High-resolution respirometry for chloroplast and mitochondrial bioenergetics in *Chlamydomonas reinhardtii* — towards biotechnology exploitations

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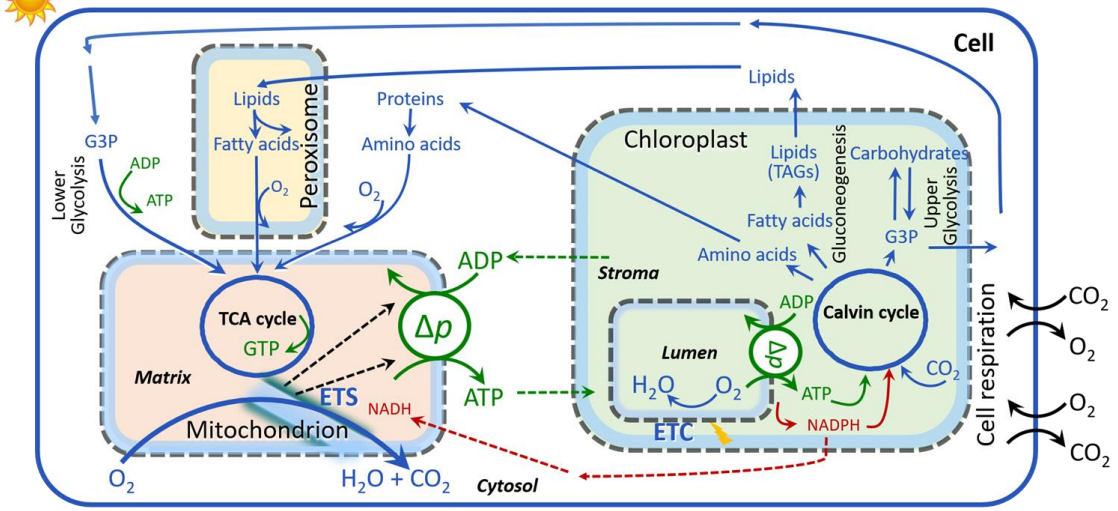
Abstract

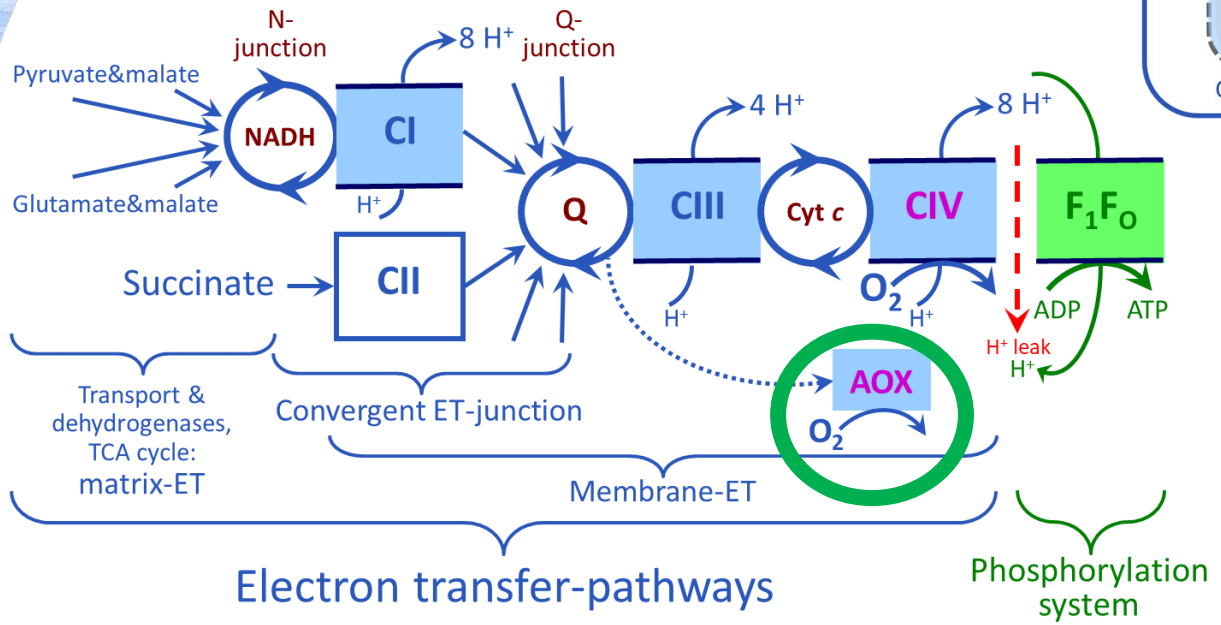
Chloroplast and mitochondrial bioenergetic control of algal growth and metabolite production is necessary to maintain metabolic integrity. In contrast to mammalian cells, algal mitochondria express alternative oxidases (AOX), which divert electron transfer from coenzyme Q (Q) away from cytochrome *c* oxidase (Complex IV, CIV) [1]. In algae, therefore, we distinguish between respiration through the Q-AOX and Q-CIV branches of the electron transfer system. A better understanding of the contribution of both branches to mitochondrial dark respiration is required to optimize algal biomass and metabolite production for biotechnological purposes. High-resolution respirometry (HRR; Oroboros O2k) is widely used to assess mitochondrial respiration and other bioenergetic parameters in the biomedical field of mitochondrial and cell research [2]. In our interdisciplinary study, we introduced a new method of studying oxygen kinetics [3,4] for the partitioning of dark respiration between the Q-AOX and Q-CIV branches in *Chlamydomonas reinhardtii* grown for biomass and lipid production [5]. This method is based on the distinct oxygen affinities of these enzymes. In addition, the multimodal approach of the Oroboros O2k was extended using the novel PhotoBiology-Module of the NextGen-O2k for determining the maximum light-saturated and inorganic carbon-saturated photosynthesis rates [6,7]. This methodology allows for increasing our current knowledge of the bioenergetics of chloroplasts and mitochondria, which is of particular importance for biotechnological exploitations. This communication is part of the NextGen-O2k project that has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement No 859770.

1. Young L et al (2013) Biochem Soc Trans 41:1305-11; 2. Doerrier C et al (2018) Methods Mol Biol 1782:31-70; 3. Meszaros AT et al (2018) Abstract Mitochondrial Medicine 2018 Hinxton UK; 4. DiMarcello M et al (2019) MitoFit Preprint Arch EA; 5. Pulz O & Gross W (2004) App Microbiol Microbiol Biotech 65(6):635-648; 6. Giordano M et al (2000) Plant Physiol 124:857-864; 7. Palmqvist K et al (1994) Plant Cell Envir 17:65-72.

Microalgae metabolic complexity

Chloroplast and mitochondrial bioenergetics are essential to maintain energy balance, metabolic homeostasis, and control algal growth and metabolite production.





Alternative oxidases, AOX, divert electron transfer away from Complex IV, causing a reduction of ATP production.

Gnaiger et al (2020) Mitochondrial physiology. Bioenerg Commun 2020.1

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Research objectives

High-resolution respirometry HRR is widely used to measure mitochondrial respiration and key bioenergetics parameters in the biomedical field.

HRR
NextGen-O2k

We apply HRR to characterize the cellular bioenergetics of *C. reinhardtii* grown for biomass and lipid production with special focus on:

- the contribution of the Q-AOX and Q-CIV branches to mitochondrial dark respiration and oxygen affinities using O_2 kinetics;
- changes in the photosynthetic pathway associated with different growth regimes.

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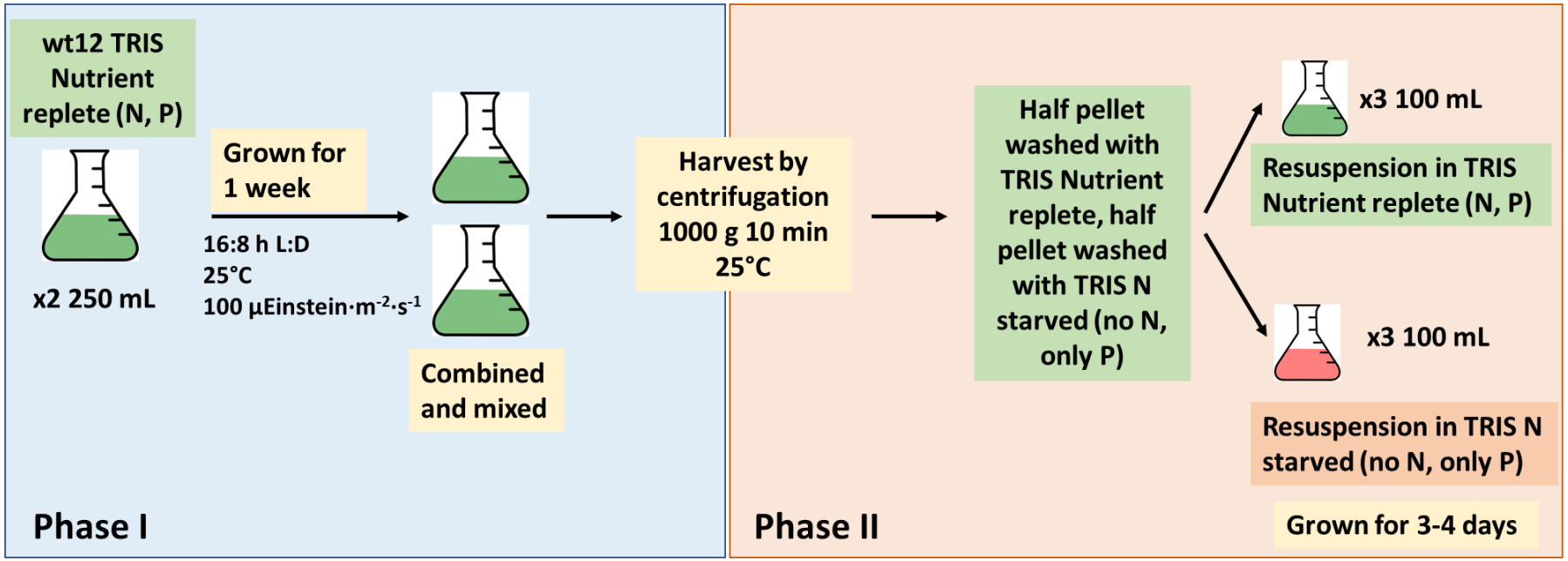
Experimental approach

C. reinhardtii wt12

- grown photoautotrophically in TRIS minimal media
- 3 days of Nitrogen starvation
- followed by HRR measurements in the NextGen-O2k



- **Nutrient replete = biomass production**
- **Nitrogen starved = lipid production**

Samples for determining cell density, chlorophyll *a* concentration, and HRR were taken at Days 0 and 3 of Phase 2.

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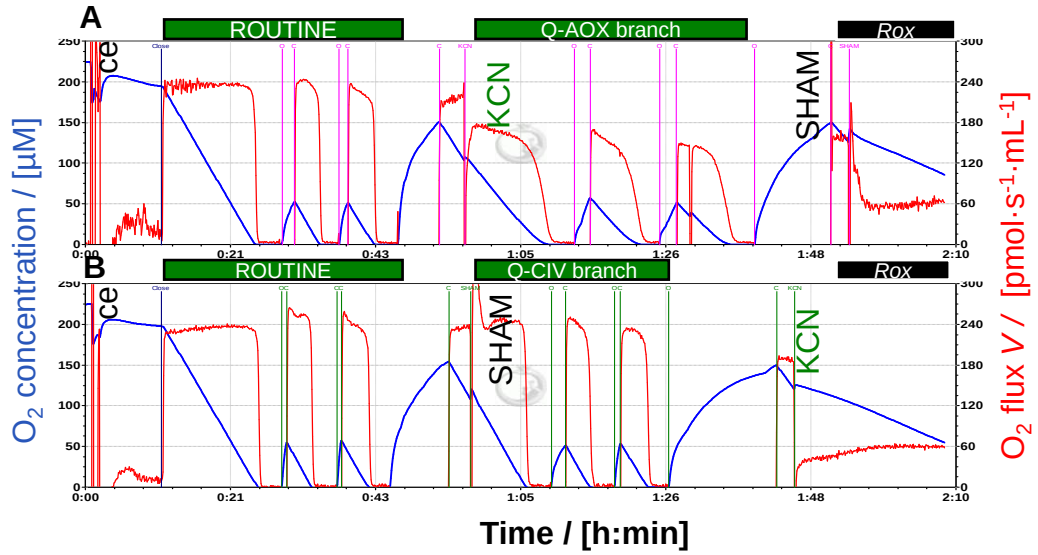
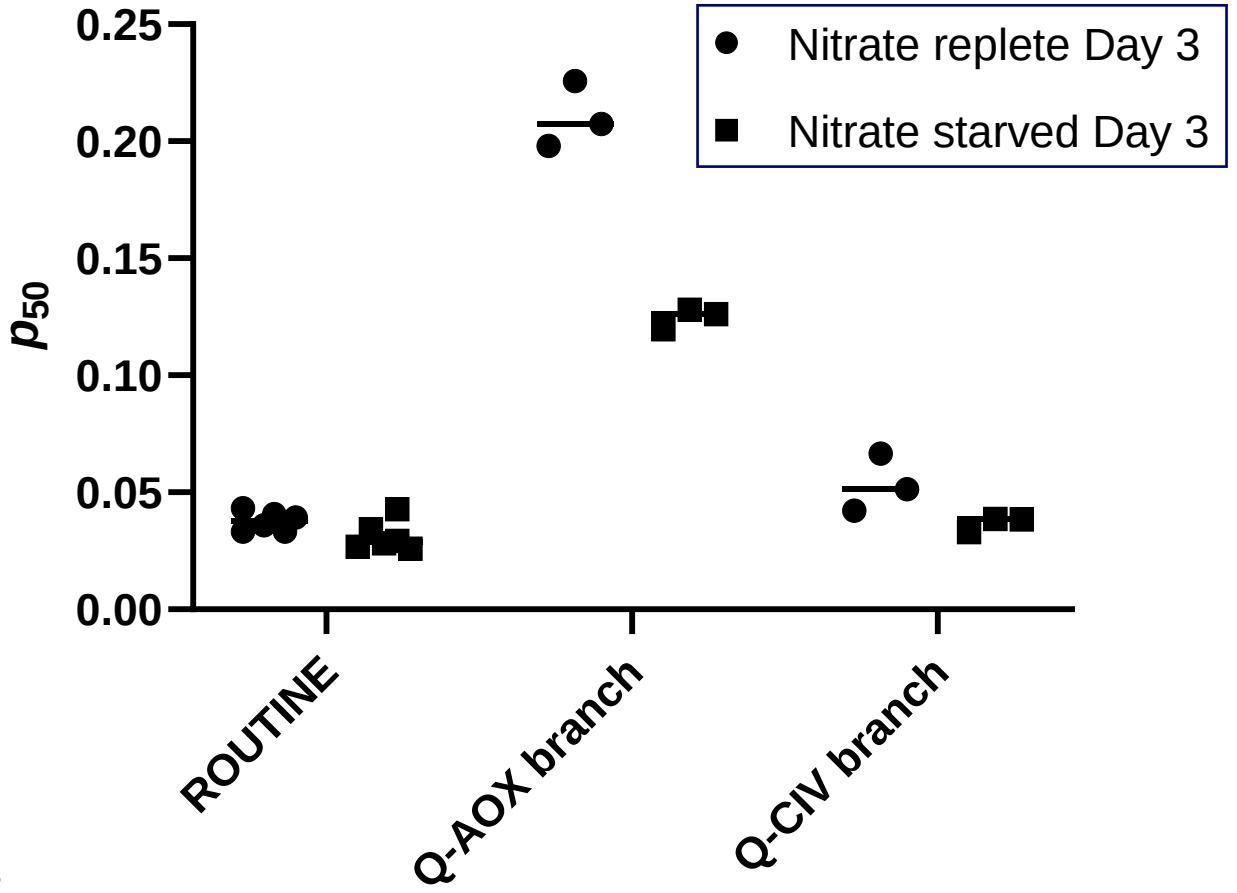
Results 1: Dark respiration, AOX, and oxygen kinetics

Nitrate starvation cultures at day 3:

- **lighter**
- **lower cell densities**



O2k traces of O_2 kinetics with repeated aerobic-anoxic transitions and re-oxygenations. (A) SUIT-022_O2_ce_D051 protocol. **(B)** SUIT-023_O2_ce_D053 protocol. www.bioblast.at



p_{50}: ROUTINE respiration, Q-AOX branch (inhibition by KCN), and Q-CIV branch (inhibition by SHAM).

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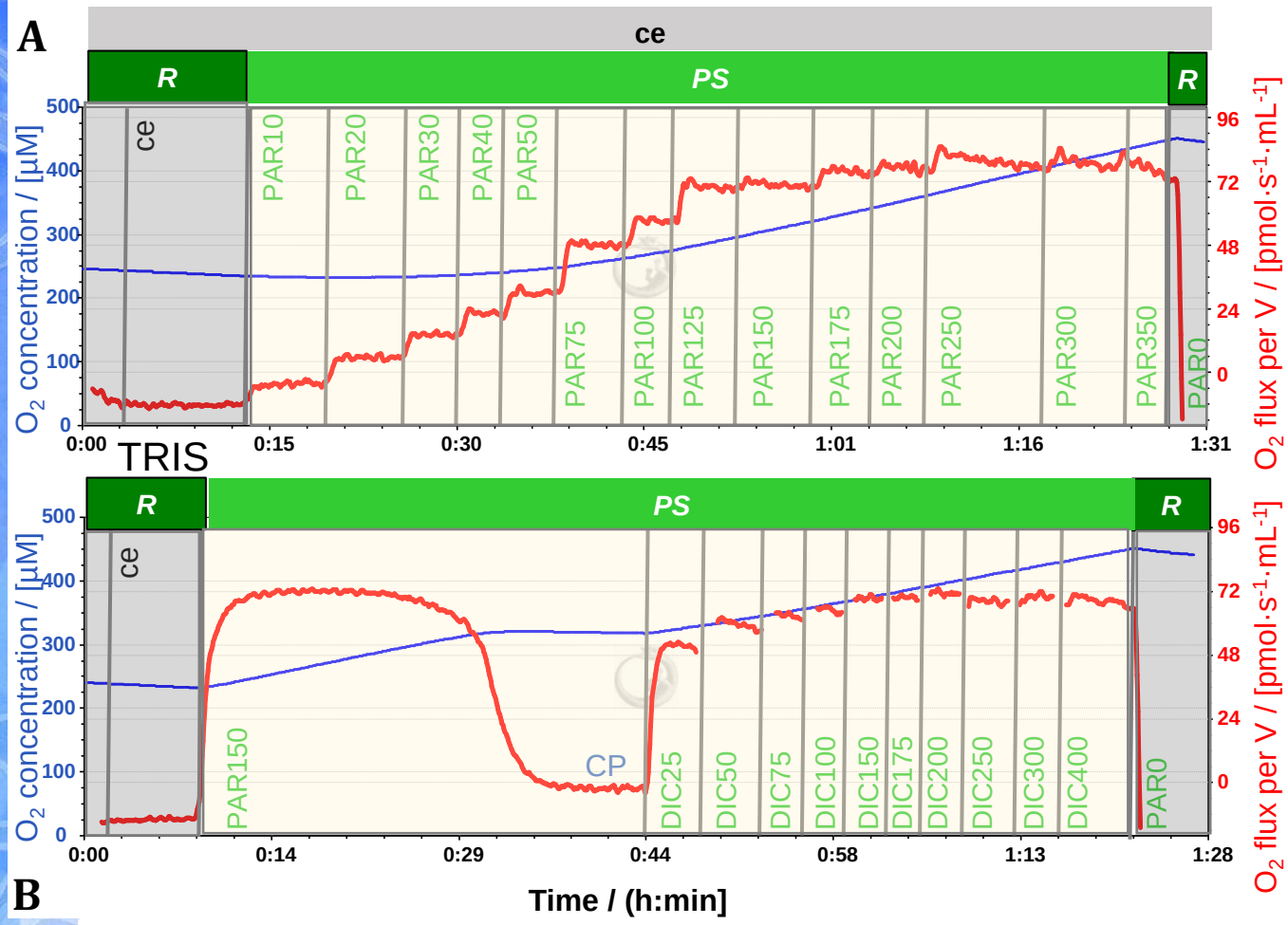
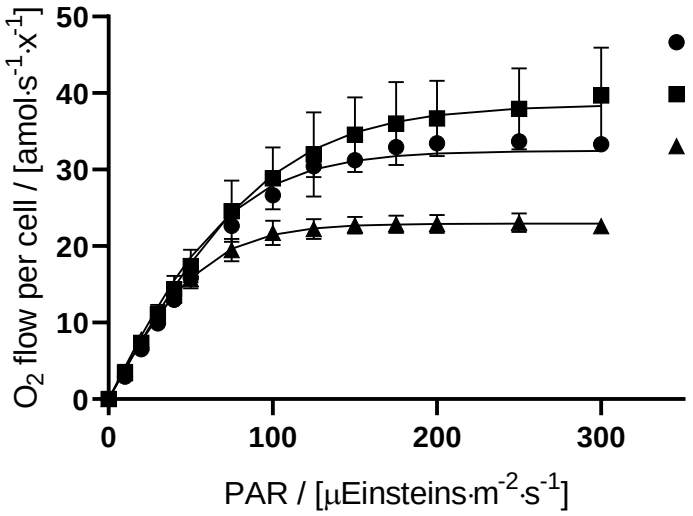
Results 2: Photosynthesis



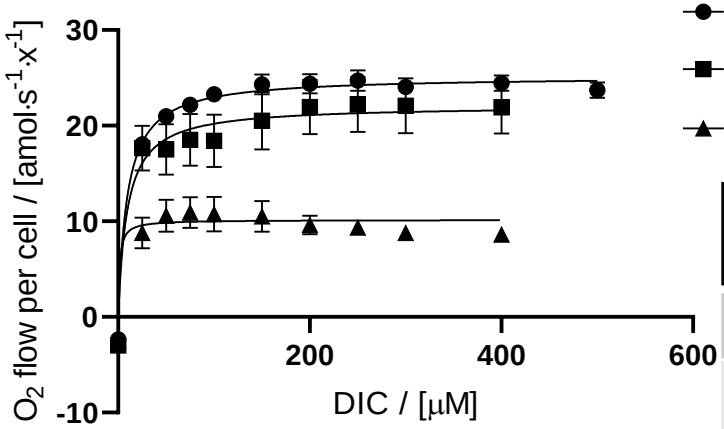
NextGen-O2k traces. (A) PI curve, SUIT-030_PB_ce_D070 protocol. **(B)** DIC curve, SUIT-030_PB_ce_D069 protocol.

PI curve



	P_{max} (95% CI) / [amol·s^{-1}·x^{-1}]
●	32.4 (1.8)
■	38.5 (0.7)
▲	22.9 (0.2)

DIC curve



	P_{max} (95% CI) / [amol·s^{-1}·x^{-1}]
●	25.2 (0.8)
■	22.1 (1.7)
▲	10.1 (0.9)

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Conclusions

1. Oxygen affinity with p_{50} ranging from 0.02 to 0.05 kPa for ROUTINE respiration, independently of nutrient treatments.

2. Inhibition of the Q-CIV branch with KCN impaired O_2 flux and p_{50} increased 4-fold in the Nitrate starved treament and 5-fold in the Nitrate replete one. The Q-AOX affinity for O_2 was 4 to 5 times lower than in Q-CIV.

3. Inhibition of the Q-AOX branch did not change the p_{50} of ROUTINE respiration.

4. O_2 kinetics by HRR provided a sensitive and fast method for quantifying the contributions of the Q-CIV and Q-AOX branches to dark respiration.

5. The NextGen-O2k PhotoBiology-Module (PB-Module) allowed to characterize the photosynthetic physiology of *C. reinhardtii* grown for biomass and lipid production.

6. Nitrate starved *C. reindhardtii* showed smaller P_{max} than the Nitrate replete cells. The photosynthetic system was compromised after 3 days of nitrogen starvation.

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Thank you!!!

Find us

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