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LIGHT-HARVESTING PIGMENTS IN PHOTOSYNTHETIC BACTERIA

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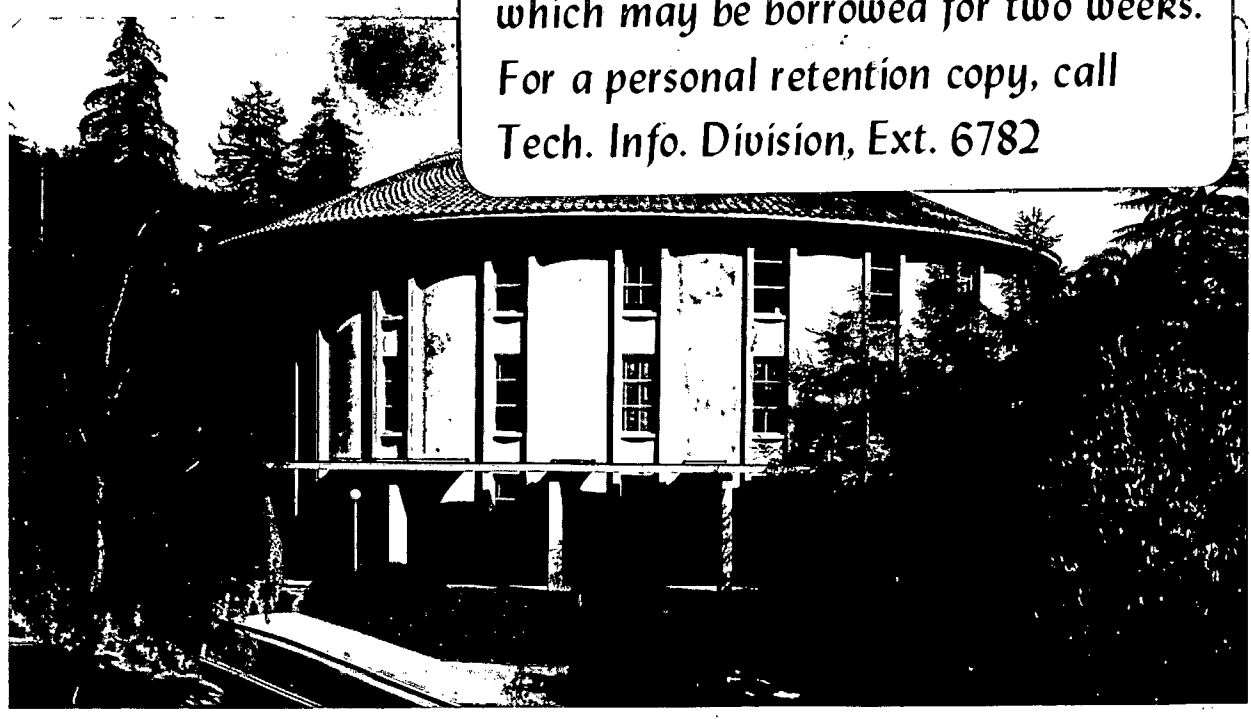
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### LIGHT-HARVESTING PIGMENTS IN PHOTOSYNTHETIC BACTERIA

John D. Bolt, C. Neil Hunter,  
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To BioScience

LIGHT-HARVESTING PIGMENTS IN  
PHOTOSYNTHETIC BACTERIA

A condensation of "Linear and Circular Dichroism and Fluorescence Polarization of the B875 Light-Harvesting Bacteriochlorophyll-Protein Complex from Rhodospseudomonas sphaeroides by John D. Bolt, C. Neil Hunter, Robert A. Niederman and Kenneth Sauer, to be published in vol. 34,

of Photochemistry and Photobiology. Bolt is currently in the Chemistry Department at Columbia University and will move shortly to E.I. du Pont, Wilmington, Delaware; Hunter is with the Department of Biochemistry, University of Bristol, Bristol BS8 1TD, UK; Niederman is with the Department of Biochemistry, Rutgers University, Piscataway, NJ 08854 and Sauer is with the Department of Chemistry, University of California, Berkeley, CA 94720. This research was supported, in part, by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Biological Energy Conversion & Conservation of the U.S. Department of Energy under Contract No. W-7405-ENG-48 (K.S.), in part, by the National Science Foundation grant PCM79-11251 (K.S.), by a U.S. Public Health Service grant GM26248 (R.A.N.) from the National Institute of General Medical Sciences. R.A.N. was the recipient of a U.S. Public Health Service Research Career Development Award GM00093-05 from the same Institute and a Postdoctoral Fellowship was provided to C.N.H. from the Charles and Johanna Busch Memorial fund by the Rutgers Bureau of Biological Research.

The major pigments of photosynthetic organisms occur in two functionally-different roles in the photon-converting membranes. The major portion (typically 99% or greater) serve as light harvesting pigments to absorb the incident radiation and transfer the resulting electronic excitation to photosynthetic reaction centers. There the second portion of the chlorophylls play an essential role in initiating the primary electron transfer (charge separation reactions). All of these pigments are thought to exist in specific complexes with proteins in the photosynthetic membranes (Sauer, 1979). The details of their organization, orientation and relation to one another are only poorly understood at present. A schematic illustration representing the organization in intracytoplasmic membranes of the purple photosynthetic bacteria Rhodospseudomonas sphaeroides is shown in Fig. 1. Photosynthetic bacteria offer an ideal class of organisms for investigating this problem. They contain only one type of reaction center complex (by contrast with higher plants, where there are at least two), and all of the light-harvesting pigments, bacteriochlorophyll (BChl) and carotenoids (Car), transfer excitation to those centers. Furthermore, methods have been developed for isolating not only the bacteriochlorophyll-containing reaction center complexes, but also several of the light-harvesting bacteriochlorophyll proteins (Sauer and Austin, 1978; Broglie, et al, 1980; Cogdell and Thornber, 1980). As described in this paper, the optical properties of the two major light-harvesting pigment-proteins designated B875 and B800-850 for Rps. sphaeroides were investigated using optical methods: absorption, circular dichroism, fluorescence polarization and linear dichroism--the latter for complexes oriented in artificial films and then stretched.

Using conventional isolation methods based on an initial detergent treatment, recently refined by Broglie, et al (1980), the two major light-harvesting components are first separated from the reaction center complexes and other membrane components and from one another. The integrity of the complexes is confirmed by observing that they retain the highly characteristic absorption band shifts of bacteriochlorophyll in vivo. Circular dichroism supports this conclusion and demonstrates that the pigment-pigment interactions within the two complexes are quite different from one another (Figs. 2 and 3). B800-850 is thought to contain 3 BChl and 1 carotenoid per monomer unit and B875 probably has 2BChl and 2 carotenoids. The relation of the different BChl molecules to one another can be characterized also by fluorescence polarization and linear dichroism studies, which confirm the differences inferred from the absorption and circular dichroism.

Earlier studies led to the conclusion that, of the two light-harvesting complexes, B875 is more closely associated with the reaction center as illustrated in Fig. 1, and that B800-850 is not only more removed spatially but also more variable in stoichiometry. Recent investigations on the related organism, Rhodospseudomonas capsulata, are suggestive that this aspect of the model may be an over-simplification (Bolt, et al., 1981). It would clearly be advantageous to know the detailed molecular structure of these pigment-protein complexes; however, suitable crystals for X-ray or electron diffraction study are not yet available. These complexes are small (20 kilodaltons)(Sauer and Austin, 1978) and, although the BChl and carotenoid molecules are not covalently attached, the complexes appear to have well-defined geometries. They may be ideal candidates for attempts at structure determination in the next stage of advancement of our understanding of the organization of photosynthetic membranes.

## Figure Captions

Fig. 1. Model of the arrangement of pigment-proteins in intracytoplasmic membranes of Rps. sphaeroides. The membranes consist of a two-dimensional array of reaction center complexes associated with two types of light-harvesting complexes, B800-850 and B875. The reaction center consists of the primary electron donor BChl, designated P870, together with an ubiquinone (UQ)-iron (Fe) complex as electron acceptor, in association with three distinct polypeptides. The B875 antenna complex may be more closely associated with the reaction centers; it consists of multiples of two BChl (B) and two carotenoids (Car) with two small polypeptides (8 and 12 kd). The B800-850 complex is more removed from the reaction centers; it consists of associations of monomer units that contain three BChl and one Car together with two small polypeptides (8 and 10 kd) (Broglie, et al., 1980; Cogdell and Thornber, 1980).

Fig. 2. Spectra of the Rps. sphaeroides B875 light-harvesting complex isolated by lithium dodecyl sulfate/polyacrylamide gel electrophoresis (Broglie et al., 1980). Top panel, absorption spectrum and fluorescence polarization,  $p$ ; middle panel, linear dichroism in stretched polyvinyl alcohol film expressed as the dichroic ratio,  $D_r$ ; bottom panel, circular dichroism spectrum. Note the different scales for the circular dichroism visible and near-IR regions.



Fig. 3. Spectra of Rps. sphaeroides B800-850 light-harvesting complex. Top panel, absorption spectrum and fluorescence polarization, p; bottom panel, circular dichorism spectrum. In this spectrum, the scales for the visible and near-IR regions are also different.

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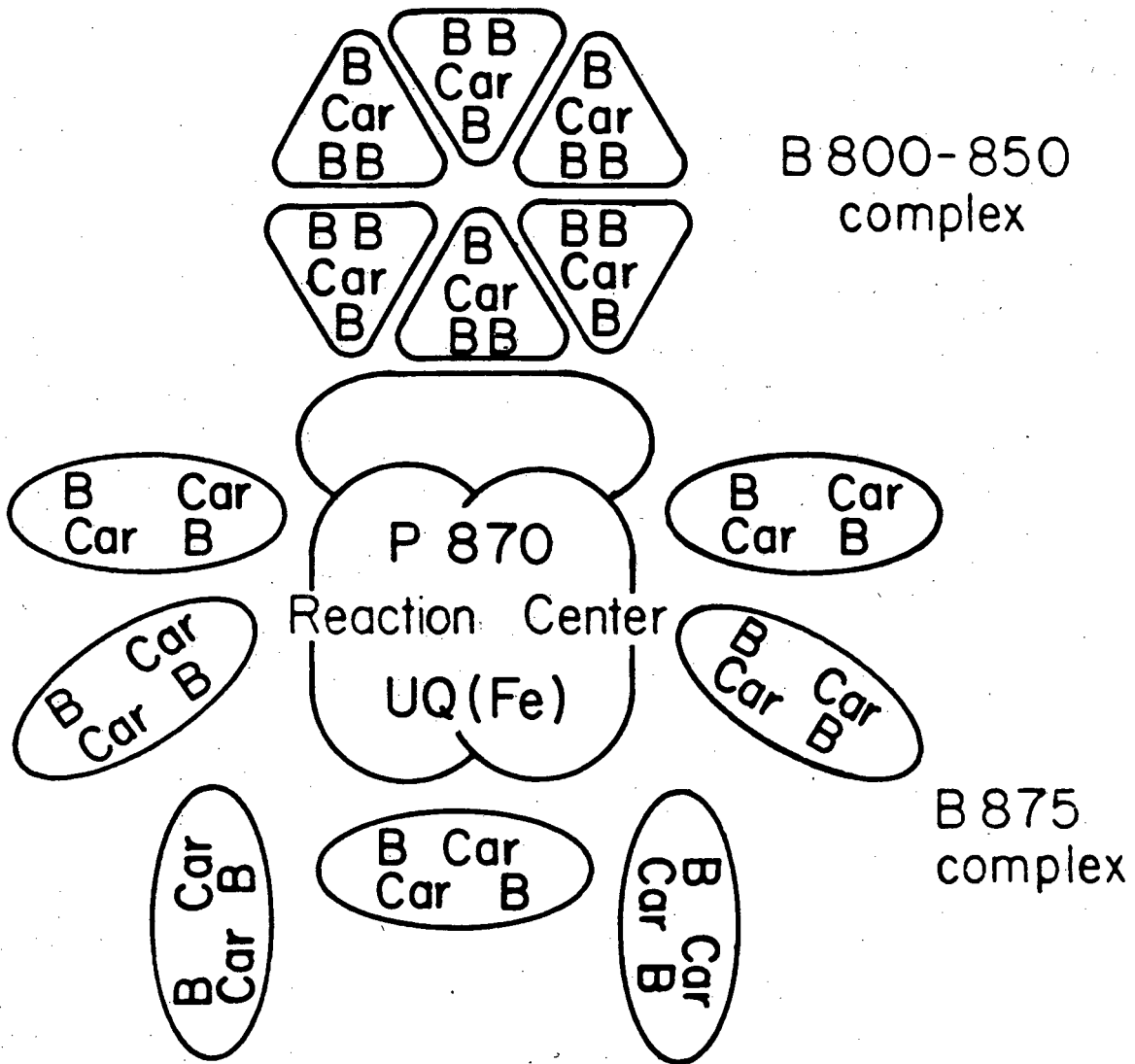


Figure 1

# Rps. sphaeroides, Wild Type

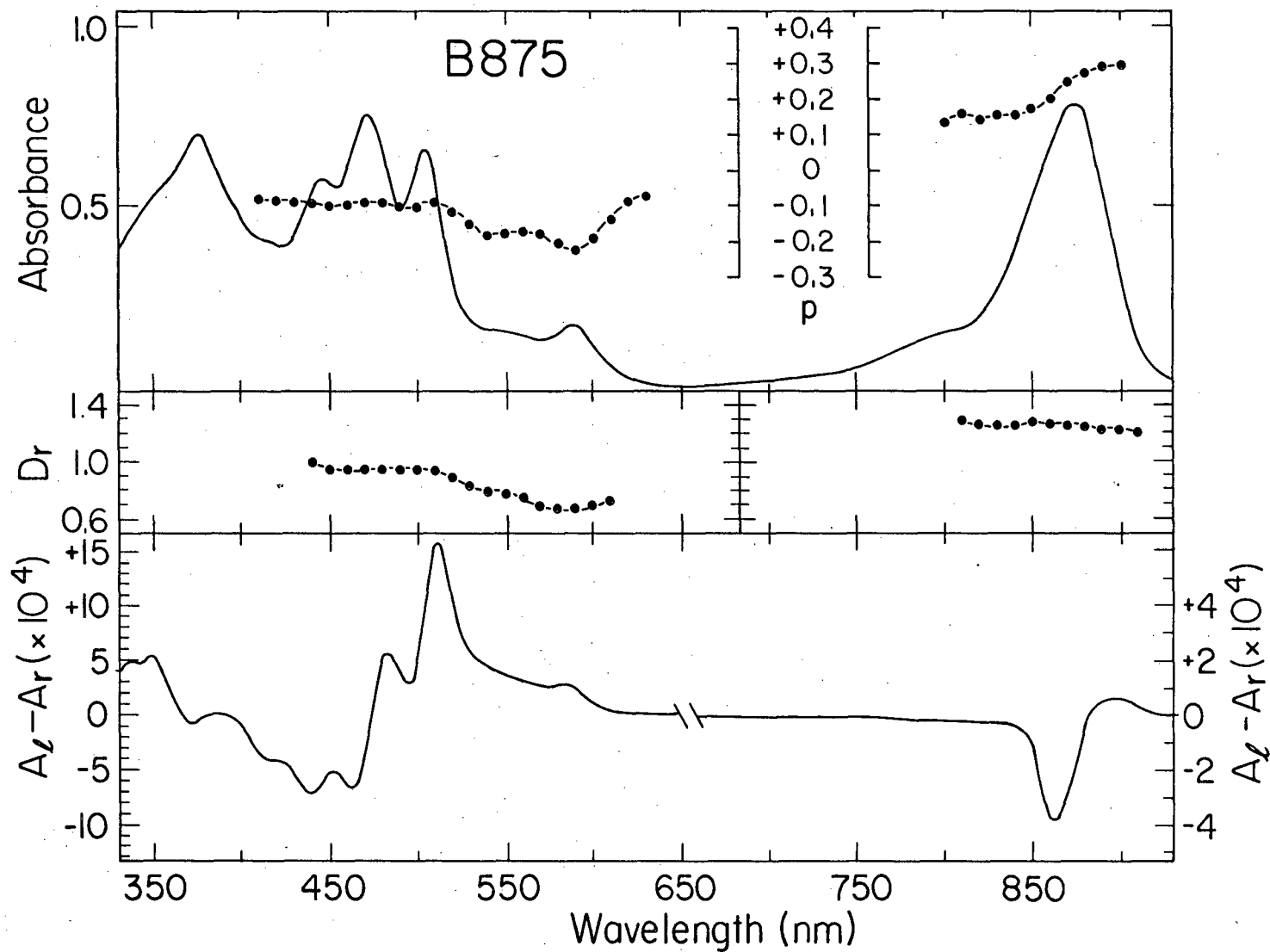


Figure 2

XBL 803-4084

# Rps. sphaeroides, Wild Type

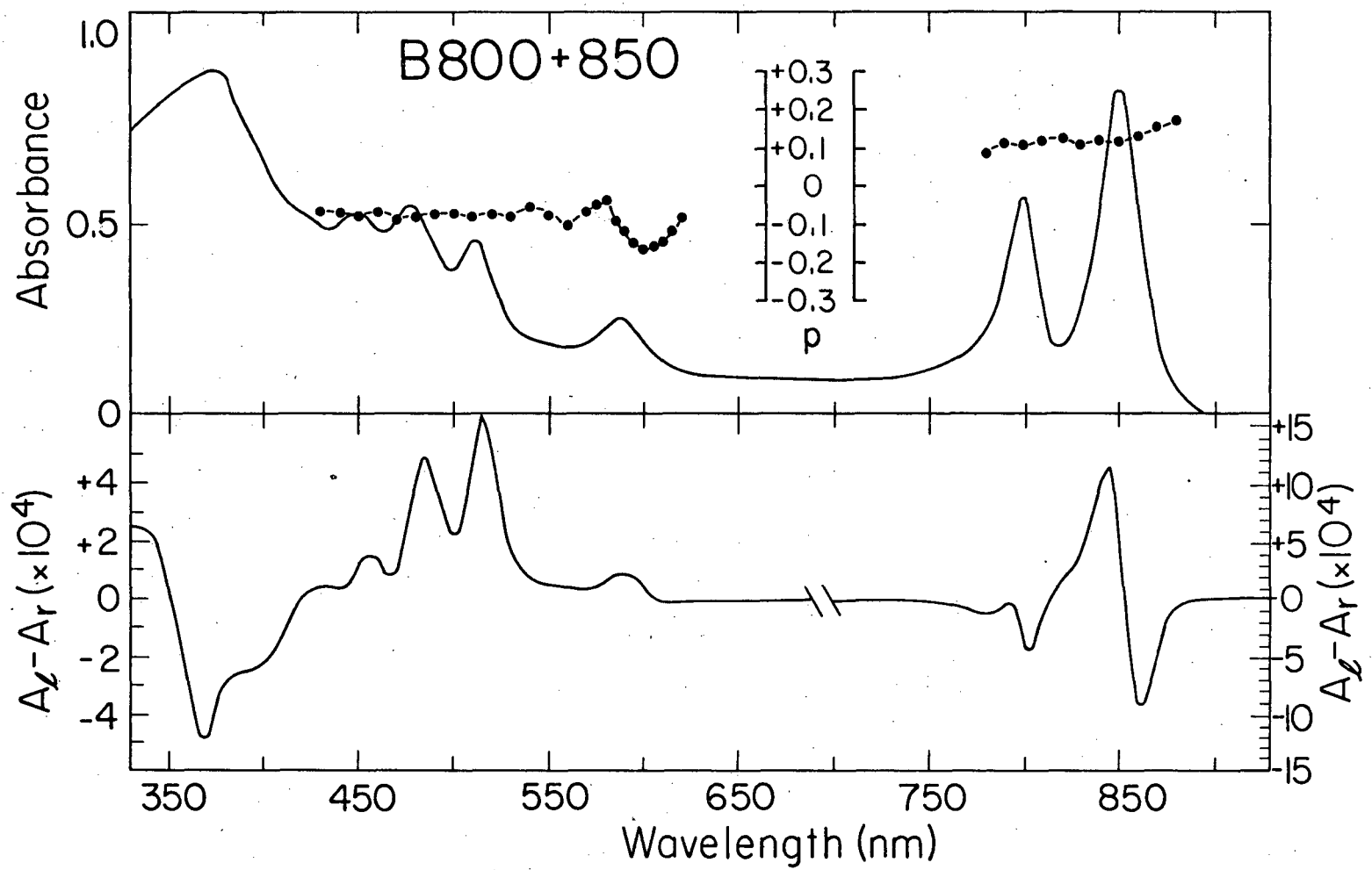


Figure 3

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