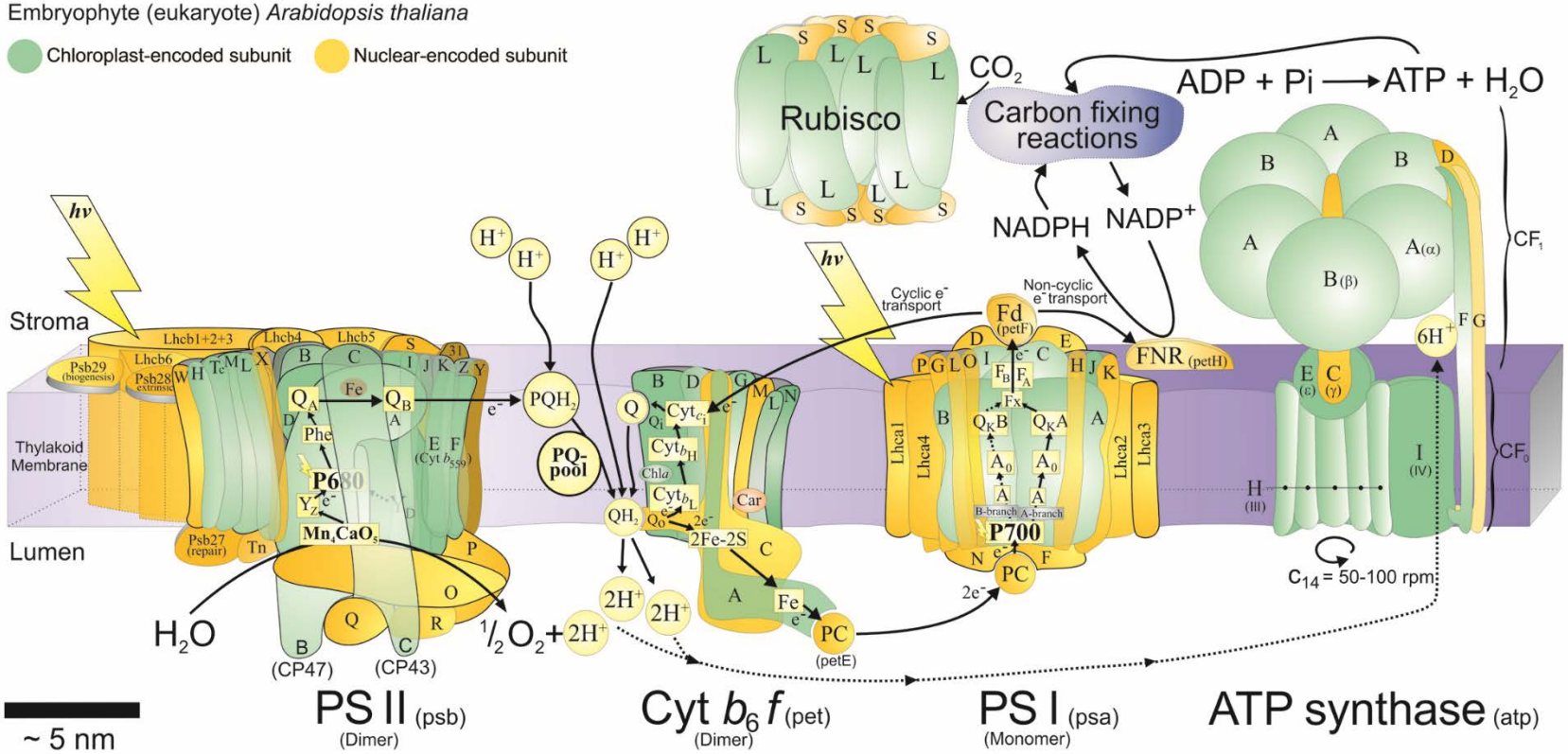
A structural phylogenetic map for chloroplast photosynthesis

John F. Allen, Wilson B. M. de Paula, Sujith Puthiyaveetil, Jon Nield
School of Biological and Chemical Sciences, Queen Mary University of London

TRENDS in Plant Science

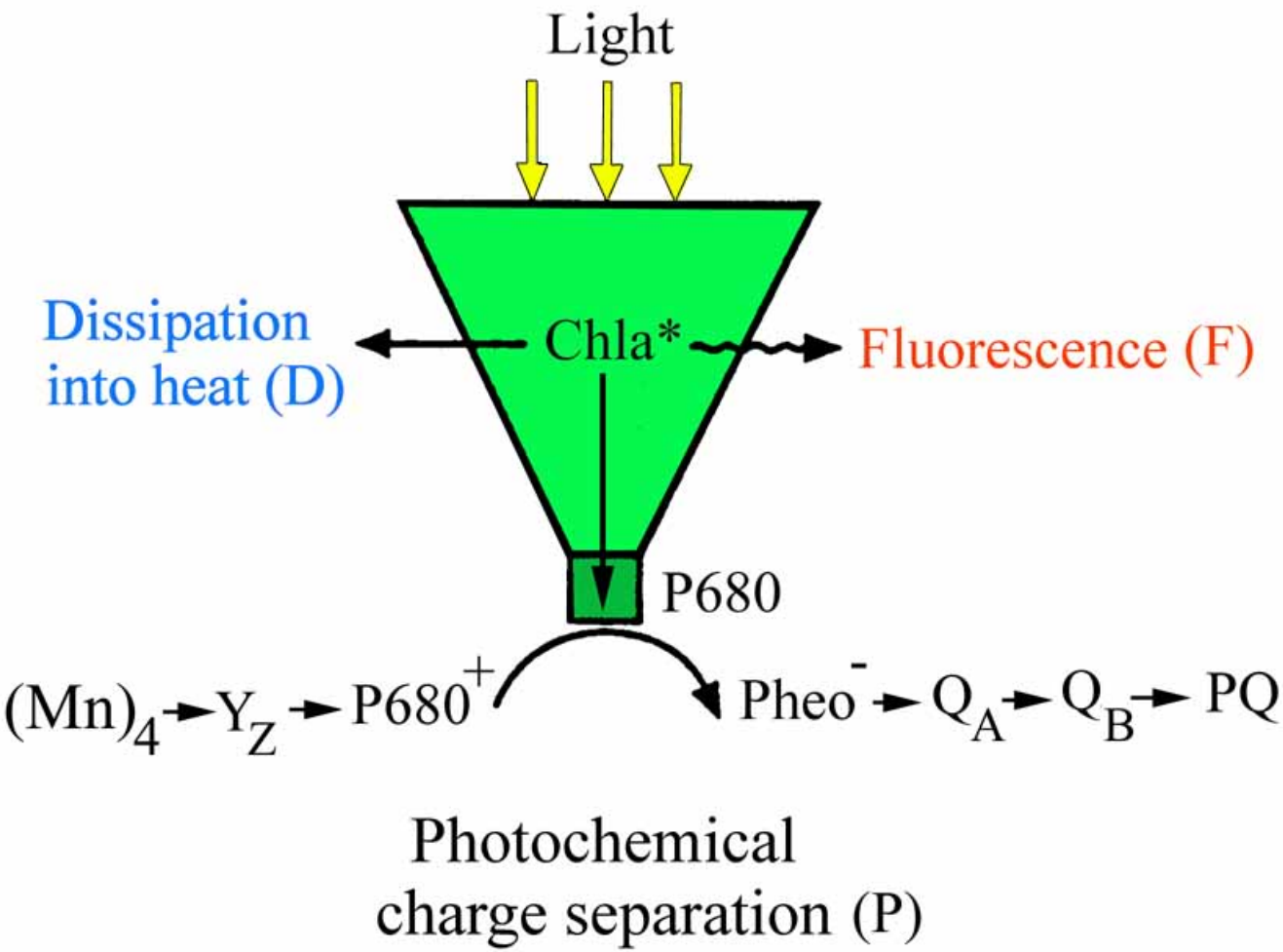
Embryophyte (eukaryote) *Arabidopsis thaliana*

Chloroplast-encoded subunit Nuclear-encoded subunit

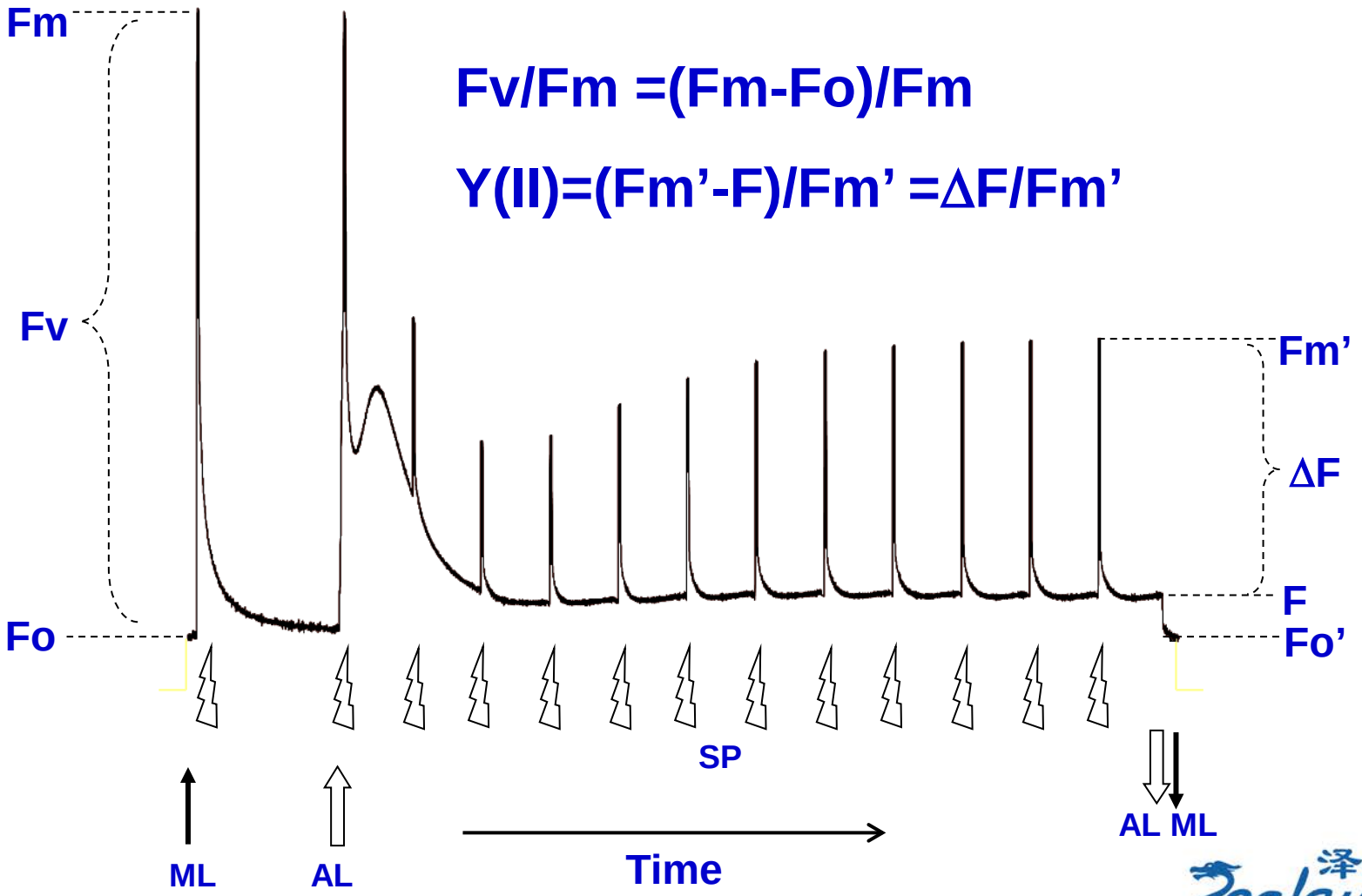


See online version for legend

- 荧光绝大部分是由PSII天线叶绿素a发出的，其总量约占吸收光能的3%左右。
- 光合作用过程的各个步骤密切偶联，任何一步的变化都会影响到PSII从而引起荧光变化，也就是说通过叶绿素荧光几乎可以探测所有光合作用过程的变化
- 叶绿素荧光是光合作用的有效探针（ Papageorgiou & Govindjee, 2004 ）


Light
Dissipation
into heat (D)
Chla*
Fluorescence (F)
P680
$(Mn)_4 \rightarrow Y_Z \rightarrow P680^+$
$Pheo^- \rightarrow Q_A \rightarrow Q_B \rightarrow PQ$
Photochemical
charge separation (P)

PAM叶绿素荧光诱导动力学曲线



$$Fv/Fm = (Fm-Fo)/Fm$$

$$Y(II) = (Fm'-F)/Fm' = \Delta F/Fm'$$

常用叶绿素荧光参数

- Fv/Fm =(Fm-Fo)/Fm : PS II的最大量子产量，反映了植物的潜在最大光合能力（光合效率）
- 当植物受到胁迫（Stress）时，Fv/Fm显著下降!
- 需充分暗适应，黎明前测最好，野外可使用暗适应夹。

- $Y(II)=\Phi_{PS\ II}=(Fm'-Fs)/Fm'=\Delta F/Fm'=qP\cdot Fv'/Fm'$:
  任一光照状态下PS II的实际量子产量（实际光合能力、实际光合效率）
  不需暗适应，不需测定Fo'，适合野外调查

常用叶绿素荧光参数

- 光化学淬灭
- qP=(Fm’-Fs)/Fv’=1-(Fs-Fo’)/(Fm’-Fo’) （基于“沼泽模型”）
- qL=(Fm’-F)/(Fm’-Fo’)·Fo’/F=qP·Fo’/F （基于“湖泊模型”）
- 即由光合作用引起的荧光淬灭，反映了光合活性的高低
- 非光化学淬灭
- qN=(Fv-Fv’)/Fv=1-(Fm’-Fo’)/(Fm-Fo)
- NPQ=(Fm-Fm’)/Fm’=Fm/Fm’-1 ，不需测定Fo’，适合野外调查
- qN或NPQ反映了植物耗散过剩光能为热的能力，反映了植物的光保护能力

常用叶绿素荧光参数

- Y(NPQ)=1-Y(II)-1/(NPQ+1+qL(Fm/Fo-1)) (Kramer et al., 2004) (需要Fo’)
- Y(NPQ)=F/Fm' - F/Fm (Genty et al., 1996; Klughammer and Schreiber, 2008)
- 调节性能量耗散的量子产量
- PS II处调节性能量耗散的量子产量。若Y(NPQ)较高，一方面表明植物接受的光强过剩，另一方面则说明植物仍可以通过调节（如将过剩光能耗散为热）来保护自身。Y（NPQ）是光保护的重要指标。

- Y(NO)=1/(NPQ+1+qL(Fm/Fo-1)) (Kramer et al., 2004) (需要Fo’)
- Y(NO)=F/Fm (Genty et al., 1996; Klughammer and Schreiber, 2008)
- 非调节性能量耗散的量子产量
- PS II处非调节性能量耗散的量子产量。若Y(NO)较高，则表明光化学能量转换和保护性的调节机制（如热耗散）不足以将植物吸收的光能完全消耗掉。也就是说，入射光强超过了植物能接受的程度。这时，植物可能已经受到损伤，或者（尽管还未受到损伤）继续照光的话植物将要受到损伤。Y（NO）是光损伤的重要指标。

Y(II)+Y(NPQ)+Y(NO)=1

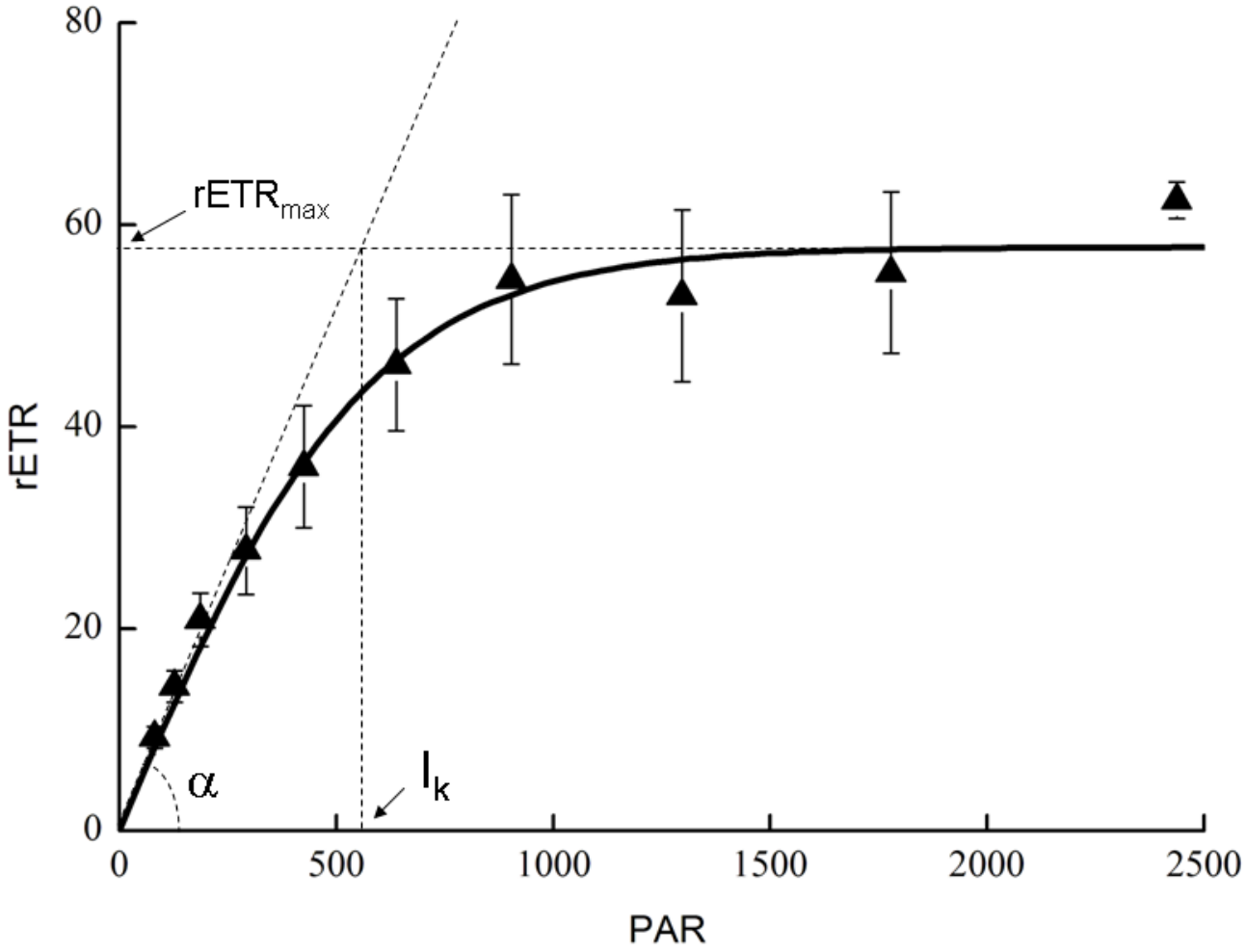
Kramer et al, Photosynth Res, 2004, 79:209-218.

常用叶绿素荧光参数

- rETR =PAR·Y(II)·0.84·0.5：光系统II的相对电子传递速率
- rETR随PAR的变化图即为光响应曲线，即使光化光的持续时间短至10 s，也可得出典型的光响应曲线，这被称为快速光曲线（Rapid Light Curves）

- ETR(II)λ =PAR(II)·Y(II)/Y(II)max：光系统II的绝对电子传递速率
- 首先需要利用MULTI-COLOR-PAM测定某个波长下的光系统II功能性光学截面积Sigma(II)λ（单位nm^2）（其中λ为波长），然后求出光系统II的量子吸收速率PAR(II)=Sigma(II)λ·L·PAR=0.6022·Sigma(II)λ·PAR。其中L为阿伏伽德罗常数，系数0.6022是将1 μmol quanta m^{-2}（即6.022×10^{17} quanta m-2）转换为0.6022 quanta nm^{-2}，PAR(II)的单位为quanta/(PSII · s)。接下来就可以计算ETR(II)λ=PAR(II) · Y(II)/Y(II)max，其中Y(II)max是经过暗适应达到稳态后的光系统II的量子产量，也就是Fv/Fm。ETR(II)的单位为electrons/(PSII · s)。

快速光曲线



拟合参数的意义

- P：光合速率，即相对电子传递速率rETR
- P_m: 最大光合速率，即最大相对电子传递速率$rETR_{max}$
- α：初始斜率，反映了光能的利用效率
- β：光抑制参数
- $I_k=P_m/\alpha$：半饱和光强，反映了样品对强光的耐受能力

差示吸收技术—P700、 P515/535 测定原理与应用

PSI的反应中心--P700

A structural phylogenetic map for chloroplast photosynthesis

John F. Allen, Wilson B. M. de Paula, Sujith Puthiyaveetil, Jon Nield
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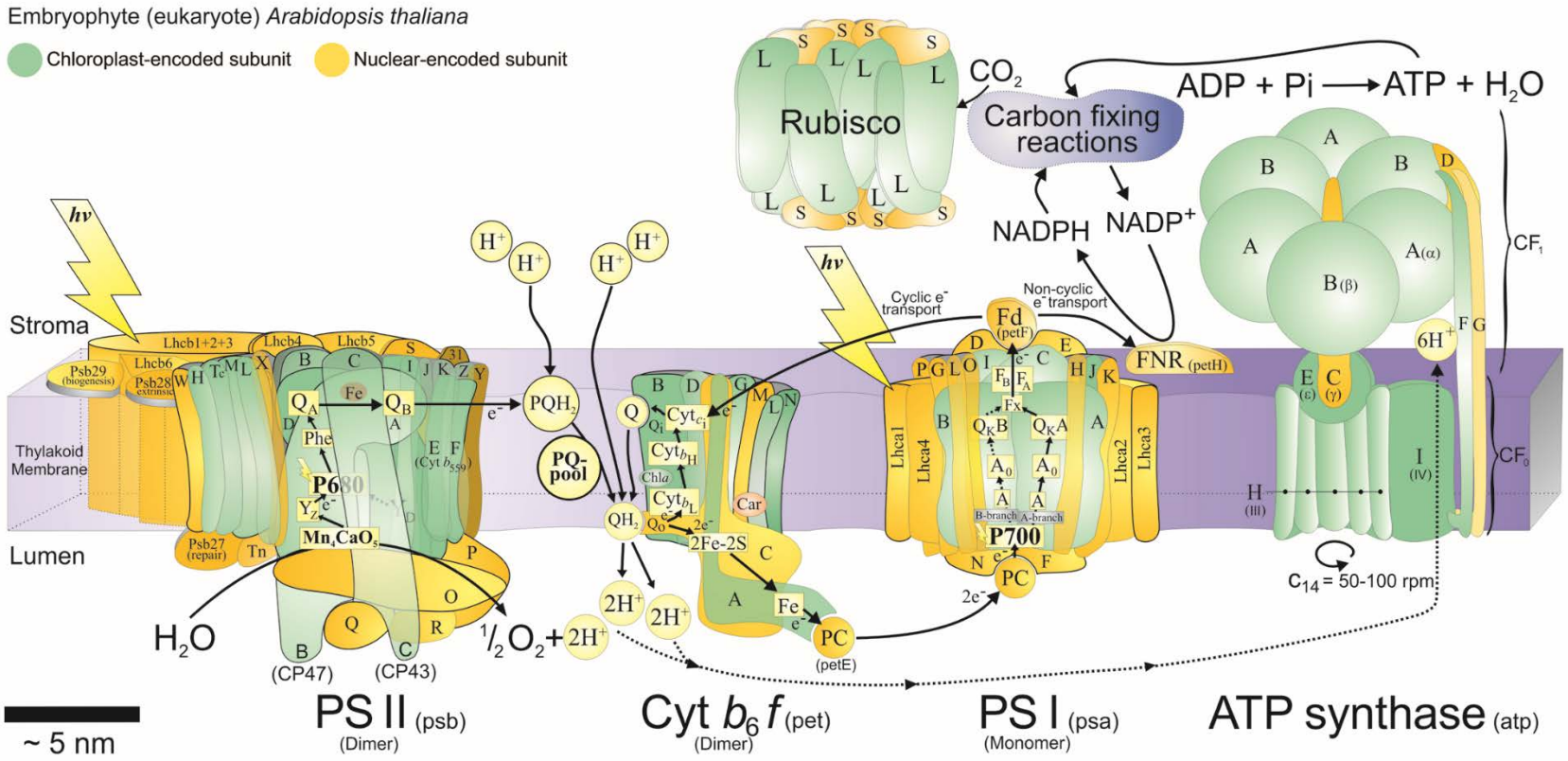
TRENDS in Plant Science



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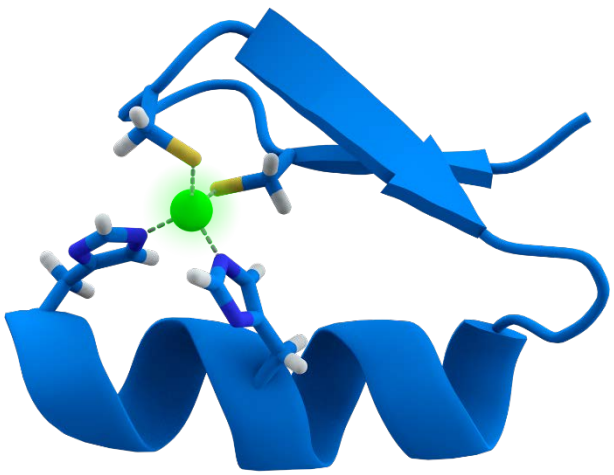
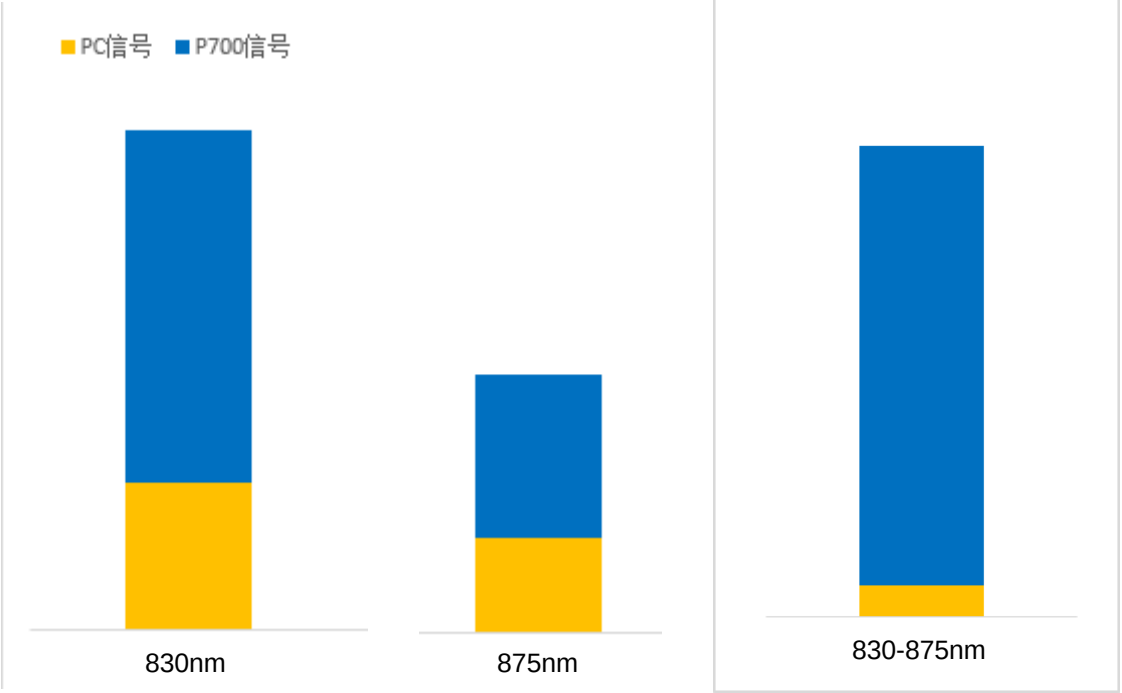
Trends in Plant Science, December 2011, Vol. 16 (No. 12)

DUAL-PAM双波长测定体系



含铜小分子蛋白 plastocyanin, PC



Plant and Cell Physiology Advance Access published April 6, 2016

Dual/KLAS-NIR

PCP
PLANT & CELL PHYSIOLOGY

Analysis of Photosystem I Donor and Acceptor Sides with a New Type of Online-Deconvoluting Kinetic LED-Array Spectrophotometer

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Julius-von Sachs Institut für Biowissenschaften, Universität Würzburg, D-97082 Würzburg, Germany

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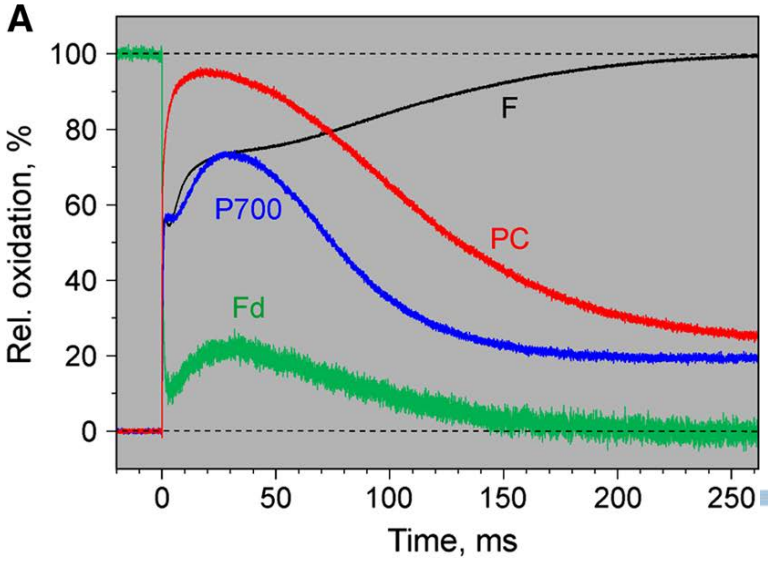
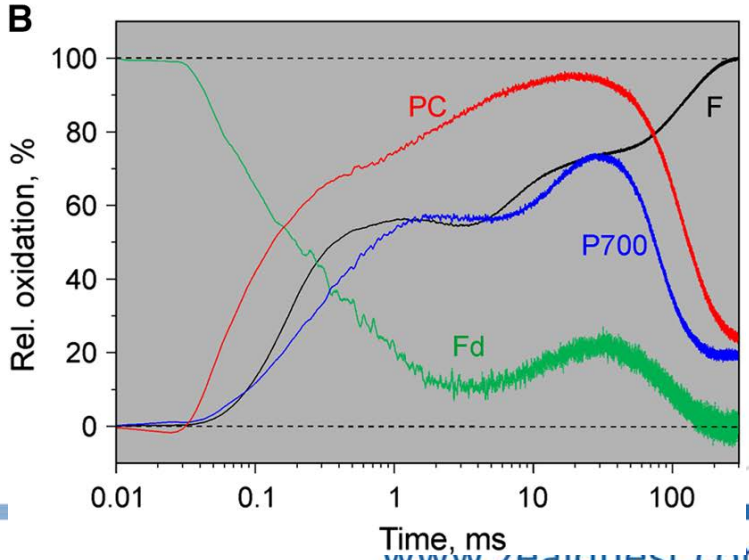
(Received January 04, 2016; Accepted February 16, 2016)

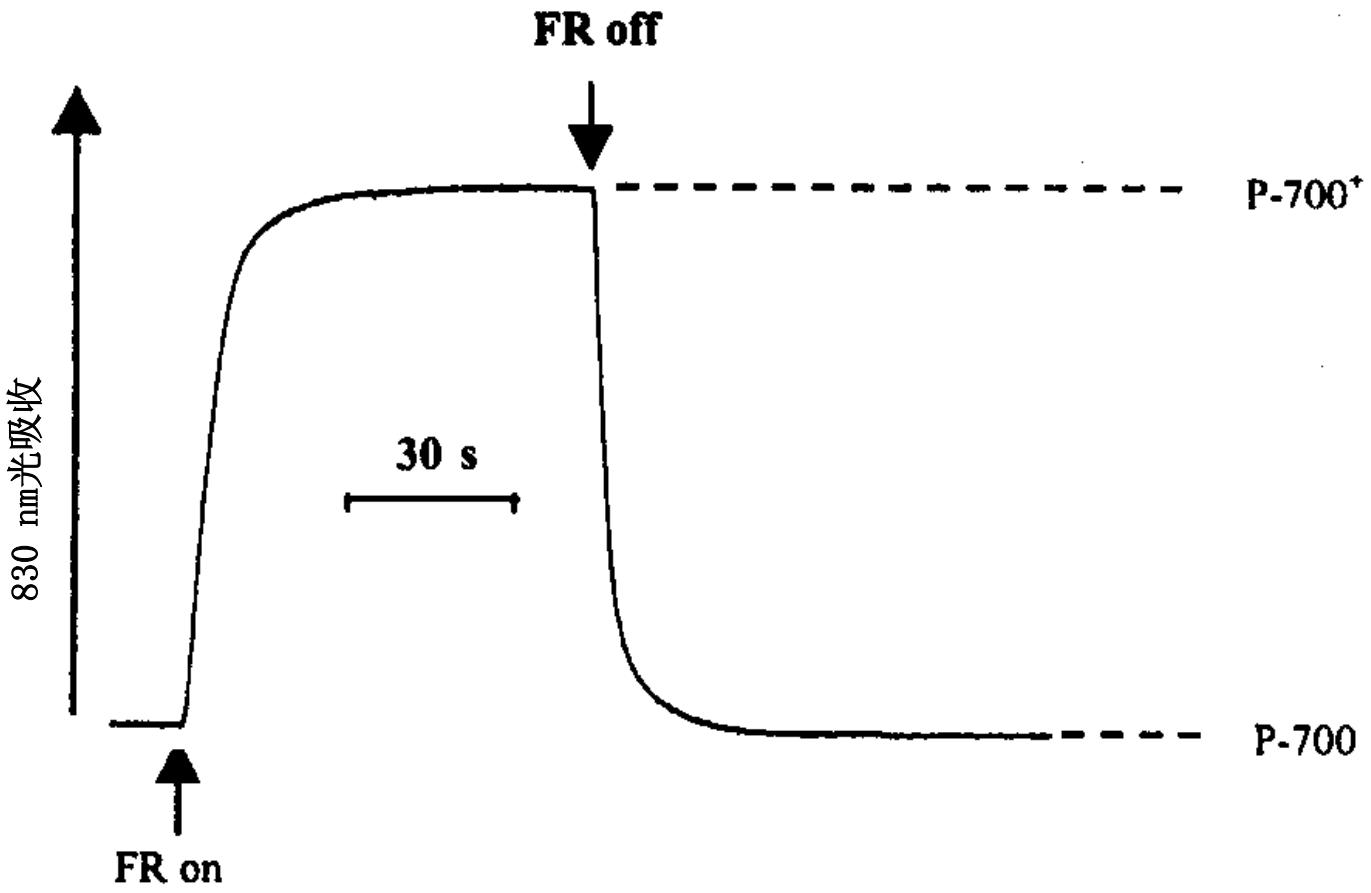
德国WALZ公司首席科学家，调制叶绿素荧光测量技术（PAM）的发明者
——乌兹堡大学Ulrich Schreiber教授





Special Focus Is

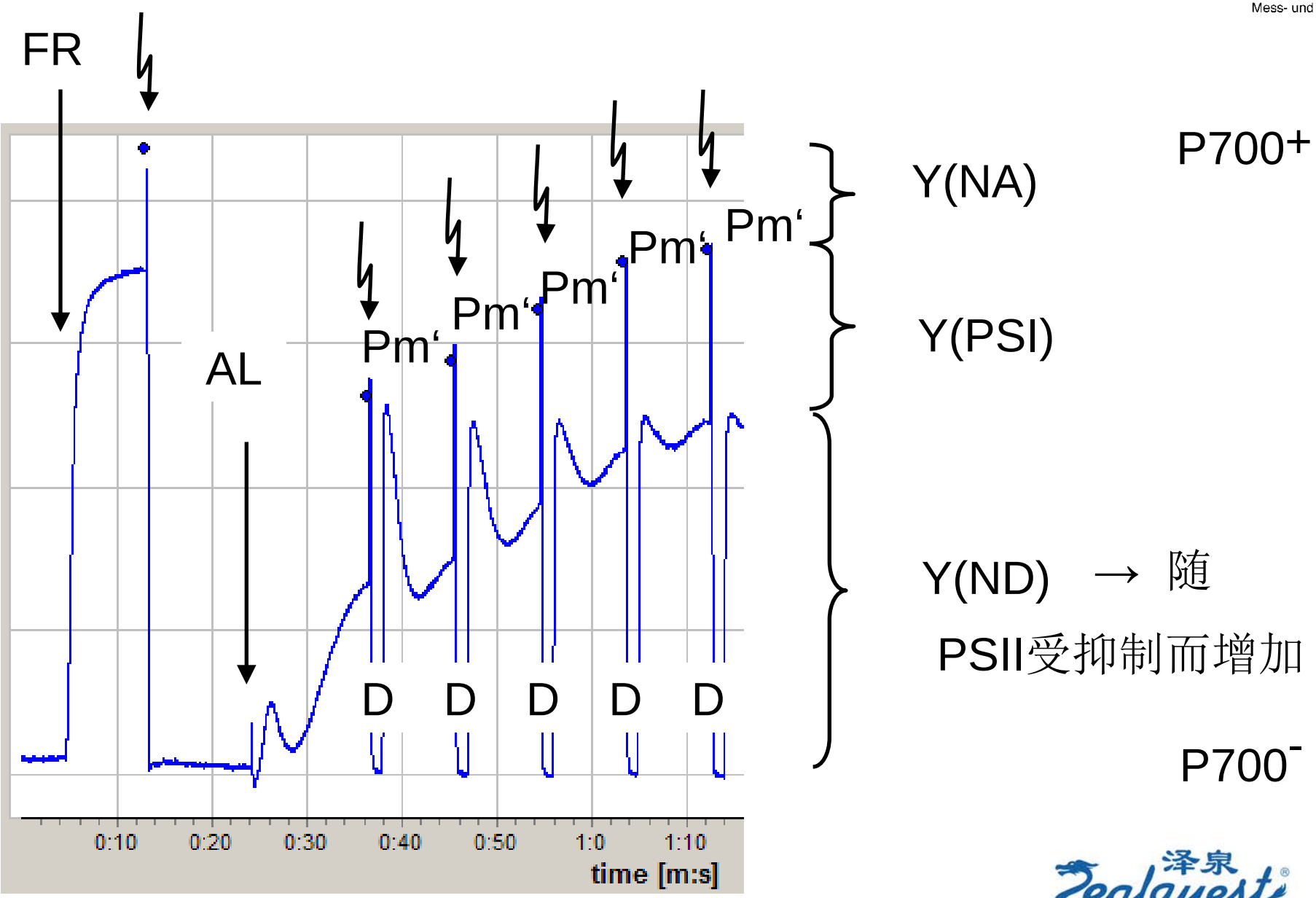
远红光诱导的P700的氧化还原动力学



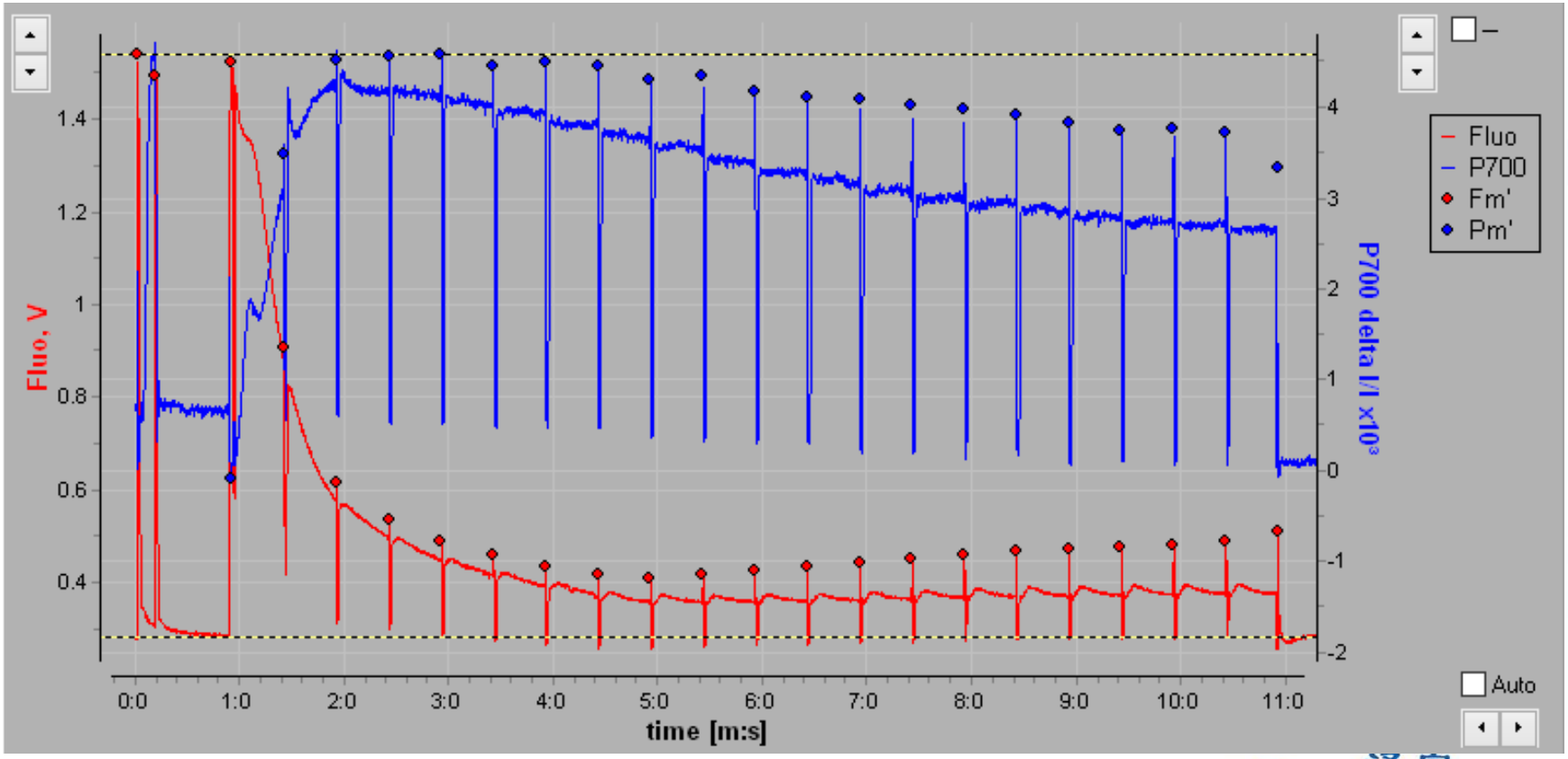
将饱和脉冲技术应用于P700的测定

The redox state of P700 provides similar information on Photosystem I as chlorophyll fluorescence on Photosystem II using a special

Saturation Pulse method



通过Dual-PAM-100可实现
荧光与P700互不干扰同步测定



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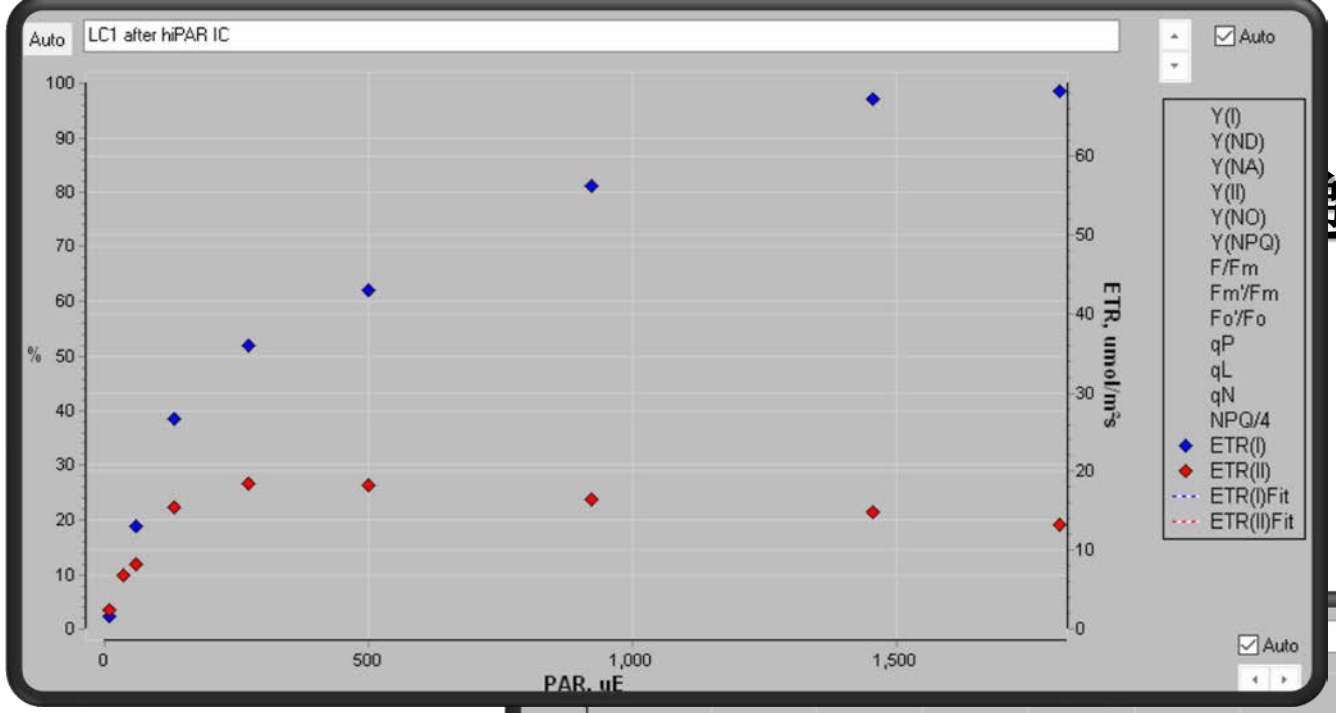
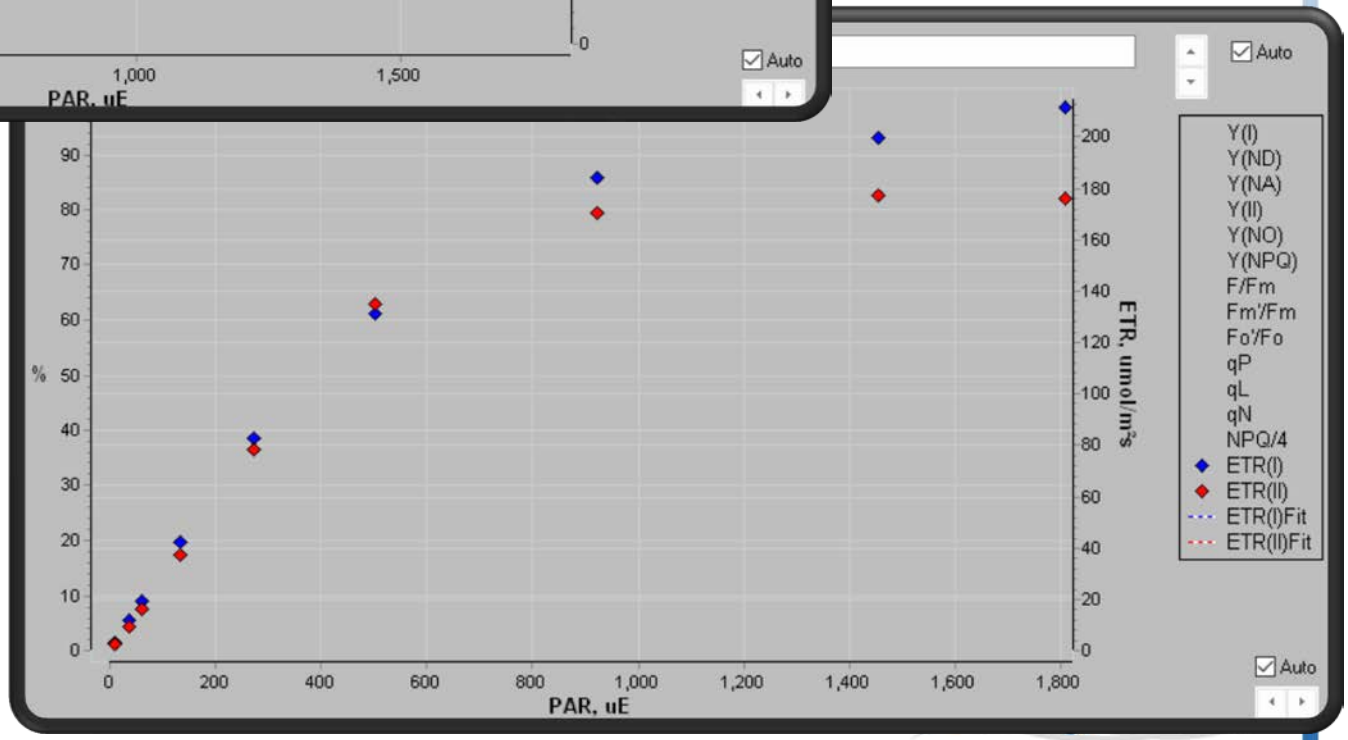
速率

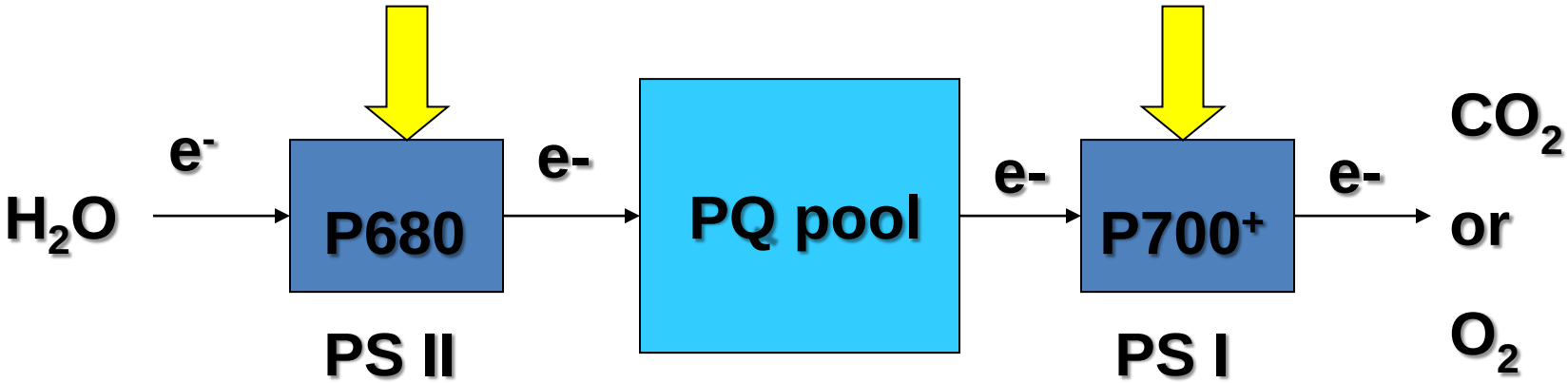

Auto
LC1 after hiPAR IC
Auto
%
ETR, umol/m²s
PAR, uE
Y(I)
Y(ND)
Y(NA)
Y(II)
Y(NO)
Y(NPQ)
F/Fm
Fm'/Fm
Fo'/Fo
qP
qL
qN
NPQ/4
ETR(I)
ETR(II)
ETR(I)Fit
ETR(II)Fit
Auto

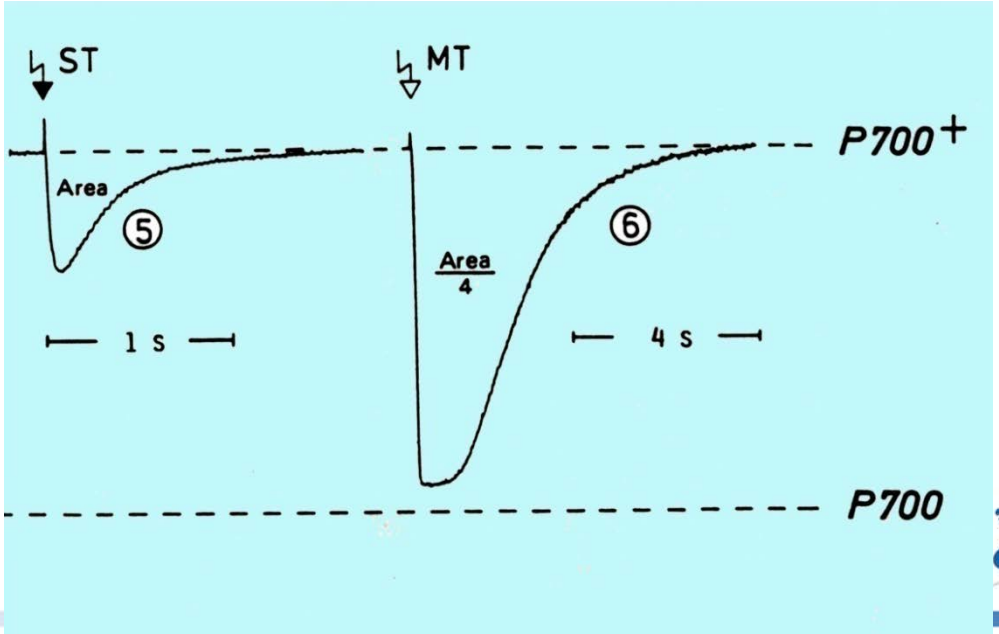
绿萝


Auto
%
ETR, umol/m²s
PAR, uE
Y(I)
Y(ND)
Y(NA)
Y(II)
Y(NO)
Y(NPQ)
F/Fm
Fm'/Fm
Fo'/Fo
qP
qL
qN
NPQ/4
ETR(I)
ETR(II)
ETR(I)Fit
ETR(II)Fit
Auto

心叶日中花

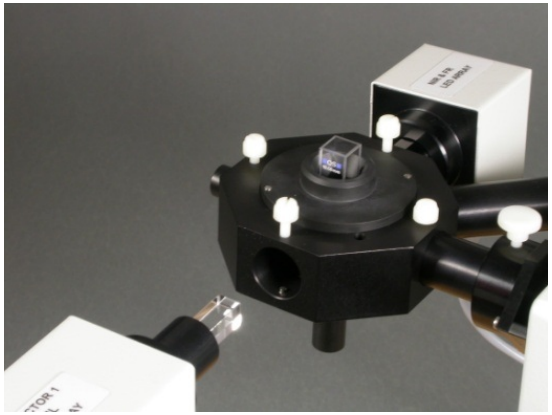
通过ST和MT测定PQ库的大小



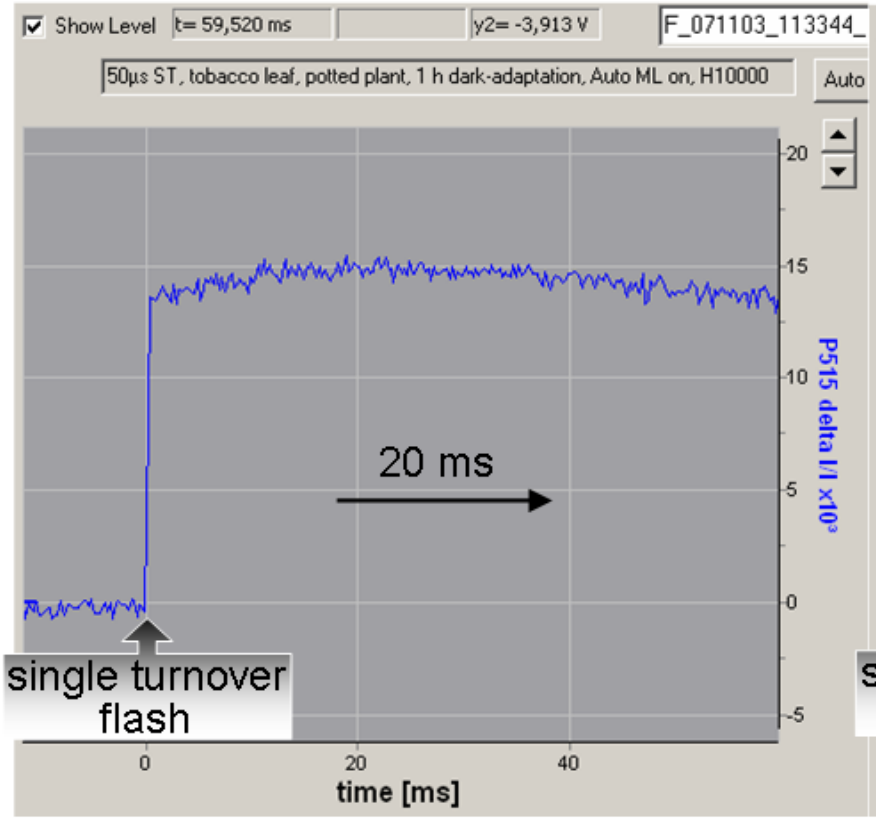


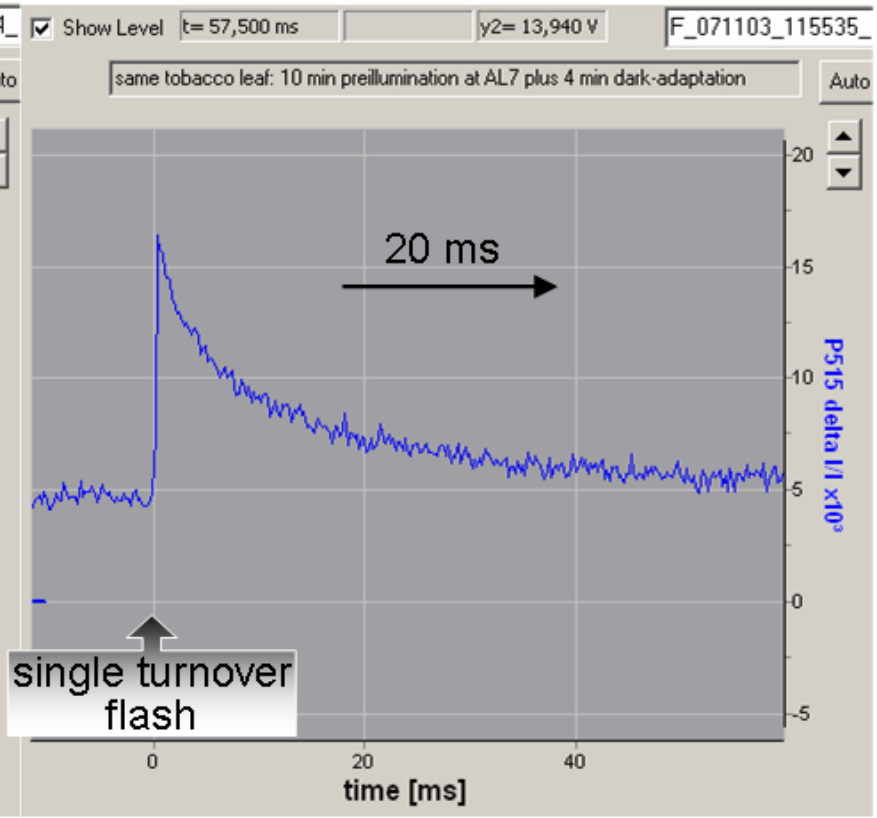
Dual-PAM-100的扩展功能

- 连接特殊的检测模块（P515/535），通过电致变色效应（ECS）测定跨膜质子梯度ΔpH、跨膜电位Δψ，和NADP的氧还状态、叶黄素循环等



ATP酶的通透性

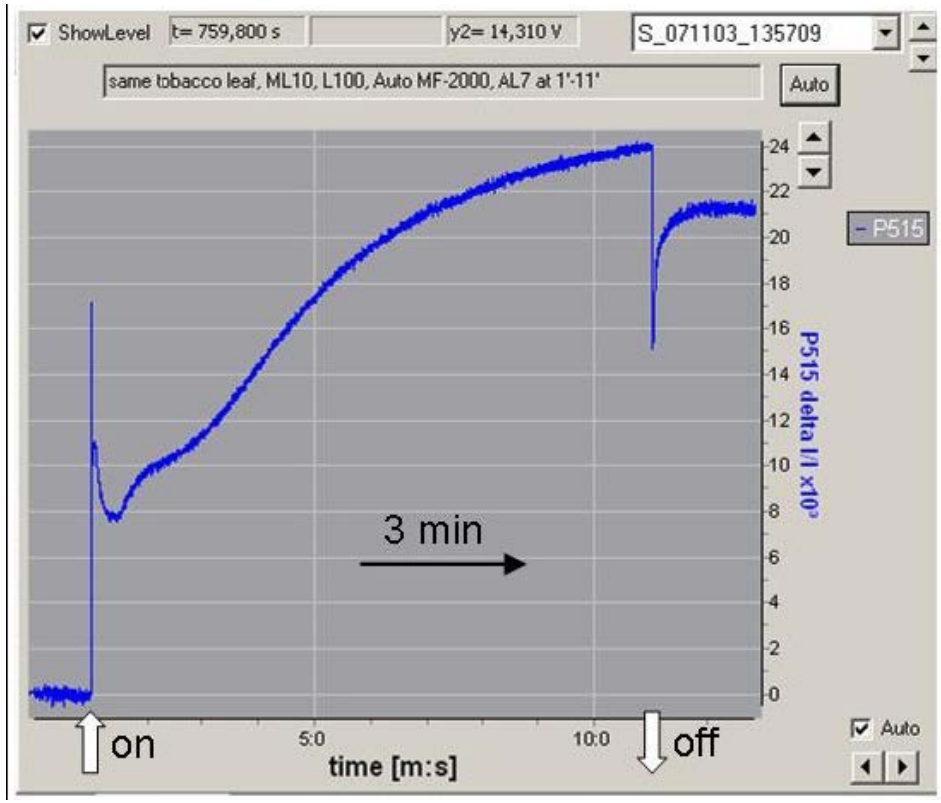


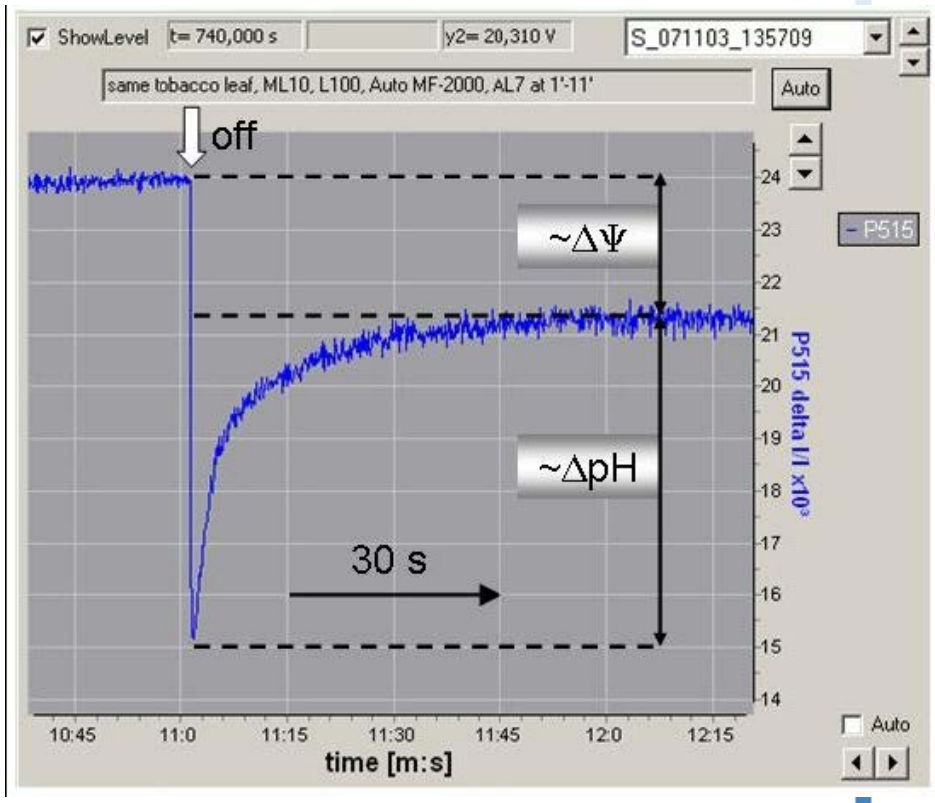


暗适应后

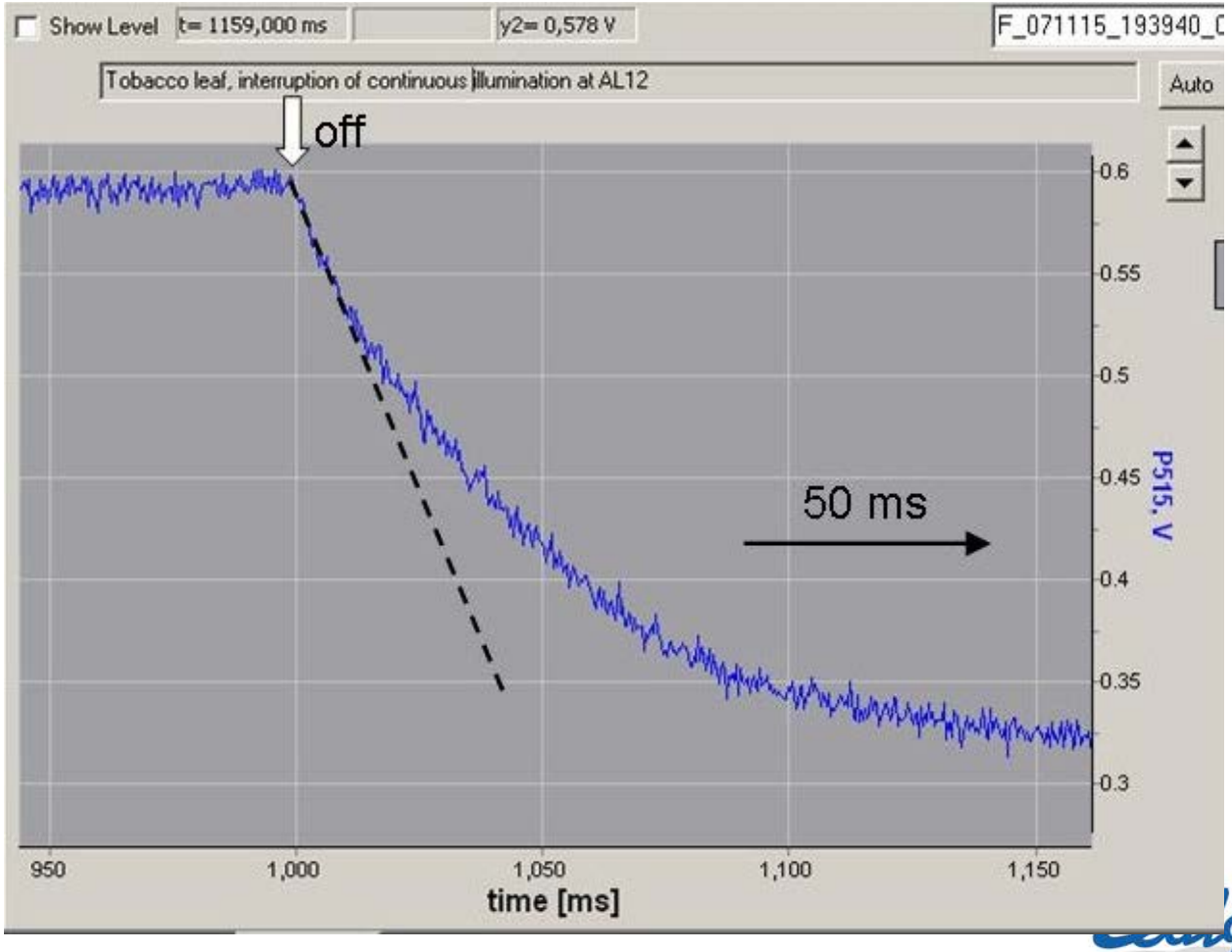
光适应后

P515信号的暗-光-暗诱导动力学

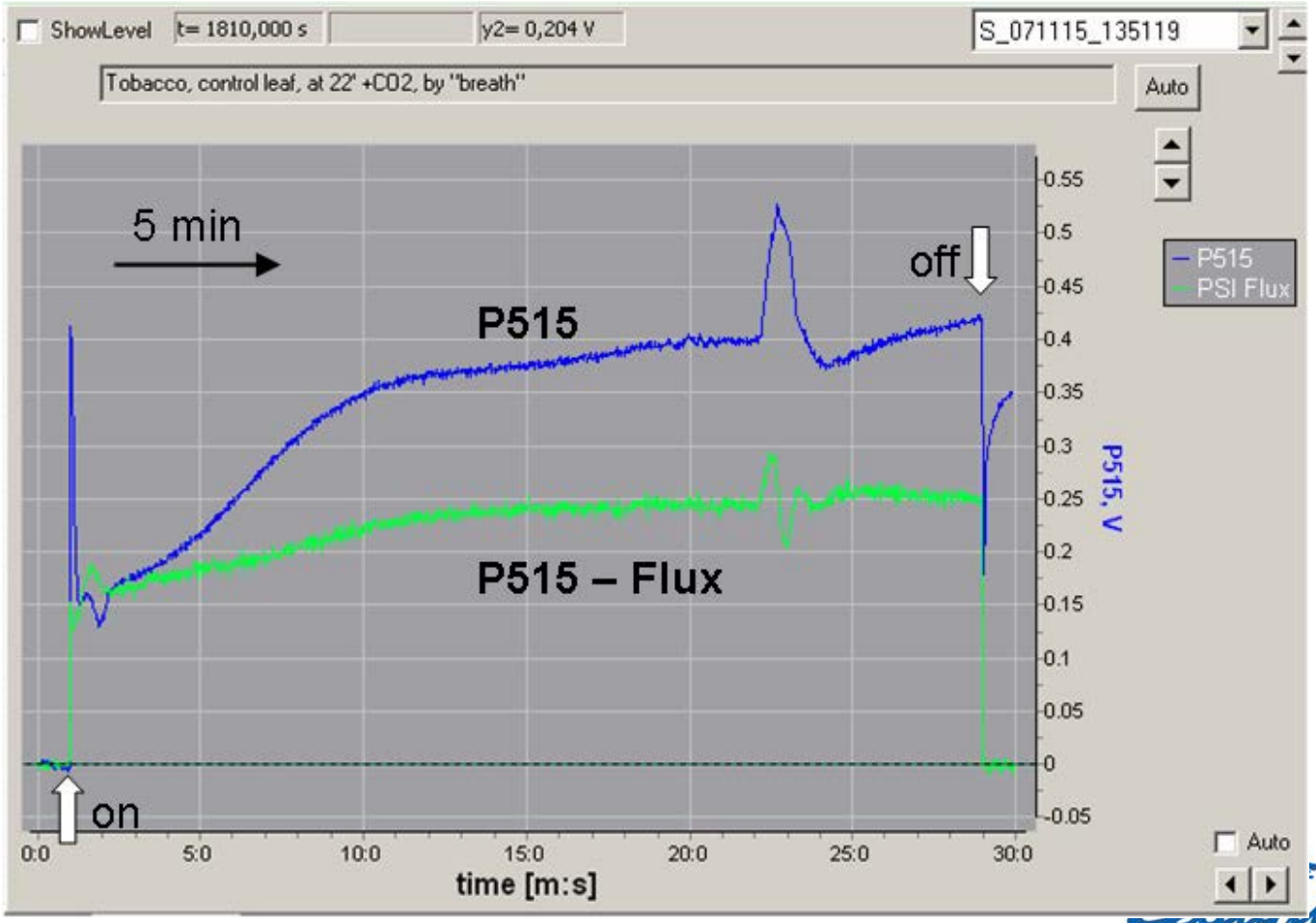




关光时P515衰减动力学曲线



Flux Mode下P515可实时监测H^+流速



小结

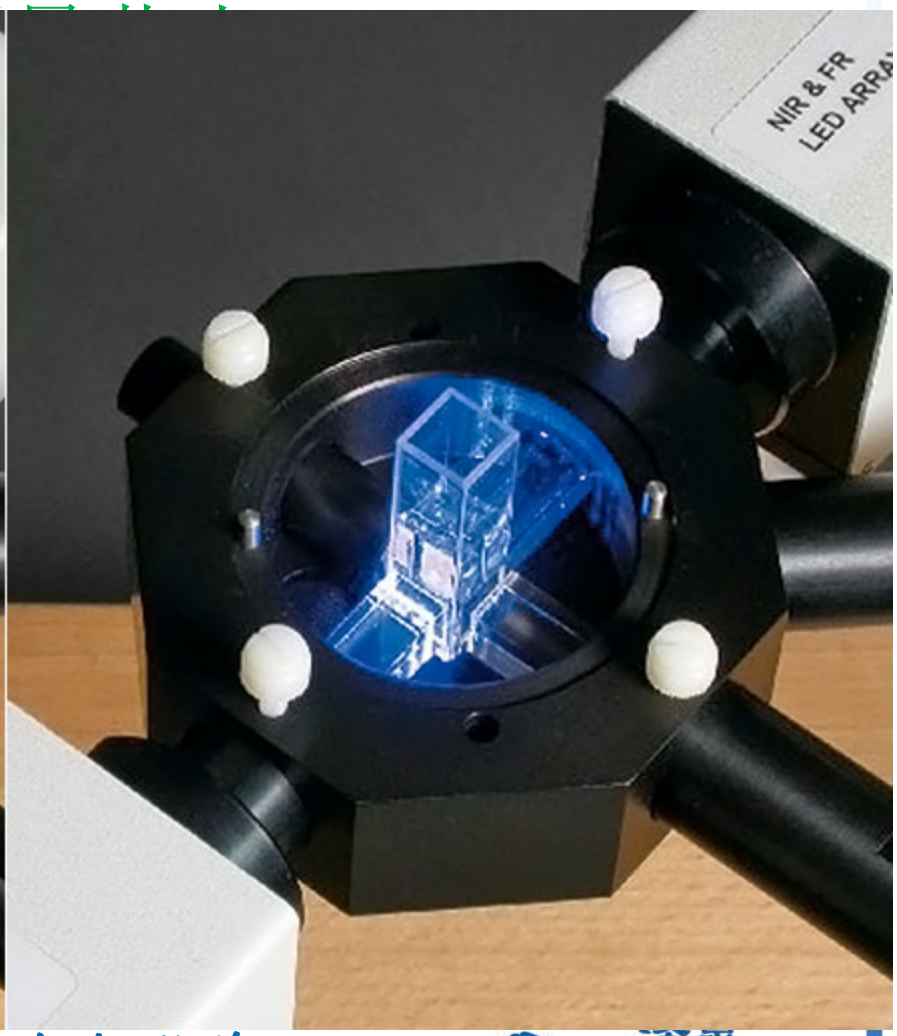
- 荧光&P700探头可测定：

P700氧化还原动力学、PQ 库、Y(ND）、Y(NA）、Y（I）、ETR（I）、同步测量所有荧光参数。

- P515/535探头可测定：

Δψ，ΔpH，pfm，ATP酶通透性，H^+流速

Dual-PAM-100



可测定叶片或微藻

更全面研究PSI和PSII的运转情况

应用实例

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低温处理时对根部保暖会阻断电子传递

High Root Temperature Blocks Both Linear and Cyclic Electron Transport in the Dark During Chilling of the Leaves of Rice Seedlings

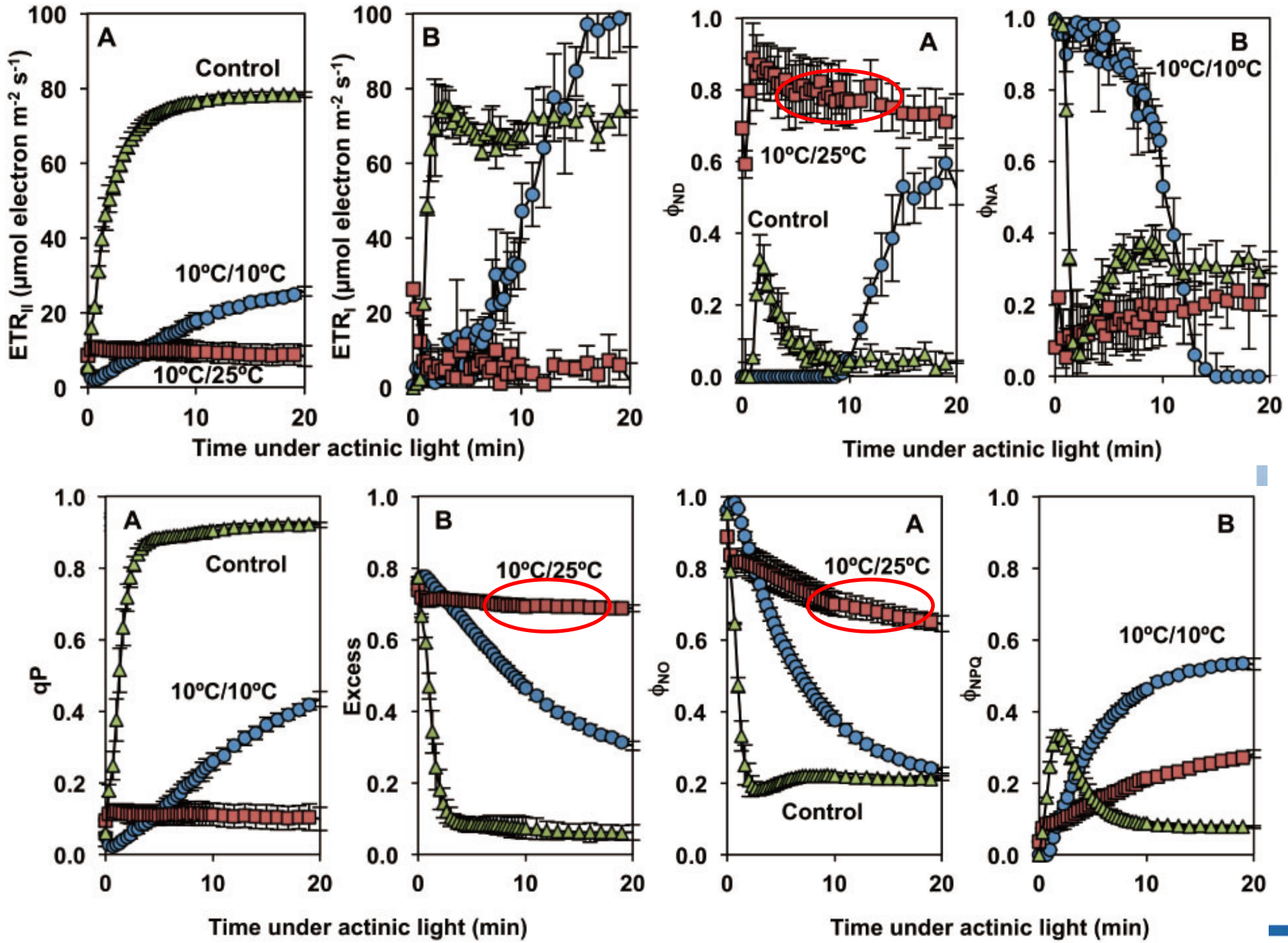
Kensaku Suzuki*, Yukimi Ohmori and Emilien Ratel

Tohoku National Agricultural Research Center, Shimo-Kuriyagawa, Morioka, Iwate, 020-0198 Japan
*Corresponding author: E-mail, suzuki@affrc.go.jp; Fax, +81-19-641-7794
(Received January 11, 2011; Accepted July 26, 2011)

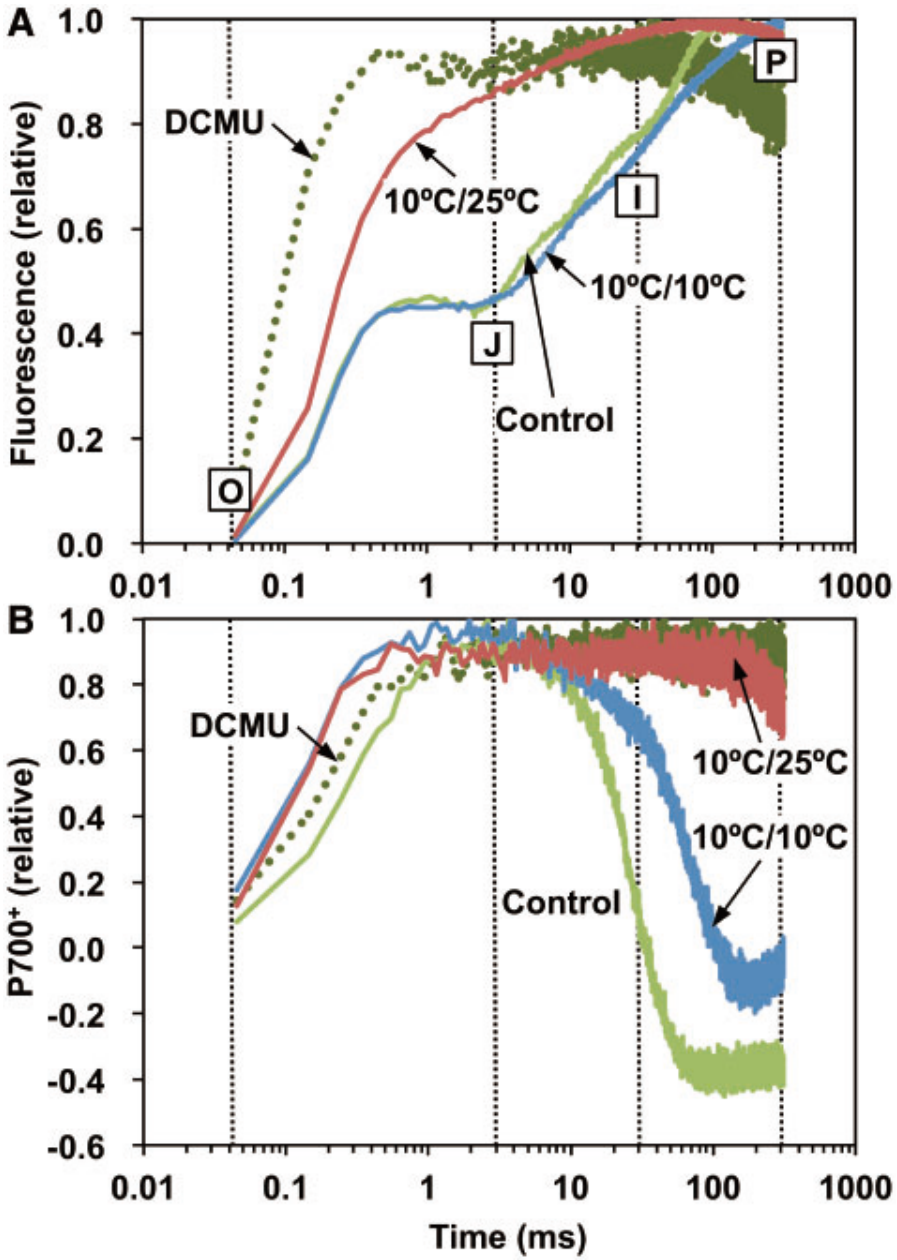
Regular Paper

The most photosynthetically active leaves of rice seedlings were severely damaged when shoots but not roots were chilled (10°C/25°C, respectively), but no such injury was observed when the whole seedling was chilled (10°C/10°C). To elucidate the mechanisms, we compared the photosynthetic characteristics of the seedlings during the dark chilling treatments. Simultaneous analyses of Chl fluorescence and the change in absorbance of P700 showed

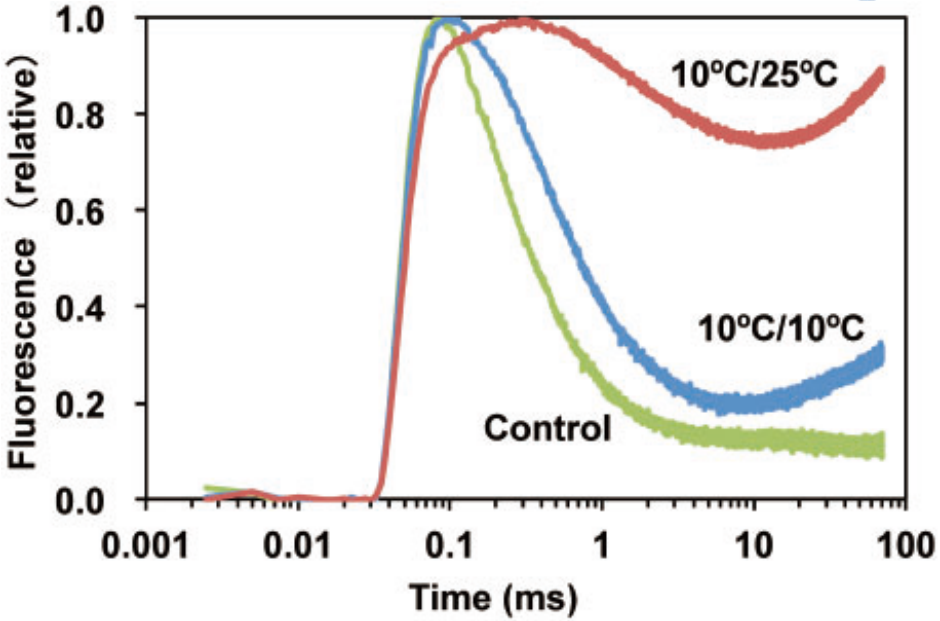
electron transport rate in PSII; F, fluorescence measured during illumination; F_m, maximum fluorescence of dark-adapted leaf; F_m', the maximum fluorescence measured during illumination; F_o, minimum fluorescence of the dark-adapted leaf; F_o', the minimum fluorescence immediately after illumination; F_P, fluorescence at the peak (P) of the fast fluorescence induction phase; F_v, variable fluorescence; F_v/F_m, maximum quantum yield of PSII; NPQ, non-


A
Control
10°C/10°C
10°C/25°C
ETR$_{II}$ (µmol electron m^{-2} s^{-1})
B
ETR$_{I}$ (µmol electron m^{-2} s^{-1})
Time under actinic light (min)
A
10°C/25°C
Control
ϕ_{ND}
B
10°C/10°C
ϕ_{NA}
Time under actinic light (min)
A
Control
10°C/10°C
qP
B
10°C/25°C
Excess
Time under actinic light (min)
A
10°C/25°C
Control
ϕ_{NO}
B
10°C/10°C
ϕ_{NPQ}
Time under actinic light (min)

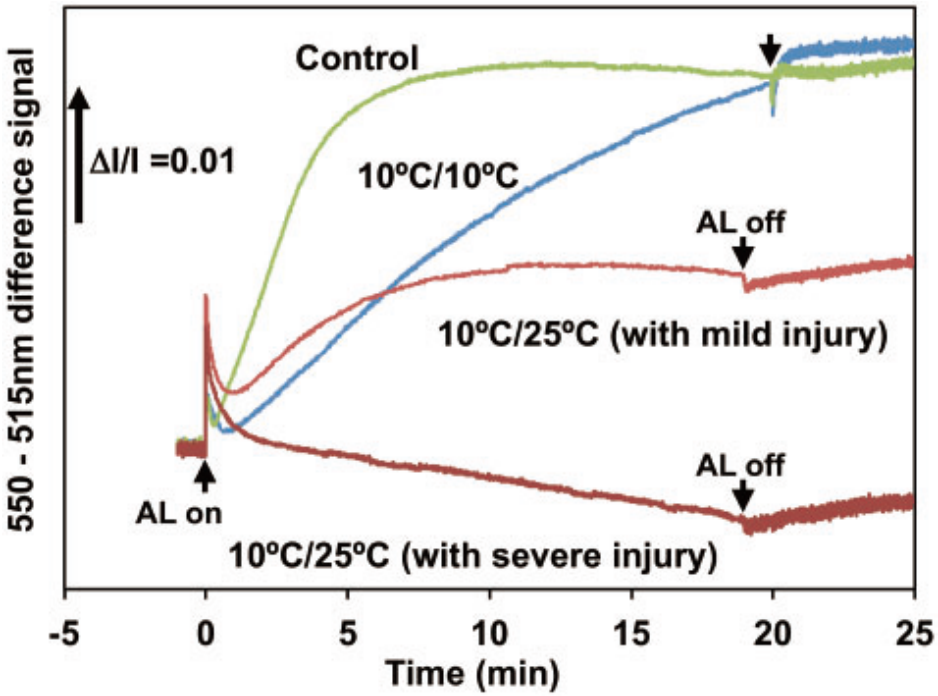
荧光及P700的快相同步测量数据

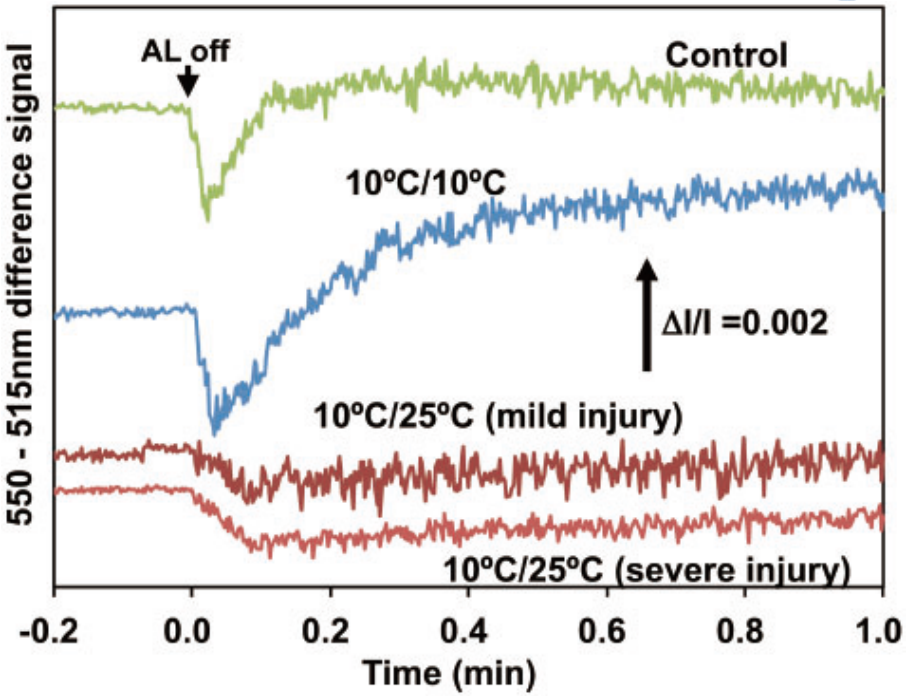


ST快相表明，QB没有PQ结合



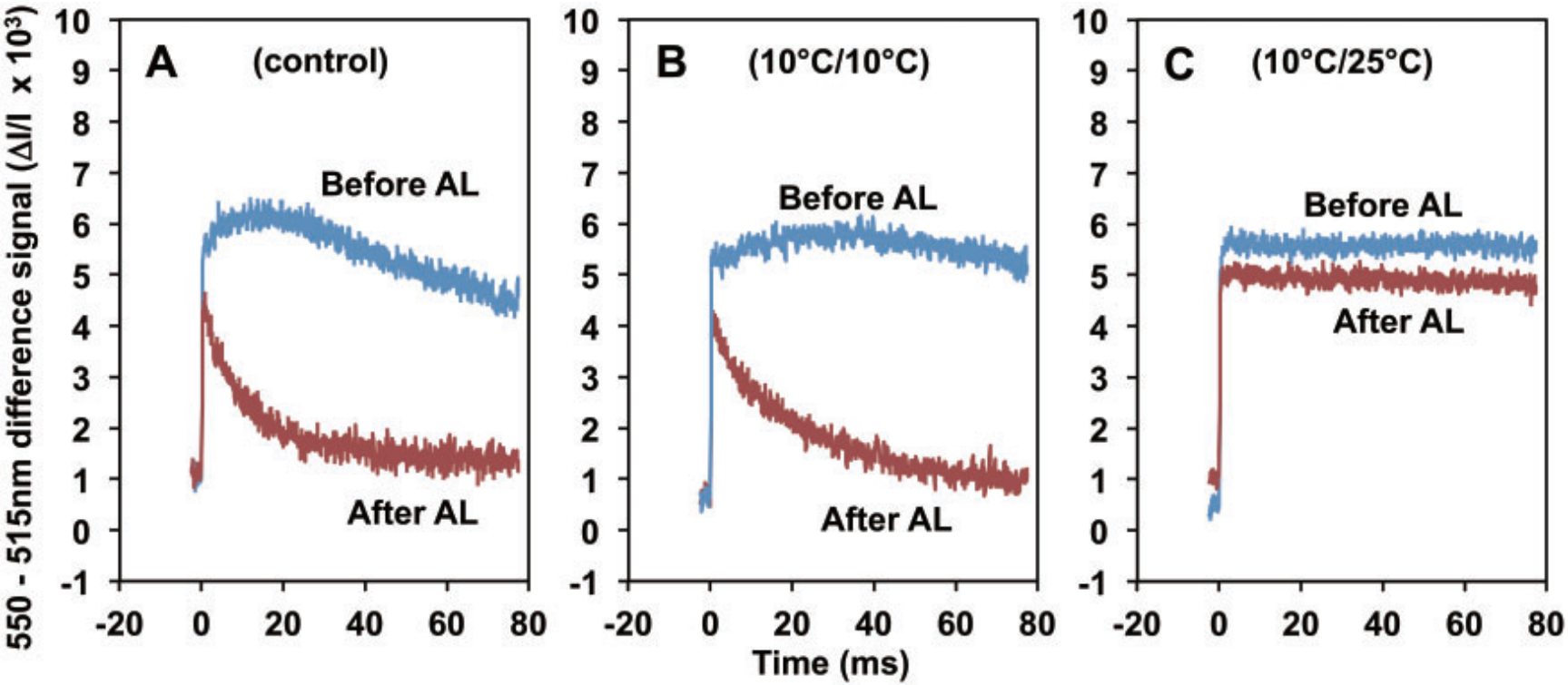
P515数据





没有**ΔpH**形成

叶绿素荧光、P700、P515/535同步测量



从P515信号的单周转快相动力学曲线可以帮助我们了解位于类囊体膜上的ATP酶的通透性。

没有PQ与QB结合

A structural phylogenetic map for chloroplast photosynthesis

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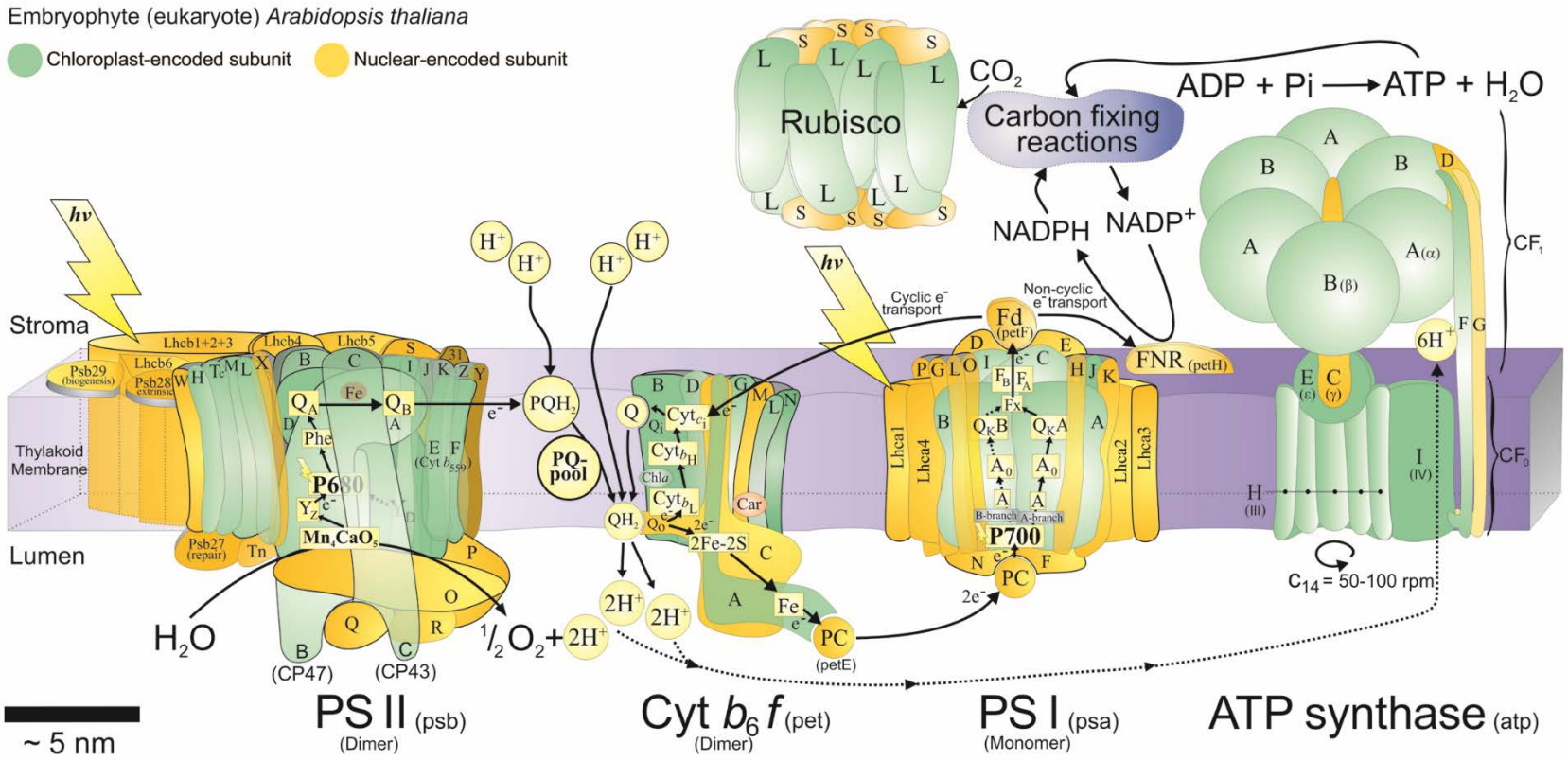
TRENDS in Plant Science



See online version for legend

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谢 谢 !