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Methods in Enzymology

Volume LIV

Biomembranes

*Part E: Biological Oxidations
Specialized Techniques*

EDITED BY

Sidney Fleischer

Lester Packer



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DEPARTMENT OF MOLECULAR BIOLOGY
VANDERBILT UNIVERSITY, NASHVILLE, TENNESSEE

Lester Packer

MEMBRANE BIOENERGETICS GROUP
DEPARTMENT OF PHYSIOLOGY-ANATOMY
UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

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ACADEMIC PRESS New York San Francisco London

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ACADEMIC PRESS, INC

111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by

ACADEMIC PRESS, INC. (LONDON) LTD.

24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

Biomembranes.

(Methods in enzymology, v. 31—)

Pt. E: edited by Sidney Colowick & Nathan O.

Kaplan; Sidney Fleischer/Lester Packer, volume editors.

Includes bibliographical references and indexes.

1. Cell membranes. 2. Cell fractionation.

3. Cell organelles. I. Fleischer, Sidney, ed.

II. Packer, Lester, ed. III. Series.

[DNLM: 1. Cell membrane. W1 ME9615K v. 31 / QH601 B6193]

QP601.M49 vol. 31-32 [QH601] 574.1'925'08s [574.8'75]

ISBN 0-12-181954-X (v. 54) 54-9110

PRINTED IN THE UNITED STATES OF AMERICA

Contributors to Volume LIV

Article numbers are in parentheses following the names of contributors.
Affiliations listed are current.

- LYLE J. ARNOLD, JR. (14), *Department of Chemistry, School of Medicine, University of California, San Diego, La Jolla, California 92093*
- DAVID P. BALLOU (7), *Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109*
- OCTAVIAN BÂRZU (26), *Department of Biochemistry, Medical and Pharmaceutical Institute, 3400 Cluj-Napoca, Romania*
- HELMUT BEINERT (10, 11, 24), *Institute for Enzyme Research, The University of Wisconsin, Madison, Wisconsin 53706*
- MAURIZIO BRUNORI (6), *C. N. R. Centre for Molecular Biology, Institutes of Chemistry and Biochemistry, Faculty of Medicine, University of Rome, Rome, Italy*
- WINSLOW S. CAUGHEY (18), *Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523*
- SUNNEY I. CHAN (19), *A. A. Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125*
- BRITTON CHANCE (9, 27), *Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104*
- RICHARD J. CHERRY (4), *Eidgenössische Technische Hochschule, Laboratorium für Biochemie, ETH-Zentrum, CH-8092 Zürich, Switzerland*
- DON DeVULT (3), *Department of Physiology and Biophysics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801*
- P. LESLIE DUTTON (23), *Johnson Research Foundation and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19174*
- HANS FRAUENFELDER (28), *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801*
- LESLIE W. -M. FUNG (13), *Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260*
- RONALD C. GAMBLE (19), *A. A. Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125*
- QUENTIN GIBSON (8), *Department of Biochemistry, Wing Hall, Cornell University, Ithaca, New York 14853*
- RAJ K. GUPTA (12), *The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111*
- CHIEN HO (13), *Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260*
- JOHN C. MAXWELL (18), *Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523*
- ALBERT S. MILDVAN (12), *The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111*
- THOMAS H. MOSS (21), *IBM Watson Research Center, Yorktown Heights, New York 10598 and Physics Department, Columbia University, New York, New York 10025*
- ECKARD MÜNCK (20), *Freshwater Biological Institute, University of Minnesota, Navarre, Minnesota 55392*
- YASH P. MYER (16), *Department of Chemistry, State University of New York at Albany, Albany, New York 12222*
- WILLIAM H. ORME-JOHNSON (10), *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706*

- NOZOMU OSHINO (27), *Nihon Schering K. K., Development and Research Department, Yodogawa-ku, Japan*
- REIKO OSHINO (27), *Biochemistry Lab, Kobe Yamate Women's College, Ikuta-ku, Kobe, Japan*
- GRAHAM PALMER (10), *Department of Biochemistry, Rice University, Houston, Texas 77001*
- P. M. RENTZEPIS (2), *Bell Laboratories, Murray Hill, New Jersey 07974*
- THOMAS G. SPIRO (15), *Department of Chemistry, Princeton University, Princeton, New Jersey 08540*
- BERT L. VALLEE (25), *Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and The Peter Bent Brigham Hospital, Boston, Massachusetts 02115*
- CLAUDE VEILLON (25), *Vitamin and Mineral Nutrition Laboratory, Nutrition Institute, Agricultural Research Center, U. S. Department of Agriculture, Beltsville, Maryland 20705*
- LARRY E. VICKERY (17), *Department of Physiology, College of Medicine, University of California, Irvine, California 92717*
- KAREN J. WIECHELMAN (13), *Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260*
- DAVID F. WILSON (1), *Department of Biochemistry and Biophysics, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104*
- GEORGE S. WILSON (22), *Department of Chemistry, University of Arizona, Tucson, Arizona 85721*
- H. T. WITT (5), *Max-Planck-Institut für Physikalische Chemie und Molekularbiologie, Technische Universität Berlin, 1000 Berlin 12, Germany*
- KWAN-SA YOU (14), *Department of Pediatrics, Duke University Medical School, Durham, North Carolina 27710*

Preface

A great deal of progress has taken place in biological oxidations and bioenergetics since "Oxidation and Phosphorylation" edited by Ronald W. Estabrook and Maynard E. Pullman (Volume X of "Methods of Enzymology") became available in 1967. To update this field five volumes on biomembranes (Volumes LII-LVI, Parts C-G, respectively) have been prepared, three dealing with biological oxidations and two with bioenergetics.

In this volume, Part E of "Biomembranes," subtitled "Specialized Techniques" we aim to bring together biochemical and biophysical techniques important to the study of biological oxidations.

We are pleased to acknowledge the good counsel of the members of our Advisory Board for these five volumes. Special thanks are also due Drs. E. Carafoli, G. Palmer, H. Penefsky, and A. Scarpa for their helpful comments on our outlines for these volumes. Valuable counsel for this volume was also provided by Drs. A. Azzi, H. Beinert, B. Chance, A. Mildvan, and G. Palmer. We were very gratified by the enthusiasm and cooperation of the participants in the field of biological oxidations and bioenergetics whose advice, comments, and contributions have enriched and made possible these volumes. The friendly cooperation of the staff of Academic Press is gratefully acknowledged.

SIDNEY FLEISCHER
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[1] Kinetic Measurements: An Overview

By DAVID F. WILSON

The shortest time interval of interest to biochemists is the approximately 10^{-15} sec required for a chromophore to absorb a photon. This is the initial event in all photoreactions, and the resulting activated singlet states may have lifetimes of up to approximately 10^{-8} sec before they decay. Molecular motion begins at approximately 10^{-14} sec with vibration along the interatomic bonds and molecular rotations being near 10^{-12} sec for small molecules (see Fig. 1). Chemistry involving bimolecular reactions in solution becomes significant only at times greater than approximately 10^{-8} sec. For example, a diffusion-limited reaction with a second-order rate constant of $10^9 M^{-1} \text{ sec}^{-1}$ will have a half-time of approximately 10^{-9} sec if both reactants are at 1 M concentrations. At more physiological concentrations of near $10^{-3} M$ or less such a reaction would have a half-time near 10^{-3} sec, and even simple protonation reactions ($K = 10^{10} M^{-1} \text{ sec}^{-1}$ to $10^{11} M^{-1} \text{ sec}^{-1}$, pH 7.0) have half-times of greater than 10^{-4} sec.

An experimentalist is concerned with measuring both the chemical species which participate in a reaction and the time course of the reaction. Thus methods must be selected which are appropriate for measuring the chemical species and which can make the required measurements with an adequate signal-to-noise ratio. When the reactions involved are of short duration (less than a few seconds), it is also necessary to use special methods for initiating the reaction in order to measure its time course. Given a completely stable or reproducible sample, the signal-to-noise ratio of the measurement increases as the square root of the number of measurements made or the amount of time over which the signal is integrated until limited by the intrinsic sensitivity of the method and/or instabilities in the measuring system.

New measuring methods are continuously being developed and known methods are continuously being improved, extending their sensitivity and the time range over which measurements can be made. In general a method with existing technology becomes applicable in a certain time range and is useful over a time range dependent on the characteristics of the method. In theory any method can be extended to any longer time period, but in practice the inherent instabilities limit measurements at longer times.

Kinetic methods used for measurements of reactions occurring with half-times of less than 3 or 4 sec are faced with the special problem of initiation of the reaction to be measured. Reagent mixing rates can be improved to give measurements in 10^{-3} to 10^{-4} sec but are unsuited for

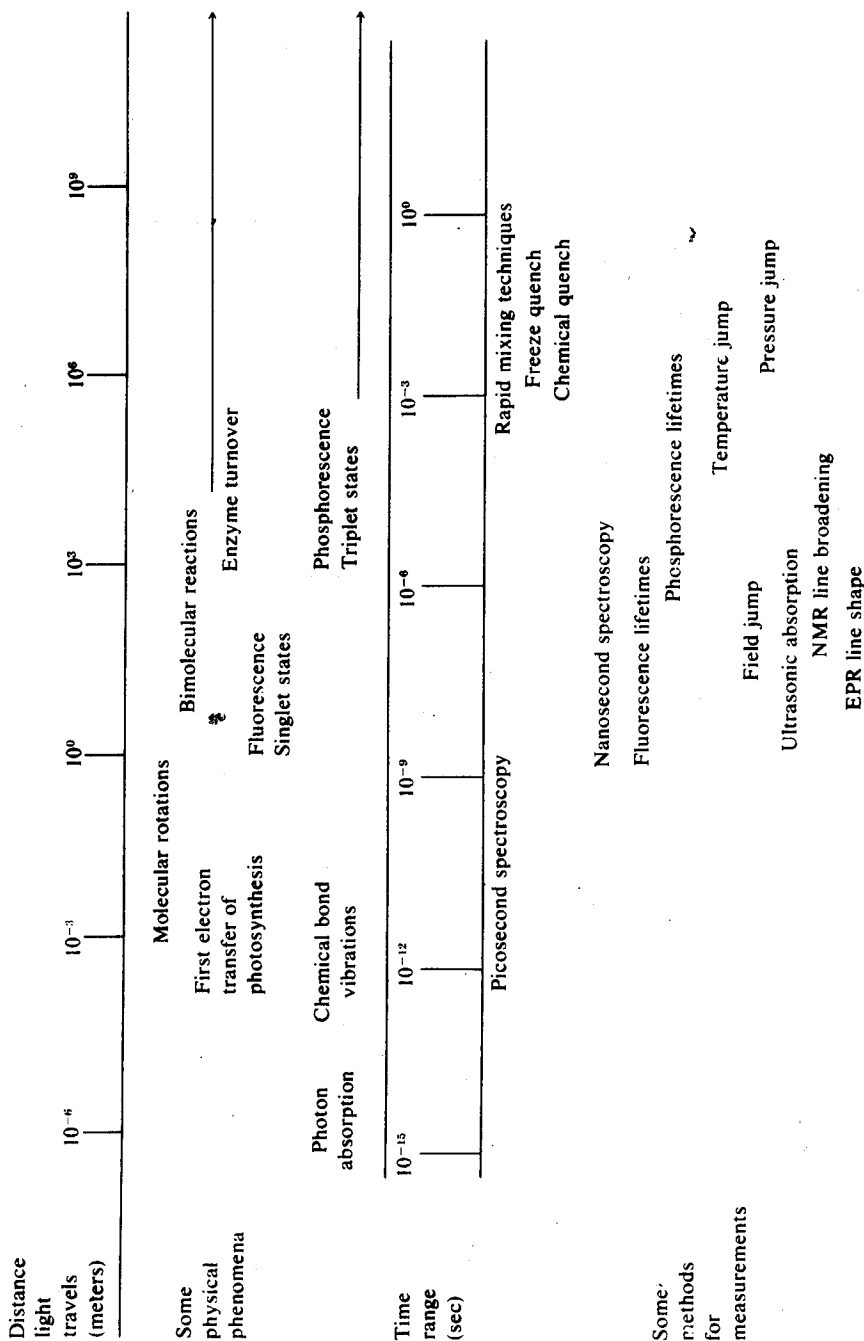


FIG. 1. See legend opposite page.

faster reactions. The methods for measuring the faster reaction rates may be called *relaxation methods*; i.e., a system at or near equilibrium is subjected to a rapid displacement from equilibrium (temperature change, light absorption, pressure change, etc.) and the rate of relaxation back to the equilibrium state is measured. An alternate approach to the study of fast reactions is to lower the sample temperature (Chance, Chapter 9; Frauenfelder, Chapter 28, this volume) and, depending on the activation energy or energies, slowing the reaction to where the available methods are applicable.

[2] Picosecond Spectroscopy in Biological Systems

By P. M. RENTZEPIS

This chapter describes the state of the art of picosecond spectroscopy and its application to biological systems. Special emphasis is given to the technological aspects and means for detection, display, and processing of data in the picosecond region. The identification and kinetics of short-lived intermediates in photosynthetic (bacteriochlorophyll) and visual (rhodopsin) processes are used as illustrations of the potential application of picosecond spectroscopy for the elucidation of the primary processes of importance in biology.

FIG. 1. A summary of some considerations which relate to the selection of methods of kinetic analysis. Light is the fundamental measure of time, traveling 3×10^{10} cm sec⁻¹. Thus time may be measured in the distance traveled by light, and the upper band of the figure lists the distance traveled by light in various time intervals. For example, light travels only 0.3 μ m in 10^{-12} sec or 0.3 mm in 10^{-10} sec. The second band of the figure lists some physical phenomena which occur in the indicated time range. Molecular rotations begin with half-times of near 10^{-12} sec for rotation of small molecules, but the time increases with increasing molecular size. Similarly, molecular singlet states are indicated as having half-lives in the general region of 10^{-7} – 10^{-8} sec, but this is only an approximation and both shorter and longer half-lives occur. The lowest band of the figure indicates a few of the experimental methods available for kinetic measurements in the indicated time ranges for their optimal utilization. The methods are usually named for the technique used to induce the reaction. The relaxation methods, for example, may be named for the manner in which the reaction is displaced from equilibrium—such as temperature jump, field jump, or pressure jump—but any technique suitable for measuring the reaction rate (dielectric dispersion, optical absorption, etc.) may be used. Other methods such as picosecond spectroscopy (Rentzepis, Chapter 2, this volume) and nanosecond spectroscopy (DeVault, Chapter 3, this volume) are named for the specialized technology required to make absorbance measurements in the indicated time range.

Picosecond spectroscopy is now about 10 years old.¹ Its use is growing continuously because one can perform experiments which reveal previously unknown and until now unobservable events of basic importance not only to biology but to chemistry and physics. This growth is also due to the vast number of ultrafast primary processes which are now accessible to experimental measurement, with relatively high sensitivity and reliability, as a result of recent technological developments which provide means not only for increased reliability in the data but also for the simultaneous display of time, spectrum, and intensity. To perform such experiments, one needs essentially the same tools as in classical spectroscopy with the additional constraints of:

1. A well-defined pulse for excitation which is of picosecond duration with well-defined shape and spectral bandwidth.
2. A clock which allows us to measure time in picosecond units.
3. A light continuum of picosecond duration and a wavelength range spanning thousands of wavenumbers to act as the absorption monitoring light.
4. An experimental laser system which can resolve the time, wavelength, and intensity parameters simultaneously while having a sensitivity approaching that of single photon counting and able to average and display the data in any desirable and meaningful manner.

We shall discuss each of these four constraints and describe the means by which each can be produced in the laboratory and then combined into the complete picosecond spectroscopy optical system shown in Fig. 1a.

The Picosecond Pulse and Its Characterization

We will not attempt a discourse on picosecond pulse generation; instead we will briefly describe the method for its generation and the measurement of pulse shape and duration. Several papers devoted to picosecond pulse generation are available, including those referred to here.²⁻⁶

The Pulses

The generation of the picosecond pulses is achieved in a cavity [Fig. 1a (2)] formed by 100% and ~50% reflecting mirrors. Between these mirrors is placed the oscillating/amplifying medium such as a flashlamp-pumped Nd³⁺

¹ P. M. Rentzepis, *Chem. Phys. Lett.* **2**, 117 (1968).

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