O_2 evolution kinetics in red-shifted Photosystem II

A4

SFB 1078

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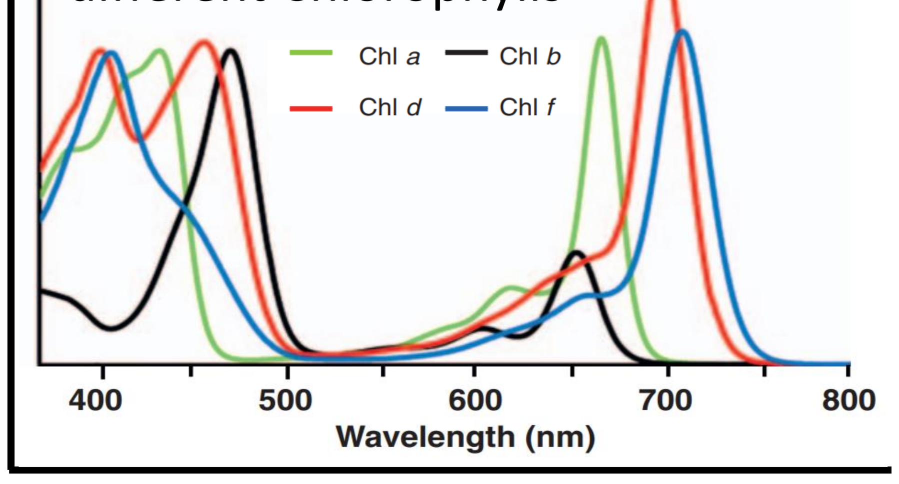
Summary

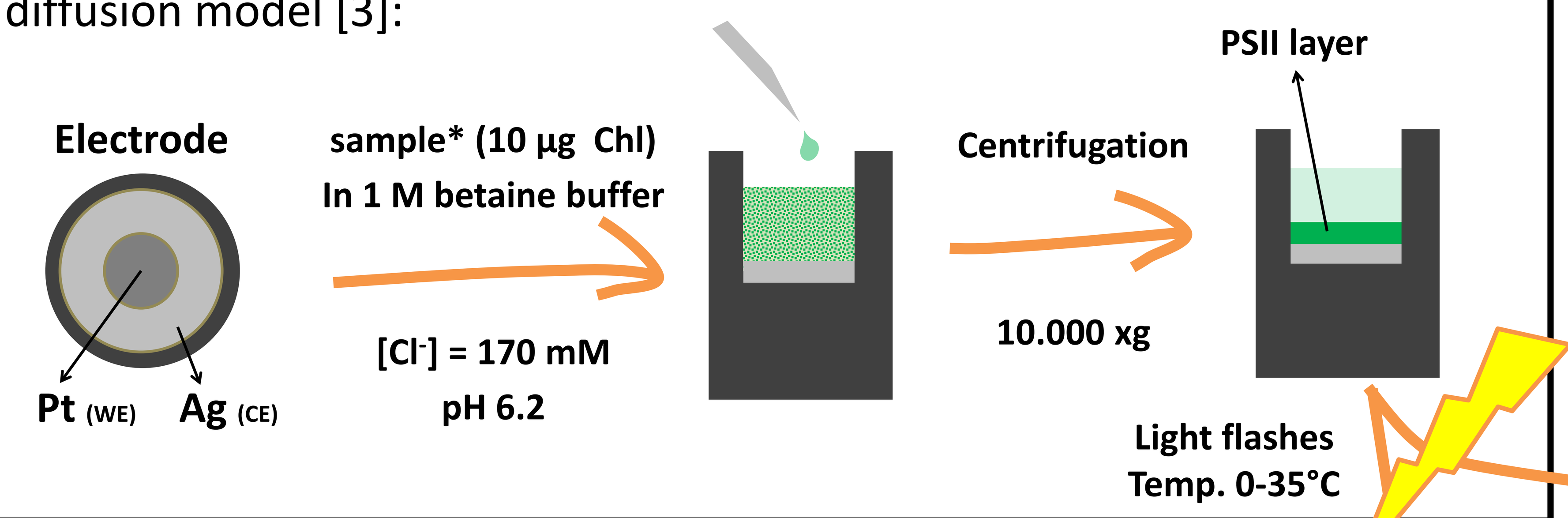
The chlorophylls in the reaction center of Photosystem II define the energy level of the initial excited state from where the subsequent photochemistry can happen. Generally, it contains chlorophyll a exclusively and is denoted as P680*. However, for *Chroococcidiopsis thermalis,* a cyanobacteria that can replace about 10% of Chl a with Chl f under red-light conditions, it was shown that Chl f is also incorporated in the reaction center lowering its initial excited state (P727*) by 110 meV [1]. Here we investigated kinetics of oxygen evolution in thylakoid membranes from *C. thermalis* grown in white and red-light conditions and compared their response to different light excitations to those of *Acaryochloris marina* (containing Chl a and d) and *Synechocystis* sp. PCC 6803 (containing only Chl a).

Absorption spectrum of different Chlorophylls [2]



Method - Time-resolved O_2 polarography

After each excitation flash, the produced O_2 is reduced by the electrode and the current is measured over time. The results are fit with a layered diffusion model [3]:



C. Thermalis E_a - FR vs. WL grown

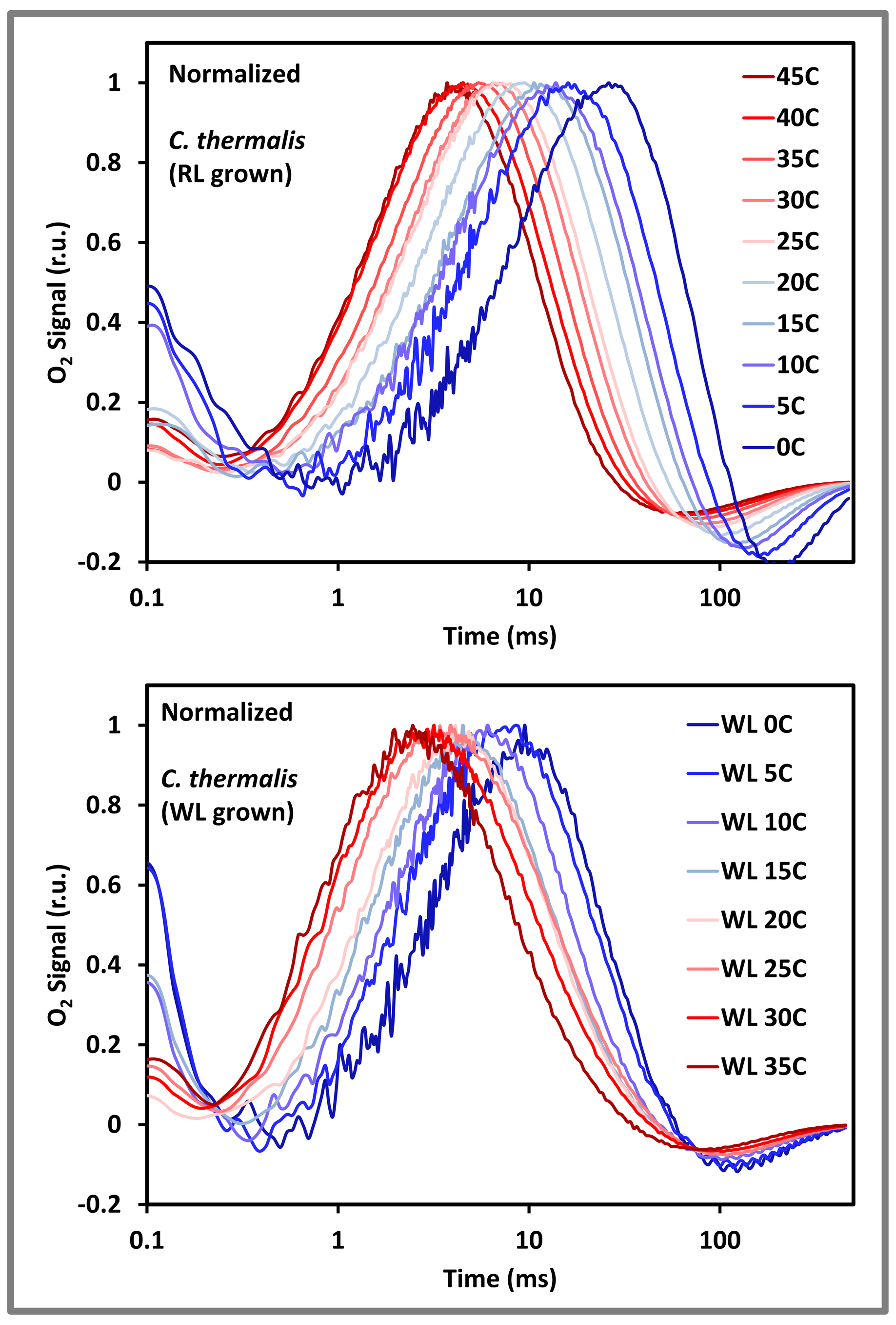
Oxygen release kinetics at different temperatures were measured for thylakoid membranes of *C. thermalis* grown in white and red-light conditions.

The kinetic traces were fit with a diffusion model [3] and the time constants of oxygen release (t_{ox}) were determined. Using the Arrhenius treatment, it was possible to estimate the activation energy (E_a) of both samples.

Arrhenius Equation

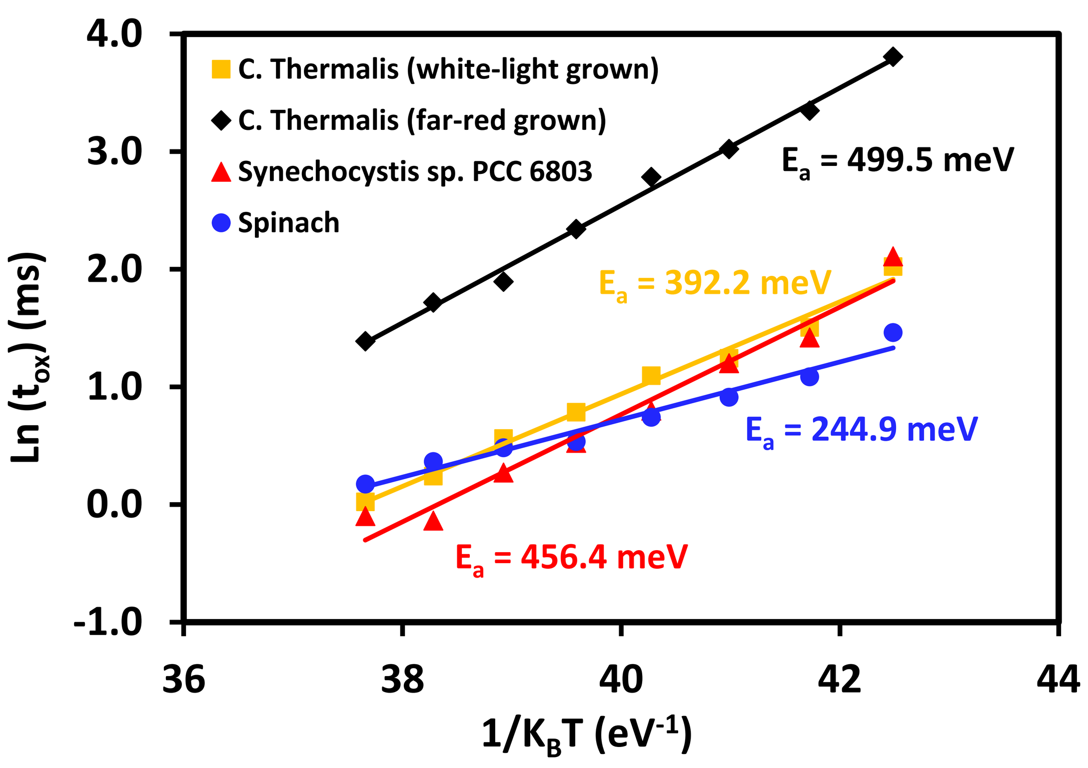
$$k = Ae^{-E_A/K_bT}$$

$$Ln(\tau) = \left(\frac{E_A}{K_bT}\right) - Ln(A)$$



The kinetic curves for the sample grown is red-light conditions (Chl f-containing) shows significantly slower kinetics.

	t_{ox} (ms)
Spinach	1.7
Synechocystis sp. PCC 6803	1.7
C. thermalis (WL grown)	2.2
C. thermalis (RL grown)	10.4

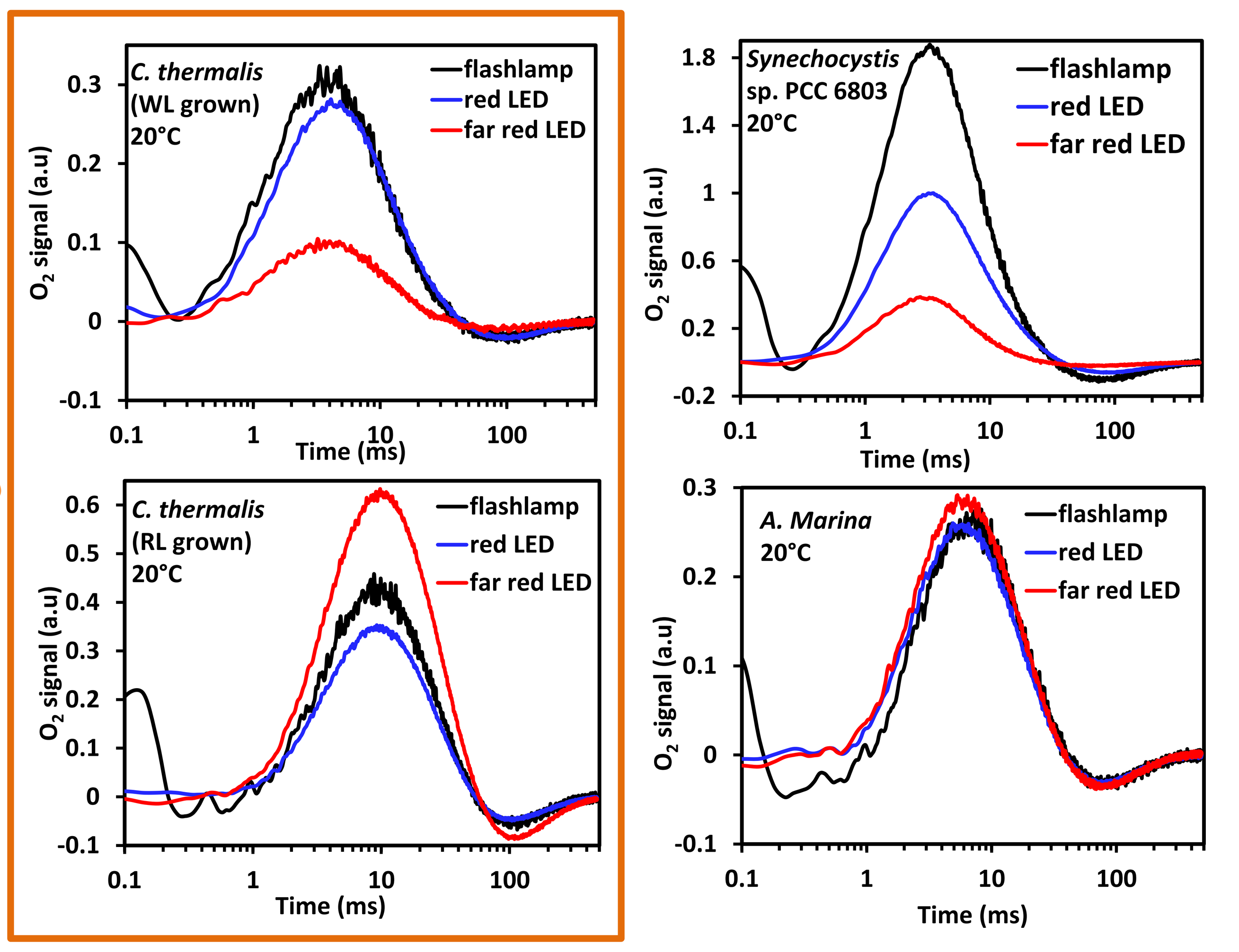


The obtained activation energy value for RL-grown *C. thermalis* was about 110 meV higher than the value obtained for WL-grown *C. thermalis.*

This energetic difference and its related slower oxygen release, alongside the absence of a significant increase in miss factor, in the Chl f containing PSII, will be key points to consider in understanding this red-shifted water oxidation.

Response to different light excitations

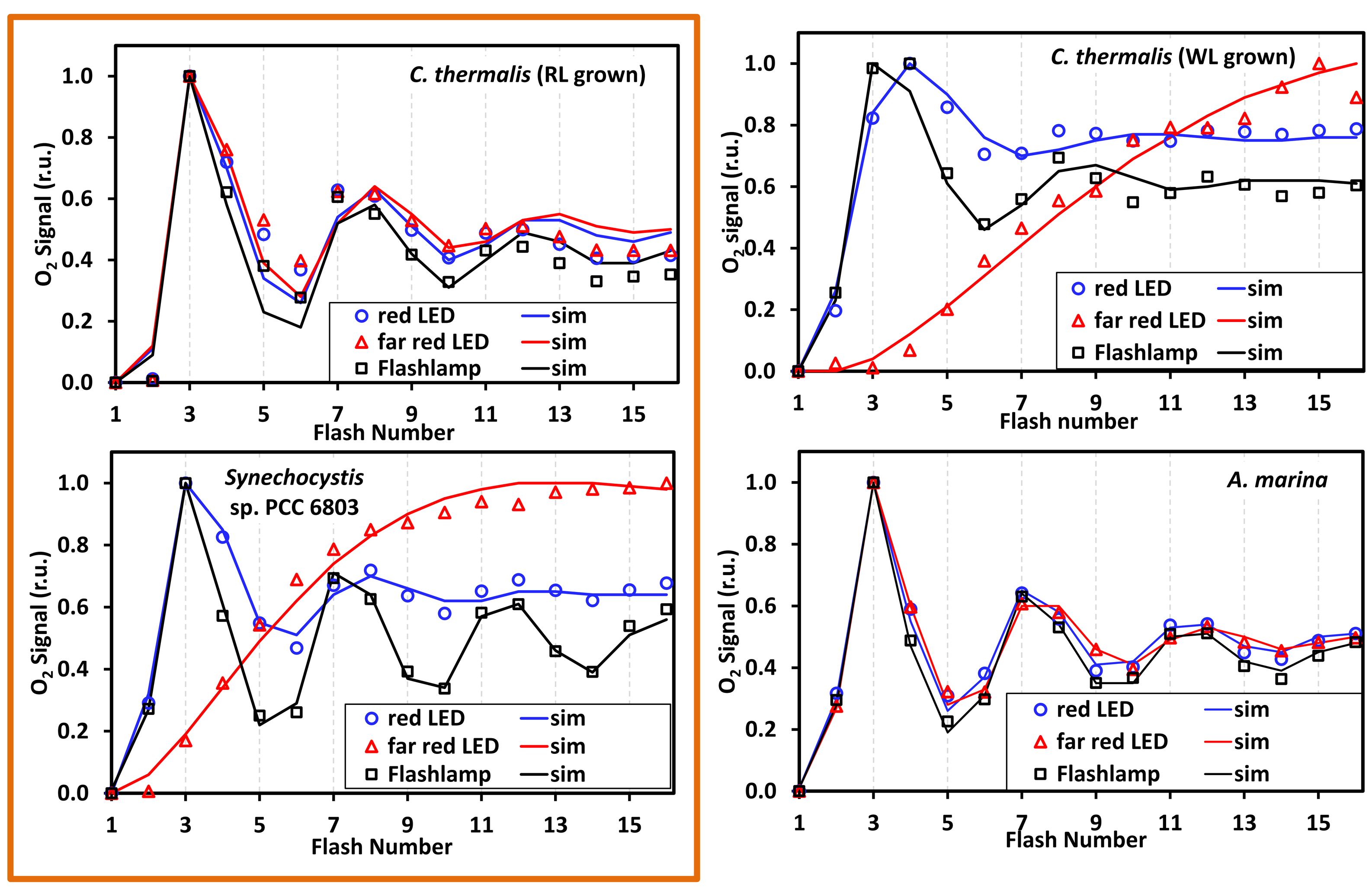
Oxygen release kinetics of different chlorophyll containing cyanobacteria were measured, each under three excitation light conditions: red LED (613 nm), far red LED (730 nm) and a flashlamp (below 570 nm).



t_{ox} at 20°C (ms)		
C. Thermalis (WL grown)		
red LED	far red LED	flashlamp
2.23	2.31	2.19
C. Thermalis (FR grown)		
red LED	far red LED	flashlamp
10.39	10.24	10.56
Synechocystis sp. PCC 6803		
red LED	far red LED	flashlamp
1.53	1.51	1.57
A. Marina		
red LED	far red LED	flashlamp
4.66	4.82	4.71

- The application of different excitation light did not change the oxygen release kinetics of any of the tested samples.
- Cyanobacteria without red-shifted chlorophylls (top panels) had a significantly lower signal when excited by 730 nm light.

Flash patterns



The samples without red-shifted chlorophylls struggled to use the far-red LED light (730 nm), which translated in the loss of the normal 4 period oscillation, with an apparent miss factor above 80%.

The slower oxygen release of RL-grown *C. thermalis* has did not translate in a significant increase in miss factor (below 20%).

References

[1] Nürnberg, D. J., Morton, J., Santabarbara, S., Telfer, A., Joliot, P., Antonaru, L. A., ... & Rutherford, A. W. (2018). Photochemistry beyond the red limit in chlorophyll f–containing photosystems. *Science, 360*(6394), 1210-1213.

[2] Chen, M., & Blankenship, R. E. (2011). Expanding the solar spectrum used by photosynthesis. *Trends in plant science, 16*(8), 427-431.

[3] Dilbeck, P. L., Hwang, H. J., Zaharieva, I., Gerencser, L., Dau, H., & Burnap, R. L. (2012). The D1-D61N mutation in Synechocystis sp. PCC 6803 allows the observation of pH-sensitive intermediates in the formation and release of O2 from photosystem II. *Biochemistry, 51*(6), 1079-1091.

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