Joliot-Type spectrophotometry: Theory and Applications

PSC Symposium

November 8th, 2019

Knoxville, TN

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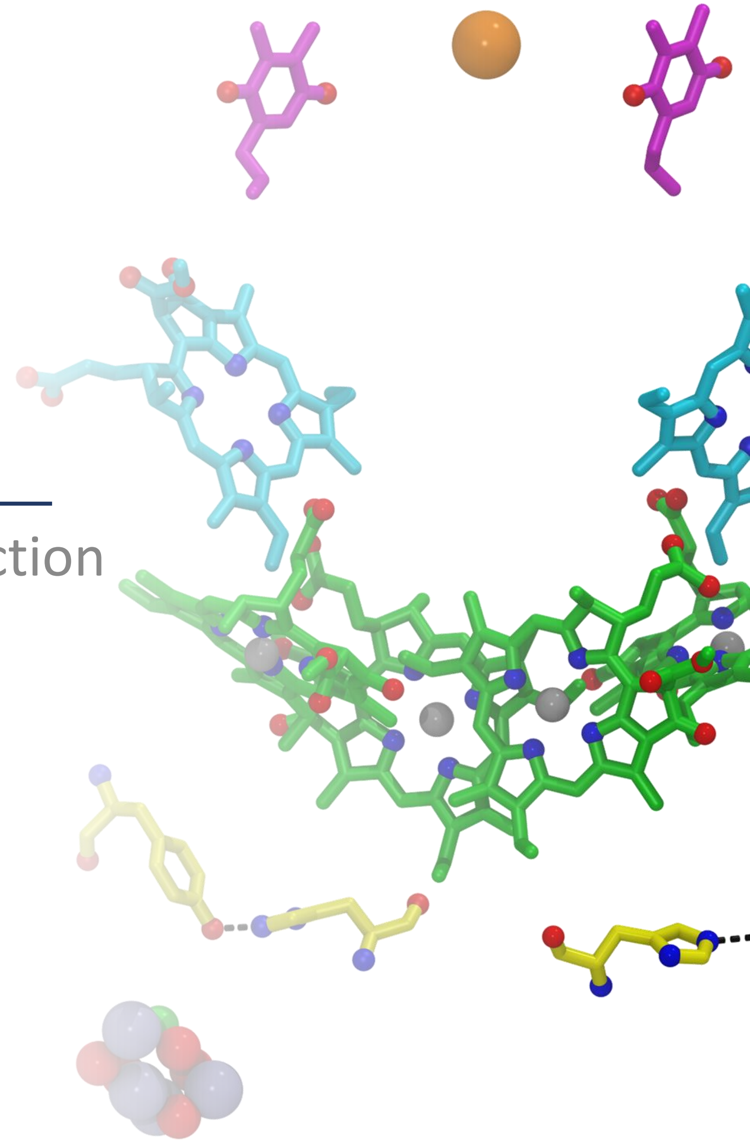
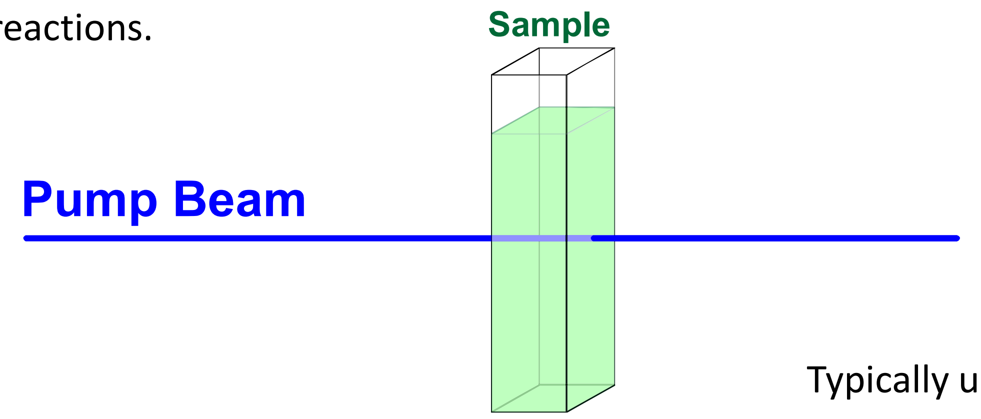
1. Introduction

Pump-Probe spectroscopy & the introduction of Joliot-type spectrophotometry



Pump-Probe spectroscopy

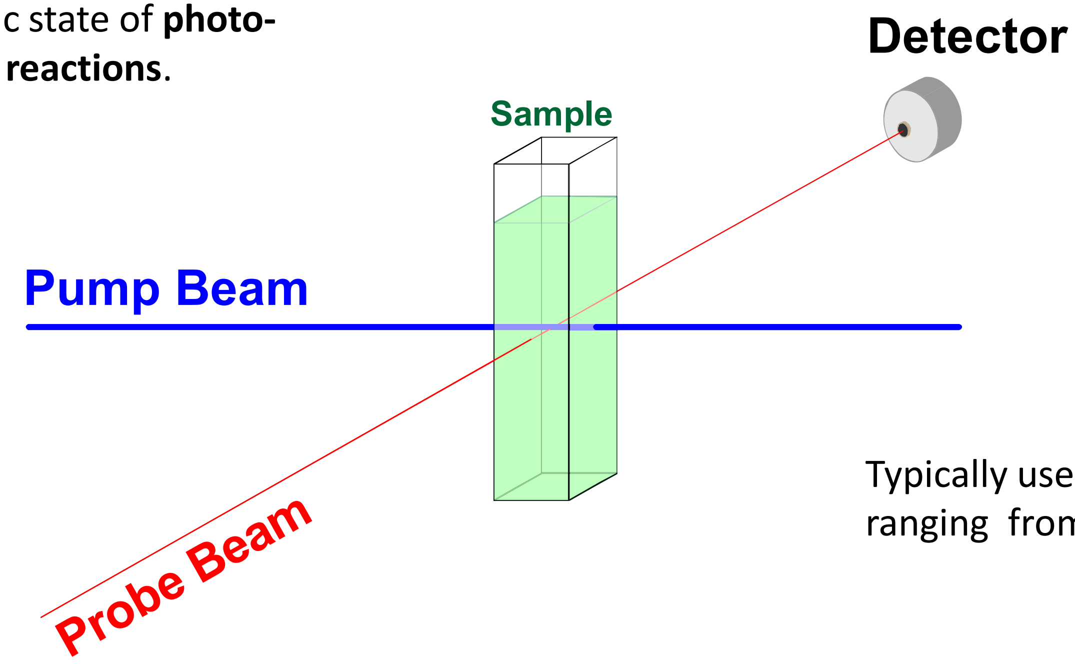
Used for measuring changes in electronic state of photo-initiated reactions.

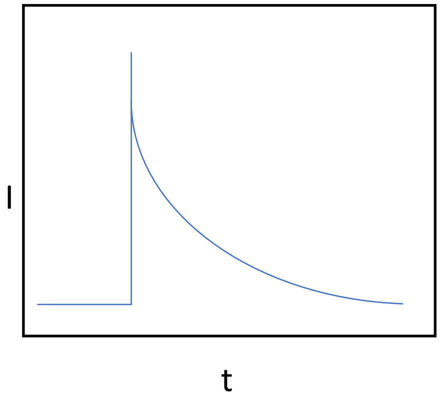


Typically used to measure rates ranging from 10^{12} - 10^{6} s^{-1}.

Pump-Probe spectroscopy

Used for measuring changes in electronic state of **photo-initiated reactions**.

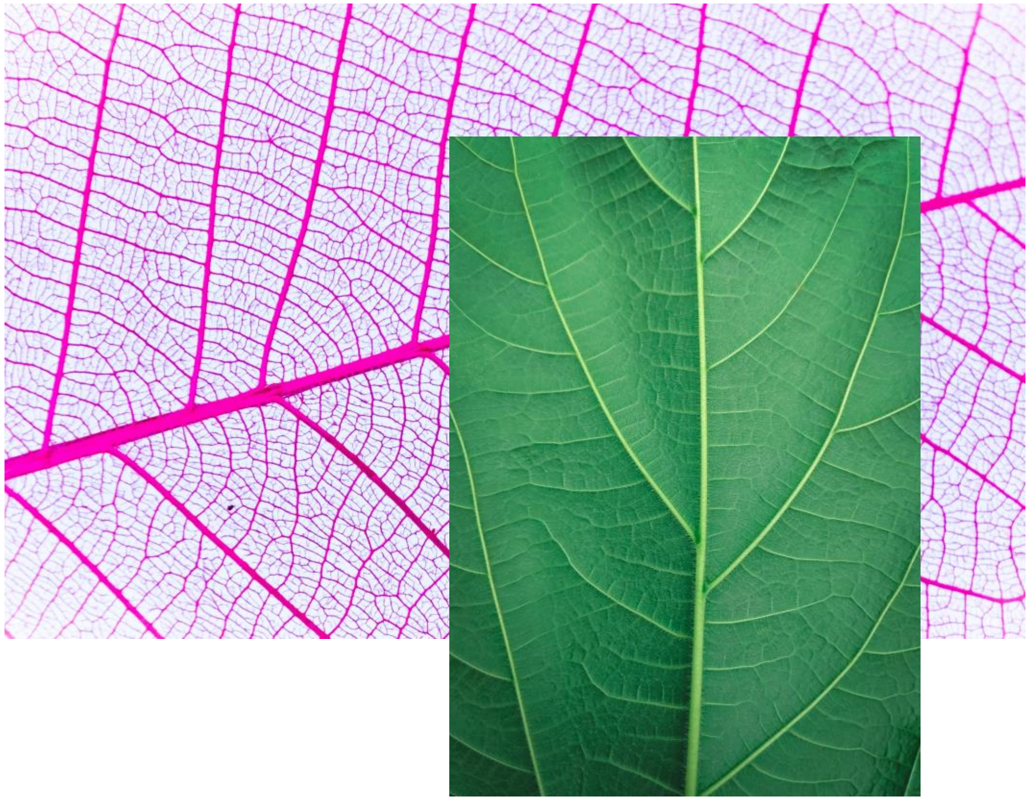




Typically used to measure rates ranging from 10^{12} - 10^{6} s^{-1}.

What is Joliot-Type Spectrophotometry?

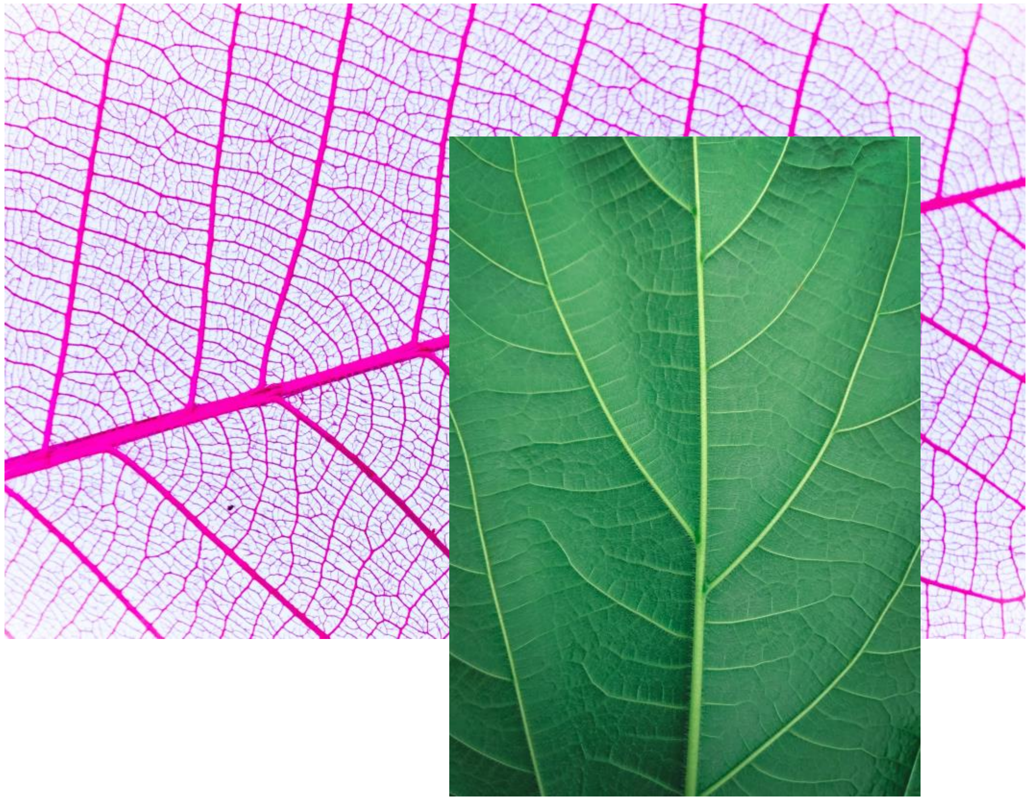
- Leaves are highly **absorbent**, highly **scattering** and **photochemically active**.
    - Difficult to get light through (**requires high light**) and easy to cause light-based artifacts in photosynthetic machinery (**requires low light**).



SpectroLogiX

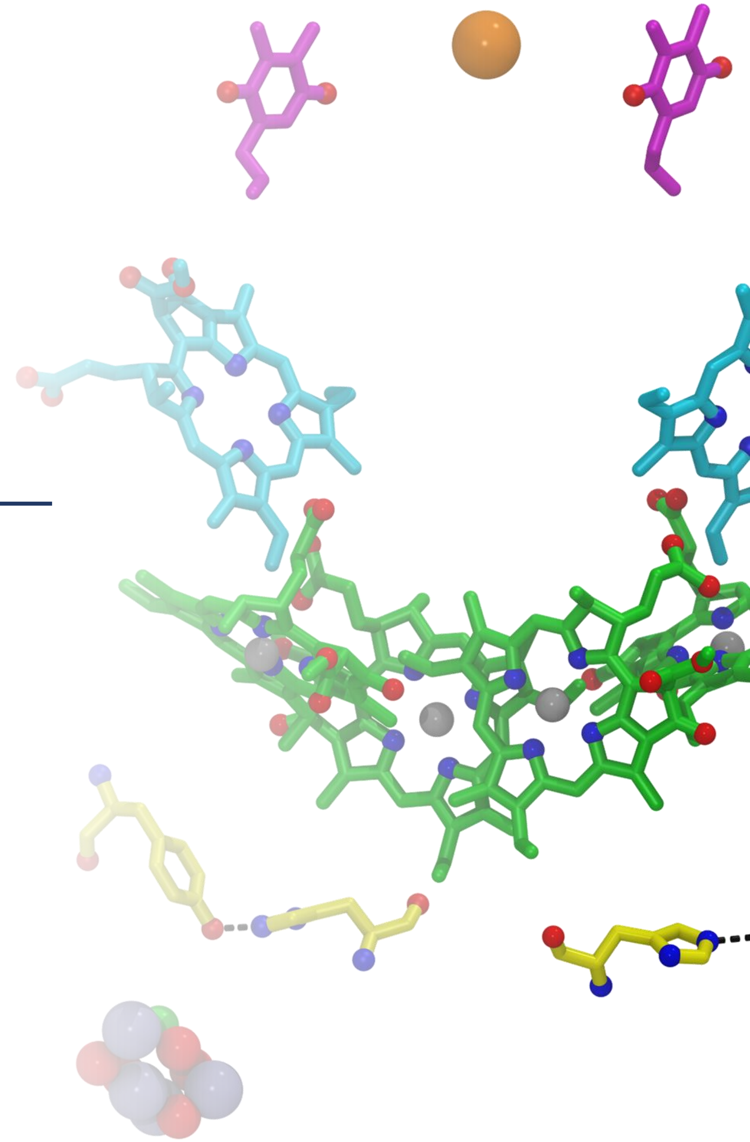
What is Joliot-Type Spectrophotometry?

- Leaves are highly **absorbent**, highly **scattering** and **photochemically active**.
  - Joliot's solution: a spectrometer to controls both **intensity** and **duration** of light.
  - Also, optics that account for **scatter**.



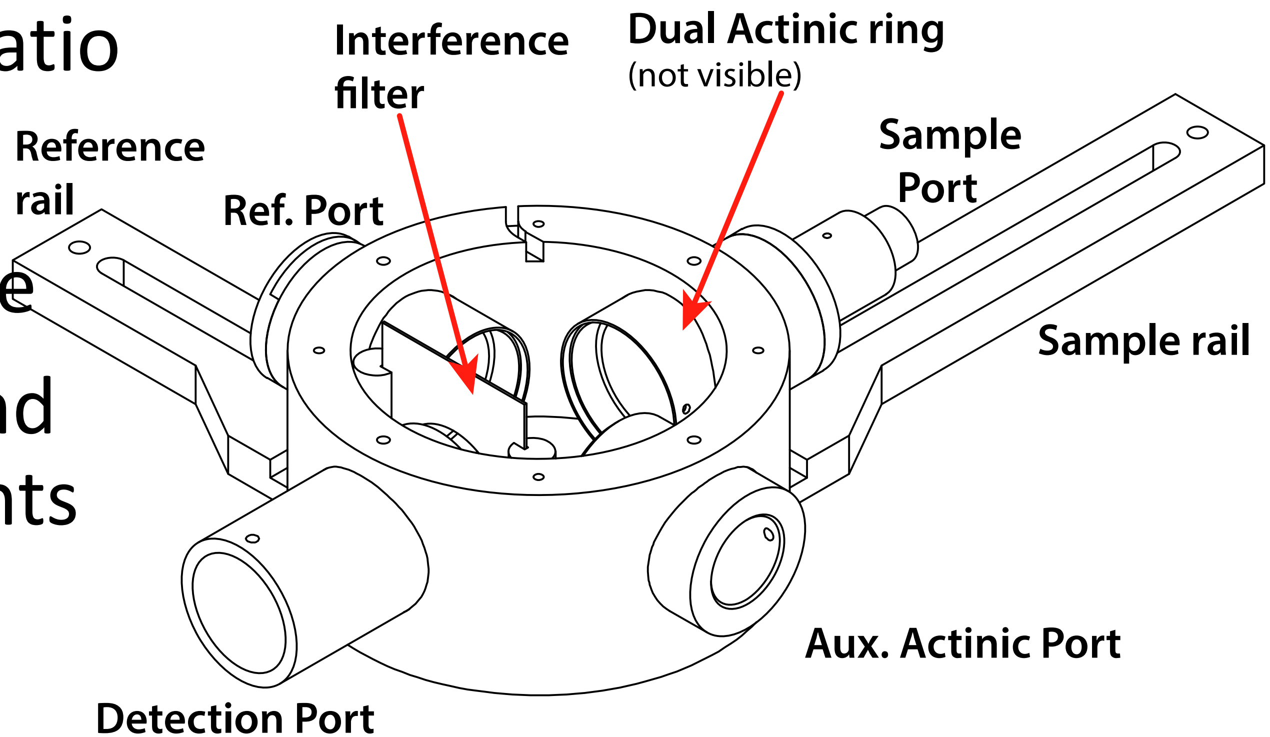
2. Technical requirements

Development of the JTS-100 & JTS-150

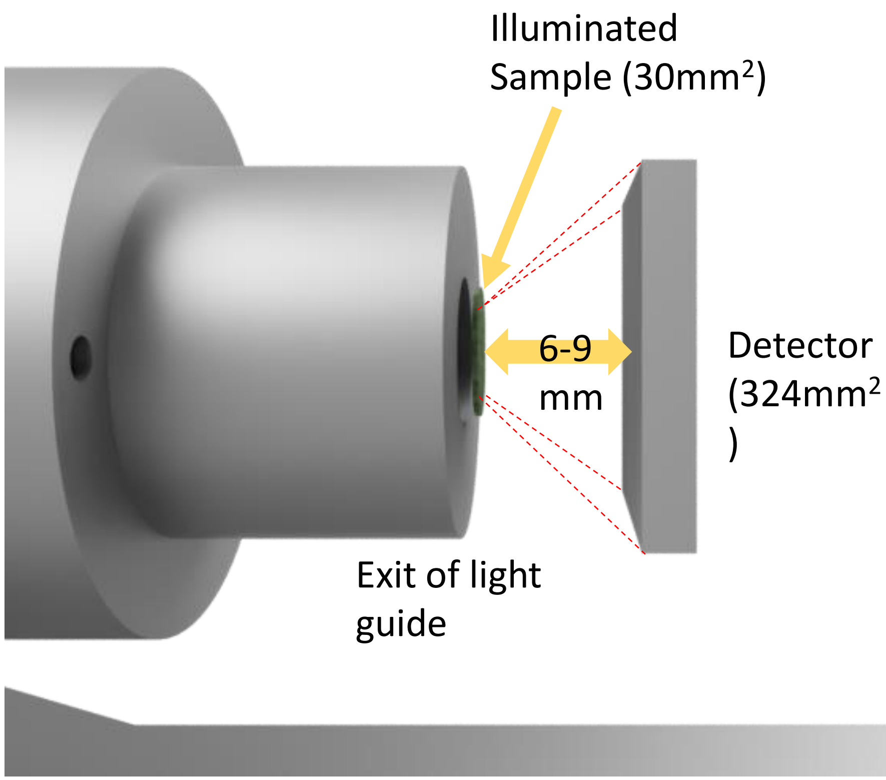


JTS-10 **Strengths: optical bench**

- Outstanding S/N ratio
- Robust optics
- Highly configurable
- Capable of Abs. and Fluo. measurements



Minimal sample to detector distance and large area diode



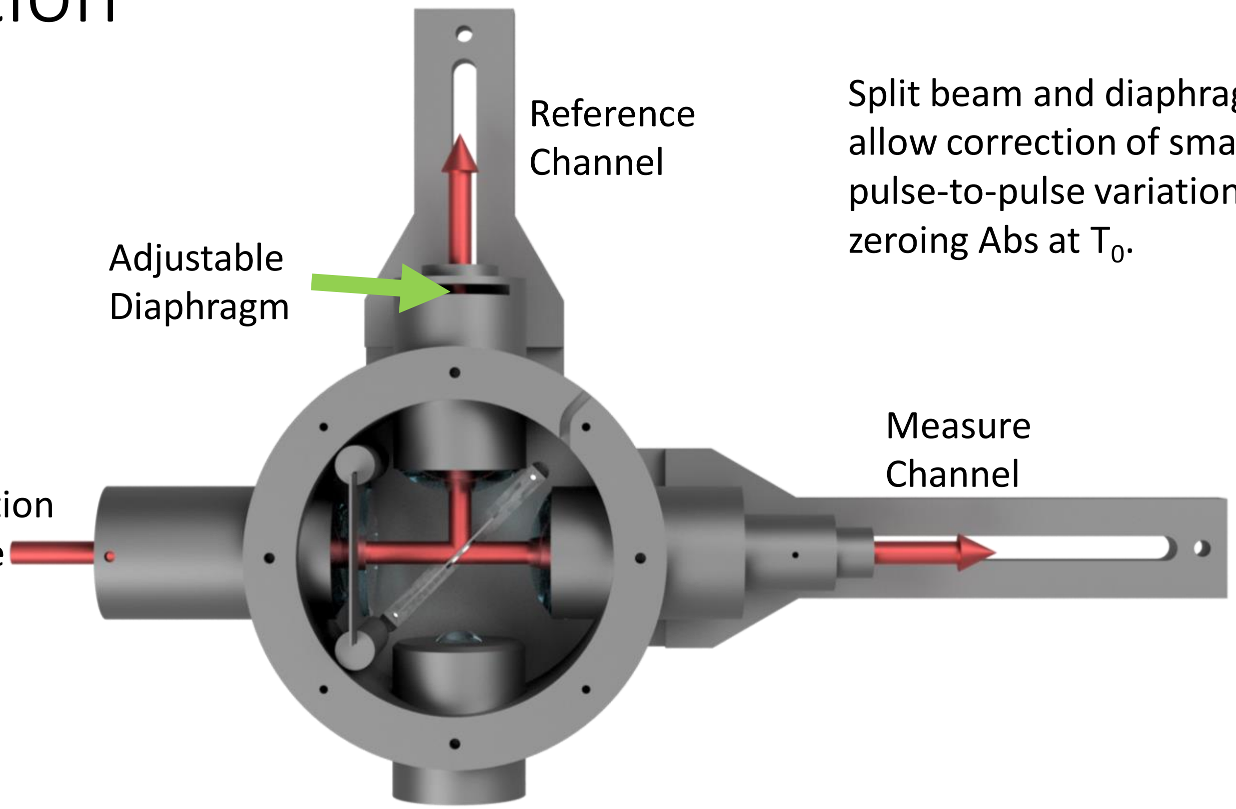
JTS spectrometers

- Large illuminated area
- Pump and Probe intersect same large area.
- Very large detector.
- Much scattered signal is still detected.

Standard Pump-Probe

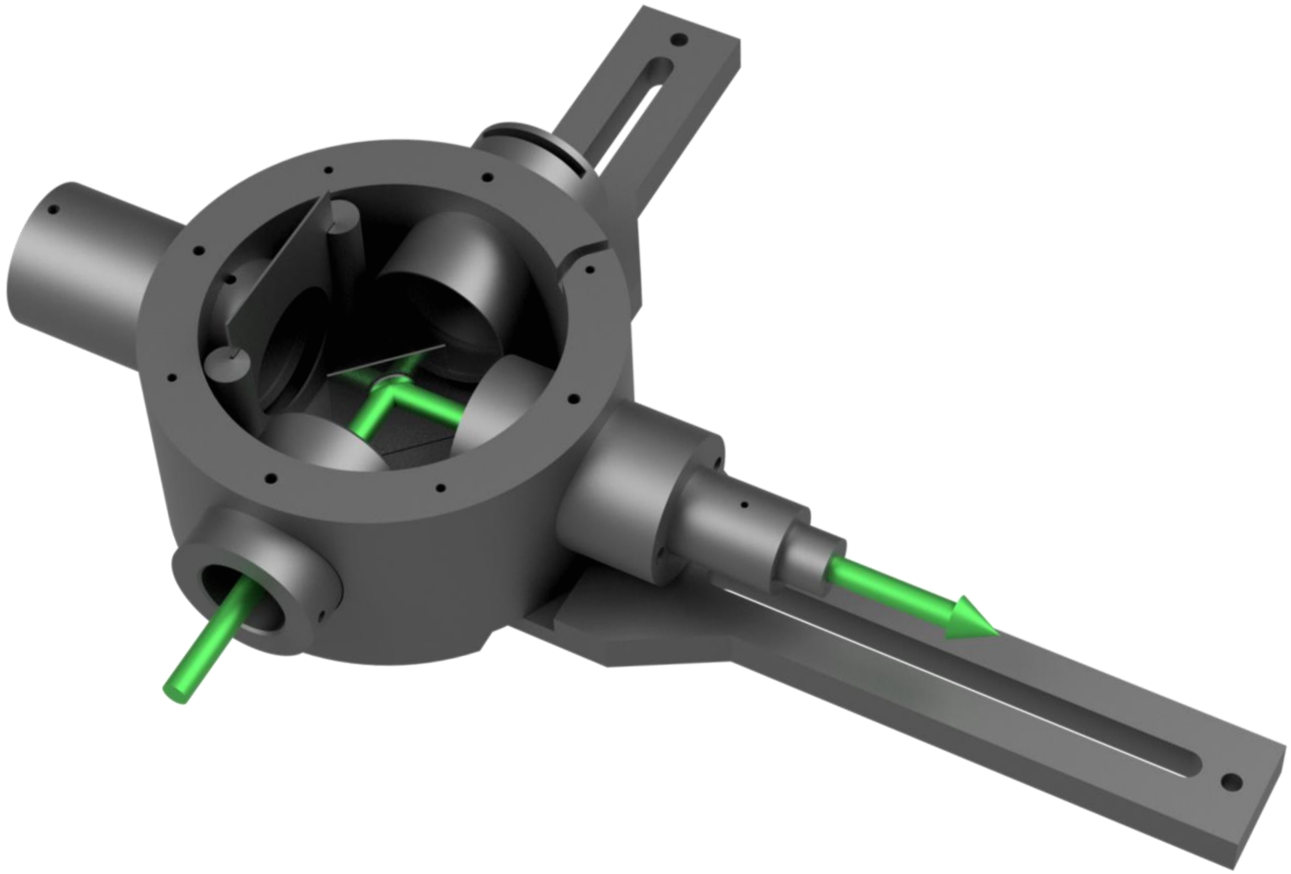
- Small diode optimized for ultrafast detection.
- Large sample-detector separation.
- Small area in pump beam.

Optical Bench: Dual beam advantages and scatter correction



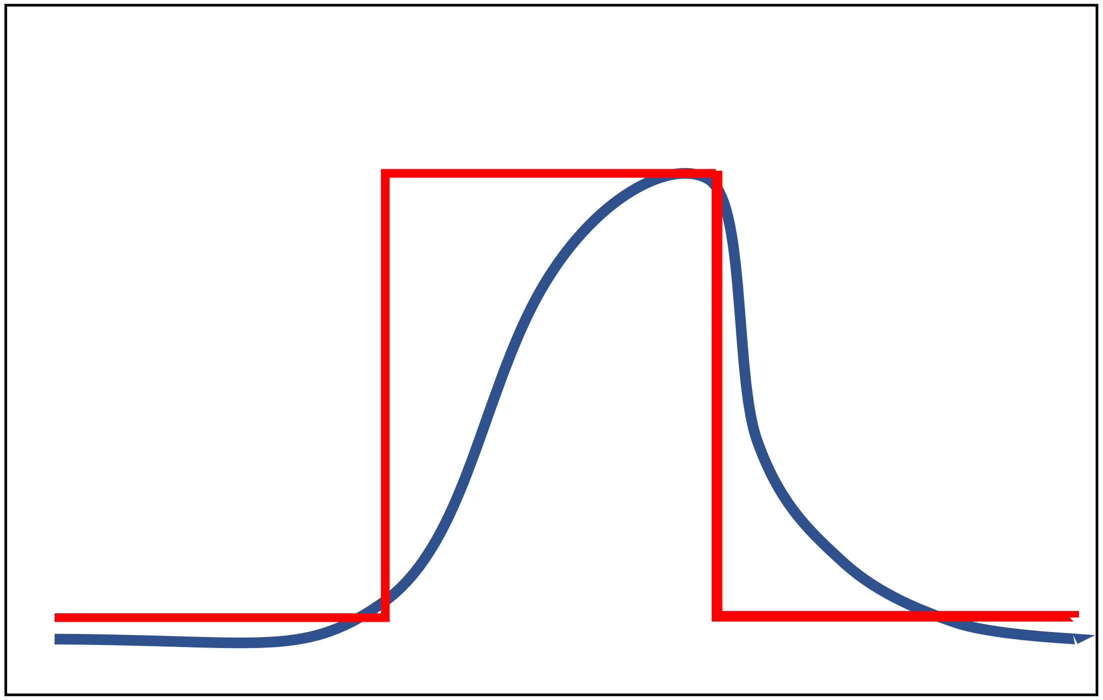
Split beam and diaphragm allow correction of small pulse-to-pulse variations and zeroing Abs at T_0.

Introducing a secondary actinic source



JTS-10 Limitations: Controller and Detectors

- Not current controlled
- Recovery from saturation slow
- Single measurement per experiment
- Adding LEDs requires custom design/engineering
- Hard to balance



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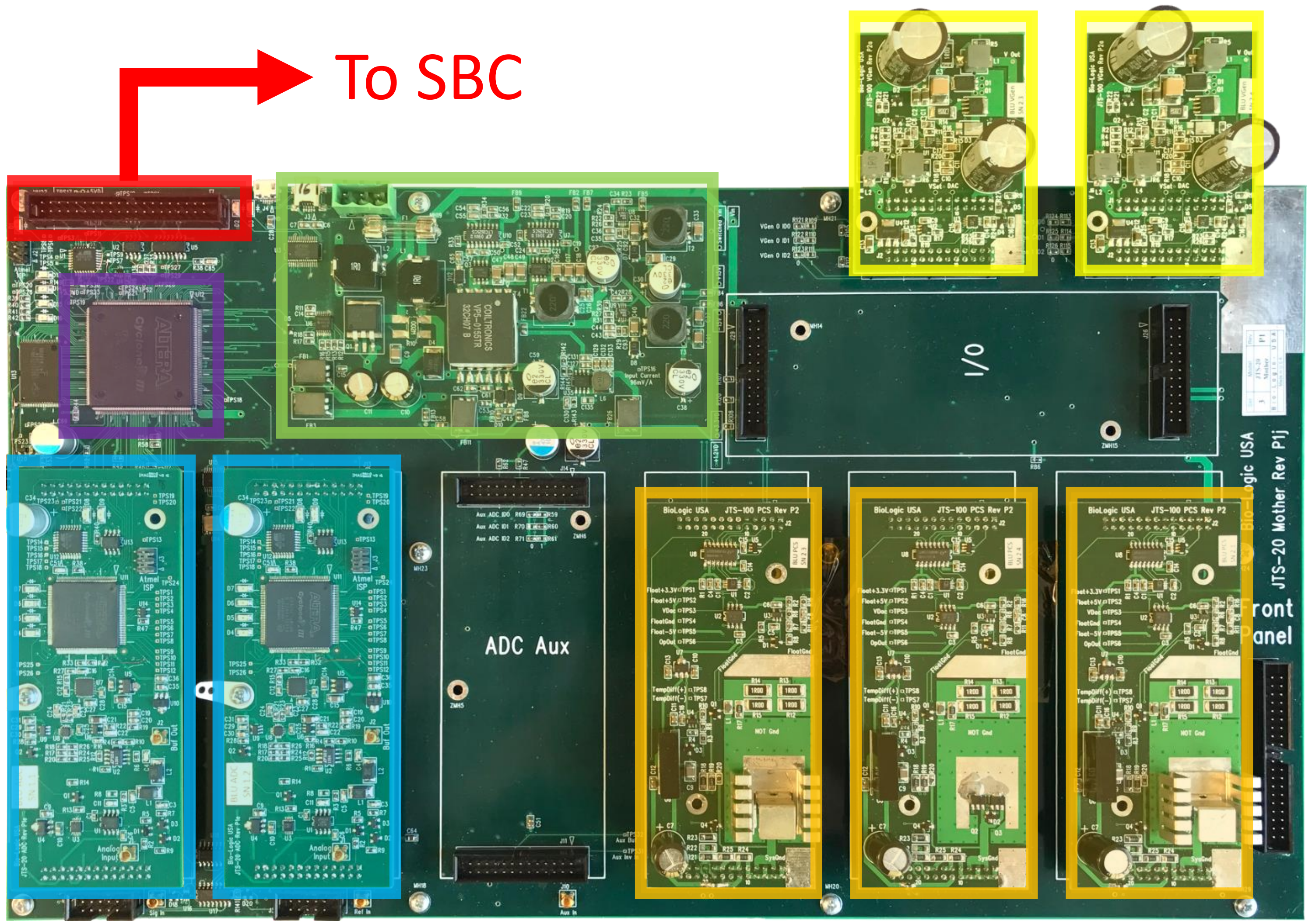
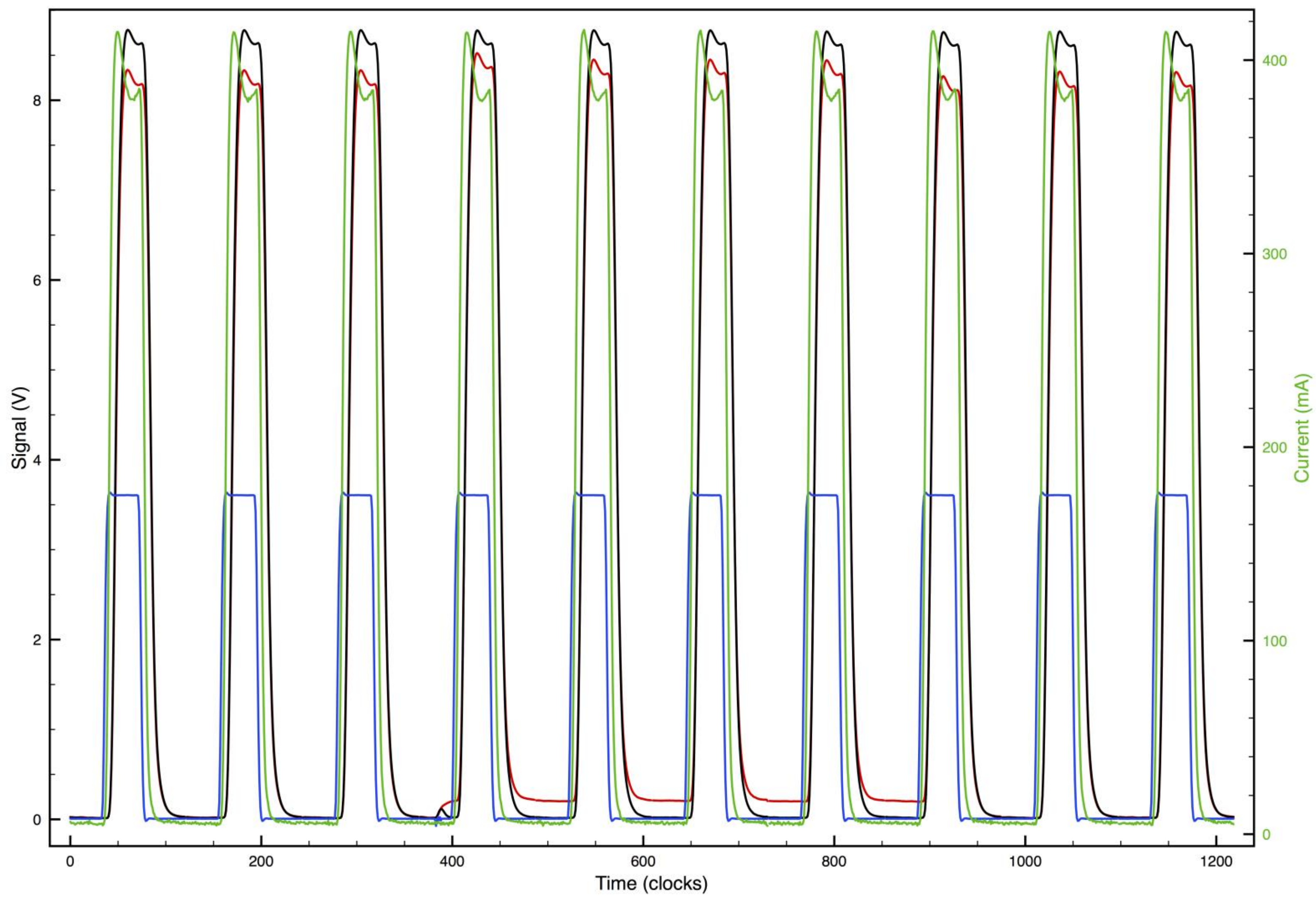
Redesigned Controller:

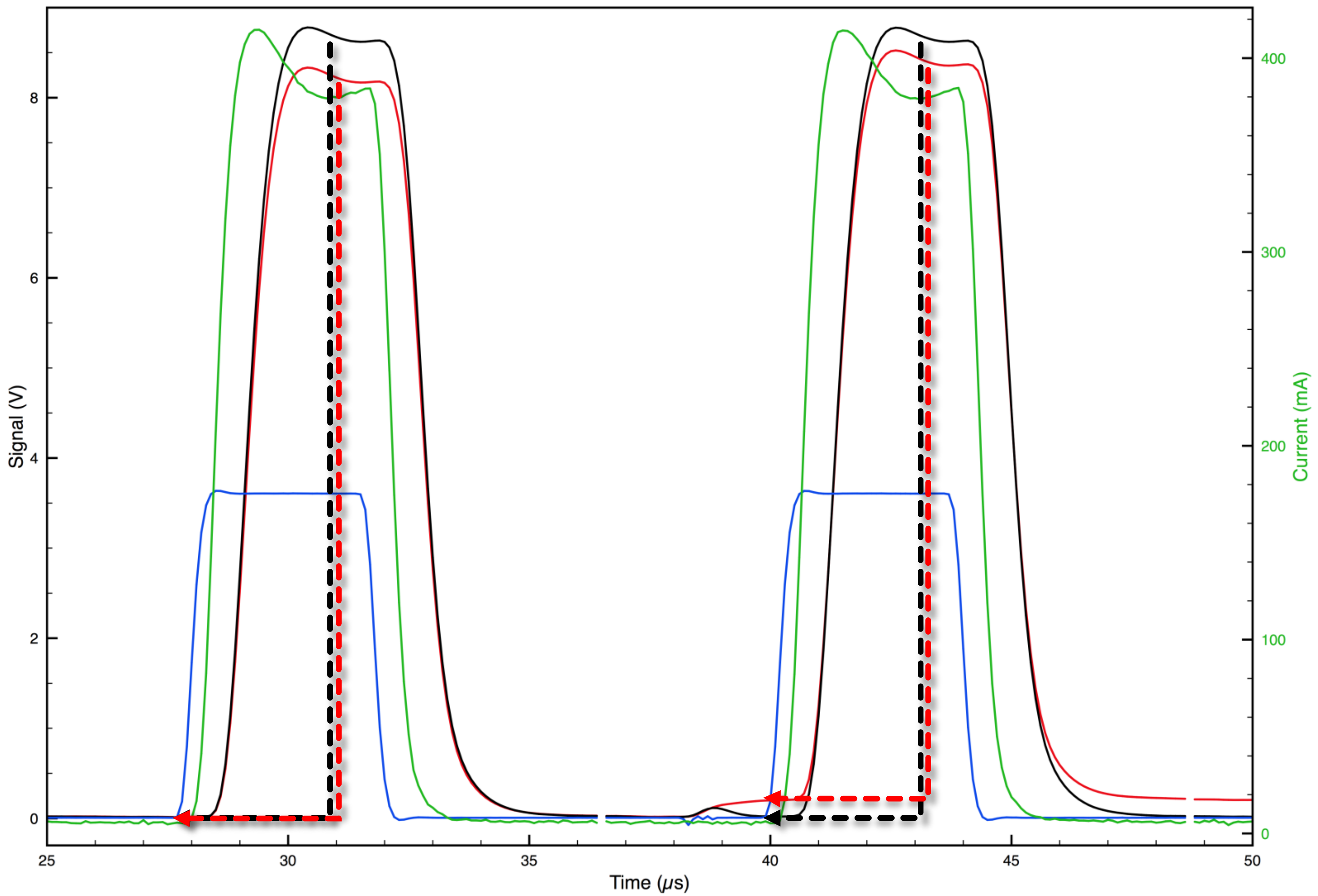
- UI served by integrated SBC
- System events at 0.1us FPGA
- Up to 3 ADCs
- Independent programmable current and voltage control



SpectroLogiX


Signal (V)
0
2
4
6
8
Current (mA)
0
100
200
300
400
Time (clocks)
0
200
400
600
800
1000
1200

Baseline corrections of during actinic period

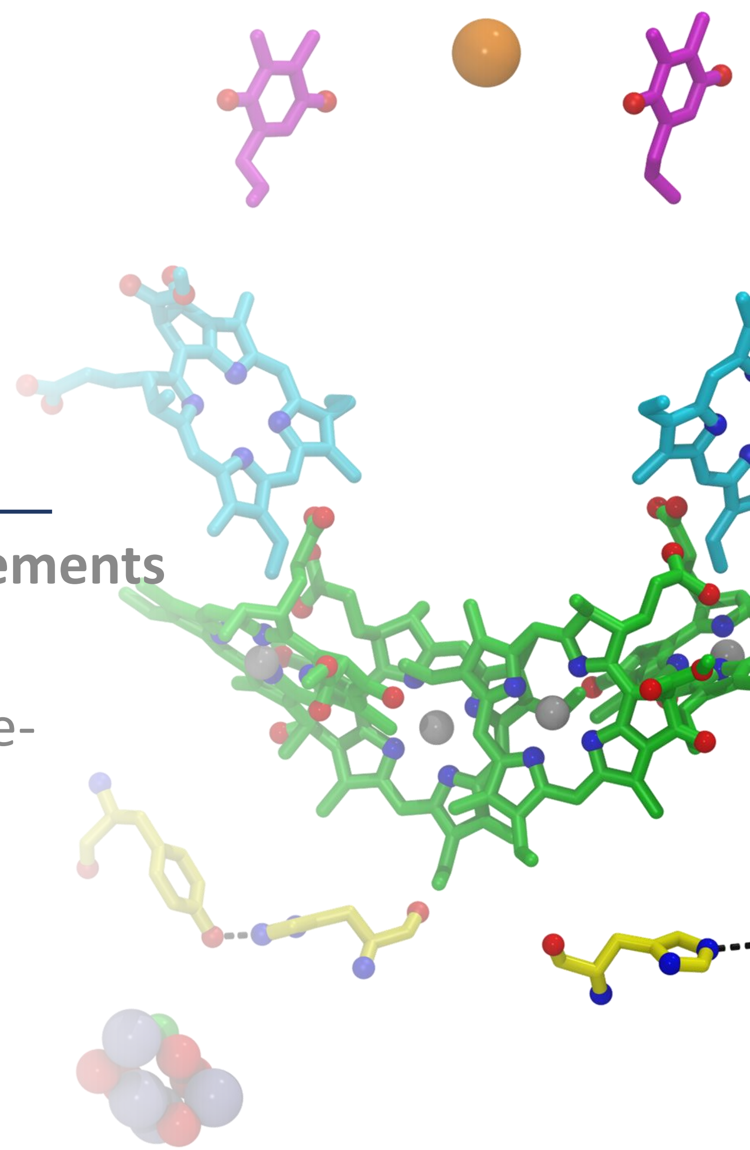


3a. Current applications

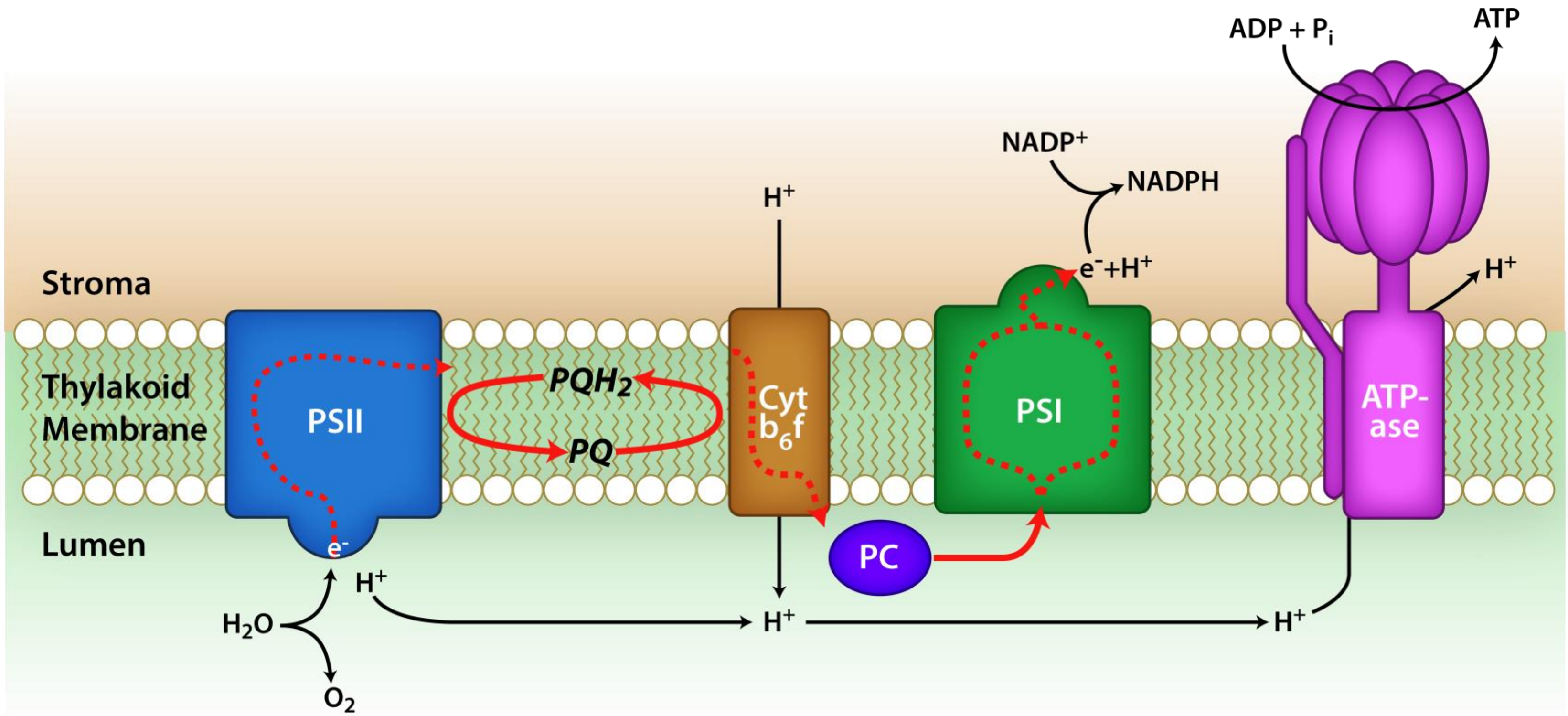
a) Background on supported PS measurements

b) Current supported PS measurements

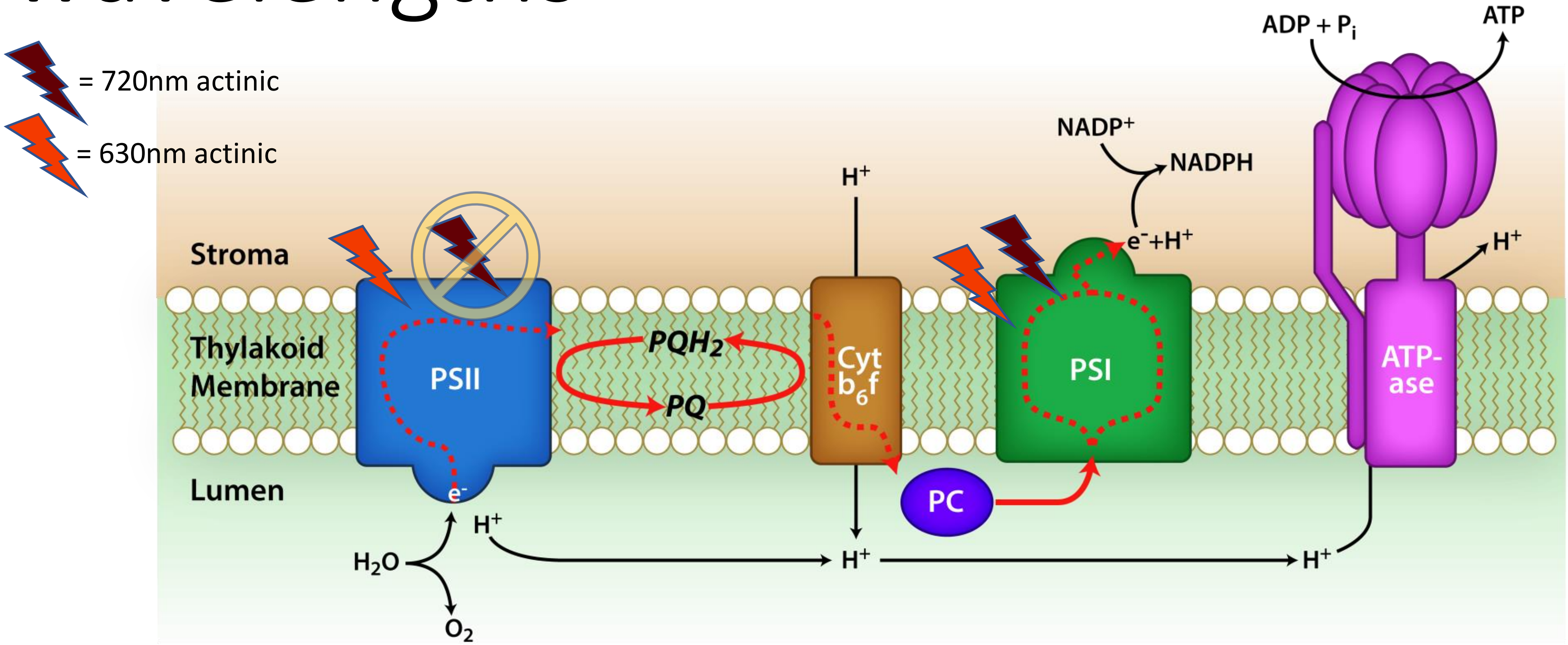
c) Simultaneous measurements of all PS e-transfers



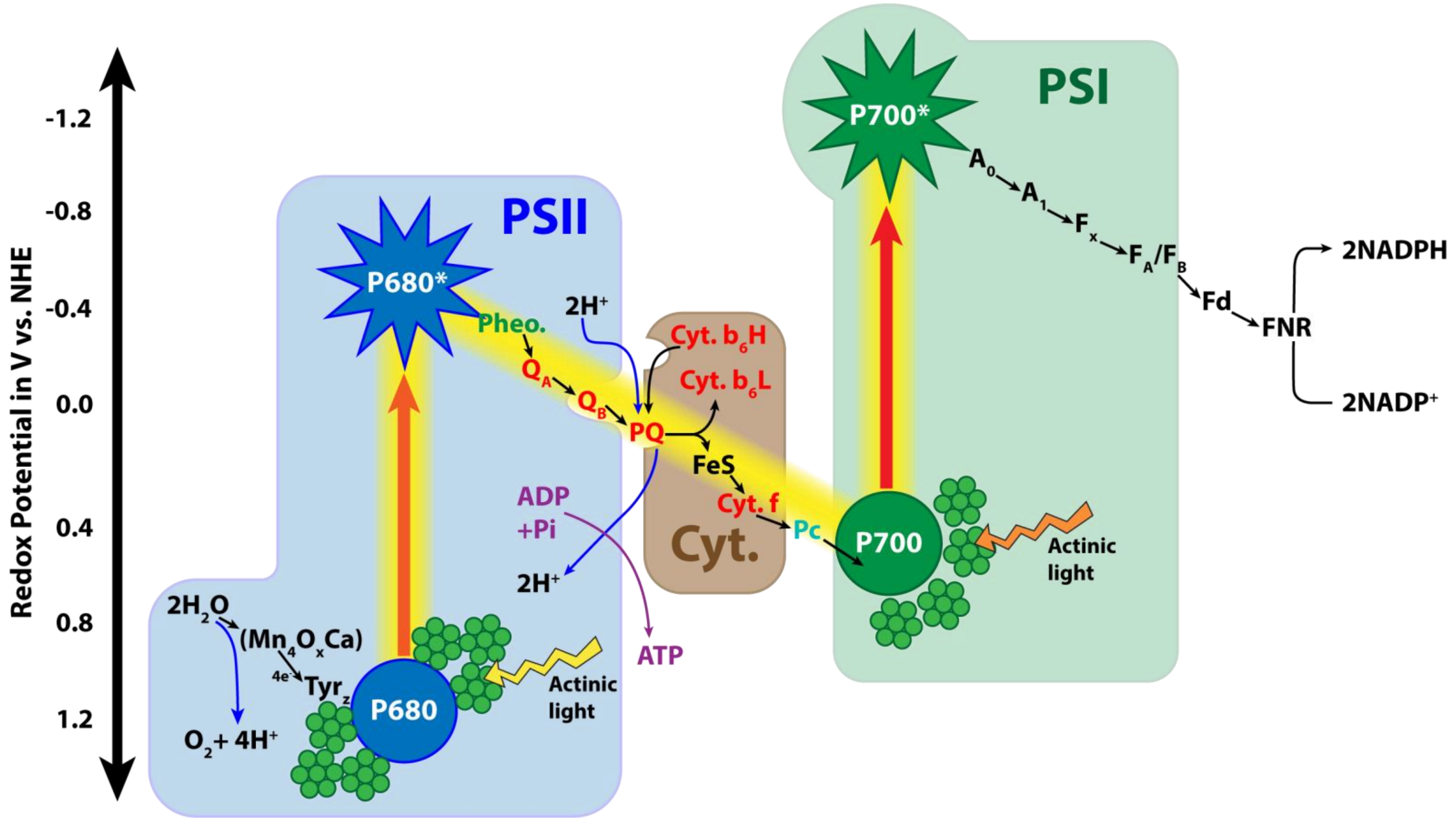
Photosynthesis: linear e⁻ flow



Selective excitation of PSI w/ long wavelengths

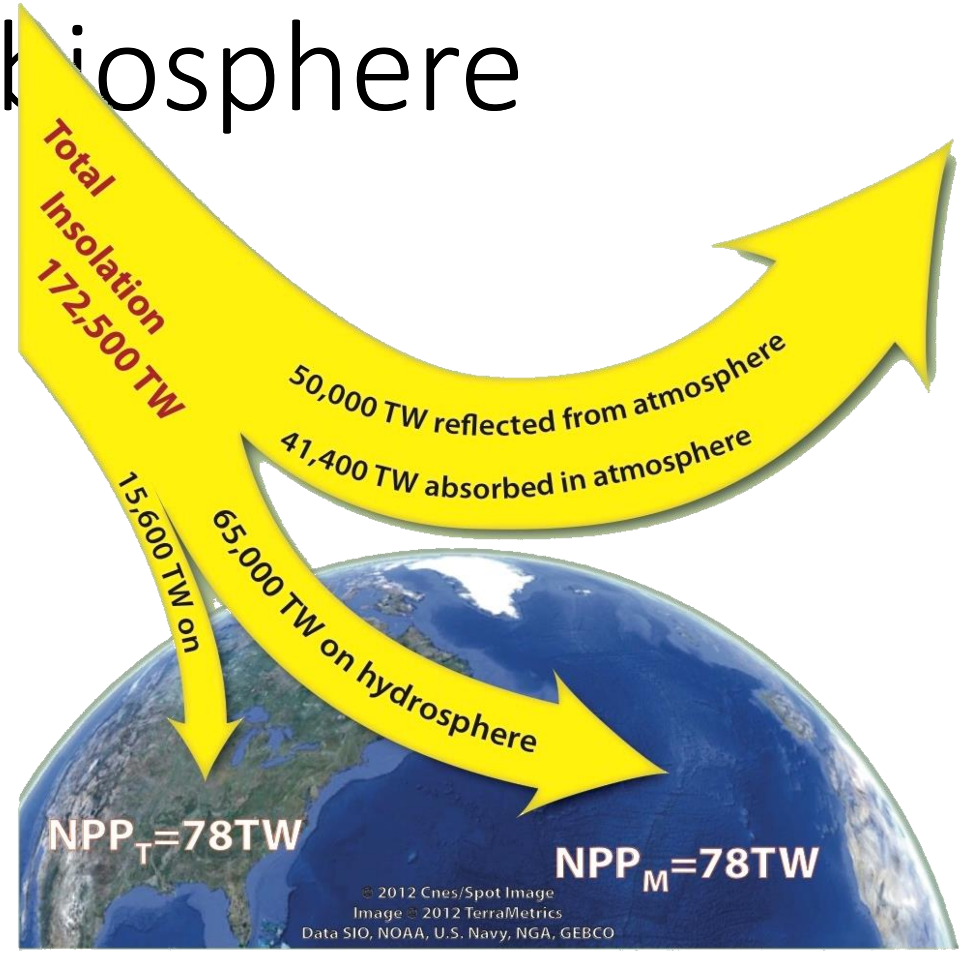


Photosynthesis: the Z-Scheme

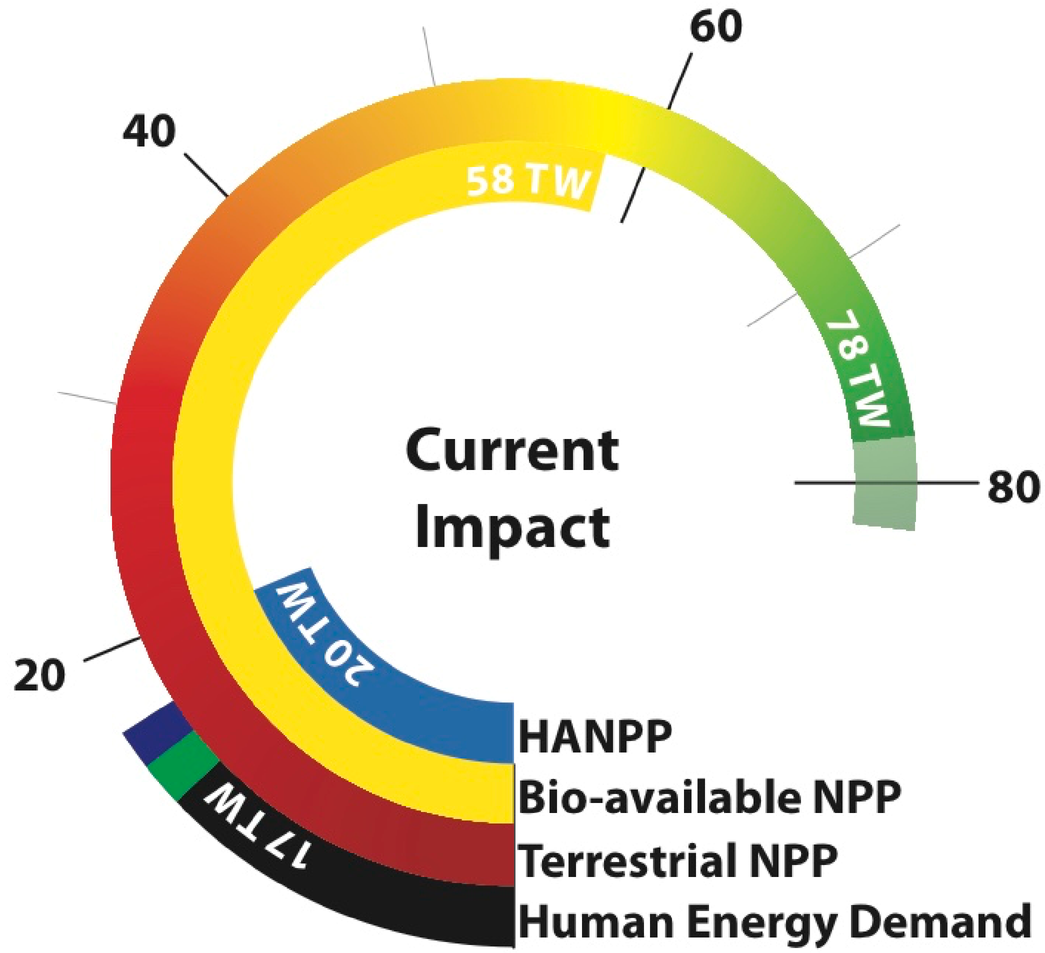


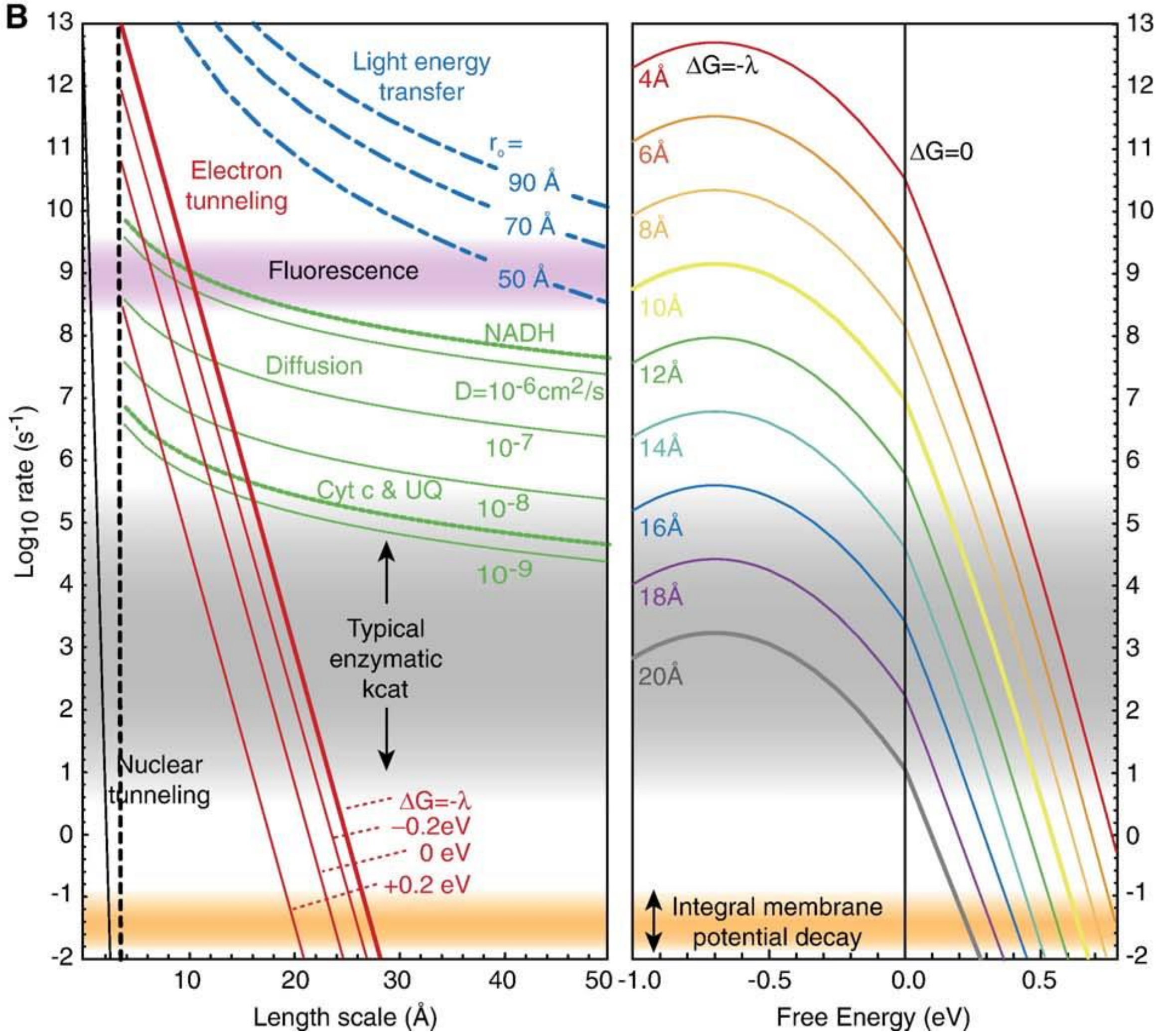
PS is the source of solar energy conversion for the biosphere

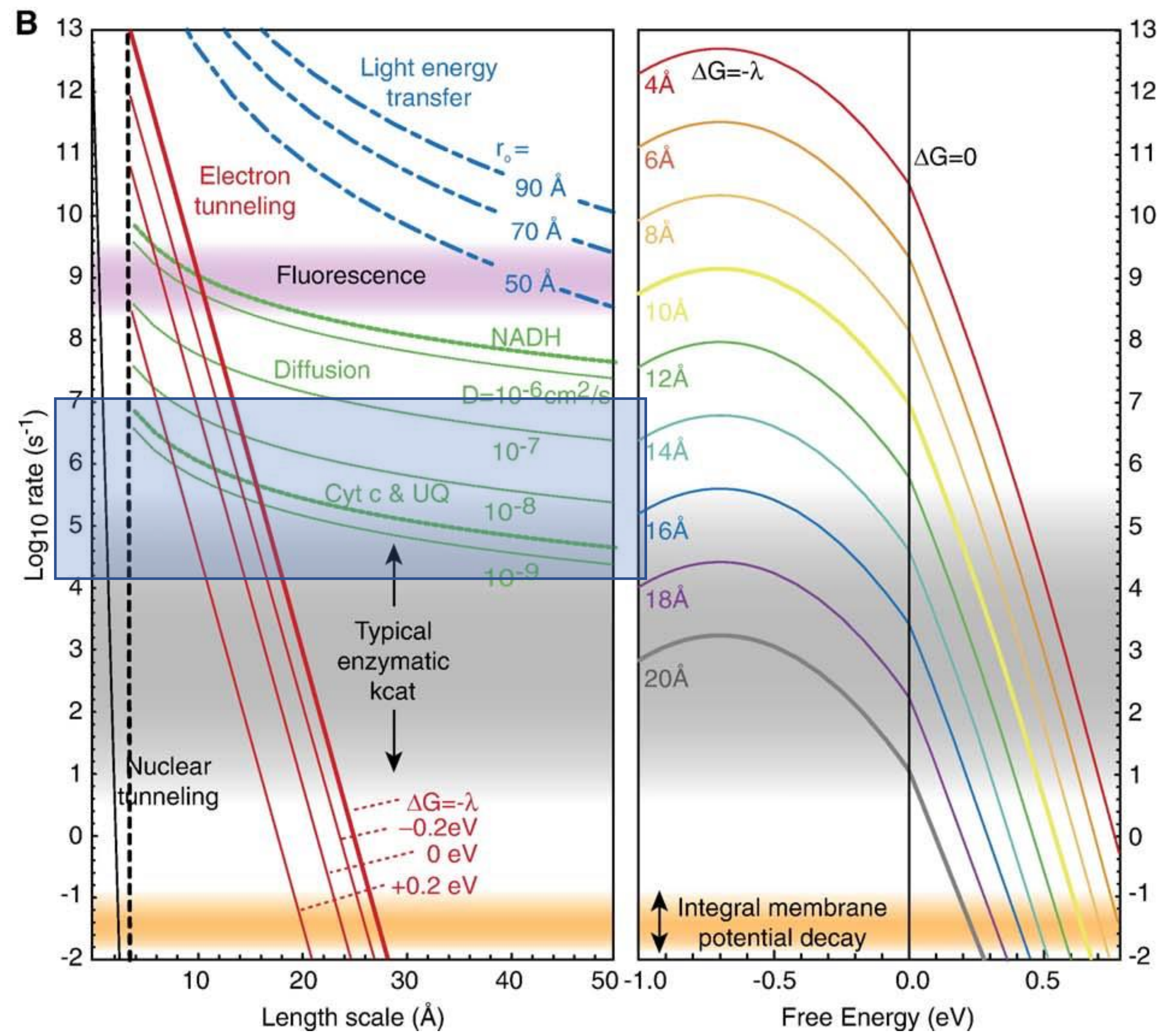
- PS converts solar energy at average rate of 156TW
- PS is major part of biogeochemical cycles for C,N,O
- To improve PS efficiency, we double the natural amount of nitrogen fixed by nature



Sherman, B. D.; Vaughn, M. D.; Bergkamp, J. J.; Gust, D.; Moore, A. L.; Moore, T. A. Evolution of Reaction Center Mimics to Systems Capable of Generating Solar Fuel. *Photosynth Res* **2014**, *120* (1–2), 59–70.

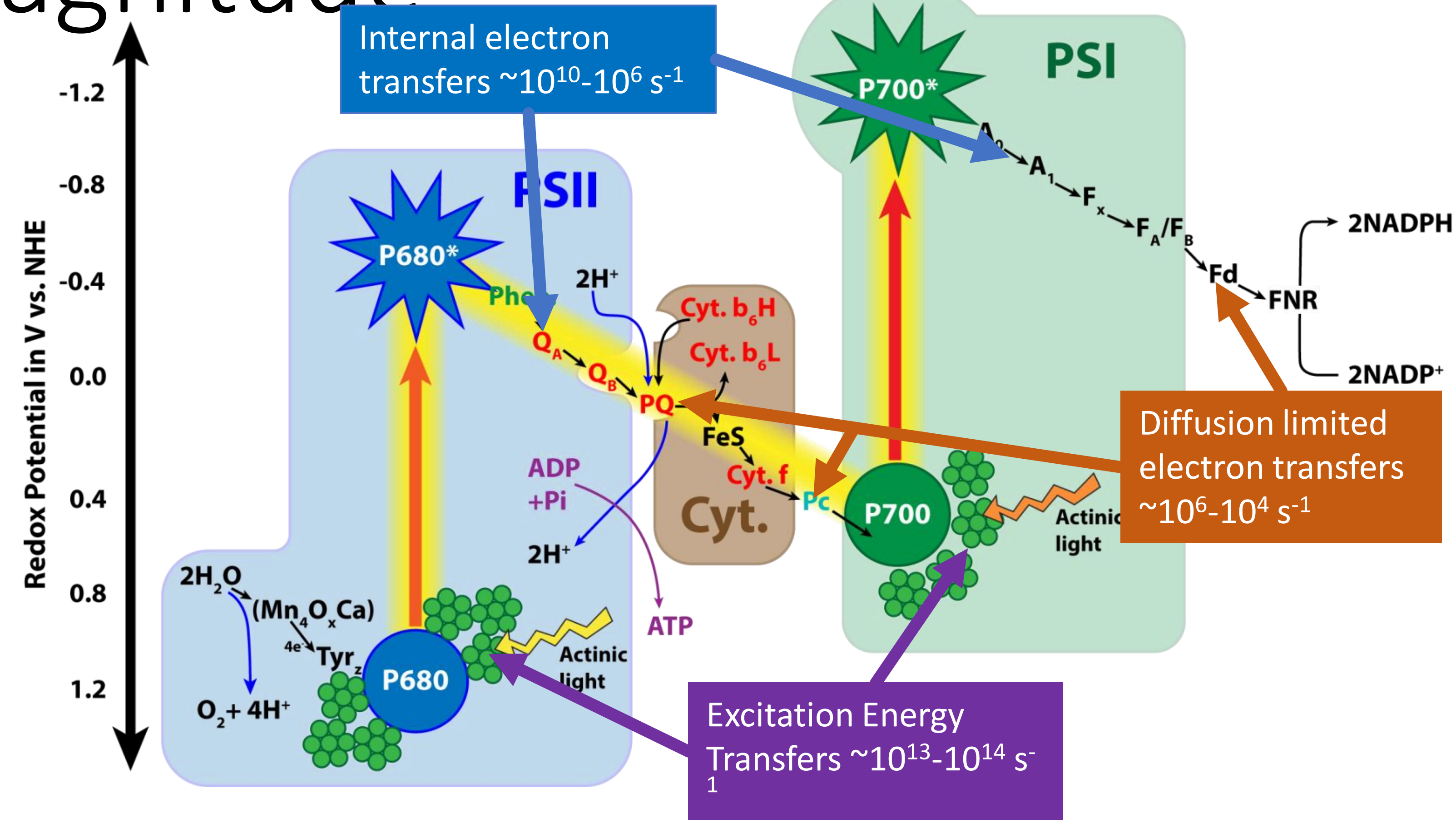

60
40
58 TW
78 TW
Current Impact
80
20 TW
20
17 TW
HANPP
Bio-available NPP
Terrestrial NPP
Human Energy Demand


B
Log10 rate (s-1)
Light energy transfer
r₀ =
90 Å
70 Å
50 Å
Electron tunneling
Fluorescence
NADH
Diffusion
D=10-6cm2/s
10-7
Cyt c & UQ
10-8
10-9
Typical enzymatic kcat
Nuclear tunneling
ΔG=-λ
−0.2eV
0 eV
+0.2 eV
Length scale (Å)
4Å
6Å
8Å
10Å
12Å
14Å
16Å
18Å
20Å
ΔG=0
Integral membrane potential decay
Free Energy (eV)

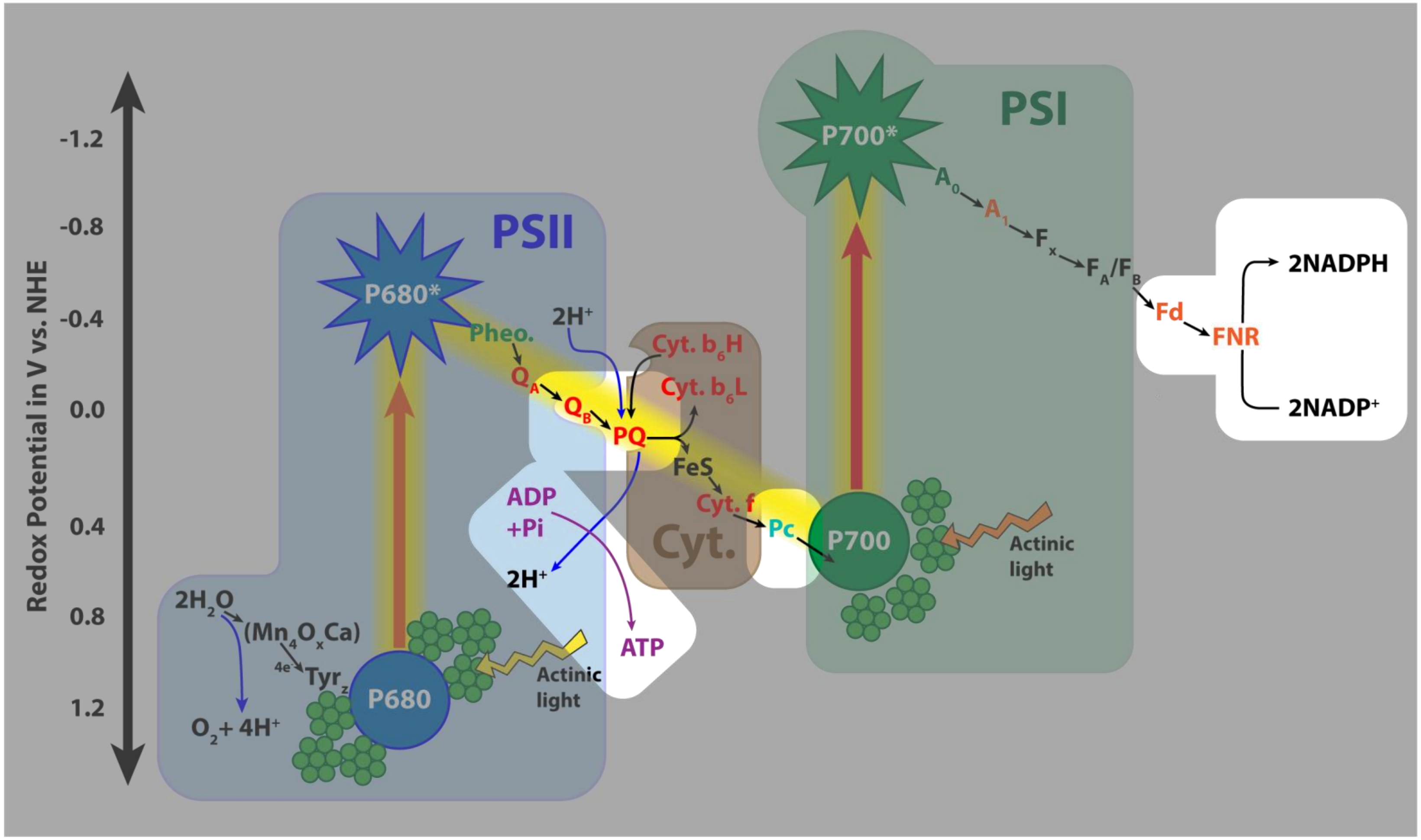


B
Light energy transfer
r_o =
90 Å
70 Å
50 Å
Electron tunneling
Fluorescence
NADH
Diffusion
D=10⁻⁶cm²/s
10⁻⁷
Cyt c & UQ
10⁻⁸
10⁻⁹
Typical enzymatic kcat
Nuclear tunneling
ΔG=-λ
−0.2eV
0 eV
+0.2 eV
Log10 rate (s⁻¹)
Length scale (Å)
4Å
ΔG=-λ
6Å
ΔG=0
8Å
10Å
12Å
14Å
16Å
18Å
20Å
Integral membrane potential decay
Free Energy (eV)

Rates span many orders of magnitude



Diffusion limits overall rate



3b. Current applications

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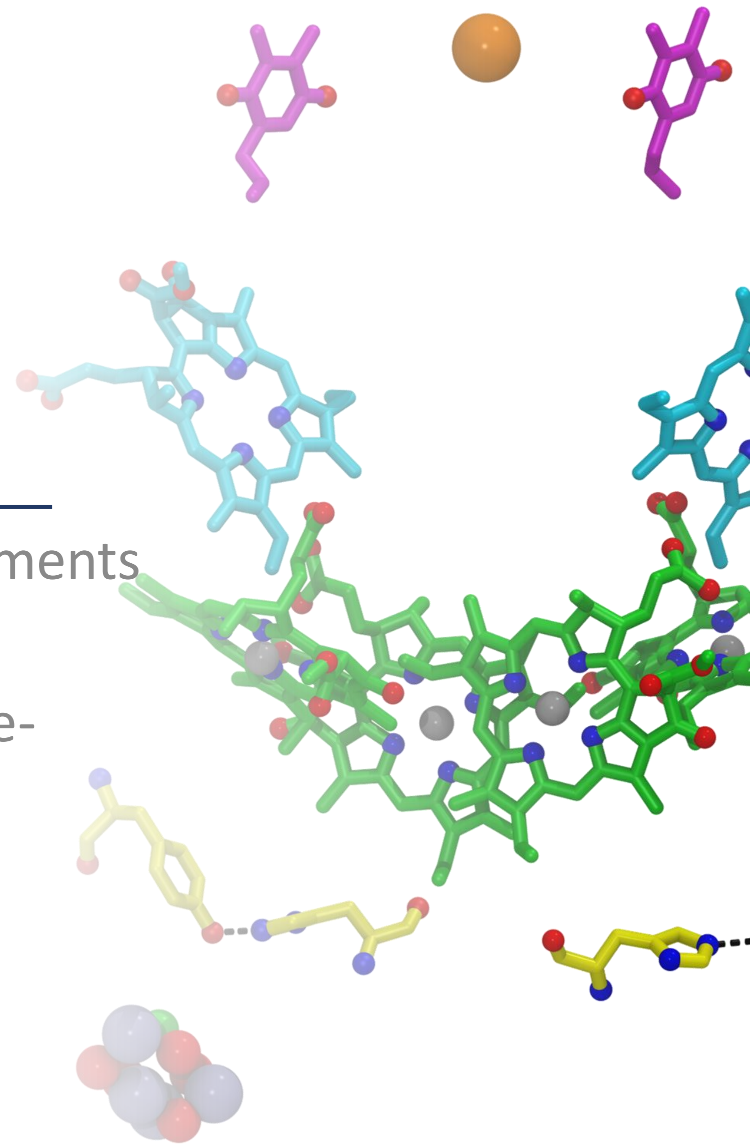

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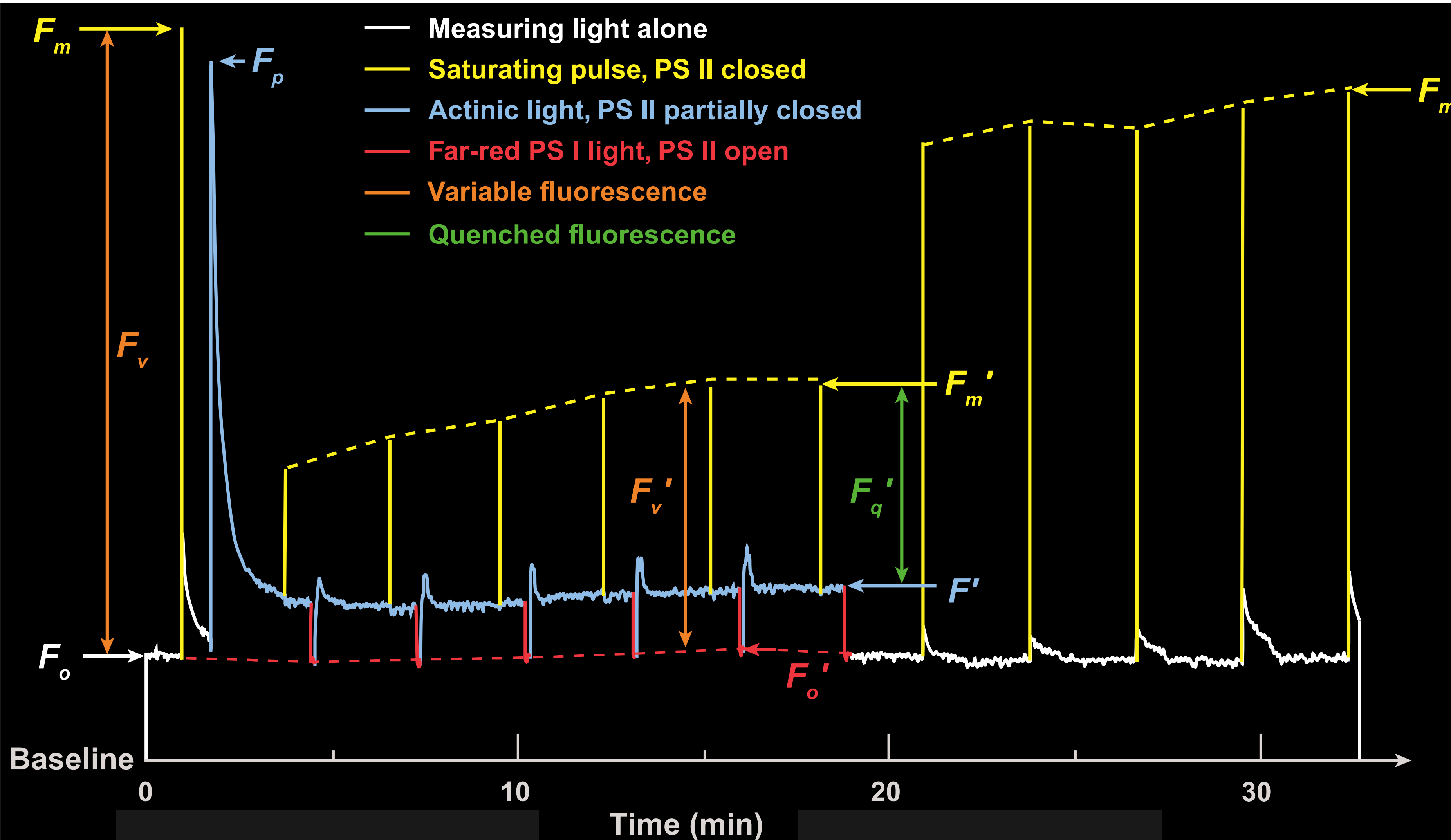
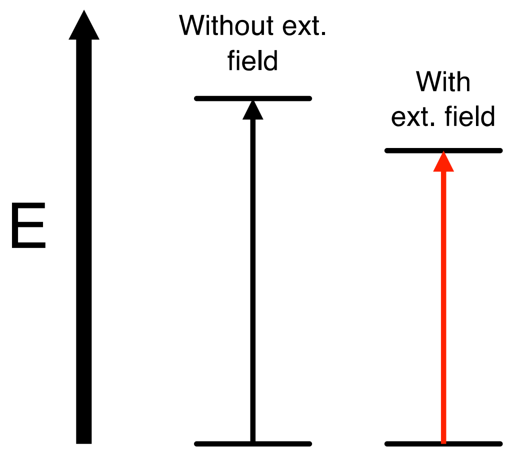
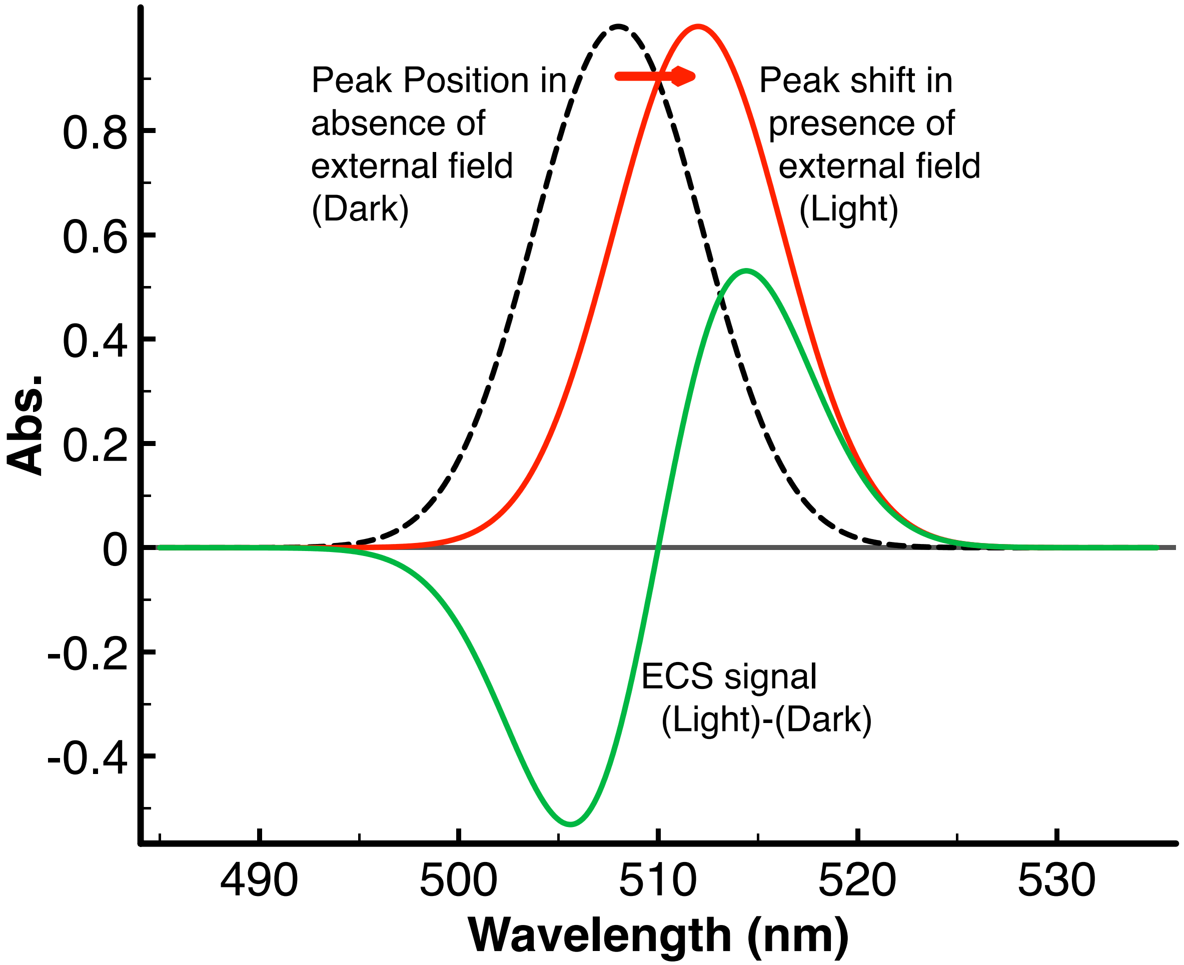


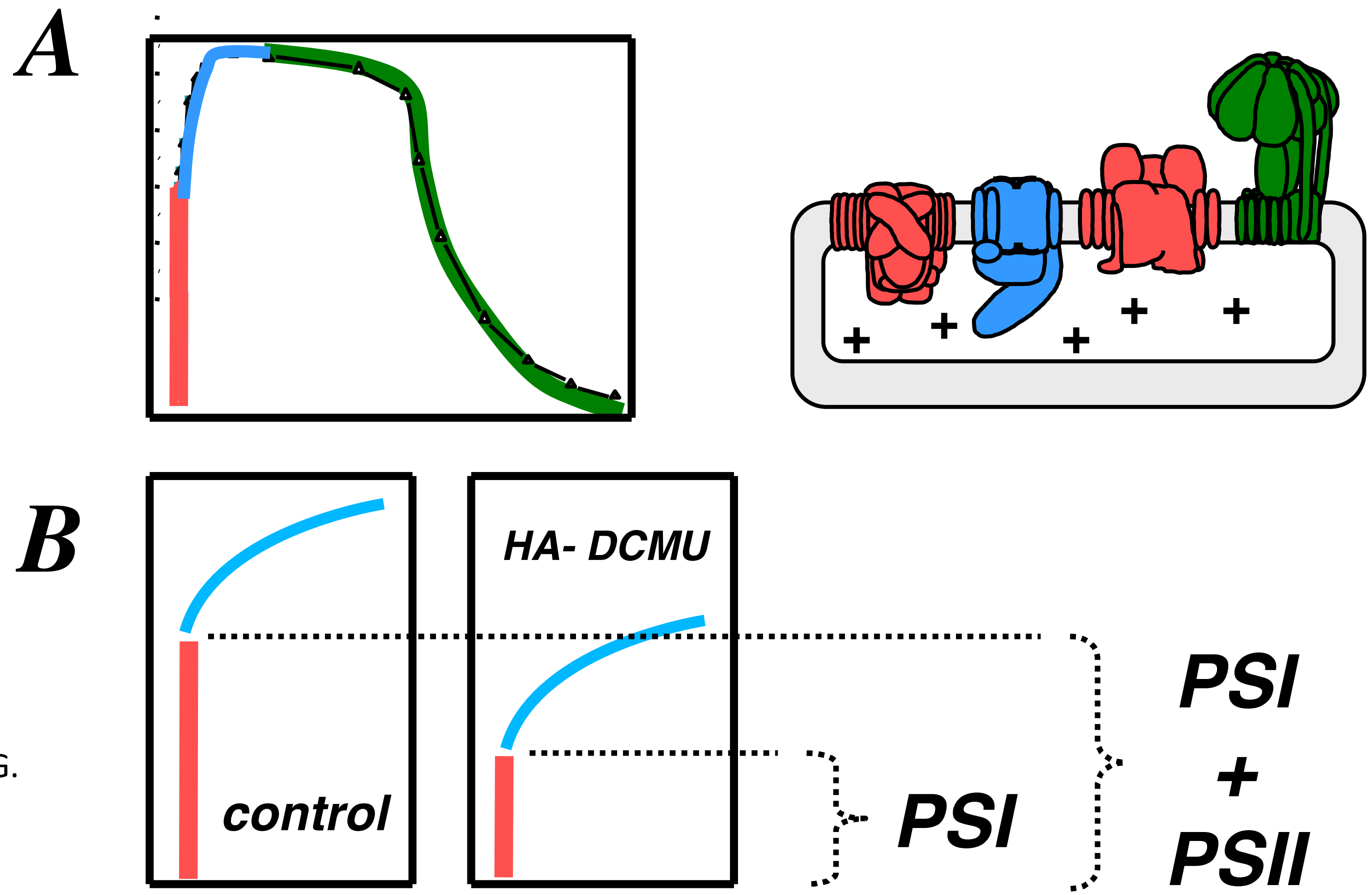
Baker, N. R. Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. *Annu. Rev. Plant Biol.* **2008**, *59* (1), 89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Basis of Electrochromic Bandshift



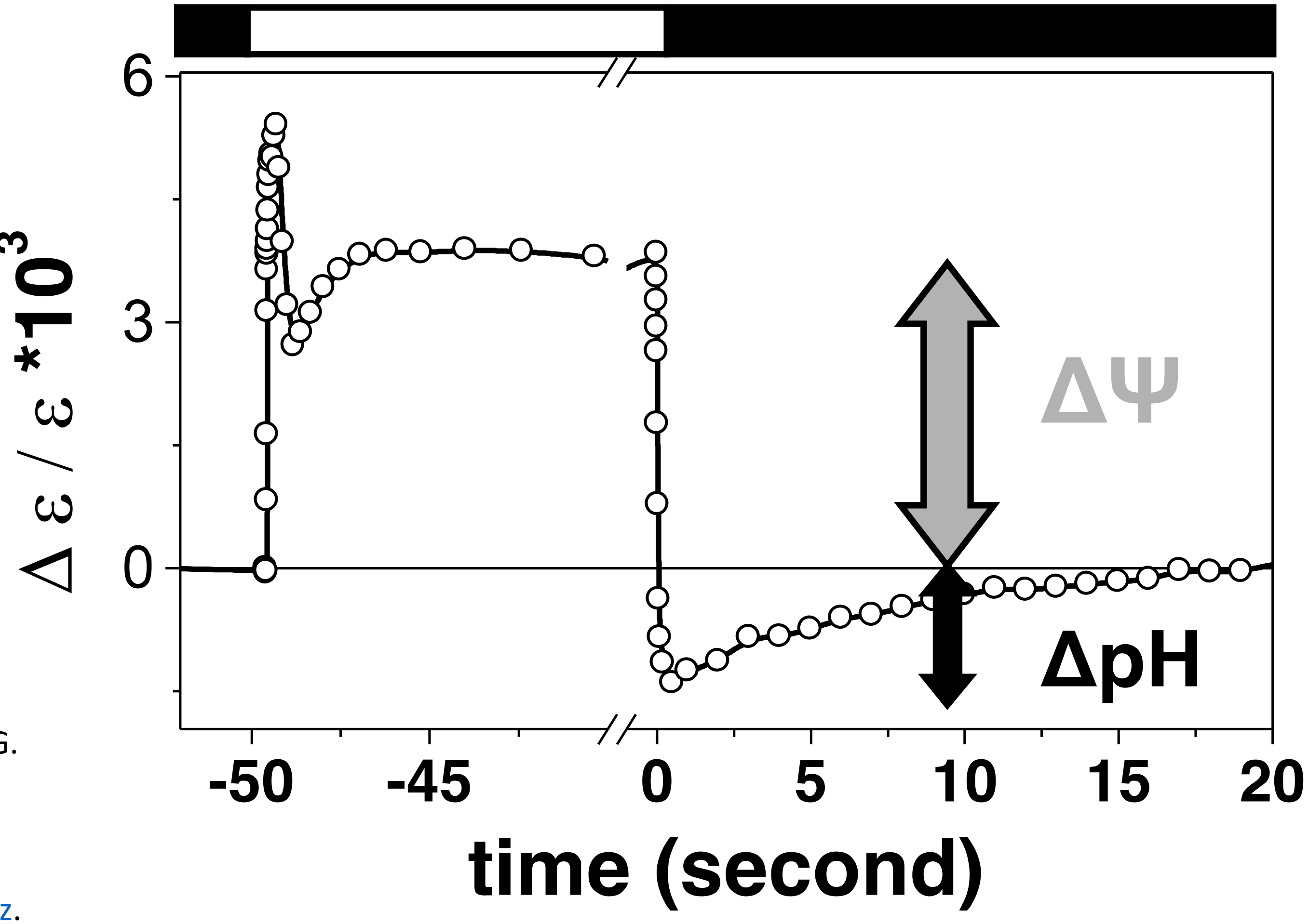


Application examples: Electrochromic shift



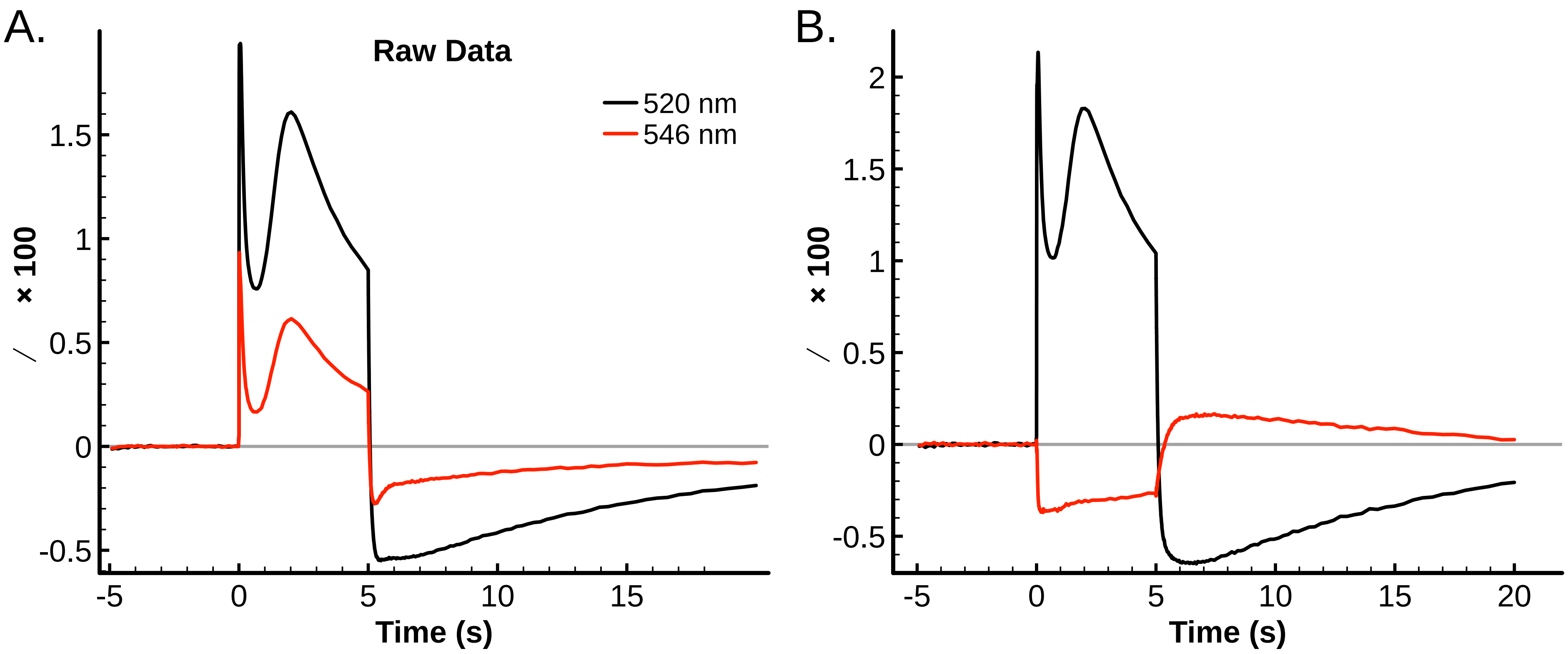
Bailleul, B.; Cardol, P.; Breyton, C.; Finazzi, G. Electrochromism: A Useful Probe to Study Algal Photosynthesis. *Photosynth Res* **2010**, *106* (1–2), 179–189. https://doi.org/10.1007/s11120-010-9579-z.

Application examples: Electrochromic shift



Bailleul, B.; Cardol, P.; Breyton, C.; Finazzi, G. Electrochromism: A Useful Probe to Study Algal Photosynthesis. *Photosynth Res* **2010**, *106* (1–2), 179–189. https://doi.org/10.1007/s11120-010-9579-z.

Example JTS-100/150 data



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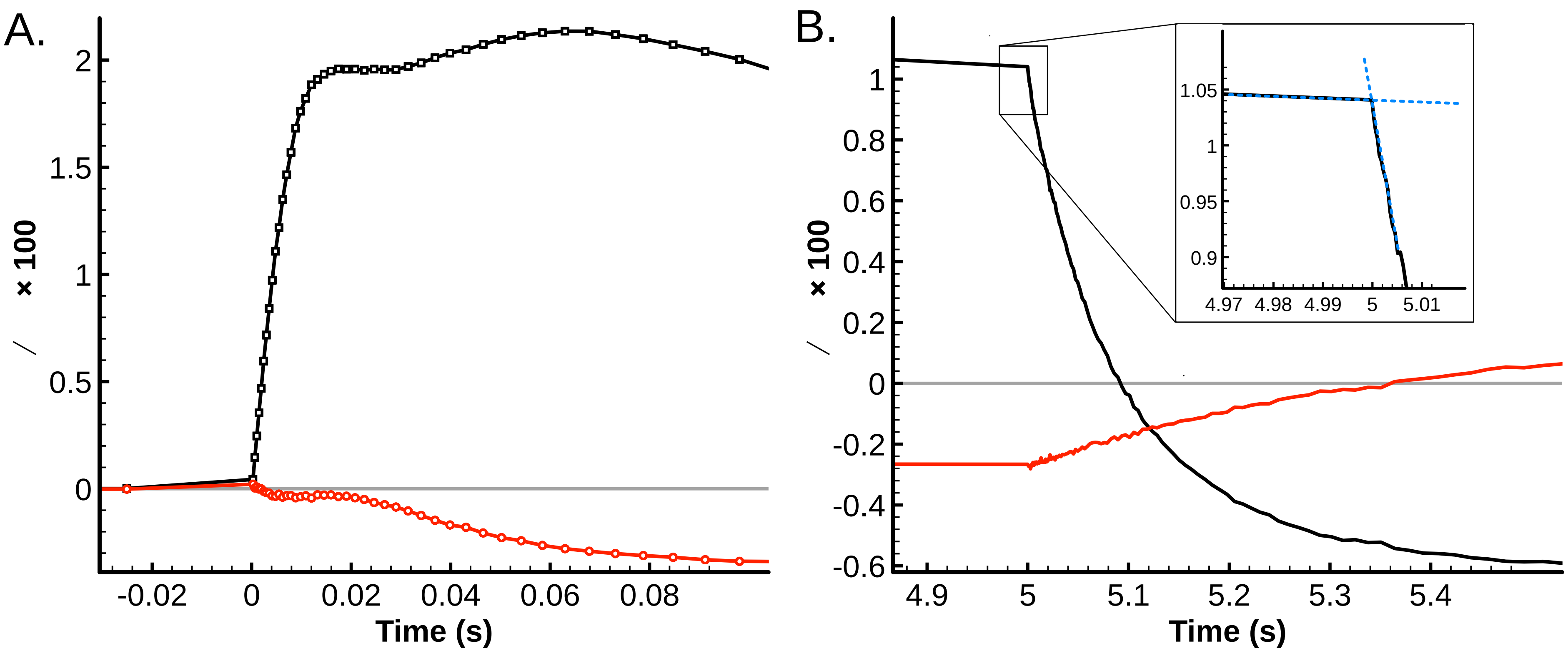
Thylakoid stacking rearrangements and ATPase activity via ECS



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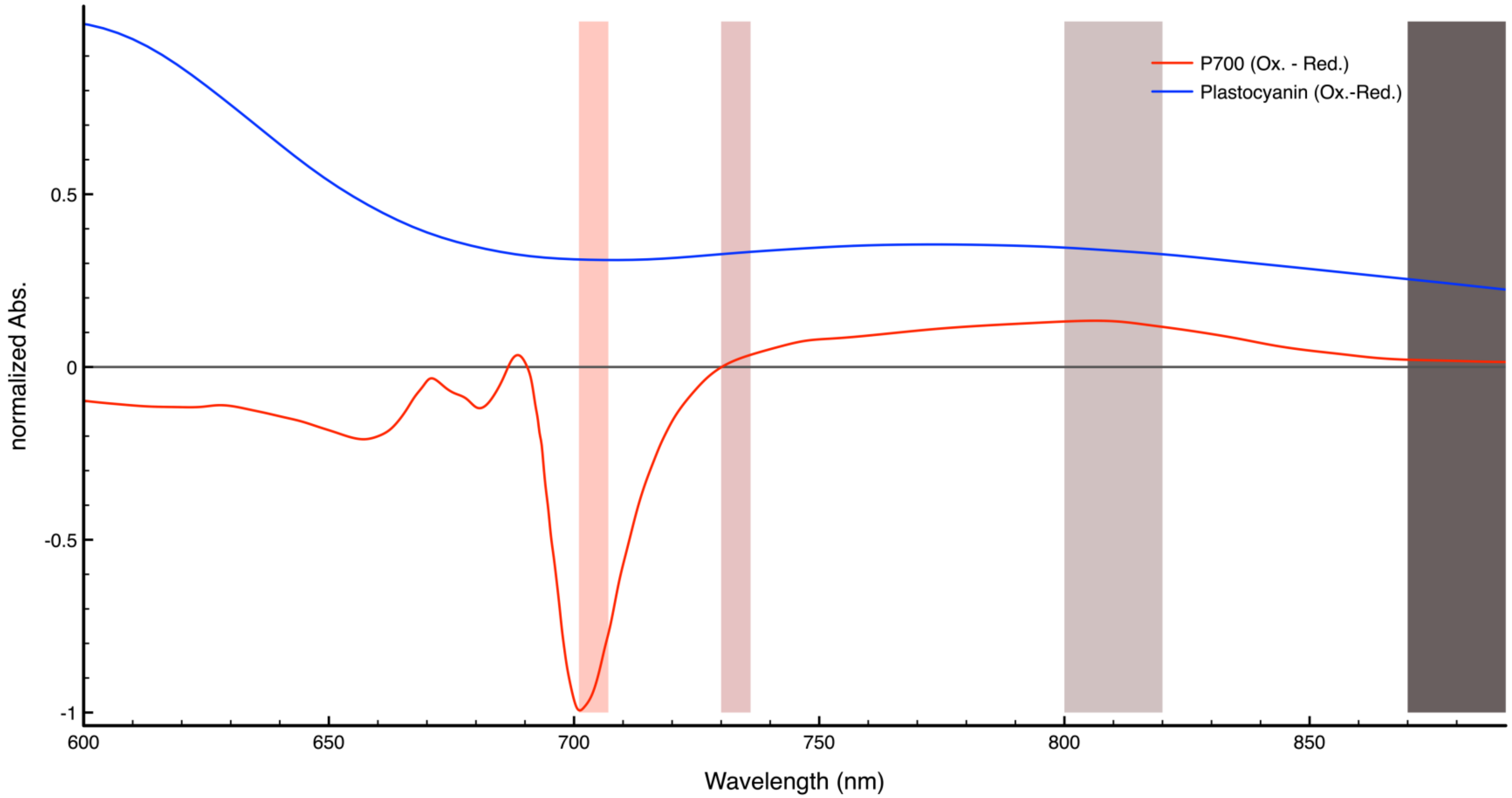
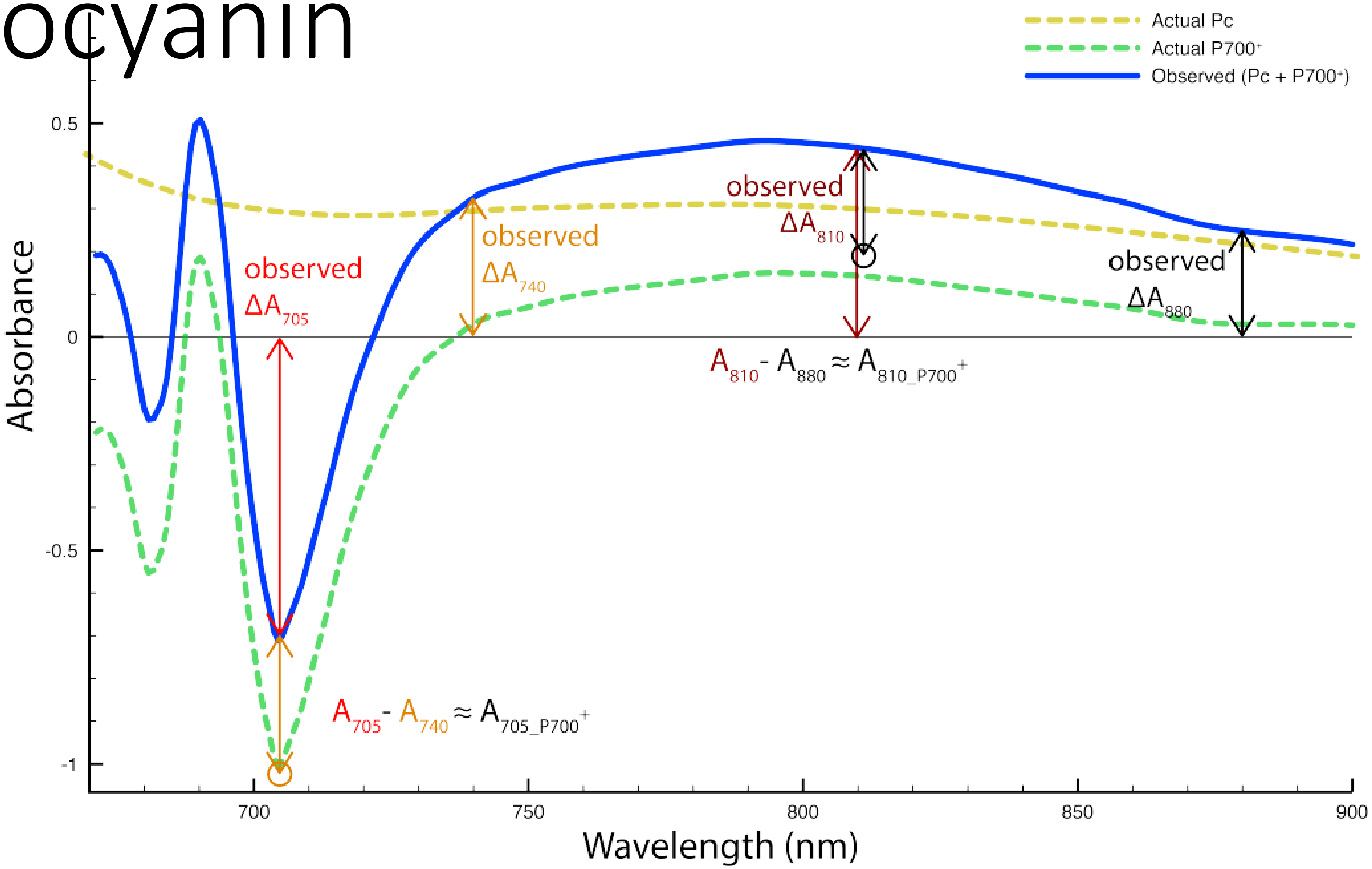
P700+ and Plastocyanin



Deconvolution of P700+ and Plastocyanin



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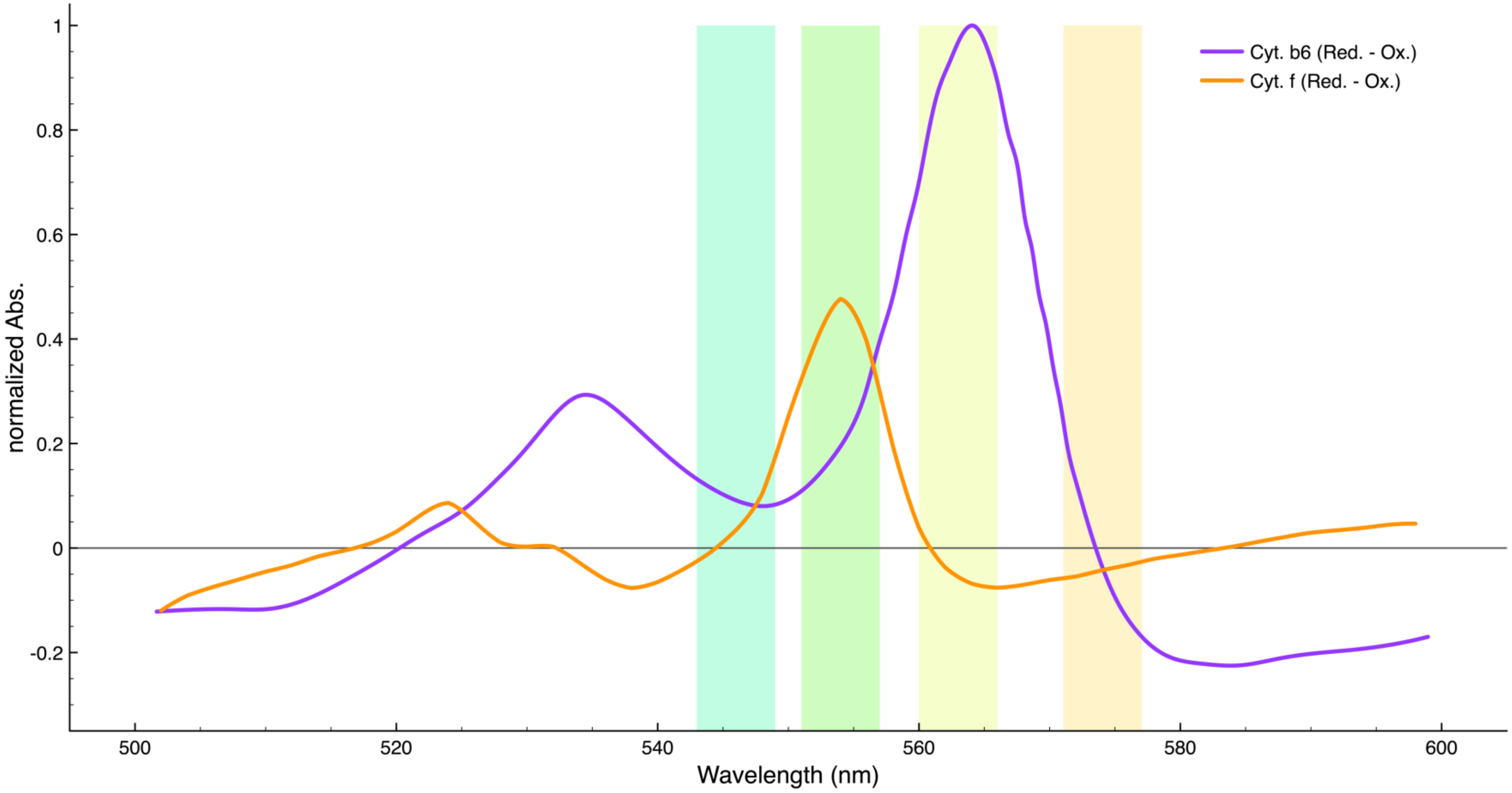
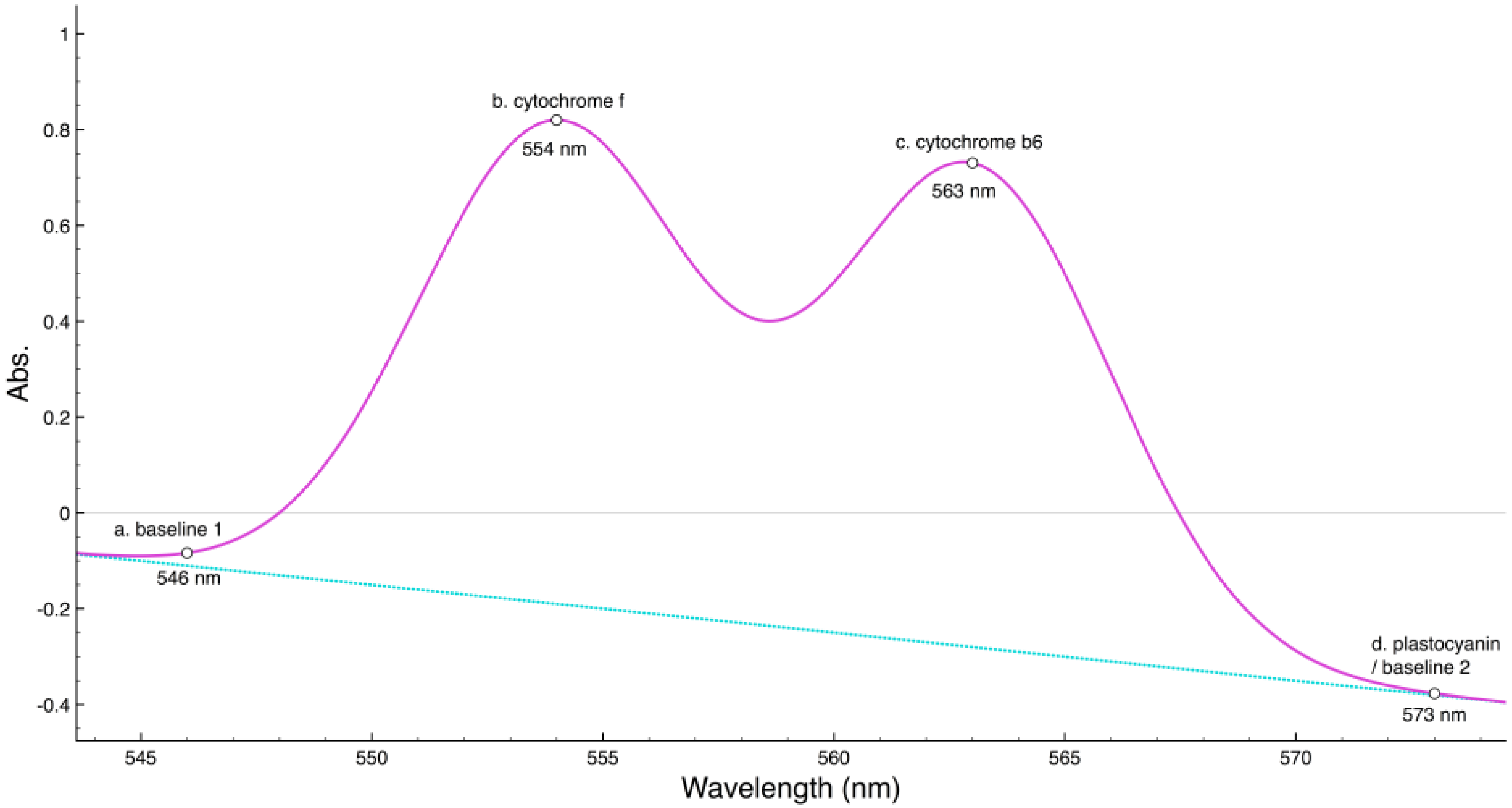
Application example: Cyt b6f



Application example:



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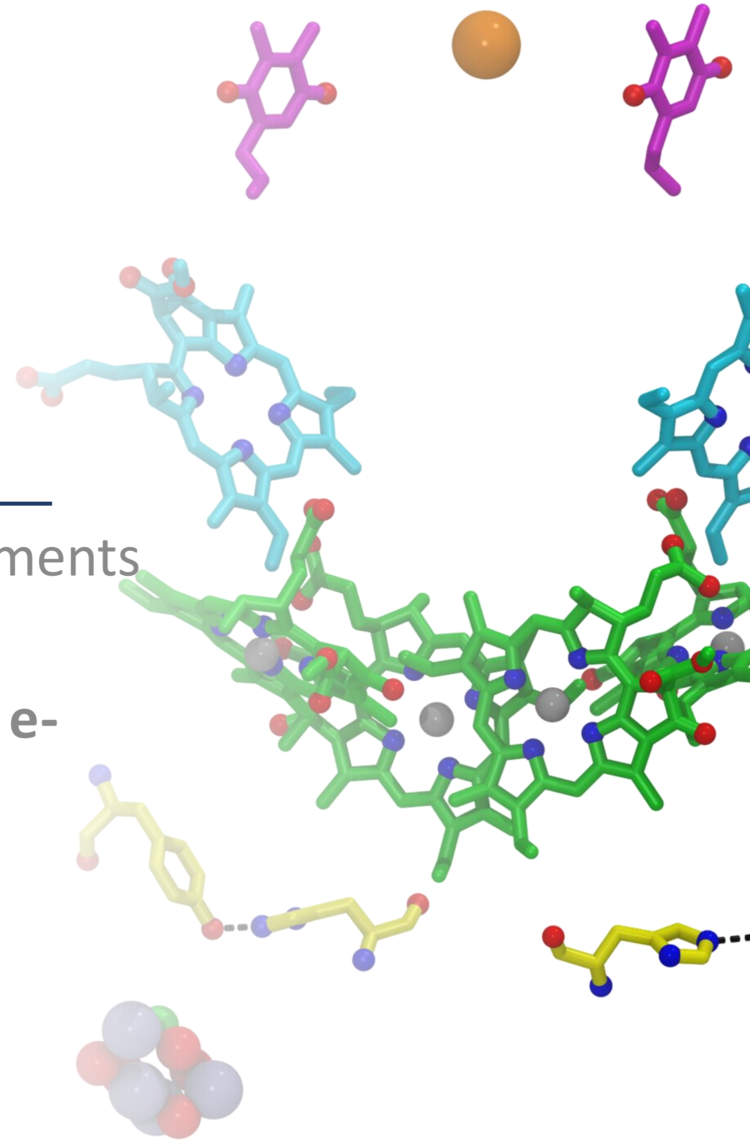
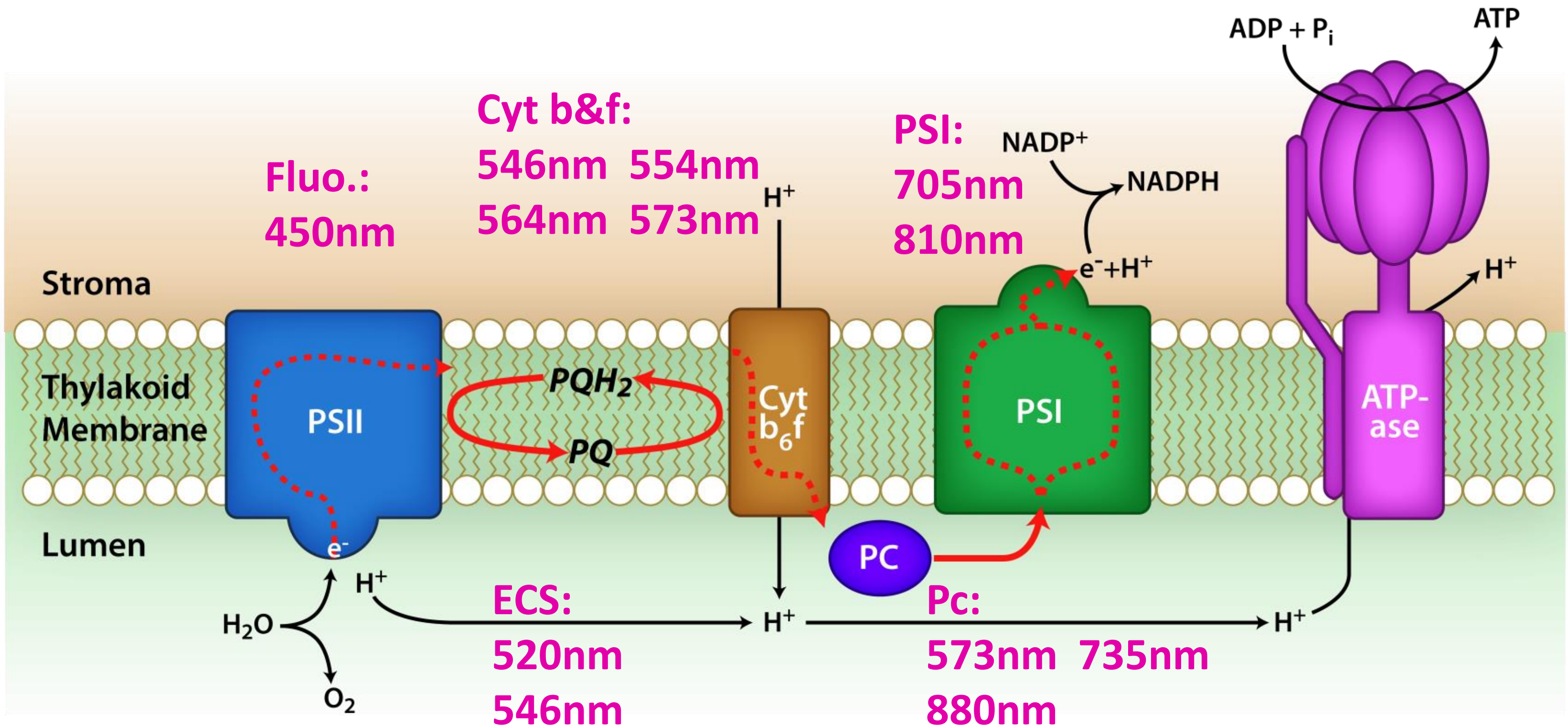
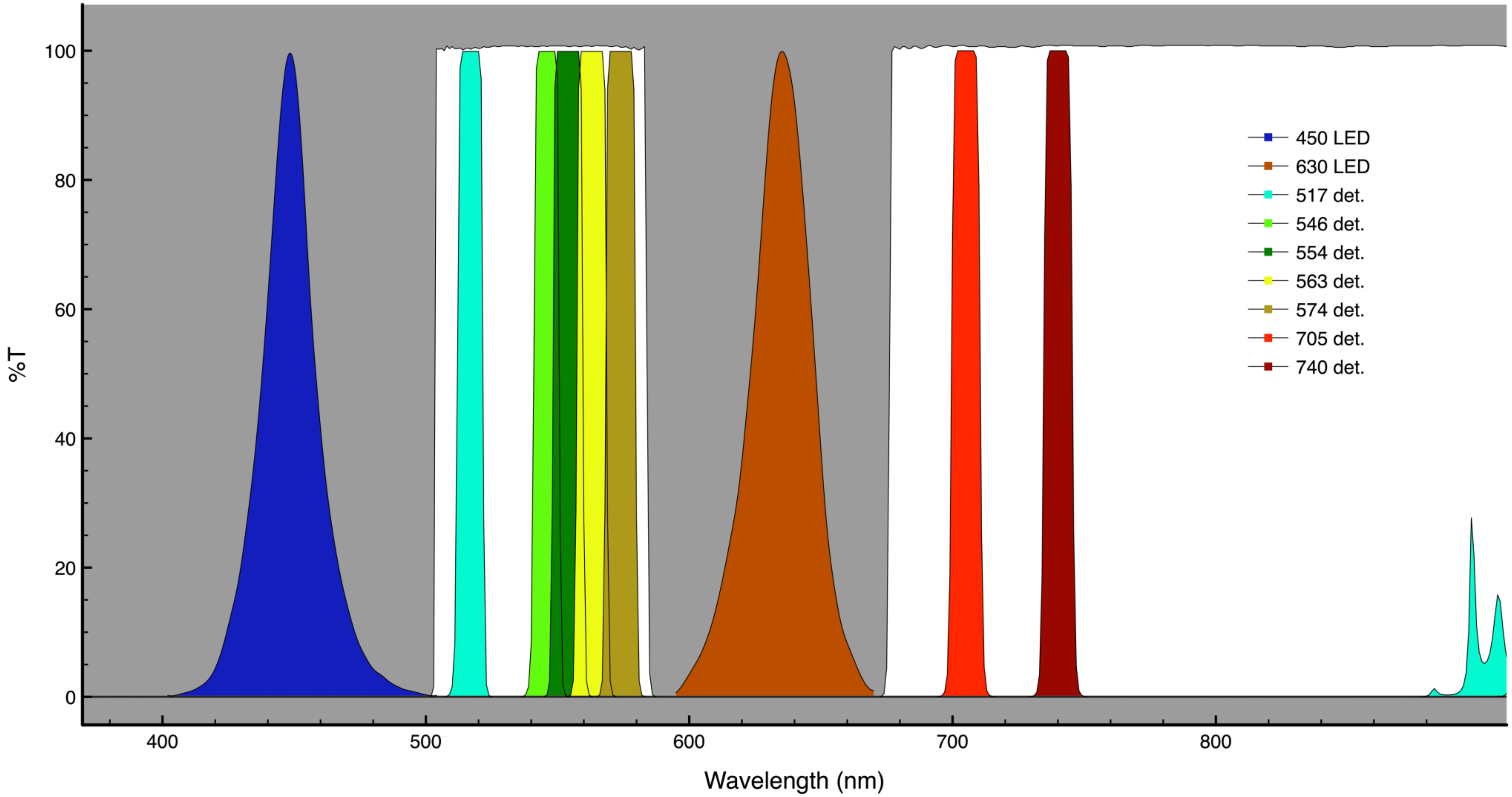


New applications: SmartLED





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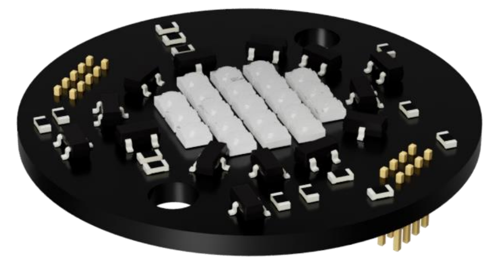
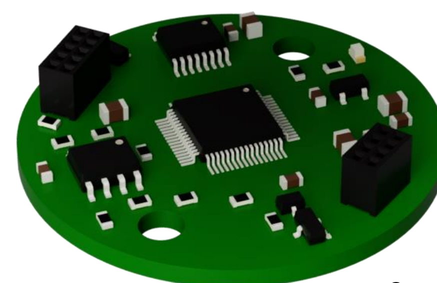
Smart-Lamp has multiple detection channels

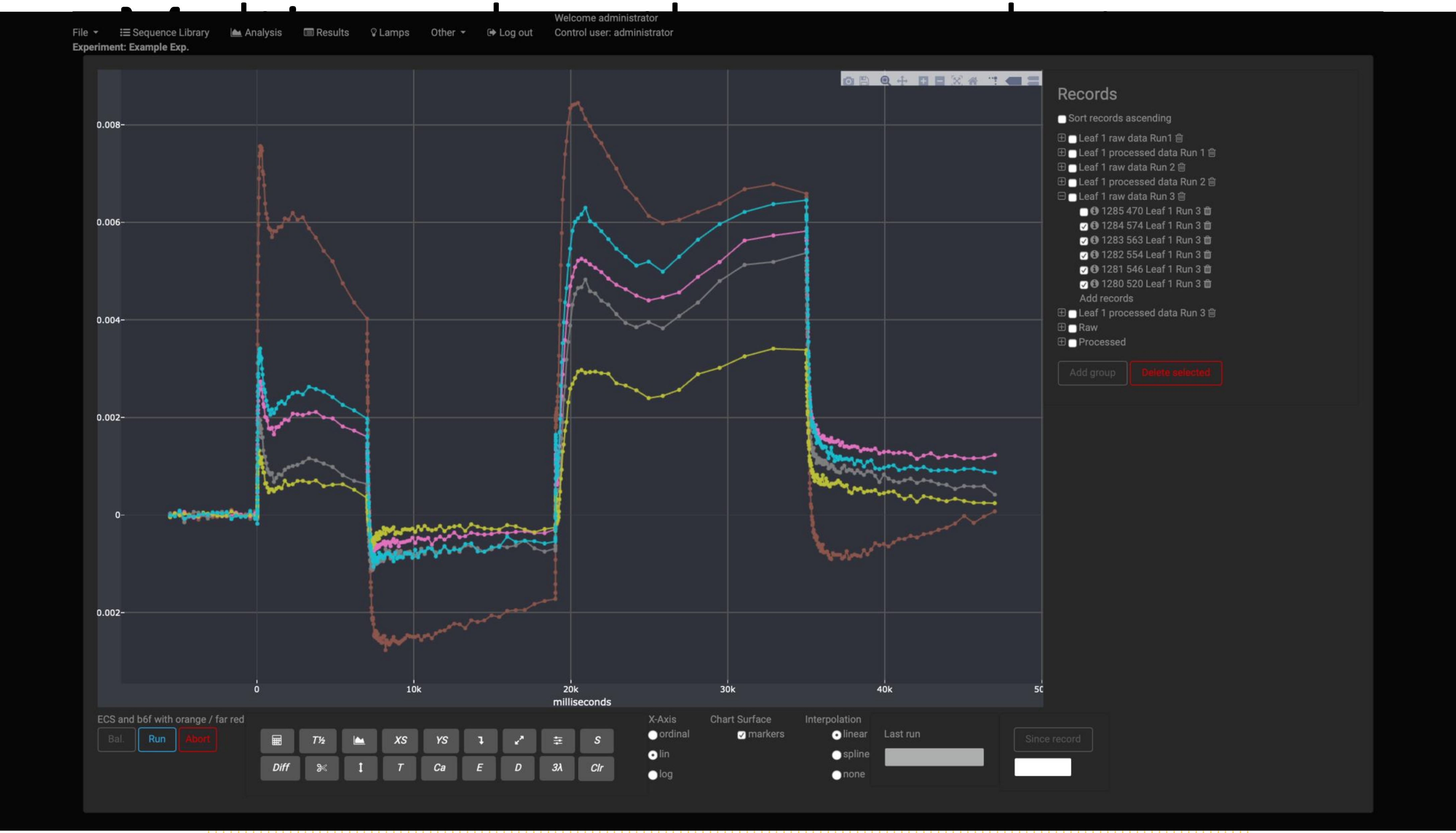
Filter plate and miniature interference filters for fine-tuning probe wavelengths



LED board with FETs for fast switching



Smart switching board functions separated from LEDs (General solution)



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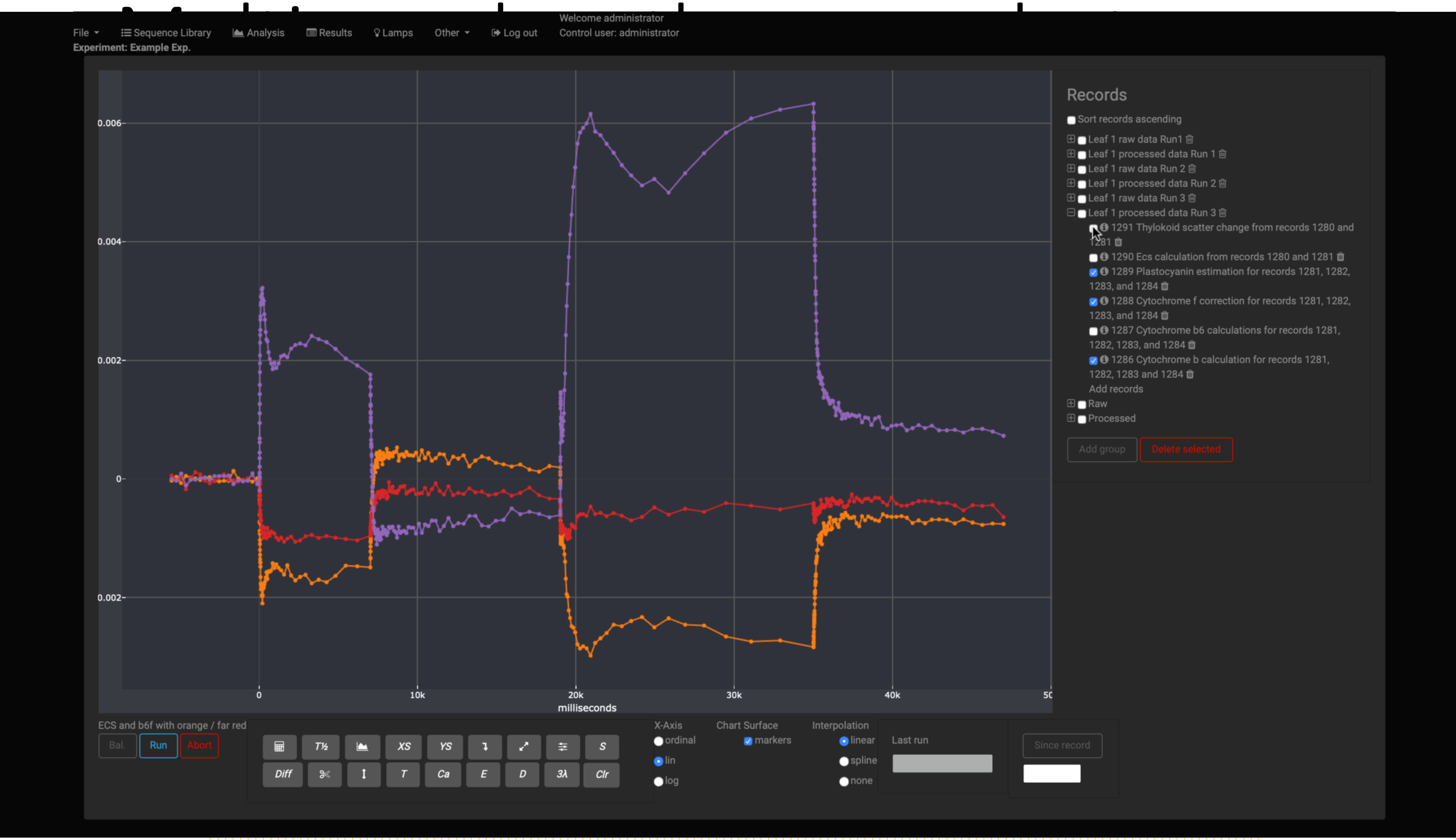
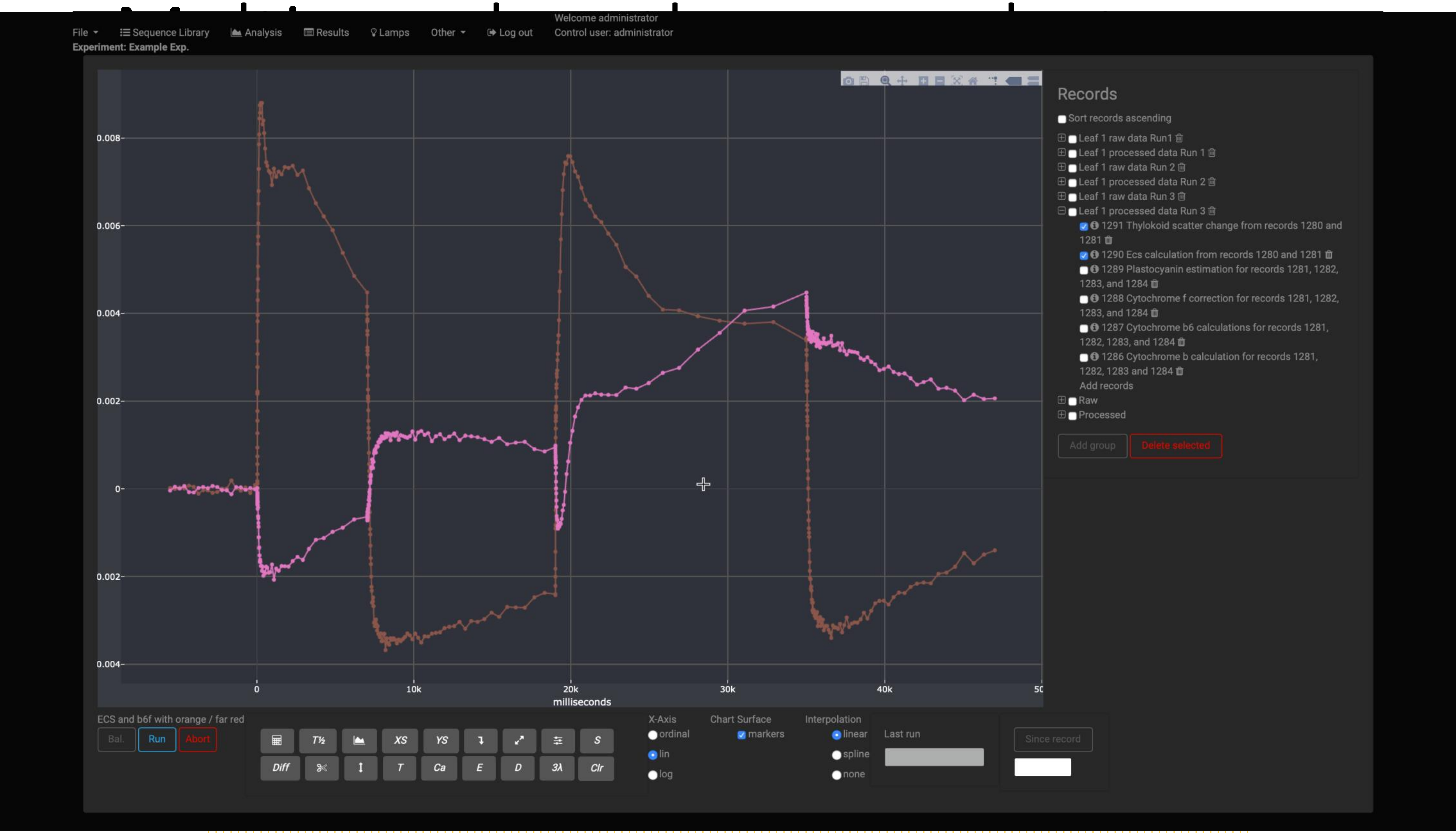

File
Sequence Library
Analysis
Results
Lamps
Other
Log out
Welcome administrator
Control user: administrator
Experiment: Example Exp.
0.006
0.004
0.002
0
0.002
0
10k
20k
30k
40k
milliseconds
Records
Sort records ascending
Leaf 1 raw data Run1
Leaf 1 processed data Run 1
Leaf 1 raw data Run 2
Leaf 1 processed data Run 2
Leaf 1 raw data Run 3
Leaf 1 processed data Run 3
1291 Thylokoid scatter change from records 1280 and 1281
1290 Ecs calculation from records 1280 and 1281
1289 Plastocyanin estimation for records 1281, 1282, 1283, and 1284
1288 Cytochrome f correction for records 1281, 1282, 1283, and 1284
1287 Cytochrome b6 calculations for records 1281, 1282, 1283, and 1284
1286 Cytochrome b calculation for records 1281, 1282, 1283 and 1284
Add records
Raw
Processed
Add group
Delete selected
ECS and b6f with orange / far red
Bal.
Run
Abort
T½
XS
YS
S
Diff
T
Ca
E
D
3λ
Clr
X-Axis
ordinal
lin
log
Chart Surface
markers
Interpolation
linear
spline
none
Last run
Since record



File
Sequence Library
Analysis
Results
Lamps
Other
Log out
Welcome administrator
Control user: administrator
Experiment: Example Exp.
0.008
0.006
0.004
0.002
0
0.002
0.004
0
10k
20k
30k
40k
milliseconds
Records
Sort records ascending
Leaf 1 raw data Run1
Leaf 1 processed data Run 1
Leaf 1 raw data Run 2
Leaf 1 processed data Run 2
Leaf 1 raw data Run 3
Leaf 1 processed data Run 3
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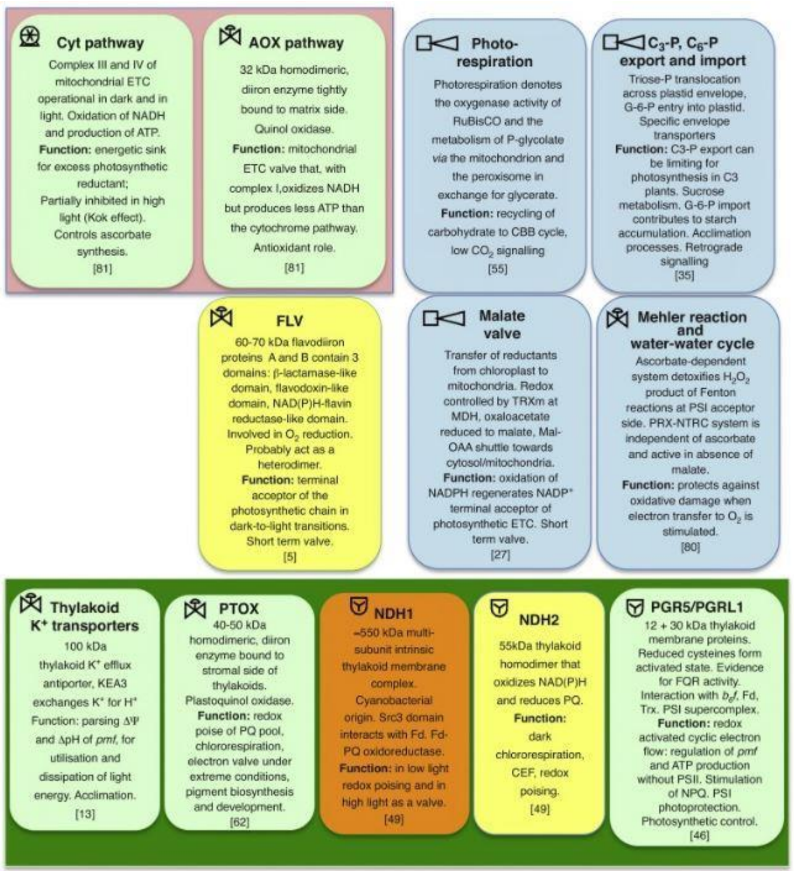
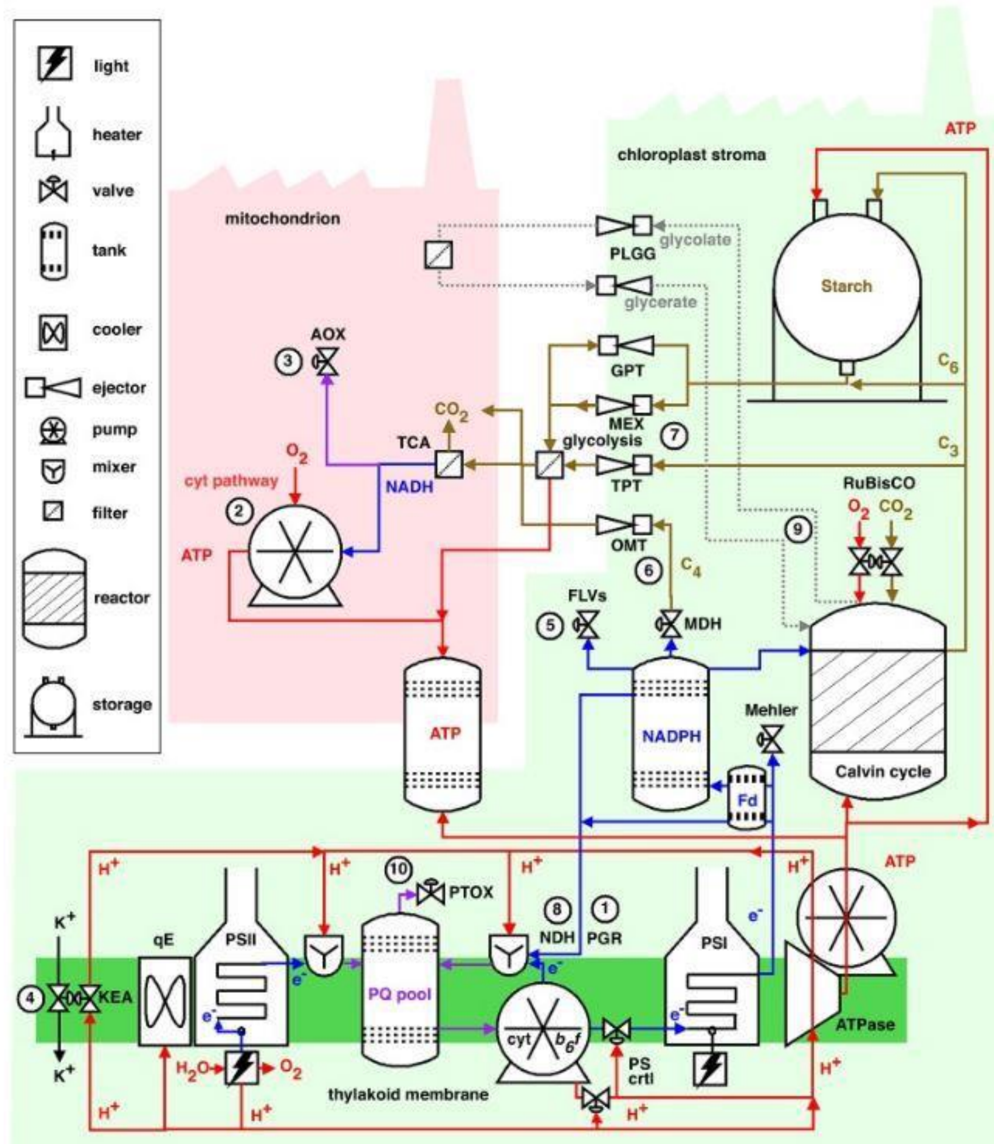
Add records
Raw
Processed
Add group
Delete selected
ECS and b6f with orange / far red
Bal.
Run
Abort
T½
XS
YS
S
Diff
T
Ca
E
D
3λ
Clr
X-Axis
ordinal
lin
log
Chart Surface
markers
Interpolation
linear
spline
none
Last run
Since record

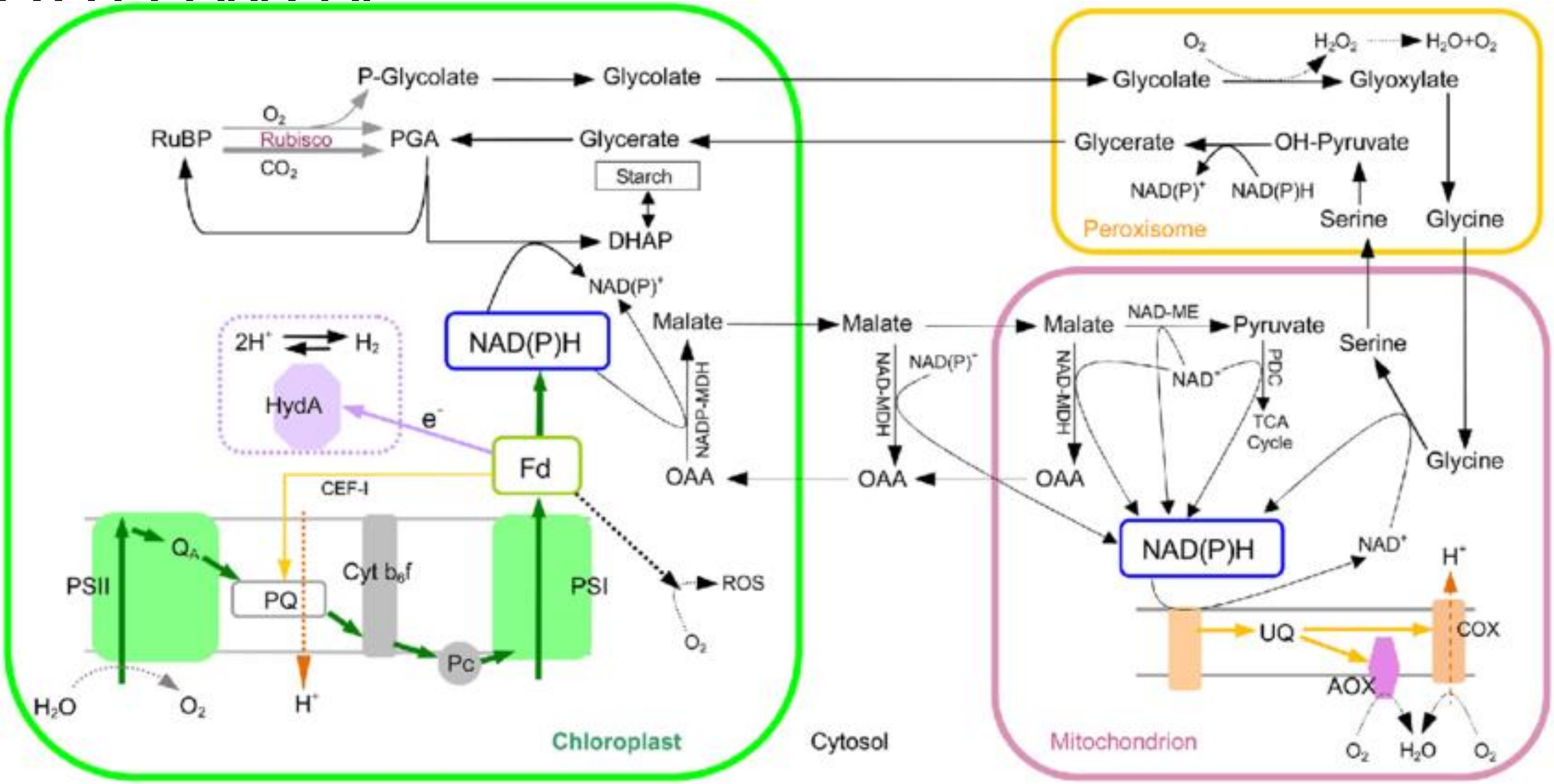
AEF is major focus of PS research





Jean Alric, Xenie Johnson. Alternative electron transport pathways in photosynthesis: a confluence of regulation. Current Opinion in Plant Biology, Elsevier, 2017, 37, pp.78-86. 10.1016/j.pbi.2017.03.014 .

Connections between PS and AOX pathway

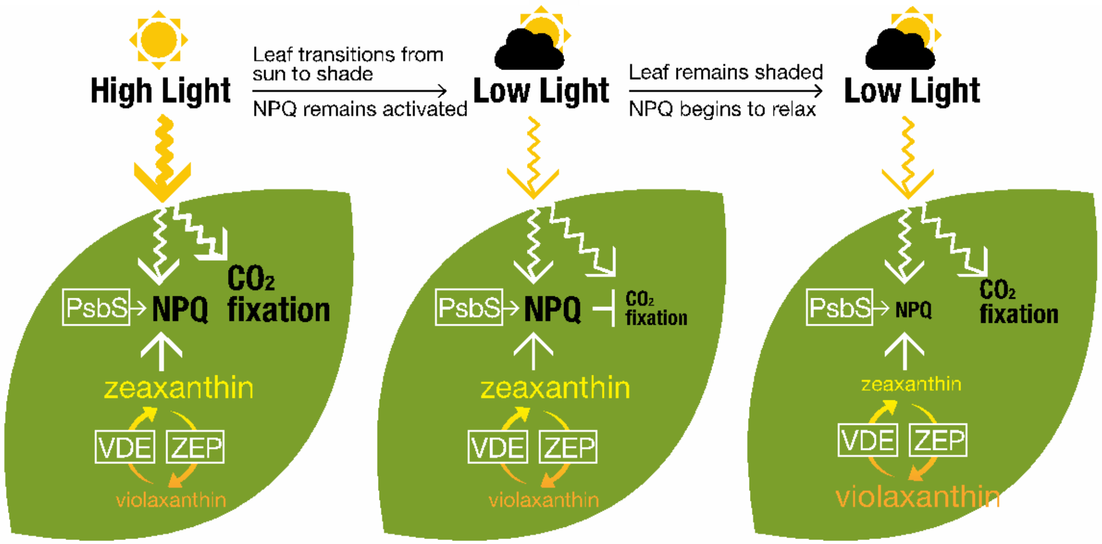


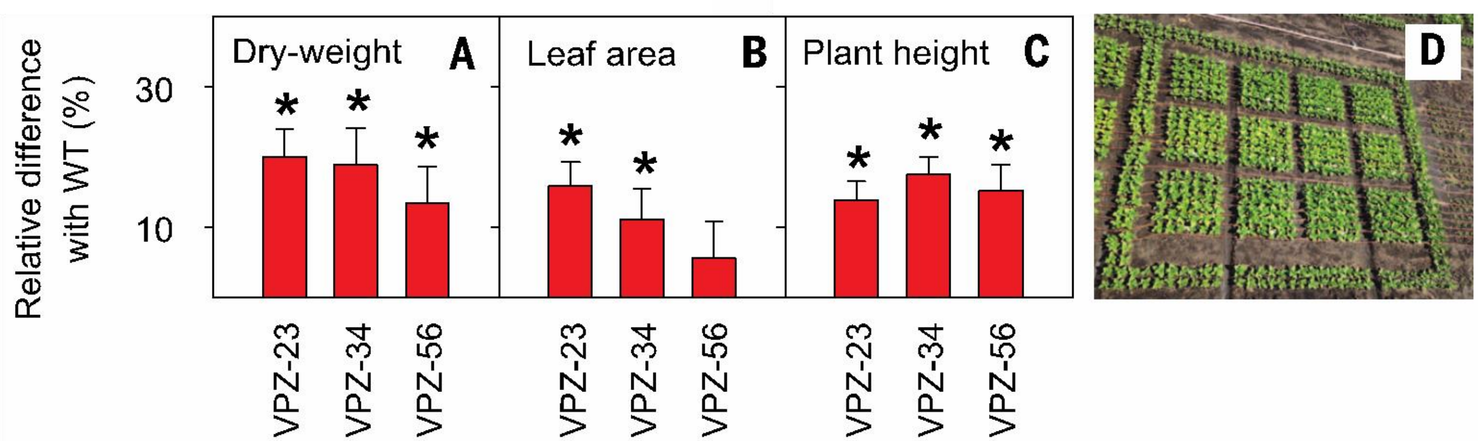
Zhang, L.; He, M.; Liu, J.; Li, L. Role of the Mitochondrial Alternative Oxidase Pathway in Hydrogen Photoproduction in Chlorella Protothecoides. *Planta* **2015**, *241* (4), 1005–1014. https://doi.org/10.1007/s00425-014-2231-y.

PLANT SCIENCE

Improving photosynthesis and crop productivity by accelerating recovery from photoprotection

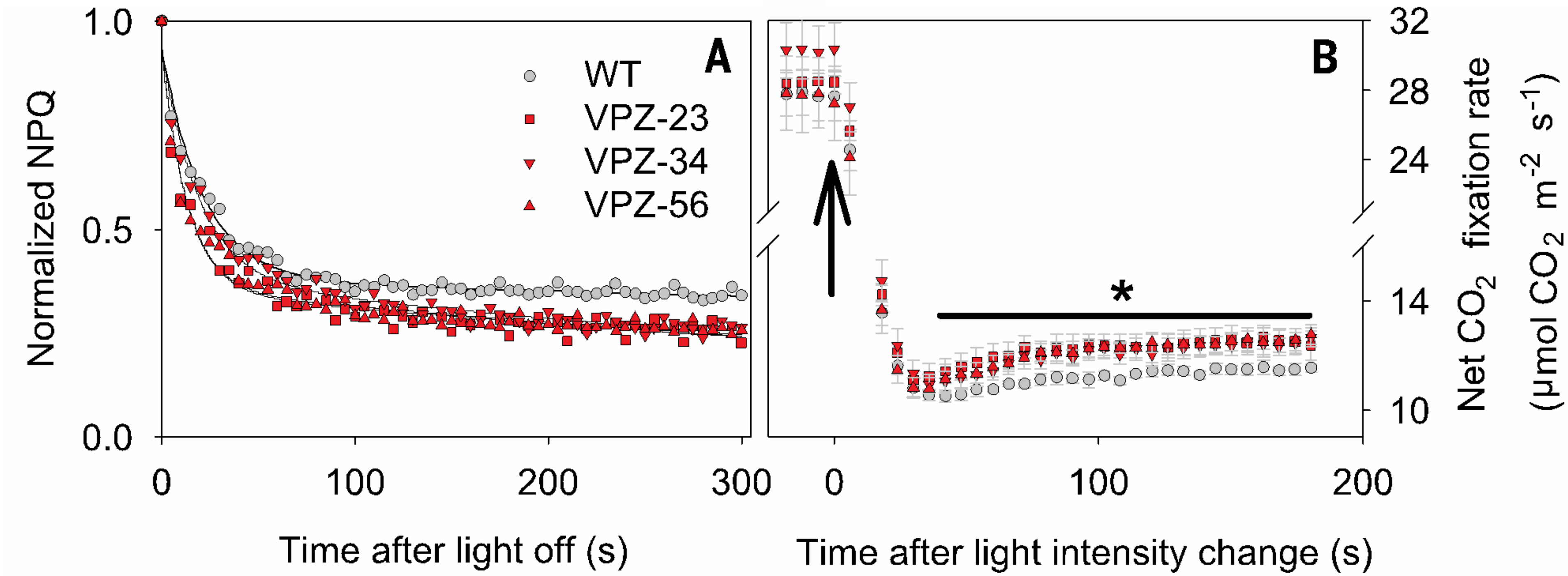
Johannes Kromdijk,[1*] Katarzyna Głowacka,[1,2*] Lauriebeth Leonelli,[3] Stéphane T. Gabilly,[3] Masakazu Iwai,[3,4] Krishna K. Niyogi,[3,4†] Stephen P. Long[1,5†]





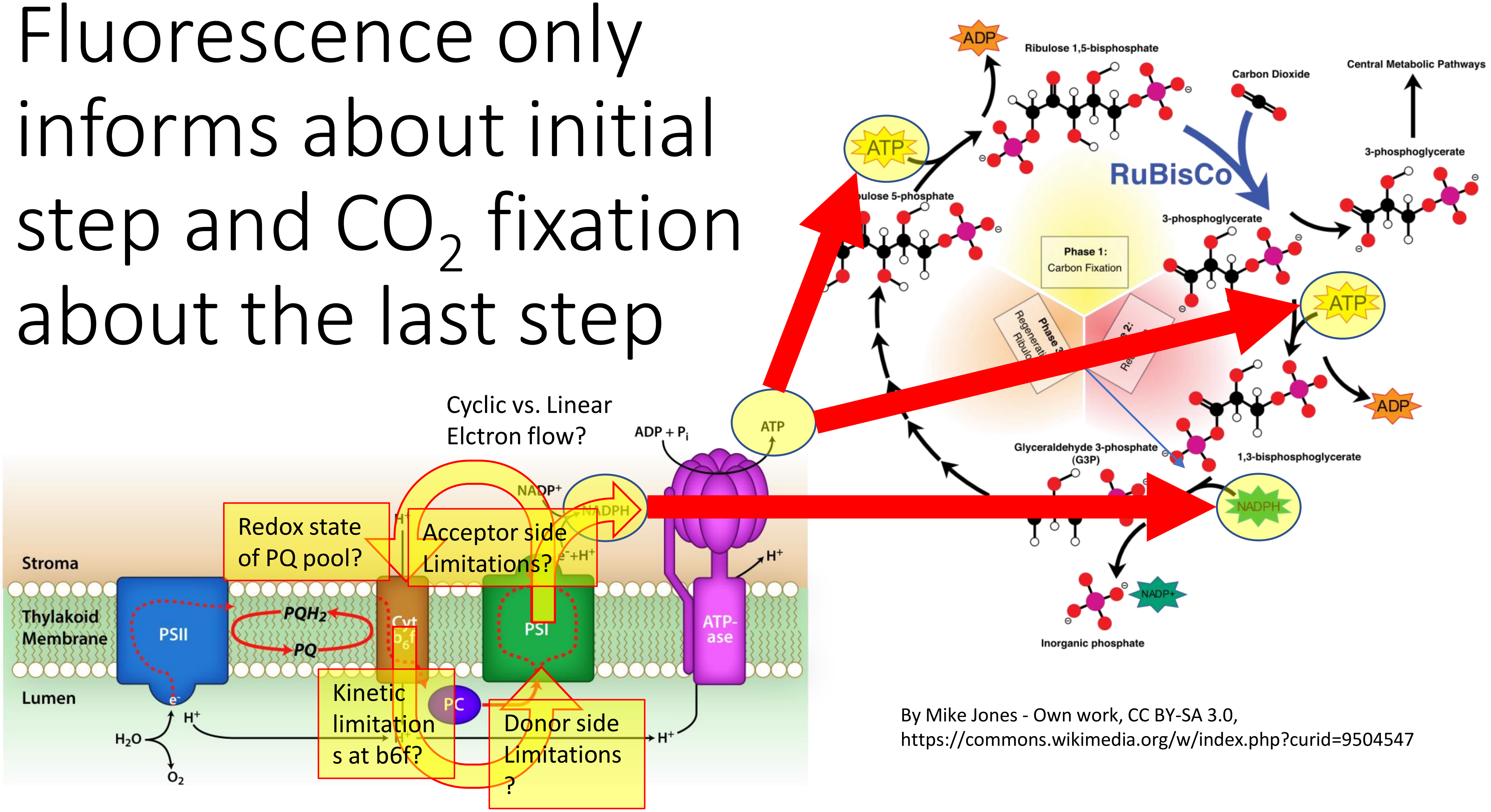
Kromdijk, J.; Głowacka, K.; Leonelli, L.; Gabilly, S. T.; Iwai, M.; Niyogi, K. K.; Long, S. P. Improving Photosynthesis and Crop Productivity by Accelerating Recovery from Photoprotection. *Science* **2016**, *354* (6314), 857–861. https://doi.org/10.1126/science.aai8878.

Fluorescence data of improved NPQ response



Kromdijk, J.; Głowacka, K.; Leonelli, L.; Gabilly, S. T.; Iwai, M.; Niyogi, K. K.; Long, S. P. Improving Photosynthesis and Crop Productivity by Accelerating Recovery from Photoprotection. *Science* **2016**, *354* (6314), 857–861. https://doi.org/10.1126/science.aai8878.

Fluorescence only informs about initial step and CO_2 fixation about the last step



SpectroLogiX

Thank you for your attention!

Questions?