

Lawrence Berkeley National Laboratory

Recent Work

Title

DISC-TO-DISC TRANSFER AS THE RATE-LIMITING STEP FOR ENERGY FLOW IN
PIIYCOBILISOMES

Permalink

<https://escholarship.org/uc/item/81c102h5>

Author

Glazer, A.N.

Publication Date

1984-07-01



Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

RECEIVED
LAWRENCE
BERKELEY LABORATORY

JUL 9 1984

Submitted to Science

LIBRARY AND
DOCUMENTS SECTION

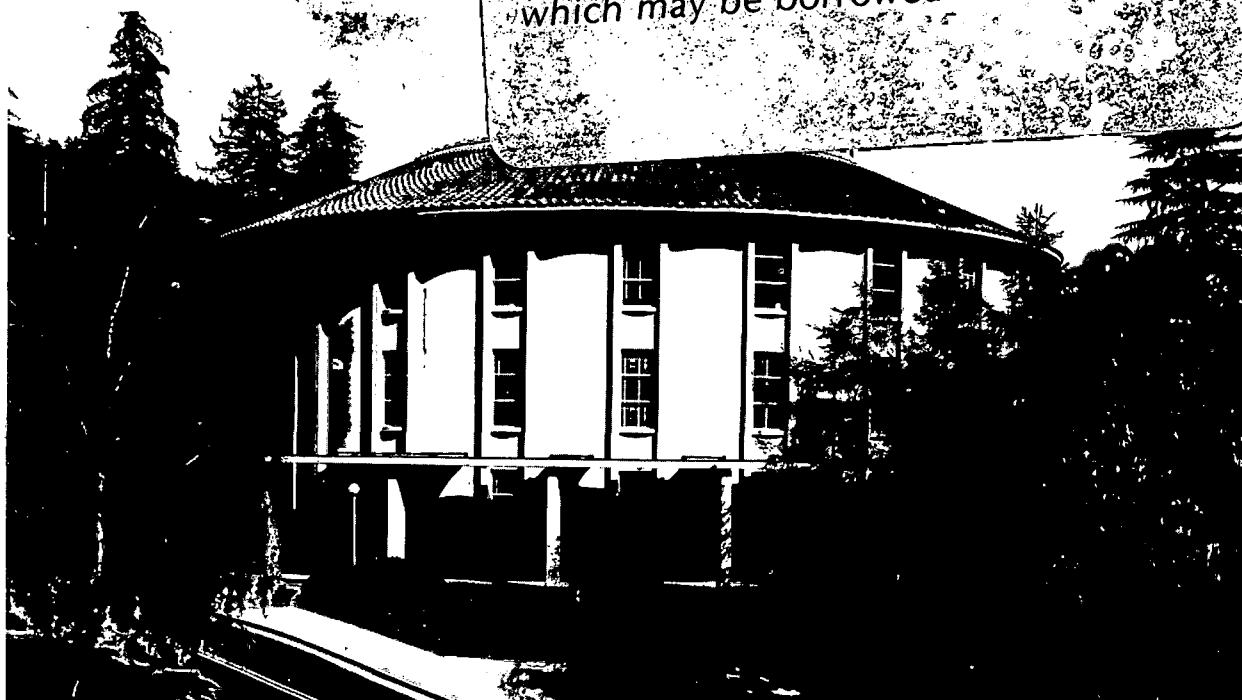
DISC-TO-DISC TRANSFER AS THE RATE-LIMITING
STEP FOR ENERGY FLOW IN PHYCOBILISOMES

A.N. Glazer, S.W. Yeh, S.P. Webb,
and J.H. Clark

July 1984

TWO-WEEK LOAN COPY

This is a Library Circulating Copy
which may be borrowed for two weeks.



LBL-18213
c. 2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

DISC-TO-DISC TRANSFER AS THE RATE-LIMITING STEP FOR ENERGY FLOW IN PHYCOBILISOMES

A. N. Glazer

Department of Microbiology and Immunology

and

Sheila W. Yeh, S. P. Webb, and J. H. Clark

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory,
and Department of Chemistry

University of California, Berkeley, CA 94720

Abstract

A broadly tunable picosecond laser source and an ultrafast streak camera were used to measure temporally and spectrally resolved emission from intact phycobilisomes and from individual phycobiliproteins as a function of excitation wavelength. Both wild-type and mutant phycobilisomes of the unicellular cyanobacterium Synechocystis 6701 were examined, as well as two biliproteins, R-phycoerythrin (240 kilodaltons, 34 bilins) and allophycocyanin (100 kilodaltons, 6 bilins). Measurements on intact phycobilisomes with known structural differences show that the addition of an average of 1.6 phycoerythrin discs in the phycobilisome rod increases the overall energy transfer time by 30 ± 5 ps. In the isolated phycobiliproteins, the onset of emission was found to be as prompt as that of a solution of rhodamine B laser dye and was independent of excitation wavelength. This imposes an upper limit of 8 ps (instrument limited) on the transfer time from "sensitizing" to "fluorescing" chromophores within these biliproteins. Together these results indicate that disc-to-disc transfer is the slowest energy transfer process within phycobilisomes. In combination with previous structural analyses, these results show that with respect to energy transfer, the lattice of ~625 light-harvesting chromophores in the Synechocystis 6701 wild-type phycobilisome functions as a linear, five-point array.

Conversion of light energy to chemical potential in biological photosynthetic systems is accomplished within macromolecular complexes composed of polypeptides and pigment molecules. A general feature of these complexes is the presence of numerous chromophores ("antenna pigments") which absorb light and transfer the excitation quantum to a special chlorophyll or bacteriochlorophyll molecule in the "reaction center" for subsequent conversion to electron flow[1]. Such complexes often contain several hundred antenna chromophores per reaction center. Since the overall quantum efficiency for the transfer of energy from the antenna to the reaction center is typically ~90%[1], energy transfer to the reaction center must compete successfully with pathways of energy dissipation within the antenna such as fluorescence and non-radiative decay. Because the majority of light-harvesting complexes are integral membrane components, information on the details of their molecular architecture is limited[2]. Consequently, the structural basis for the highly efficient energy transfer in these systems is not understood. However, an opportunity to correlate the structure and function of one light-harvesting system is provided by the phycobilisome, an antenna complex that efficiently funnels light energy collected over a broad spectral region and a large spatial area to the reaction center of photosystem II in cyanobacteria and red algae[3].

Phycobilisomes are peripheral membrane complexes which can be readily isolated without perturbing their structural and functional properties[3]. Phycobilisome morphology depends on organismal origin. These particles have molecular weights ranging from 7×10^6 to 15×10^6 daltons, contain between 300 and 800 tetrapyrrole (bilin) chromophores, and absorb light over much of the visible spectrum. Energy absorbed by any of these chromophores is efficiently transferred to terminal energy acceptors within the particle via energetically favorable radiationless pathways[3]. Phycobilisomes consist of distinct structural domains: rods made up of stacked discs which radiate from a central core. They are constructed of phycobiliproteins,

a family of brilliantly colored, water-soluble, oligomeric proteins which carry various covalently attached bilin prosthetic groups[4]. The structures of several phycobilisomes have been described in recent reviews[4,5].

The structure of the Synechocystis 6701 phycobilisome used in these experiments is known in considerable detail. Furthermore, a number of well-characterized phycobilisomes with different, known structures are available from mutant strains for comparative studies[5,6]. Diagrams of the structures of the phycobilisomes from Synechocystis 6701 and from the mutant strain CM25[6] are shown in Fig. 1. The structures of the two particles are identical except that the phycoerythrin complexes that form the outer portions of the rod subassemblies are absent in the mutant phycobilisome.

The major species involved in the energy transfer pathway in Synechocystis 6701 phycobilisomes are outlined in Fig. 1. Previous studies of Synechocystis 6701 wild-type phycobilisomes[4-6] have indicated that if each disc is considered as a single chromophoric unit, a maximum of five disc-to-disc transfers is required to convey the excitation energy to the terminal emitters:

1 2 3 4 5

PE(30.5K) → PE(31.5K) → PC(33.5 K) → PC(27K) → APC COMPLEXES → TERMINAL ACCEPTORS,

where PE(30.5K), PE(31.5K), etc., refer to disc complexes which make up the rod substructures of the phycobilisome (see Fig. 1).

It is known that the phycobiliprotein complexes that make up the individual discs have fluorescence lifetimes of ~2 ns[7,8]. For the purpose of making a rough calculation, we assume that all of the transfers are equally efficient. For a five-step process, the 90% overall energy transfer efficiency observed[9] would require a 98% quantum transfer efficiency at each step. Given that each transfer competes with a fluorescence lifetime of ~2 ns, a transfer efficiency of 98% implies that the overall time constant for the disc-to-disc transfer process must be ~40 ps.

To test the validity of this estimate, we have examined the kinetics of emission from intact phycobilisomes with picosecond resolution. For these studies, as well as for the measurements on individual phycobiliproteins presented below, the excitation source was the output of a tunable, picosecond, optical parametric source (OPS)[10] pumped by the third harmonic of a single, amplified pulse from a passively mode-locked Nd:YAG laser system (Quantel, YG400). An ultrafast streak camera (Hadland Photonics, IMACON 500) was used to time-resolve the emission. A detailed description of this apparatus will be presented elsewhere[11]. Spectral filtering of the emission through 10 nm bandpass (FWHM) interference filters allowed discrimination between the various components of the phycobilisome or phycobiliprotein emission. The broad tunability (450-630 nm) of the OPS was exploited to provide selective excitation at any point in any of the absorption bands of the chromophores of the samples examined with the exception of the peak of the 650 nm band of allophycocyanin. Power densities used ranged from $<10^{12}$ to 10^{14} photons/cm².

Synechocystis 6701 wild-type and CM25 mutant phycobilisomes were prepared from exponentially growing cyanobacterial cultures by previously described procedures[6]. Samples were stored in 0.75 M NaK-phosphate buffer (pH 8.0), 0.75 M sucrose at 4°C. Both steady-state emission spectra and time-resolved measurements (vide infra) indicated that the preparations were essentially free of dissociated material.

The wild-type and CM25 phycobilisomes differ (see Fig. 1) in that the mutant lacks phycoerythrin discs at the ends of the rods, whereas the wild-type particles contain an average of 1.6 phycoerythrin discs per rod[6]. The polypeptide compositions of the two phycobilisomes are identical with respect to all other components, as are their absorption and circular dichroism spectra above 600 nm. Electron micrographs of CM25 exhibit shorter rods than wild-type particles, but otherwise their morphology is equivalent to that of the wild-type structures[6]. Excitation of either phycobilisome at 570 nm results in virtually identical steady-state

emission spectra with maxima at 676 nm. In the wild-type phycobilisomes, 570 nm light is absorbed by phycoerythrin (75%), phycocyanin (21%), and allophycocyanin (4%), whereas in CM25 phycobilisomes 83% of 570 nm light is absorbed by phycocyanin and virtually all of the remainder by allophycocyanin. The availability of both wild-type and mutant phycobilisomes offers a unique opportunity for comparative studies to determine the time required for energy absorbed by the phycoerythrin discs to be transferred to the phycocyanin discs.

The results of picosecond kinetics studies on these phycobilisomes are shown in Fig. 2. Data shown in this figure represent the accumulation of signal from 500 laser shots. The smooth curves are computer-generated fits to the data. Parameters for the fits were obtained through an iterative "convolute-and-compare" technique[11]. Fig. 2a shows the time-resolved emission from the terminal energy acceptors of the phycobilisome at 680 nm following excitation at 570 nm. The best fits to the wild-type phycobilisome data give an average exponential risetime of 56 ± 8 ps. The CM25 data give an average best-fit exponential risetime of 25 ± 4 ps[12]. The important result is that the difference in emission delay time between the two structures is 30 ± 5 ps[13]. We take this time to be a direct measure of the average transit time for the energy from the outermost phycoerythrin discs to the closest phycocyanin disc in the phycobilisomes. Within experimental error, a decrease in the excitation power density by a factor of ten did not change the observed time[14].

To ensure that the results from these two phycobilisomes can be compared, we performed an experiment for which the results should be independent of the structural differences between the two phycobilisomes. We tuned the excitation wavelength to the 620 nm absorption maximum of phycocyanin so that the phycocyanin complexes of both the mutant and wild-type phycobilisomes were directly excited. The temporal profiles of 680 nm emission from the terminal acceptors upon 620 nm excitation are shown in Fig. 2b. As is readily apparent from the striking

similarity of the profiles, there is no difference between the rates at which phycocyanin discs in the mutant and wild-type structures funnel quanta to the terminal acceptors. The average best-fit exponential risetime for wild-type phycobilisomes is 28 ± 4 ps, and that for CM25 phycobilisomes is 25 ± 4 ps.

As a further, independent measure of the transfer time between phycoerythrin and phycocyanin discs, emission from phycoerythrin in wild-type phycobilisomes was selectively excited at 530 nm and selectively detected at 570 nm. A typical time-resolved emission trace, Fig. 3, gives a best-fit decay constant of 27 ps. An important aspect of the curve shown in Fig. 3 relates to sample purity and reproducibility. The data in Fig. 3 were obtained from a 10-month-old sample. This sample showed a 2.5% contribution of long-lived emission arising from phycoerythrin that cannot undergo rapid energy transfer because it is no longer associated into a phycobilisome. In fresh (less than one-week-old) samples, this long-lived emission was below the detection limit ($<0.1\%$). The presence of small amounts ($<2.5\%$) of dissociated phycobilisomes had no detectable effect on the rapidly decaying component of the phycoerythrin emission from intact particles. The average time constant for this decay, obtained from data taken on five different preparations at two different power levels ($\sim 10^{12}$ and $\sim 10^{13}$ photons/cm²) over a period of seven months, was 28 ± 4 ps.

It should be noted that all of the independent measurements reported here are internally consistent. For example, if the observed times for transfer from phycocyanin to the terminal acceptor are taken into account, the measured phycoerythrin fall time predicts that the overall transfer time from an intact wild-type phycobilisome should be 55 ± 10 ps, compared to the observed 56 ± 8 ps. Further, if it is assumed that C-phycoerythrin in situ in Synechocystis 6701 phycobilisomes would have a lifetime of ~ 1.5 ns (the value found for the isolated biliprotein[8]) in the absence of energy transfer, the observed time

constant indicates a 98.2% efficiency for transfer to phycocyanin. A similar estimate was made by Porter and co-workers[9] for the efficiency of transfer from the phycoerythrin of Porphyridium cruentum phycobilisomes.

From the comparative studies on intact phycobilisomes described above, we have determined the average time delay for energy transfer associated with the addition of an average of 1.6 phycoerythrin discs to otherwise unchanged rods. It is important to emphasize that the measured time delay is the sum of all time delays between the initial excitation of a phycoerythrin chromophore and the arrival of the excitation quantum at an adjacent phycocyanin disc, and to point out that the phycoerythrin discs themselves are complicated structures. For example, R-phycoerythrin carries 34 bilin chromophores within a $(\alpha\beta)_6\gamma$ disc-shaped assembly of dimension $110 \times 60 \text{ \AA}$ [4]. This phycoerythrin hexamer represents an individual disc within the rod substructure. The bilin chromophores within a given biliprotein fall into two classes, sensitizing (s) and fluorescing (f), based on the absorption and emission spectra of the discs[7]. For example, as seen in Fig. 4 for R-phycoerythrin, there are several maxima in the absorption spectrum. The emission spectrum of R-phycoerythrin shows only a single peak at 578 nm. The two higher energy peaks in the absorption spectrum are assigned to s chromophores, while the lowest energy absorption peak corresponds to f chromophores. These assignments are supported by steady-state polarization measurements[7], which showed that randomly polarized emission resulted from blue excitation, while substantial polarization was observed upon red excitation. These observations provide evidence for energy flow between the s and f chromophores within the discs. As noted above for the overall energy transfer process, since this intradisc energy transfer competes efficiently with the fluorescence lifetime of the s chromophores, the internal s to f transfer time must be very short.

There are thus two distinct types of energy transfer events that can influence the time required for a quantum initially absorbed in a phycoerythrin disc to appear in a phycocyanin disc: intradisc s to f transfer and interdisc phycoerythrin to phycocyanin transfer. For phycobilisomes with more than one phycoerythrin disc per rod, interdisc phycoerythrin to phycoerythrin transfer must also be considered. To provide a foundation for the understanding of energy transfer in phycobilisomes, we examined the s to f energy transfer process within the individual phycobiliproteins Gastroclonium coulteri R-phycoerythrin and Anabaena variabilis allophycocyanin. The properties of R-phycoerythrin were noted above. Allophycocyanin carries 6 bilins per $(\alpha\beta)_3$ trimer and is a component of the core.

Exposure of phycobilisomes to near-neutral-pH solutions of moderate ionic strength (0.05-0.2 M) leads to dissociation of the particles[3,4]. Depending on the phycobilisome and the particular biliprotein, certain dissociation products of the phycobilisome retain the aggregation state and, either wholly or in large measure, the spectroscopic properties which they possessed within the native structure[4]. R-Phycoerythrin and allophycocyanin satisfy two important criteria. First, of the biliproteins studied to date, their aggregation state and spectroscopic properties are least altered by the isolation procedures. Second, they represent biliproteins which are at the two extremes of bilin content[4]. R-Phycoerythrin appears to fully retain the spectroscopic characteristics it exhibits within phycobilisomes. Allophycocyanin has a 650 nm absorbance ~20% lower and a 620 nm absorbance ~5% higher than that calculated for this trimer within the intact phycobilisome[15]. All biliprotein preparations used in these studies were obtained by previously described procedures[16] and were purified to homogeneity. All solutions used for spectroscopic measurements were monodisperse as judged from absorption and emission characteristics.

By monitoring the effect of excitation wavelength on the temporal profile of emission from the f chromophores, the rate of energy transfer within a phycobiliprotein can be directly determined. Such data are shown in Fig. 4. For comparison, the emission profile of rhodamine B, known to have an essentially instantaneous risetime on this timescale[17], is also shown in Fig. 4. Typical fits are shown superimposed on the data. Pertinent results are compiled in Table 1. Examination of these temporal profiles reveals that for R-phycoerythrin, there is no delay in the rise of emission with blue excitation compared to the rise with red excitation. Similar results were found for allophycocyanin (see Table 1). Our measurements put an upper limit of 8 ps (instrument limited) on the transfer from s to f chromophores. This finding is consistent with previous studies of isolated phycobiliproteins[8] and with the following observations: a spectrally sharp onset of fluorescence at 565 nm in the steady-state spectrum and a lack of detectable emission at 550 nm which could be attributed to s chromophores.

This result has important bearing on the interpretation of energy transfer dynamics in intact phycobilisomes. We have shown that communication of energy between s and f chromophores within individual discs is essentially instantaneous on the timescale of these experiments. In combination with the results on intact phycobilisomes described above, this indicates that disc-to-disc transfer must be the rate-limiting energy transfer process in these phycobilisomes.

If disc-to-disc transfer is the rate-limiting step, the kinetics of energy transfer in phycobilisomes can be modeled in a straightforward manner. In this model, it is assumed that all disc-to-disc transfer times within rods are equivalent. With this model, seemingly disparate results obtained by others on various intact phycobilisomes can be predicted solely on the basis of known phycobilisome structures. Gillbro et al.[18] recently compared the kinetics of energy transfer in the phycobilisomes of Synechococcus 6301 and a mutant, AN112, obtained from one of our

laboratories (ANG). The difference between the wild-type and mutant particles is limited to the rod substructures, which have an average of three phycocyanin discs for the wild type and one for the mutant[19]. On 620 nm excitation of these structures, the recovery of absorption at 620 nm was 84 ± 8 ps for the wild type and 43 ± 8 ps for the mutant[18]. The kinetic model predicts that the 41 ± 16 ps difference with a change in rod length of two discs arises from a disc-to-disc transfer time of 26 ± 8 ps, whereas a disc-to-disc transfer time of 24 ± 4 ps is predicted on the basis of the results presented here for Synechocystis 6701.

As reported above for wild-type Synechocystis 6701 phycobilisomes with an average of 1.6 phycoerythrin and 2 phycocyanin discs per rod, we find a risetime of 56 ± 8 ps for terminal acceptor emission at 680 nm on 570 nm excitation and a C-phycoerythrin fall time of 28 ± 4 ps. In a study of the hemidiscoidal phycobilisome Rhodella violacea which is morphologically similar to Synechocystis 6701 but which has a different rod biliprotein composition, Holzwarth et al.[20] reported a risetime of 55 ps for 672 nm fluorescence upon 571 nm excitation, a B-phycoerythrin recovery time in a photobleaching experiment of 28 ps, and a B-phycoerythrin emission fall time of 34 ps. In studies on phycobilisomes from Nostoc sp., which have nearly identical rod morphology to that of Synechocystis 6701, Wong et al.[8] found a B-phycoerythrin fall time of 34 ± 13 ps. From these comparisons, we feel that the qualitative features of the model proposed here are likely to hold for phycobilisomes of varying morphology and biliprotein composition.

References and Notes

1. R. K. Clayton, Photosynthesis: Physical Mechanisms and Chemical Patterns (Cambridge University Press, Cambridge, 1980).
2. A. N. Glazer, Annu. Rev. Biochem. 52, 125 (1983).
3. E. Gantt, Annu. Rev. Plant Physiol. 32, 327(1981); N. Tandeau de Marsac, Bull. Inst. Pasteur 81, 201 (1983); A. N. Glazer, Annu. Rev. Microbiol. 36, 173(1982).
4. A. N. Glazer, in The Biochemistry of Plants, M. D. Hatch and N. K. Boardman, eds. (Academic Press, New York, 1982), Vol. 8, pp.51-96; A. N. Glazer, Biochim. Biophys. Acta 768, 29(1984).
5. A. N. Glazer, D. J. Lundell, G. Yamanaka, and R. C. Williams, Ann. Microbiol. (Inst. Pasteur) 134B, 159(1983); J. C. Gingrich, D. J. Lundell, and A. N. Glazer, J. Cell. Biochem. 22, 1 (1983).
6. R. C. Williams, J. C. Gingrich, and A. N. Glazer, J. Cell Biol. 85, 558(1980); J. C. Gingrich, L. K. Blaha, and A. N. Glazer, ibid 92, 261(1982); J. C. Gingrich, R. C. Williams, and A. N. Glazer, ibid 95, 170(1982).
7. R. E. Dale and F. W. J. Teale, Photochem. Photobiol. 12, 99(1970); F. W. J. Teale and R. E. Dale, Biochem. J. 116, 161(1970); J. Grabowski and E. Gantt, Photochem. Photobiol. 28, 39(1978).
8. D. Wong, F. Pellegrino, R. R. Alfano, and B. A. Zilinskas, Photochem. Photobiol. 33, 651(1981); I. Yamazaki, M. Mimuro, T. Murao, T. Yamazaki, K. Yoshihara, and Y. Fujita, Photochem. Photobiol. 39, 233(1984).
9. G. Porter, C. J. Tredwell, G. F. W. Searle, and J. Barber, Biochim. Biophys. Acta 501, 232(1978); G. F. W. Searle, J. Barber, G. Porter, and C. J. Tredwell, ibid 501, 246(1978); P. Hefferle, M. Nies, W. Wehrmeyer, and S. Schneider, Photobiochem. and Photobiophys. 5, 41(1983).
10. D. M. Guthals, C. C. Hayden, H. Nathel, D. W. Anthon, and J. H. Clark, J. Opt. Soc. Am. 73, 1897 (1983).
11. S. P. Webb, S. W. Yeh, and J. H. Clark, Rev. Sci. Inst. (in preparation).
12. It is important to realize that the complex nature of the system under study implies that single, or even multiple[9] exponential fits to the data represent a substantial oversimplification. Known structural heterogeneities for Synechocystis 6701 (see Fig. 1) include single and double C-phycoerythrin discs within different rods and the fact that of the three core units, one is attached to four rods, while each of the other two core units is attached to a single rod. It should also be emphasized that the spectral separation between the various phycobiliproteins is good, but not perfect (vide supra). Single exponential fits to the data condense all these parallel decay channels into one. Even multiple exponential fits with excellent statistical validity will not necessarily yield accurate values for the true rate parameters. The approach taken in this work is to fit the data with appropriate single or multiple exponentials to obtain rates which are descriptive of the overall energy transfer process, and to test the validity of such determinations with independent experiments and by comparison with previous work.

13. Note that the uncertainty in the difference of these two values is smaller than the error in the minuend. The major source of error in either of the two separate measurements arises from the inaccuracy in the determination of the true starting point of the emission. This source of error is removed in the relative measurement.
14. Previous work [F. Pellegrino, D. Wong, R. R. Alfano, and B. A. Zilinskas, Photochem. Photobiol. 34, 691(1981)] has shown that the observed fluorescence lifetimes in similar systems are strongly power dependent. Extensive studies of the power dependence of the fluorescence kinetics were carried out to ensure that the present results are completely free of any artifacts due to excitation intensity. The present work was performed at excitation power densities up to 1000 times lower than the previously observed threshold for the onset of power-dependent effects.
15. D. J. Lundell and A. N. Glazer, J. Biol. Chem. 258, 902 (1983).
16. D. A. Bryant, A. N. Glazer, and F. A. Eiserling, Arch. Microbiol. 110, 61(1976); V. T. Oi, A. N. Glazer, and L. Stryer, J. Cell Biol. 93, 981(1982).
17. C. V. Shank, E. P. Ippen, and O. Teschke, Chem. Phys. Lett. 45, 291(1977).
18. T. Gillbro, A. Sandström, V. Sundström, and A. R. Holzwarth, FEBS Lett. 162, 64(1983); T. Gillbro, A. Sandström, and V. Sundström, in Ultrafast Phenomena, eds. D. H. Auston and K. B. Eisenthal, Springer, New York, 1984 (in press).
19. G. Yamanaka, A. N. Glazer, and R. C. Williams, J. Biol. Chem. 255, 11004(1984).
20. A. R. Holzwarth, J. Wendler, and W. Wehrmeyer, Photochem. Photobiol. 36, 479 (1982).
21. Work in the Department of Microbiology supported by National Science Foundation Grant 82-08158; work in the Laboratory of Chemical Biodynamics and Department of Chemistry supported by the Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U. S. Department of Energy under Contract No. DE-AC03-76SF00098. Sheila W. Yeh is a National Science Foundation Pre-doctoral Fellow. J. H. Clark is an Alfred P. Sloan Research Fellow and a Henry and Camille Dreyfus Teacher-Scholar. We thank Ms. Crystal Chan for the preparation of the phycobillosomes used in this study, as well as Dr. Dennis Guthals and Mr. Howard Nathel for generously sharing their expertise in the construction and operation of the OPS.

Table 1. Best-fit emission risetimes for isolated phycobiliproteins and the rhodamine B control.

Sample	Excitation wavelength (nm)	Detection wavelength (nm)	Best-fit risetime (ps)
R-phycoerythrin	470	600	6.6 ^a
	496	600	2.2 ^b
	540	600	6.5 ^c
	565	600	4.6 ^d
Allophycocyanin	532	670	0.2
	575	670	6.9
	619	670	5.5
Rhodamine B	470	600	7.0 ^e
	470	600	0.8
	530	600	7.4
	530	600	8.7
	530	600	7.7

^aCurve a, Fig. 4.

^bCurve b, Fig. 4.

^cCurve c, Fig. 4.

^dCurve d, Fig. 4.

^eCurve Rho B, Fig. 4.

Figure Captions

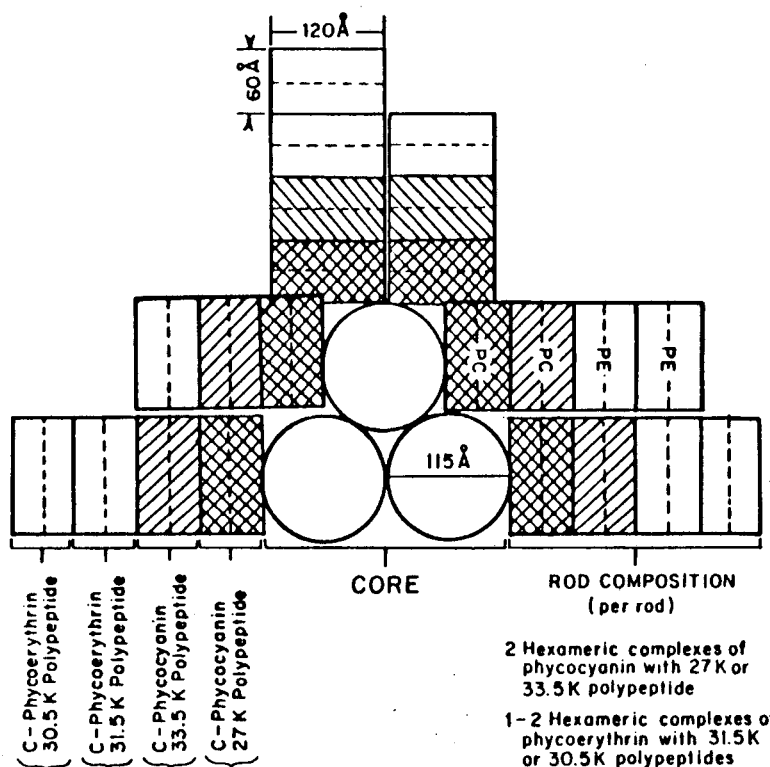
Fig. 1. Diagrammatic representation of the structure of Synechocystis 6701 and mutant strain CM25 phycobilisomes. Each disc is a hexameric biliprotein aggregate held together and joined to its nearest neighbor proximal to the core by a linker polypeptide, X, where $X = 30.5K, 31.5K, \text{etc.}$, and the numbers refer to the molecular weight of the protein in kilodaltons. Thus the composition of each disc is $(\alpha\beta)_6X$. The number of bilins associated with each biliprotein in these phycobilisomes and the direction of energy transfer are also shown. The asterisk indicates that the bilin content given for allophycocyanin includes two bilins which are each attached to a separate copy of an 18.5 kilodalton polypeptide that forms a part of the core of this phycobilisome[6].

Fig. 2. Time-resolved 680 nm emission from terminal acceptors of Synechocystis 6701 (thick trace) and CM25 (thin trace) phycobilisomes. Excitation wavelengths were 570 nm (a) and 620 nm (b). The smooth curves are computer-generated fits to the data with risetimes of 47 ps (Synechocystis 6701, a), 21 ps (CM25, a), and 25 ps (b).

Fig. 3. Time-resolved emission from C-phycoerythrin in intact Synechocystis 6701 phycobilisomes for excitation at 530 nm and detection at 570 nm. The smooth curve is the best double-exponential fit to the data with fall times of 27 ps (97.5% amplitude) and 1.5 ns (2.5% amplitude).

Fig. 4 Effect of excitation wavelength on emission risetime for isolated R-phycoerythrin. Excitation wavelengths are shown in comparison to the R-phycoerythrin absorption spectrum (top). Rising portion of time-resolved, 600 nm emission from R-phycoerythrin for excitation at 470 nm (a), 496 nm (b), 540 nm (c), and 565 nm (d); and of 600 nm emission from rhodamine B in ethanol for excitation at 470 nm (Rho B). Smooth curves show computer-generated fits to the data with risetimes of 6.6 ps (a), 2.2 ps (b), 6.5 ps (c), 4.6 ps (d), and 7.0 ps (Rho B).

SYNECHOCYSTIS 6701 WILD TYPE



SYNECHOCYSTIS 6701 STRAIN CM 25

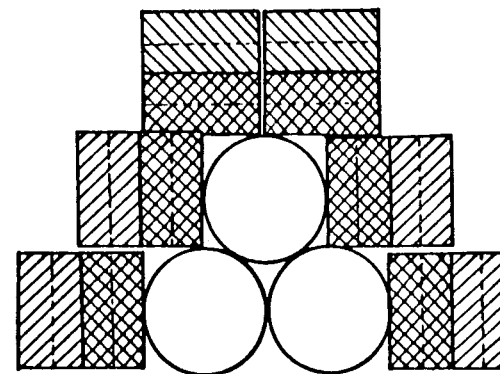
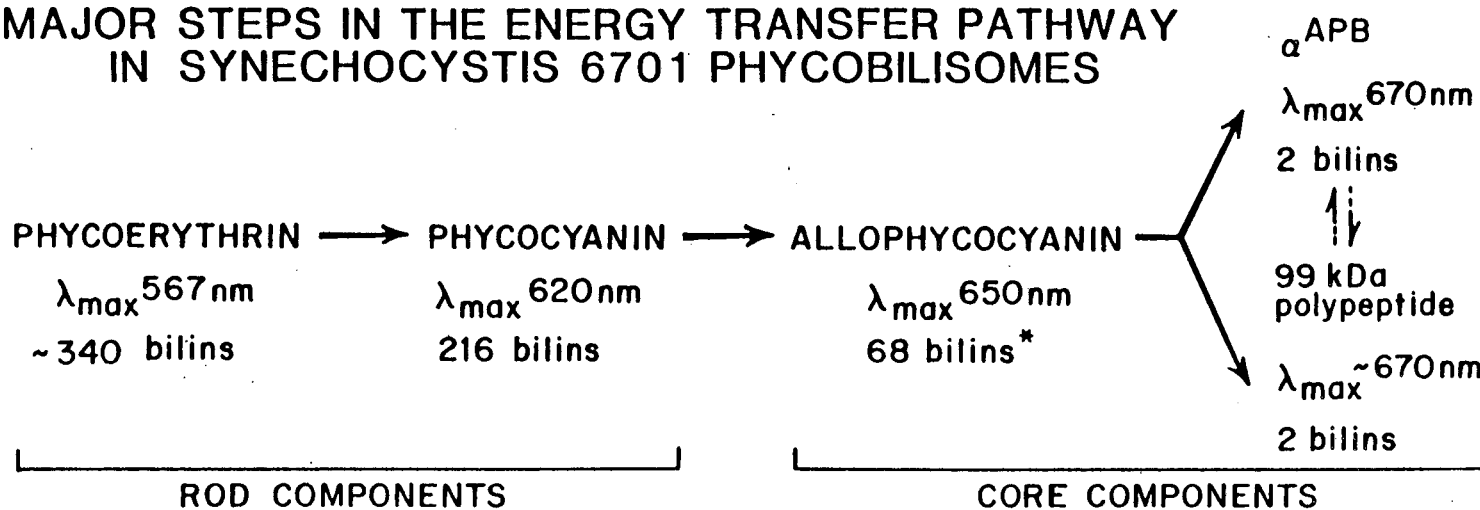


Fig. 1

MAJOR STEPS IN THE ENERGY TRANSFER PATHWAY IN SYNECHOCYSTIS 6701 PHYCOBILISOMES



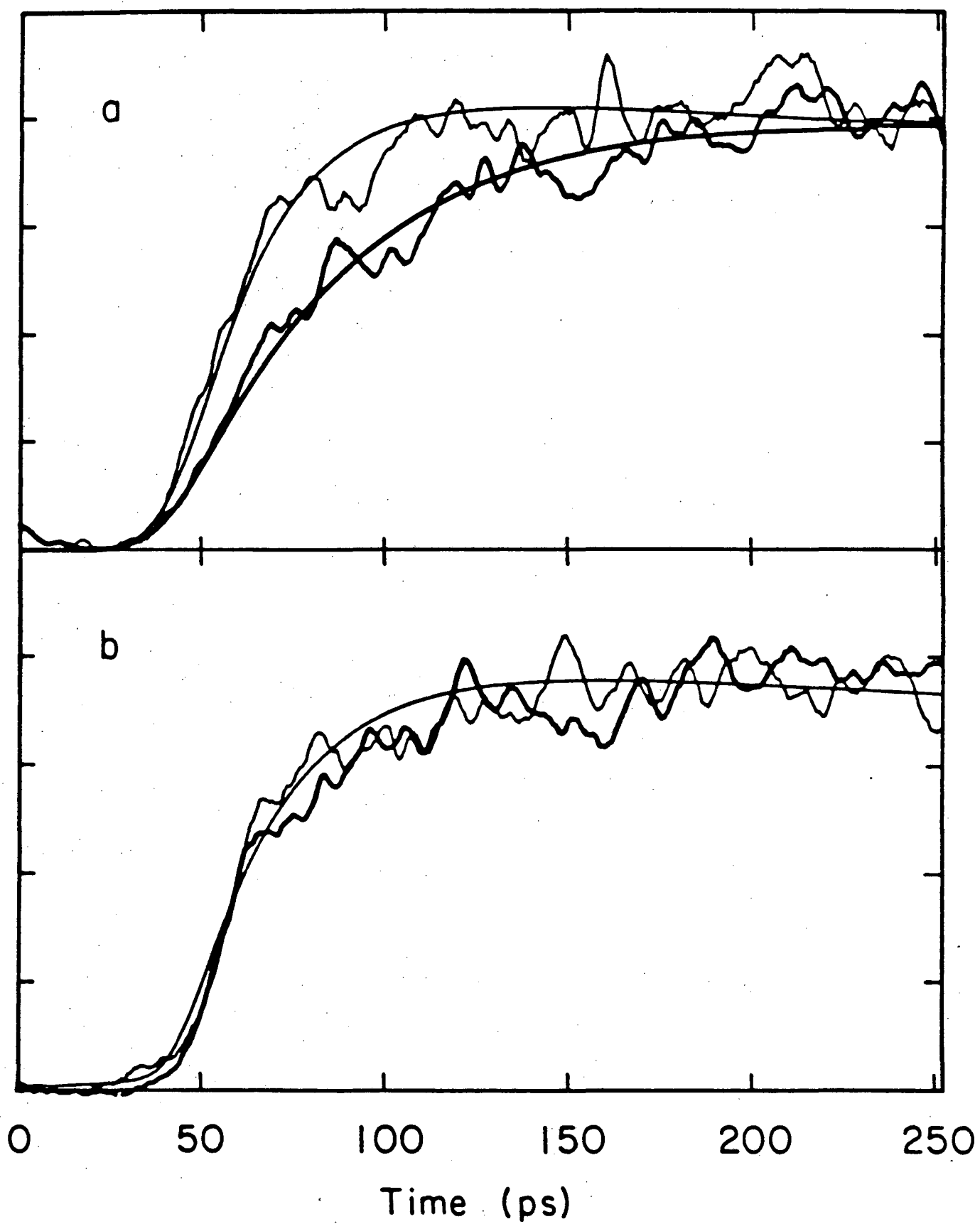


Fig. 2

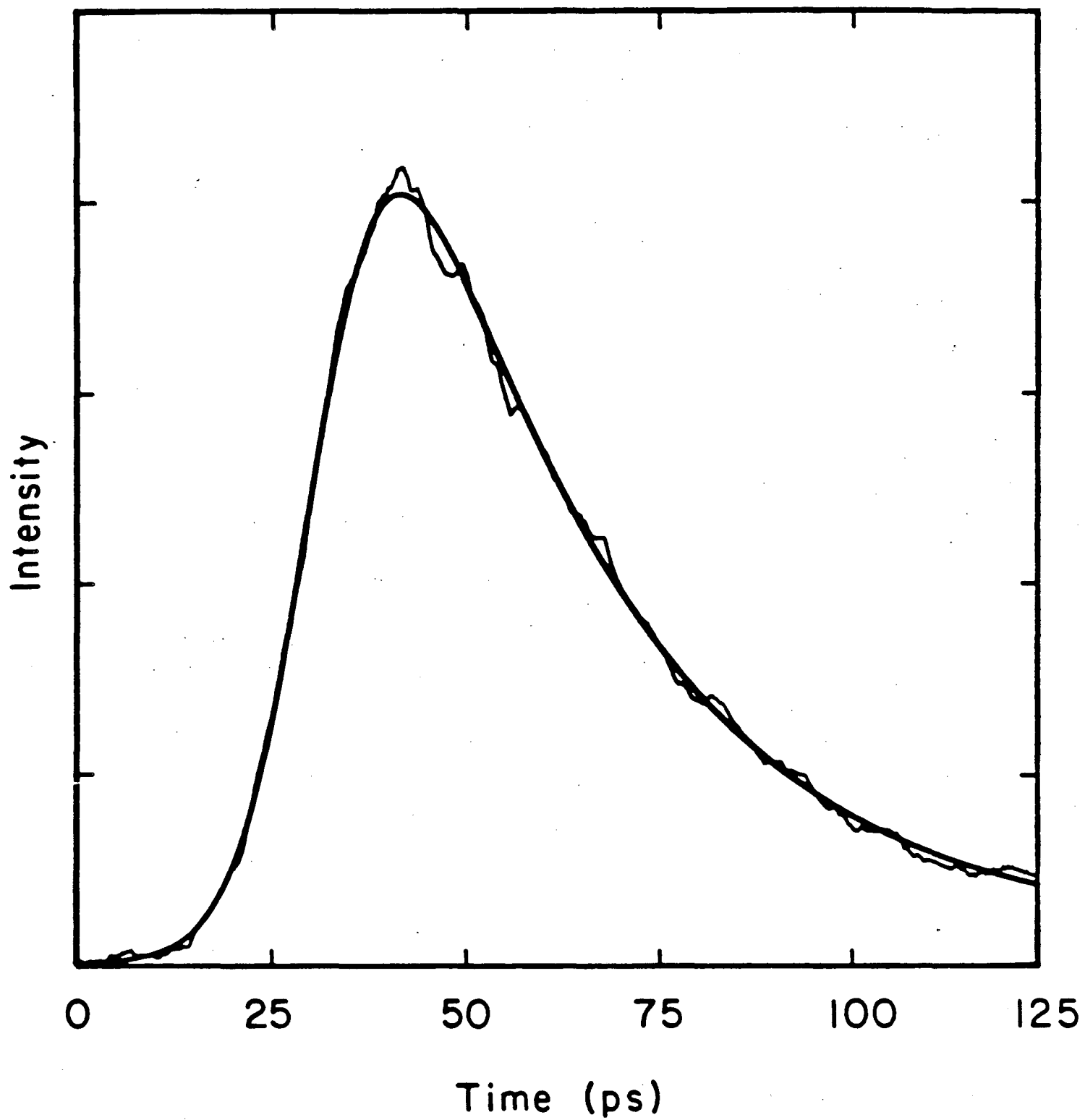


Fig. 3

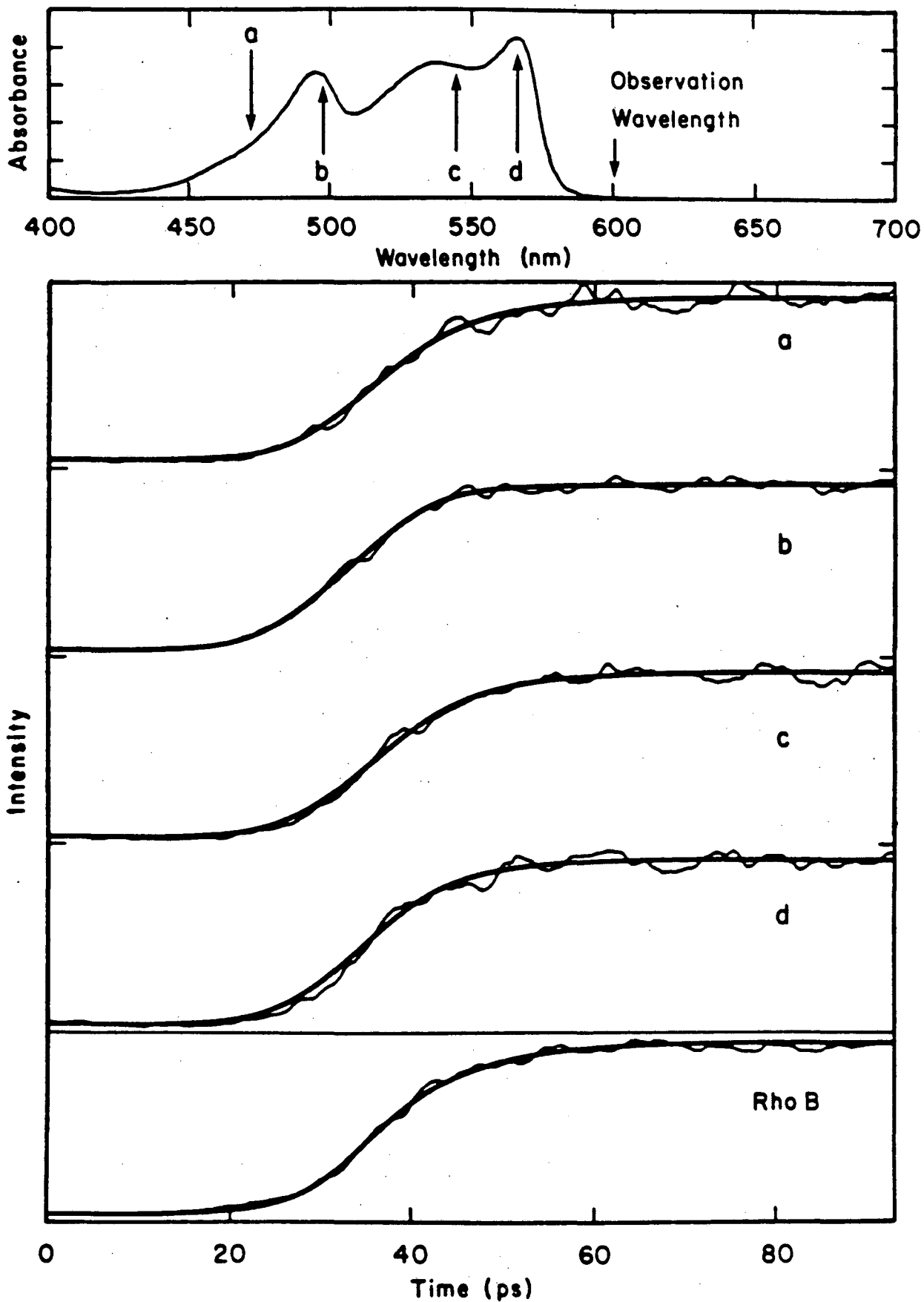


Fig. 4

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

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