Biophysical labeling methods in molecular biology

Physical labels such as stable nitroxide radicals, luminescent and photochromic chromophores, so-called Mössbauer atoms, and electron-dense assemblies of heavy atoms have proved to be effective tools in solving many problems at the molecular level in biological systems. These physical labels are used as "molecular rulers" to measure the distances between chosen groups and to measure the size, form, and microrelief of objects. By providing information about these factors, the label provides information that can help the scientist to understand the structures of membranes, nucleic acids, enzymes, and proteins and how they function.

This volume covers all aspects of this field: the theoretical bases, the experimental techniques, and how to interpret the resulting data. It also critically discusses some recent results obtained with these techniques and gives an analysis of likely developments in the future.

Biophysical labeling methods in molecular biology

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Preface

About 200 years ago the German poet and philosopher J. W. Goethe noted that Nature is not only a great artist but also a skillful master. The contemporary generation of scientists who work in the field of molecular biology can appreciate the external beauty of nature, the internal perfection of biological structures and physicochemical processes taking place in nature, and the enormous difficulty of studying them.

Modern molecular biology faces extremely complicated experimental problems. Proteins, biological membranes, nucleic acids, polysaccharides, and other ingredients of a biological cell interact, form sophisticated structures, and accomplish numerous catalytic, regulatory, and other functions. Many of the specific problems to be solved arise in the study of these systems.

In investigations of biological systems, one uses a broad arsenal of physical and chemical methods. Of particular importance in this arsenal is the approach of selective modification of biological objects with various labels capable of providing information on their structure, molecular dynamics, and mechanisms of actions. The necessity of such an approach is caused by the specificity of biological systems. Rather than seek complete information, a researcher usually aims to learn the main structural and dynamic properties important in the functional activity of a system.

These days the method of physical labeling is used to solve many structural problems in biophysical and biochemical laboratories all over the world. The most popular methods use spin and fluorescent labels and probes. The achievements in this field in the 1970s have been summarized in a number of monographs and reviews. However, since then considerable progress has been made; in addition to the scientific areas that seem relatively conventional by now, new ones have appeared. In particular, methods of triplet, photochromic, electron scattering, and Mössbauer labeling have been put forward and developed in the author's laboratory at the Institute of Chemical Physics (Chernogolovka). A method of total labeling of protein and other object surfaces originated at the same institute.

It has proven to be particularly effective to combine various types of

labels. Consistent use of this approach has led to the solution of a number of complicated problems such as deciphering the active centers of enzymes (nitrogenase, cytochrome P-450, photosynthetic reaction centers) and elucidating the structure and molecular dynamics of proteins, membranes, and nucleic acids.

In the author's opinion, methods developed in various laboratories to solve specific problems are of general importance and may prove to be useful for studying a wide range of biological objects.

In the first five chapters of the present monograph, the general experimental and theoretical grounds are expounded for various methods using physical labels and probes (spin, fluorescent, triplet, photochromic, Mössbauer, and electron scattering), as well as the technique of total labeling. Also elucidated therein are the main principles of chemically modifying the objects under study to accommodate or contain the labels. The concluding chapters consider the principal results in the field of enzymatic catalysis, molecular biology, and biophysics obtained by means of the method of physical labeling.

The author believes that the effort involved in writing this monograph will have been worthwhile if it arouses interest in biophysical labeling and makes it easier for chemists, biochemists, and biophysicists to understand the principles of this method to such an extent that they will use it creatively to solve their own scientific problems.

The author has used a three-level approach to describe some of the topics, taking into account the variations in the educational training of his readers:

1. a qualitative consideration of the phenomenon on which the method is based,
2. presentation of formulas, figures, and schemes in their simplest and most suitable form for practical application,
3. a more rigorous physical and mathematical substantiation.

Greater attention has been paid to the first two levels.

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Abbreviations

ANS	1,2,4-triaminonaphtholsulfonic acid
ATP	adenine triphosphate
BSA	bovine serum albumin
DAF	delayed annihilation fluorescence
DHSPC	dihydrostearylphosphatidylcholine
2D-ELDOR	two-dimensional ELDOR
DMPE	dimyristoylphosphatidylethanolamine
DMPG	dimyristoylphosphatidylglycerol
DMPC	dimyristoylphosphatidylcholine
DPPC	dipalmitoylphosphatidylcholine
ELDOR	electron–electron double resonance
ENDOR	electron–nuclear double resonance
ESEM	electron spin-echo method
ESE MT	electron spin echo with magnetization transfer
EXAFS	extended X-ray absorption fine structure
FL	fluorescent label
FP	fluorescent probe
FRAP	fluorescence recovery after photobleaching
FRAT	free-radical assay technique
HF	high frequency
HFI	hyperfine interaction
HSA	human serum albumin
Ig	immunoglobulin
IR	ion-relaxator
MESL	mercarbide electron-scattering label
MW	microwave
NMR	nuclear magnetic resonance
NMME	nuclear magnetic modulation effect
NQI	nuclear quadrupole interaction
NR	nitroxide radical
PCMB	*p*-chloromercuribenzoate
PE	phosphatidylethanolamine
PI	phosphatidylinositol

PM	paramagnetic metal
PS	phosphatidylserine
RC	reaction center
SL	spin label
SP	spin probe
SR	sarcoplasmic reticulum
SR ESR	saturation recovery ESR
ST ESR	saturation transfer ESR
TCTA	2,4,6-trichloro-1,3,5-triazine
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TEMPOLE	4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl
TEMPONE	4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl
TL	triplet label
TP	triplet probe
TTLT	total tritium labeling technique
A_{iso}	isotropic HFI constant
$A_{x,y,z}$	anisotropic HFI constant
a_B	Bohr radius
α_e	electrostatic factor
$\alpha_{e,g}$	polarizability
β_e	electron Bohr magneton
β_n	nuclear Bohr magneton
D	Debye unit
$D_{R,\perp,\parallel}$	rotational diffusion coefficient
D_{tr}	translational diffusion coefficient
Δ	line width of ESR spectrum
ΔF_0	standard free energy
$\Delta F^{\neq}$	free energy of activation
ΔF_r	reorganization energy
ΔH_{pp}	peak-to-peak line width
$\Delta H_{\frac{1}{2}}$	line width between the points of maximum slope
ΔH_L	Lorentzian line width
ΔH_G	Gaussian line width
$\Delta \nu_{max}$	spectral shift
$\Delta \varphi_m$	transmembrane potential
δ	chemical shift
E	electric field strength
ε_0	dielectric constant
ε	extinction coefficient
f_g	geometric steric factor
g	g-factor
g_e	g-factor of a free electron
γ_e	electron gyromagnetic ratio

γ_n	nuclear gyromagnetic ratio
H	Hamiltonian
H_m	modulation amplitude
H_1	MW amplitude
H_z	strength of a magnetic field
h	Planck constant
η	viscosity
I	spectral line intensity
I_f	fluorescence intensity
I_n	magnetic quantum number of nucleus
I_{ph}	phosphorescence intensity
J	exchange integral
$j(\omega)$	correlation function
K_Q	Stern-Volmer constant
K_q	quenching rate constant
k_{cl}	collision rate constant
k_e	spin exchange rate constant
k_{tr}	electron transfer rate constant
æ	nonadiabaticity coefficient
$æ_{D,A}$	spin wave function
λ	line width
λ'	spin-orbital coupling constant
$\bar{M}$	transition dipole moment
M_r	molecular mass
m	magnetic quantum number
m_i	quantum number
μ	ionic strength
μ_e	magnetic moment of an electron
μ_{el}	electron dipole moment
N_A	Avogadro number
n	refraction index
ν_c	correlation frequency
ν_L	Larmor frequency
ν_m	modulation frequency
ν_r	resonance frequency
P	degree of polarization
p	*p*-orbital
Q	quadrupole moment
ρ	spin density
S	order parameter
S_i	excited singlet state
S_{jj}	overlap integral
s	*s*-orbital

σ	charge density
T_c	transition temperature
T_i	excited triplet state
T_{1e}	electron spin–lattice relaxation time
T_{2e}	electron transverse relaxation time
T_{1n}	nuclear spin–lattice relaxation time
T_{2n}	nuclear transverse relaxation time
T_p	passage time
τ_D	Debye relaxation time
τ_c	correlation time
τ_{ch}	characteristic time
τ_{col}	collision time
τ_f^*	fluorescence lifetime
τ_M	chemical exchange time
τ_{ph}^*	phosphorescence lifetime
τ_r	electric dipole relaxation time
τ_R	rotation diffusion time
τ_{rs}	residence time
V	voltage
V'	resonance integral
V_2'	absorption ESR spectrum with a 90° phase shift
φ	electric potential
φ_0	quantum yield
$\varphi_{D,A}$	wave function
ω_L	Larmor frequency