



POSTER #10

**PHENOTYPIC VARIABILITY IN RESPONSE TO OXIDATIVE STRESS UNVEILS
A LINK BETWEEN CHLOROPLAST REDOX DYNAMICS
AND CELL FATE IN MARINE DIATOMS**

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Diatoms are photosynthetic microorganisms of great ecological and biogeochemical importance, contributing about 40% of marine primary production. They form vast blooms that are frequently characterized by “boom and bust” dynamics. These dynamics include rapid proliferation of the population followed by a coordinated demise, which has been suggested to involve programmed cell death. However, the molecular basis and cellular mechanisms that underline the ecological success of diatoms are still underexplored. Recent studies performed in our lab demonstrated how subcellular oxidation patterns in response to environmental stress conditions may play a pivotal role in cell fate determination in the diatom *Phaeodactylum tricornutum*. Here, we aim to further investigate the phenotypic variability within diatom populations in response to oxidative stress. To this end, we combined flow cytometry and microfluidic fluorescence imaging to measure organelle-specific oxidation dynamics at the single-cell level using the redox-sensitive sensor roGFP2. The chloroplast targeted roGFP2 exhibited a bi-stable oxidation pattern in response to oxidative stress, revealing two distinct sub-populations (Figure 1A, C). Cell death was subsequently induced in the oxidized sub-population, while the reduced sub-population survived the stress (Figure 1B, D). We further characterized an early phase of pre-commitment to cell death in response to oxidative stress, after which there was an irreversible induction of a cell death cascade. Chloroplast oxidation preceded the commitment to cell death, and was used as a novel cell fate predictor. We propose that intra species phenotypic variability among individual diatom cells may provide an ecological strategy to cope with rapid environmental fluctuations in the marine ecosystem.

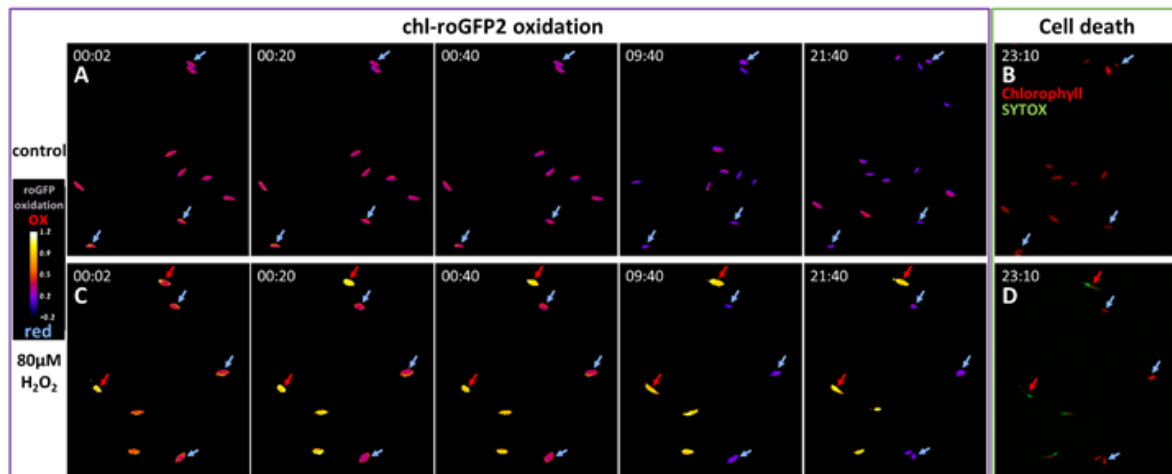


Figure 1. **Microfluidics *in vivo* imaging of chloroplast roGFP2 oxidation and mortality in response to oxidative stress.** roGFP2 oxidation of chl-roGFP2 cells was imaged over time using microfluidics and epifluorescence microscopy. Cells were imaged following treatment with either fresh media (control; A,B) or 80 μ M H₂O₂ (C,D). To quantify cell death, cells were stained with Sytox 22.5 hours post treatment. Two sub populations of oxidized (red arrows) and reduced (blue arrows) cells were observed in the treated cells. A,C: Representative frames of the calculated roGFP2 oxidation degree at different times following treatment in pseudo color. B,D: An overlay of Sytox staining (green, dying cells) and chlorophyll AF (red) at 23:10 hours post treatment. A-D: For visualization only part of the field is shown, the same field is shown for both roGFP oxidation and Sytox staining. Timestamp represents the time post treatment, hh:mm.