Real-Time Fluorescence Lifetime-Resolved Images of individual cells of Wild Type and NPQ mutants of *Chlamydomonas reinhardtii*

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**Abstract**

The chlorophyll (Chl-a) fluorescence from single cells of wild type (WT) and non-photochemical quenching (NPQ) mutants (NPF1 and NPF2) of *Chlamydomonas reinhardtii* was obtained with a new high-speed instrument for measuring fluorescence time-resolved images (see instrumentation). The mutants NPF1 and NPF2 used in this work (Kargel et al., 1997; acclimated to high light intensity) were grown as described previously (Holub et al., 2006) and were used in their wild-type and phase-modulated actinic light conditions. As a result of the NPF1 and NPF2 experiment, strong changes of Chl-a fluorescence in the NPF mutants in comparison with the WT phase are observed (see Figure 1). The lifetime of Chl-a from the WT is a measure for the total lifetime of Chl-a, whereas the lifetime of Chl-a from the NPF mutants will be decreased. The fluorescence lifetime of Chl-a in WT is used to determine the energy distribution used for the phase-modulated actinic light images. A second experiment was performed on single cells in the WT and NPF1 and NPF2 mutants.

This work is supported by the NSF NMP-0555255 and NSF DMR-0607844.

**Non-Photochemical Quenching of Chlorophyll a Fluorescence**

Photoprotection by NPQ of Chlorophyll a (Chl-a) fluorescence of PMS is correlated with the accumulation of NPF2 (Kargel et al., 2006) during the light-dark-light photodyl cycle (see e.g. Demmig-Adams et al., 1995). A lifetime change of one of the most important Chl-a fluorescence lifetimes of the reactions are initiated by the actinic light (Kargel et al., 1997). Both the fluorescence lifetime of the actinic light in the actinic light condition (Holub et al., 2006) and the actinic light condition (Kargel et al., 1997) were observed by fluorescence imaging (see Figure 1). The lifetime of the actinic light in the actinic light condition (Kargel et al., 1997) was observed by fluorescence imaging (see Figure 1).

**Materials and Methods**

A new high-speed instrument was developed for real-time imaging applications and would require major changes in order to develop different measurement parameters. The NPF1 and NPF2 mutants were obtained with a new high-speed instrument for measuring fluorescence time-resolved images (see Instrumentation). The mutants NPF1 and NPF2 used in this work (Kargel et al., 1997) were grown as described previously (Holub et al., 2006) and were used in their wild-type and phase-modulated actinic light conditions. As a result of the NPF1 and NPF2 experiment, strong changes of Chl-a fluorescence in the NPF mutants in comparison with the WT phase are observed (see Figure 1). The lifetime of Chl-a from the WT is a measure for the total lifetime of Chl-a, whereas the lifetime of Chl-a from the NPF mutants will be decreased. The fluorescence lifetime of Chl-a in WT is used to determine the energy distribution used for the phase-modulated actinic light images. A second experiment was performed on single cells in the WT and NPF1 and NPF2 mutants.

**Image Differences in lifetime of Chl-a fluorescence from WT and NPQ-mutant cells**

Strong differences in the fluorescence intensity (between WT/NPF1 and NPF2 mutants) of Chl-a fluorescence from single cells were observed in the present study (see Figure 1). The fluorescence intensity was determined by measuring the photon flux (photon flux measurements) and the photon flux (photon flux measurements) of the different mutants. The mutants NPF1 and NPF2 used in this work (Kargel et al., 1997) were grown as described previously (Holub et al., 2006) and were used in their wild-type and phase-modulated actinic light conditions. As a result of the NPF1 and NPF2 experiment, strong changes of Chl-a fluorescence in the NPF mutants in comparison with the WT phase are observed (see Figure 1). The lifetime of Chl-a from the WT is a measure for the total lifetime of Chl-a, whereas the lifetime of Chl-a from the NPF mutants will be decreased. The fluorescence lifetime of Chl-a in WT is used to determine the energy distribution used for the phase-modulated actinic light images. A second experiment was performed on single cells in the WT and NPF1 and NPF2 mutants.

**Summary**

A new high-speed instrument for measuring fluorescence time-resolved images in real time has been developed and applied for investigating differences in lifetime of Chl-a of *Chlamydomonas reinhardtii* (Kargel et al., 1997). The fluorescence lifetime of Chl-a in WT is used to determine the energy distribution used for the phase-modulated actinic light images. A second experiment was performed on single cells in the WT and NPF1 and NPF2 mutants.