Title

PICOSECOND FLUORESCENCE KINETICS IN SPINACH CHLOROPLASTS AT LOW TEMPERATURE

Authors

Nairn, J.A.
Reisberg, P.
Sauer, K.

Publication Date

1981-12-01

LBL-13786
Preprint

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

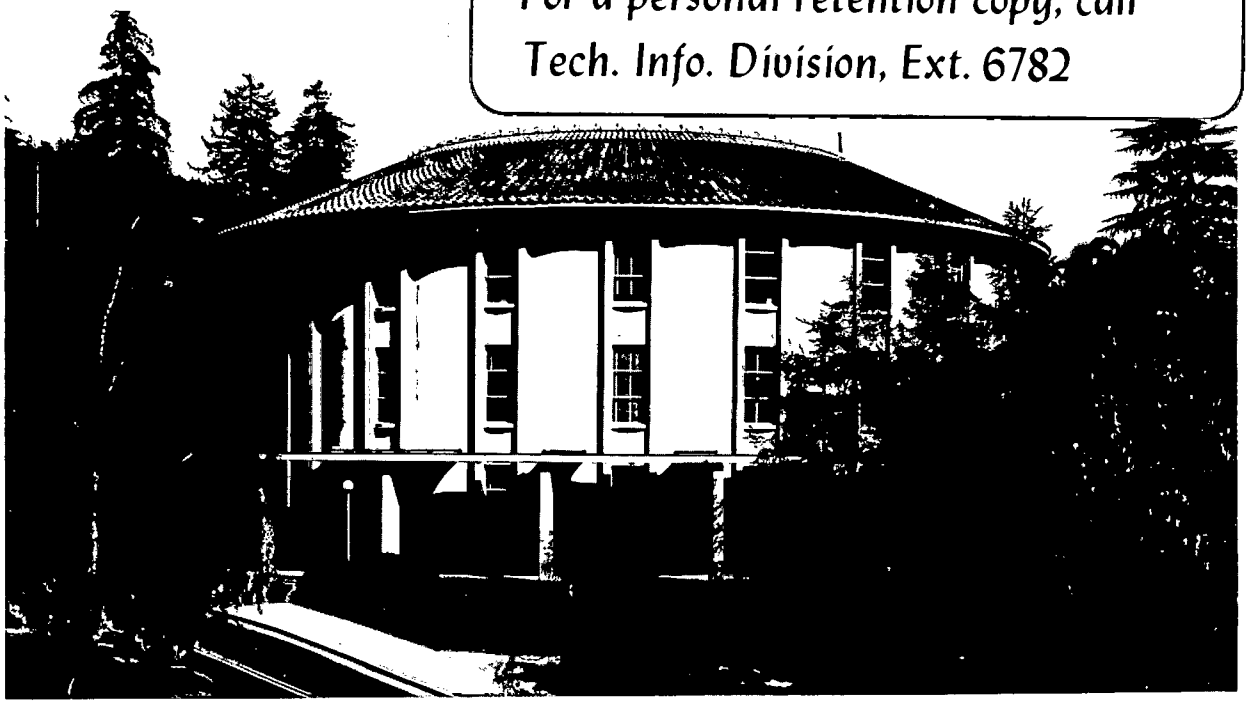
CHEMICAL BIODYNAMICS DIVISION

Submitted to FEBS Letters

PICOSECOND FLUORESCENCE KINETICS IN SPINACH CHLOROPLASTS AT LOW TEMPERATURE

John A. Nairn, Paul Reisberg,
and Kenneth Sauer

December 1981



Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

DISCLAIMER

PICOSECOND FLUORESCENCE KINETICS IN SPINACH CHLOROPLASTS AT LOW TEMPERATURE

John A. Nairn, Paul Reisberg, and Kenneth Sauer

Department of Chemistry and Laboratory of Chemical Biodynamics
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720 USA

Keywords: chloroplasts, fluorescence, fluorescence lifetimes, low temperature fluorescence, photosynthesis, picosecond

This work was supported in part by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Biological Energy Conversion and Conservation of the U.S. Department of Energy under Contract No. W-7405-ENG-48; and in part by a National Science Foundation Grant PCM 79-11251; and an Award from the U.S. Public Health Service.

1. Introduction

The fluorescence decay kinetics for spinach chloroplasts at room temperature has been found in recent studies to be characterized by three exponential components [1-3]. The slow phase (1-2ns) is "delayed" fluorescence arising from chlorophyll (Chl) antenna molecules excited upon charge recombination between, P_{680}^{+}, the oxidized primary electron donor in photosystem II (PS II), and I^{-}, the reduced primary electron acceptor, which is believed to be a pheophytin molecule [4-7]. The recombination occurs with a high yield when the secondary electron acceptor Q [8] is reduced [1-7]. Two faster phases of fluorescence decay are due to excitation that is lost prior to reaching the reaction center. Of these, the more rapid one (50-100ps) reflects the excitation transfer time from the Chl a antenna of PS II (Chl a_2) to the reaction center of PS II, and the less rapid one (400-750ps) arises, in part, from excitation transfer from the Chl a/b light-harvesting antenna (Chl a/b LH) to the reaction center of PS II. These two light-harvesting Chl components make up the PS II antenna in Butler's tripartite model [9]. The interpretation of the three fluorescence decay components is based on the assumption that the room temperature fluorescence is mostly from the PS II antenna; that is, from the Chl a_2 antenna and the Chl a/b LH antenna. PS I fluorescence may make a small contribution in addition, but this should not substantially effect the interpretation of the three components as outlined above (For details see Ref. [3]).

The room temperature fluorescence emission spectrum from spinach chloroplasts is broad and featureless with a peak at 680-685nm and a tail extending to beyond 700nm [10]. The spectra of the three fluorescence decay components are similar to each other [1]. Thus, it is not possible to study the fluorescence decay from the different components of the photosynthetic unit

independently by varying the detection wavelength. In contrast, at 77K, the fluorescence emission spectrum, while still broad, is resolved into three peaks at 685nm, 695nm, and 735nm [10,11]. From measurement of the fluorescence emission spectra of purified subchloroplast particles at 77K, it has been proposed that the origin of these three peaks are the Chl a/b LH antenna (685nm), the Chl a_2 antenna (695nm), and PS I (735nm) [11].

Picosecond resolution of the fluorescence decay kinetics as a function of wavelength at 77K allows a selective study of the fluorescence properties of different parts of the photosynthetic unit. The fluorescence decay at short wavelengths ($\lambda \leq 680$nm) is characterized by three exponential decay components which resemble those at room temperature. At long wavelengths ($\lambda \geq 710$nm), the fluorescence decay is described by one exponential rise component and two exponential decay components. The resolvable risetime is 50-100ps, and the major decay component has a lifetime of about 3ns. The other component, with a lifetime of 400-600ps, is a small fraction of the total decay and appears to be associated with the tail of the short wavelength fluorescence.

2. Materials and Methods

Broken spinach chloroplasts were isolated by the method described previously [1,2]. Measurements were done on chloroplast samples with a Chl concentration of 18 µg/ml. The chloroplasts were suspended in 10mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-NaOH buffer (pH 7.5) containing 0.1M sucrose, 5mM $MgCl_2$, 5mM NaCl, 12.5µM 3-(3',4'-dichlorophenyl)-1,1-dimethyl urea (DCMU), and 1.25mM NH_2OH. DCMU and NH_2OH were added to assure that all of the PS II reaction centers were closed by reduction of the secondary electron acceptor Q. The sample was illuminated while being cooled to low temperature (77K) by immersion in liquid nitrogen in an optical dewar.

Fluorescence lifetimes were measured with a single-photon timing system which has a Spectra Physics synchronously-pumped mode-locked dye laser system as an excitation source. The laser output pulses have a full-width half-maximum duration of about 15ps at the excitation wavelength of 620nm. The fluorescence detection wavelength was selected by placing an interference filter with a bandwidth of $\pm$5nm in front of the photomultiplier. Details of the single-photon timing system and the methods of numerical analysis are described elsewhere [1,12,13]. All of the data analyses presented here result from a resolution of the fluorescence decay kinetics into a sum of exponentials. That is, the time dependence of the fluorescence decay is given by

$$F(t) = \sum_{i=1}^{3} \alpha_i \exp(-t/\tau_i) \qquad (1)$$

where α_i and τ_i are the amplitude and the lifetime of the i^{th} component. The yield of the i^{th} component is equal to $\alpha_i\tau_i$; this yield represents the total number of photons emitted in the i^{th} phase. We estimate that our system can resolve fluorescence lifetimes as short as 25ps [1].

3. Results

The fluorescence decay components from spinach chloroplasts at 77K for detection wavelengths $\leq$ 680nm are summarized in Table I. These components are similar to the three components observed in spinach chloroplasts at room temperature that are suspended in the same buffer; i.e., spinach chloroplasts in the presence of Mg^{+2}, DCMU, and NH_2OH. Two apparent differences between the room and low temperature results are that the slow and middle phases are faster at 77K and that the relative yield of the slow phase is 40-50% less at 77K.

At wavelengths $\geq$ 710nm, we observe a very different fluorescence decay, which is characterized by three different kinetic components. One component is

a risetime of 50-100ps and the other two are decays of 400-600ps and 2200-3200ps. The lifetimes of these three components are plotted as a function of emission wavelength in Fig. 1.

At the longest wavelengths (λ>740nm), the 400-600ps phase is less than 3% of the total fluorescence yield. The resolved rise and slow decay components then predominate have approximately equal amplitudes which are opposite in sign. This fact suggests that the rise and slow decay can be assigned to a single pigment bed of Chl. The observable rise of 50-100ps is the time required for energy transfer to this pigment bed, which then fluoresceses at long wavelengths and decays with a 3ns lifetime. In Fig. 2 is plotted the yield of the fluorescence emitted from the long wavelength pigment bed of Chl, the yield of the 400-600ps component, and the total yield as a function of emission wavelength. The yield of the fluorescence emitted from the long wavelength pigment bed of Chl follows the fluorescence spectrum beyond 710nm, and in this wavelength region the yield of the 400-600ps component decreases to a low level.

At intermediate wavelengths it was not possible to fit the data adequately with either three or four kinetic components, presumably owing to overlap of the short wavelength and the long wavelength emission components. The resulting decay is multiphasic and complex.

4. Discussion

We propose that there are five exponential kinetic components in the low temperature emission of spinach chloroplasts in the presence of Mg^{+2}, DCMU, and NH_2OH. Three of these components are observable at 670nm and at 680nm. These are a slow component with a lifetime of 1500-1600ps, a middle component with a lifetime of about 300ps, and a fast component with a lifetime of 100-150ps. The

weighted mean lifetime of the short wavelength decay is 750-900ps. This range is in agreement with short wavelength measurements on pea chloroplasts by Wong et al. [14]. A two-component fit to our data is in agreement with the two component analysis of 685nm fluorescence from pea chloroplasts at 77K by Beddard et al. [15]. The fourth and fifth components that we observe predominate at long wavelengths and are best characterized beyond 750nm, where they are > 95% of the total decay. The fourth kinetic component is a risetime of about 100ps, and the fifth is a decay with about a 3200ps lifetime. The long wavelength decay is in reasonable agreement with literature values from pea chloroplasts [14,16,17] and from bean leaf [18] which range between 2100ps and 3100ps. A similar risetime of 135ps at 735nm in spinach chloroplasts illuminated at short wavelengths was reported by Campillo et al. [19], but Butler et al. [18] reported that the long wavelength fluorescence rise in pea chloroplasts illuminated at short wavelengths is less than the 50ps resolution of their system. We find that a long wavelength fluorescence risetime between 50ps and 150ps is reproducible.

At wavelengths between 685nm and 700nm each of the above five components is present to a large enough extent that the data cannot be fit with only three or four components. At wavelengths between 710nm and 735nm, the long wavelength components are sufficiently dominant that three-exponential fits describe the data. The 400-600ps component of the long wavelength fits, which monotonically decreases in yield for wavelengths $\geq$ 730 nm, is probably the tail of the short wavelength fluorescence. It is slower than the middle phase measured at short wavelengths because some of the short-wavelength slow phase is averaged into it. Analogously, the long-wavelength slow decay component lifetime decreases at wavelengths less than 740nm because some of the short wavelength slow phase probably is averaged into it.

The fluorescence decay components at 670nm and 680nm are similar to the room temperature decay components. We have studied the temperature dependence of the short wavelength components and the absence of an abrupt transition in their temperature dependence suggests that the origin of the 670nm and 680nm components at 77K is the same as the origin of the room temperature components. Our analysis of the room temperature fluorescence postulated that the slow component is "delayed" fluorescence which arises from excited Chl antenna molecules populated after a charge recombination in the PS II reaction center [1,3]. This model accounts for the observed decrease in the relative yield of the slow phase at 77K; at low temperatures, the energetically uphill charge recombination and energy transfer back to the Chl antenna becomes less probable.

The fact that the amplitudes of the rise and of the slow decay of the long wavelength fluorescence are equal but opposite in sign means that the Chl pigment bed responsible for the long wavelength emission receives most of its excitation through relatively slow (100ps) energy transfer and not by direct absorption of the excitation pulse by the pigment molecules that subsequently emit. This pigment bed probably contains a small number of Chl molecules, which is consistent with the assignment of the long wavelength fluorescence to a Chl trap, C705, located in PS I [17,18,20]. The risetime of about 100ps reflects the time required for energy to transfer from the bulk Chl antenna to C705. In future studies we hope to determine whether there is a "variable" component of PS I fluorescence that is distinct from that resulting from excitation transfer controlled by the state of the PS II reaction center.

ACKNOWLEDGEMENTS

This work was supported in part by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Biological Energy Conversion and Conservation, U. S. Department of Energy under contract No. W-7405-ENG-48, and in part by a National Science Foundation Grant PCM 79-11251. One of us (P. R.) wishes to acknowledge a National Research Service Award from the U. S. Public Health Service.

REFERENCES

1. Haehnel, W., Nairn, J. A., Reisberg, P., and Sauer, K. (1981) Biochim. Biophys. Acta, in press.

2. Nairn, J. A., Haehnel, W., Reisberg, P., and Sauer, K., in preparation.

3. Reisberg, P., Nairn, J. A., Haehnel, W., and Sauer, K., in preparation.

4. Klevanik, A. V., Klimov, V. V., Shuvalov, V. A., and Krasnovskii, A. A. (1977) Dokl. Akad. Nauk, SSSR 236, 241-244.

5. Klimov, V. V., Klevanik, A. V., Shuvalov, V. A., and Krasnovskii, A. A. (1977) FEBS Lett. 82, 183-186.

6. Klimov, V. V., Allakhverdiev, S. I., and Paschenko, V. Z. (1978) Dokl. Akad. Nauk, SSSR 242, 1204-1207.

7. Shuvalov, V. A., Klimov, V. V., Dolan, E., Parson, W. W., and Ke, B. (1980) FEBS Lett. 118, 279-282.

8. Duysens, L. M. N., and Sweers, H. E. (1963) in: Microalgae and Photosynthetic Bacteria (Japanese Society of Plant Physiologists, eds.) pp 353-372, Univ. of Tokyo Press, Tokyo.

9. Butler, W. L. (1978) Ann. Rev. Plant Physiol. 29, 345-378.

10. Boardman, N. K., Thorne, S. W., and Anderson, J. M. (1966) Proc. Natl. Acad. Sci. USA 56, 586-593.

11. Satoh, K., and Butler, W. L. (1978) Plant Physiol. 61, 373-379.

12. Turko, B., Nairn, J. A., and Sauer, K., in preparation.

13. Nairn, J. A. (1982) "Orientation and Picosecond Energy Transfer Studies on Chlorophyll in the Photosynthetic Membrane", PhD Thesis, Univ. California, Berkeley.

14. Wong, D, Merkelo, H., and Govindjee (1979) FEBS Lett 104, 223-226.

15. Beddard, G. S., Fleming, G. R., Porter, G., Searle, G. F. W., and

Synowiec, J . A. (1979) Biochim. Biophys. Acta 545, 165-174.

16. Butler, W. L., Tredwell, C. J., Malkin, R. and Barber, J. (1979) Biochim. Biophys. Acta 545, 309-315.

17. Tusov, V. B., Koratovskii, B. N., Paschenko, V. Z., and Rubin, L. B. (1980) Dokl. Akad. Nauk SSSR 252, 1500-1504.

18. Butler, W. L., and Norris, K. H. (1963) Biochim. Biophys. Acta 66, 72-77.

19. Campillo, A. J., Shapiro, S. L., Geacintov, N. E., and Swenberg, C. E. (1977) FEBS Lett 83, 316-320.

20. Satoh, K., and Butler, W. L. (1978) Biochim. Biophys. Acta 502, 103-110.

TABLE I

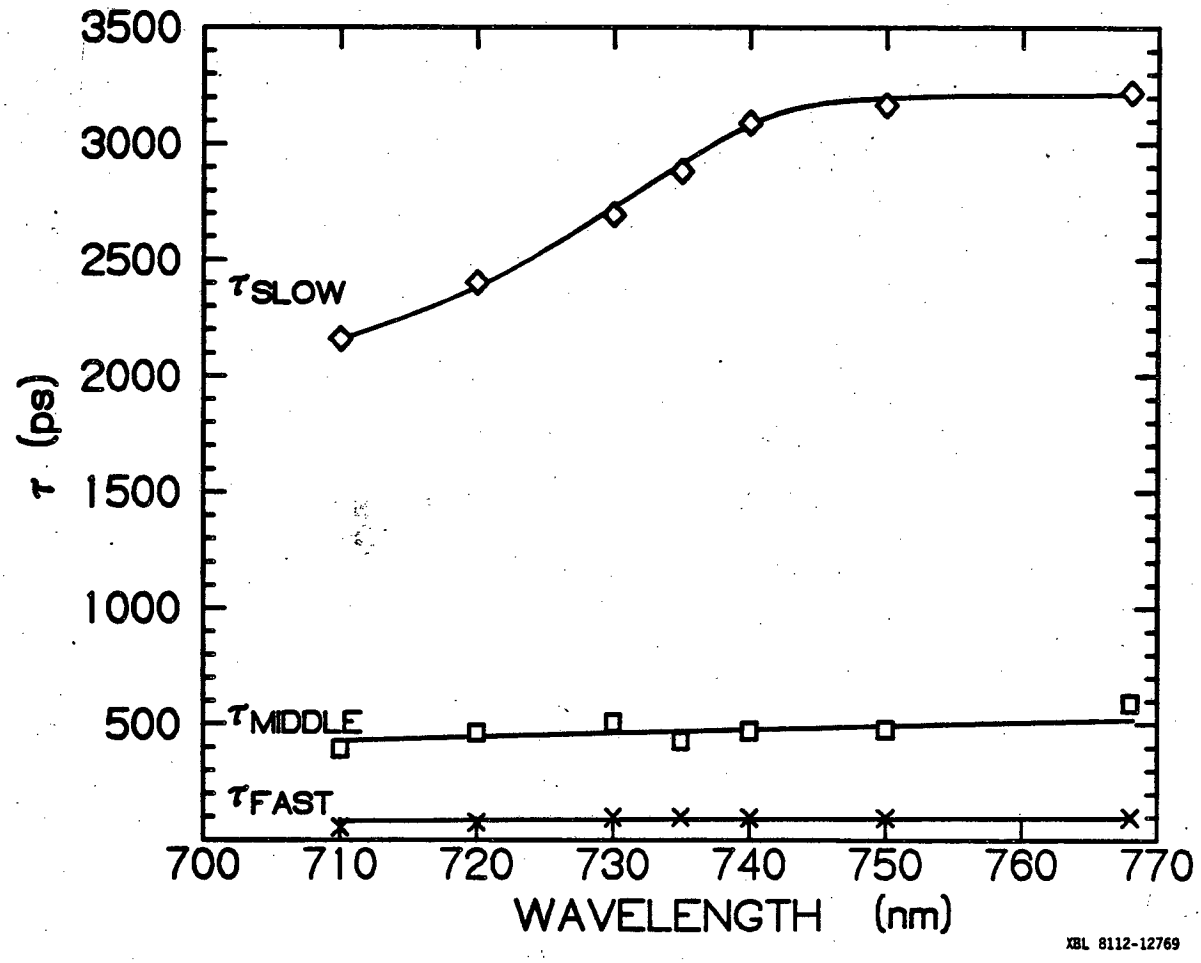
Amplitudes (α), lifetimes (τ), and fluorescence yields (ϕ) of the three fluorescence decay components in spinach chloroplasts at 77K in the presence of 5 mM $MgCl_2$, 12.5 μM DCMU, and 1.25 mM NH_2OH. Amplitudes and yields are in percent of the total.

λ(nm)	α	τ(ps)	ϕ
670	8	1590	40
	38	310	40
	54	110	20
680	13	1520	48
	49	320	39
	38	140	13

FIGURE LEGENDS

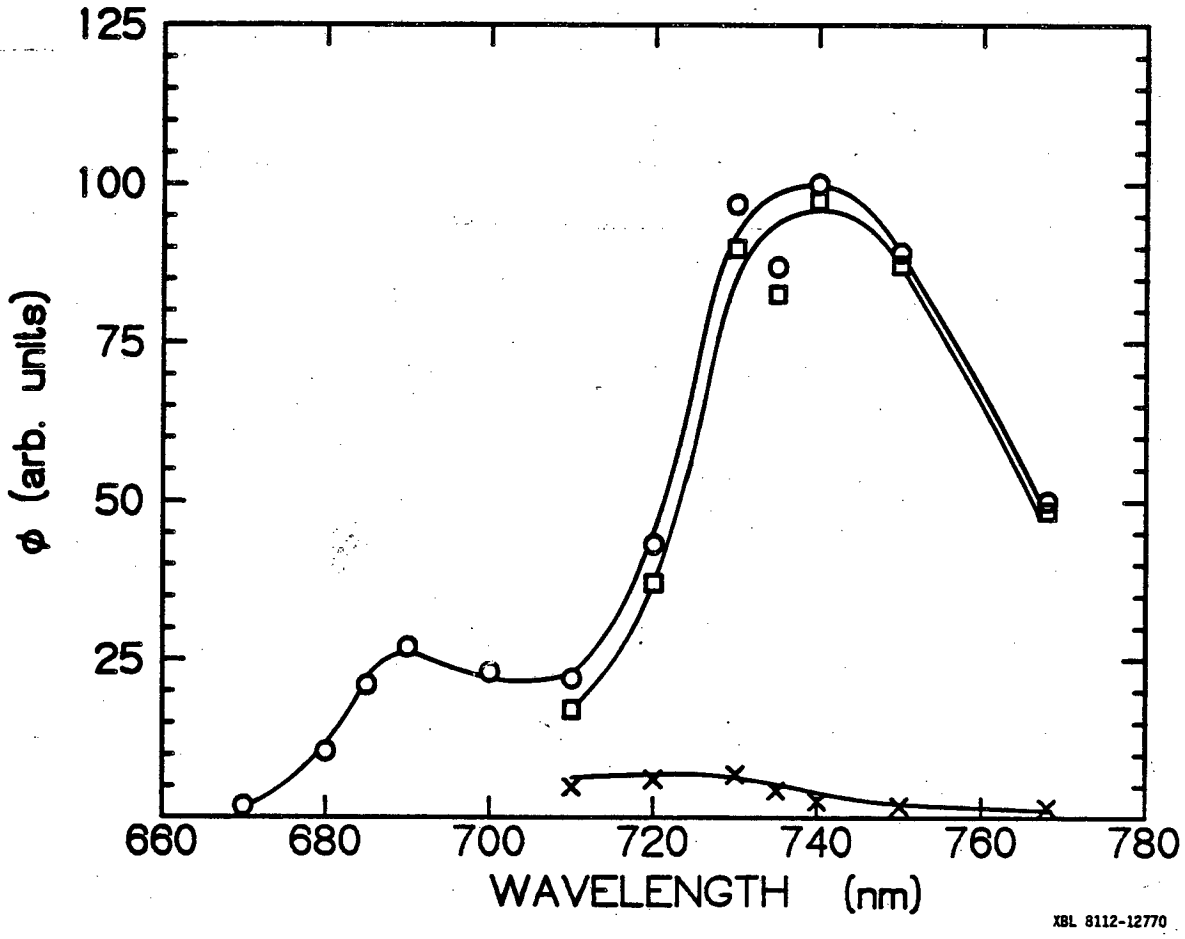
Figure 1: Lifetimes of the three components in the long wavelength fluorescence decay at 77K in spinach chloroplasts as a function of the wavelength of emission.

Figure 2: Total fluorescence yield (0), yield of the fluorescence emitted from the long wavelength pigment bed of Chl (□), and yield of the 400-600ps component (X) in spinach chloroplasts at 77K as a function of the wavelength of emission.



Nairn et al.

Figure 1



Nairn et al.

Figure 2

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720