Springer Series in Materials Science 292

Gertz I. Likhtenshtein

Nitroxides

Brief History, Fundamentals, and Recent Developments

Springer

Springer Series in Materials Science

Volume 292

Series Editors

Robert Hull, Center for Materials, Devices, and Integrated Systems,
Rensselaer Polytechnic Institute, Troy, NY, USA
Chennupati Jagadish, Research School of Physical, Australian National University,
Canberra, ACT, Australia
Yoshiyuki Kawazoe, Center for Computational Materials, Tohoku University,
Sendai, Japan
Jamie Kruzic, School of Mechanical & Manufacturing Engineering,
UNSW Sydney, Sydney, NSW, Australia
Richard M. Osgood, Department of Electrical Engineering, Columbia University,
New York, USA
Jürgen Parisi, Universität Oldenburg, Oldenburg, Germany
Udo W. Pohl, Institute of Solid State Physics, Technical University of Berlin,
Berlin, Germany
Tae-Yeon Seong, Department of Materials Science & Engineering,
Korea University, Seoul, Korea (Republic of)
Shin-ichi Uchida, Electronics and Manufacturing, National Institute of Advanced
Industrial Science and Technology, Tsukuba, Ibaraki, Japan
Zhiming M. Wang, Institute of Fundamental and Frontier Sciences - Electronic,
University of Electronic Science and Technology of China, Chengdu, China

The Springer Series in Materials Science covers the complete spectrum of materials research and technology, including fundamental principles, physical properties, materials theory and design. Recognizing the increasing importance of materials science in future device technologies, the book titles in this series reflect the state-of-the-art in understanding and controlling the structure and properties of all important classes of materials.

More information about this series at http://www.springer.com/series/856

Gertz I. Likhtenshtein

Nitroxides

Brief History, Fundamentals, and Recent Developments

Springer

Gertz I. Likhtenshtein
Department of Chemistry
Ben-Gurion University of the Negev
Beersheba, Israel

Institute of Problems of Chemical Physics
Russian Academy of Science
Chernogolovka, Moscow Region, Russia

ISSN 0933-033X ISSN 2196-2812 (electronic)
Springer Series in Materials Science
ISBN 978-3-030-34821-2 ISBN 978-3-030-34822-9 (eBook)
https://doi.org/10.1007/978-3-030-34822-9

© Springer Nature Switzerland AG 2020
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

It is known that there are delicate links and fine parallels between an art and science. Both these spheres of human endeavor involve a unique combination of professional skill and creative search. Sometimes, an intuitive line of a great poet or philosopher may be likened to the opening of a new horizon in science. Thus, the composer Maurice Ravel in his famous "Bolero" allegorically depicts the process of birth and development of an epochal discovery that gives rise to many advantages. Like that opening musical movement, the first publication on synthesis a nitroxide by Fremi in 1825 initiated, after a long lag period, the birth and rapid development of a novel class of stable nitroxide radicals which happened to be extremely important in both aspects, basic and applied.

At present, the science of nitroxides widely extended her "hands" to chemistry, physics, and biology. The application of nitroxides ranges from use as spin labels and antioxidants in biological studies, charge carriers for energy storage, basis for magnetic materials' mediators in polymerization reactions, functional spin probes for pH, oxygen, and thiol levels to catalysts in chemical and electrochemical oxidation reactions.

Classical and modern physical chemistry and chemical physics, chemical kinetics, organic, inorganic, and quantum chemistry provide an arsenal of physical methods and establish a basis for the investigation of structure and action mechanism of processes involving nitroxides.

This book embraces all principal aspects of structure and physicochemical action mechanisms of nitroxides. It is a view of nitroxides by a physicochemist with long-term experience in the area. The book is not intended to provide an exhaustive survey of each topic but rather a discussion of their theoretical and experimental background and recent developments. The literature of nitroxides is so vast, and many scientists have made important contribution in the area that it is impossible in the space allowed for this book to give a representative set of references. In fact, for each section in this area one can write several books. The author apologizes to those he has not been able to include.

Research on nitroxide which combines their fundamental importance for human welfare and intellectual fascination for investigation will promote solving exciting and complicated problems in chemistry, biology, and physics.

Chapter 1 of the monograph is a brief outline of 175 years history of nitroxides. Chapters 2 and 3 form the theoretical and experimental chemical background for nitroxide numerous application. Chapter 4 is a general survey of fundamentals of electron spin resonance and nuclear magnetic resonance, and main physical methods directly related to nitroxide. Advantages in design and use of nitroxide biradicals are reviewed in Chap. 5. Chapter 6 presents a review on the use of tethered nitroxide–fluorophore molecules as probes of redox status, antioxidant activity, oxidative stress, and free radical reaction. Nitroxide-mediated polymerization (NMP) is the subject of Chap. 7. Chapter 8 describes role nitroxides as the base for magnetic materials. Involving nitroxide radicals and their derivatives in biological processes is in focus of Chap. 9. Applications of nitroxides as spin labels and probes constitute the contents of Chap. 10. Chapter 11 is devoted to the use of nitroxyls to solve some of the physicochemical problems.

The monograph is intended for scientists and engineers working in the fields of chemistry, physics, and biology in which nitroxyls currently find or would find their application. Book, as a whole, and separate chapters can be used as a subsidiary manual for instructors, graduate and undergraduate students of university chemistry, physics, and biophysics departments.

Department of Chemistry, Ben-Gurion University of the Negev and Institute of Problem of Chemical Physics, Russian Academy of Science provided excellent conditions for writing this book for which the author is extremely grateful.

Moscow Region, Russia/Beersheba, Israel Gertz I. Likhtenshtein

Contents

About the Author

Prof. Gertz I. Likhtenshtein received his Ph.D. and his Doctor of Science from the Semenov Institute of Chemical Physics at the Russian Academy of Science in Moscow, where he was appointed to the position of Head of Laboratory of Chemical Physics of Enzyme Catalysis in 1966, becoming a full professor in 1976. In 1992, he moved to the Department of Chemistry at the Ben-Gurion University of Negev, Israel, as a full professor in charge of the Laboratory of Chemical Biophysics and has been an Emeritus since 2003. He has authored eleven scientific books and around 390 papers, and his awards include the Medal of the Exhibition of Economic Achievement, the USSR Diploma of Discovery, the USSR State Prize, the V. V. Voevodsky International Price for Electron Spin Resonance, the N. M. Emanuel Price for Biophysical Chemistry, and the Diploma of the Israel Chemical Society. Professor Likhtenshtein is a Foreign Member of the Academy of Science of Republic Tadjikistan and was a member of the International ESR Society, the American Biophysical Society, the Israel Chemical Society, and the Israel ESR Society. His recent main scientific interests focus on the analysis of biologically important molecules.

Chapter 1
Nitroxides: 170 Years of History

1.1 Long Preamble of a Tale (1845–1960)

As an each way, a long road of the 174 years history of stable radicals bearing a >N—O group has started with the first step. This step has been made in 1845 by Edmond Frémy (1814–1894), a director the Muséum national d'histoire naturelle, who was one of understanding chemists of nineteenth century [1]. Edmond Frémy published numerous articles in the *Annales de Chimie et de Physique*, seven volumes *of Traité de chimie générale,* and ten volumes *Encyclopédie Chimique*, in collaboration with several other scientists. But his main advantage, which left memories in the chemical history, was a synthesis and characterization of disodium nitrosodisulfonate (potassium nitrosodisulfonate, Frémy's salt (Fig. 1.1a).

A synthesis of potassium nitrodisulfonate,occurring by the following scheme:

$$HNO_2 + 2\,HSO_3^- \rightarrow HON(SO_3)_2{}^{2-} + H_2O$$
$$3\,HON(SO_3)_2{}^{2-} + MnO_4^- + H^+ \rightarrow 3\,ON(SO_3)_2{}^{2-} + MnO_2(s) + 2\,H_2O$$
$$2\,ON(SO_3)_2{}^{2-} + 4\,K^+ \rightarrow K_4\left[ON(SO_3)_2\right]_2(s)$$

now can be readily performed in a high school student chemical laboratory. Fremy's salt is long-lived radical in water and other solvents in unaerobic conditions. Nevertheless, a rapid and highly exothermic decomposition of this compound occurs spontaneously in air that is a serious limitation for its applications.

Next step in the area was done by O. Piloty and B. G. Schwerin in 1901 [2] who prepared the first organic radical containing >N—O. The radical was named as "porphyrexide" (Fig. 1.1b). Heinrich Wieland and Moriz Offenbacher reported in

© Springer Nature Switzerland AG 2020

G. I. Likhtenshtein, *Nitroxides*, Springer Series in Materials Science 292,
https://doi.org/10.1007/978-3-030-34822-9_1

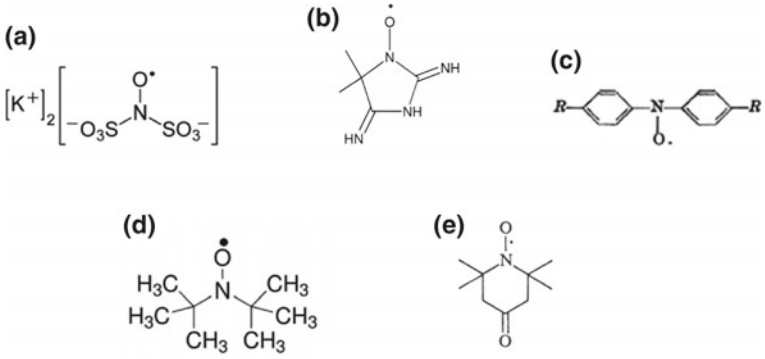
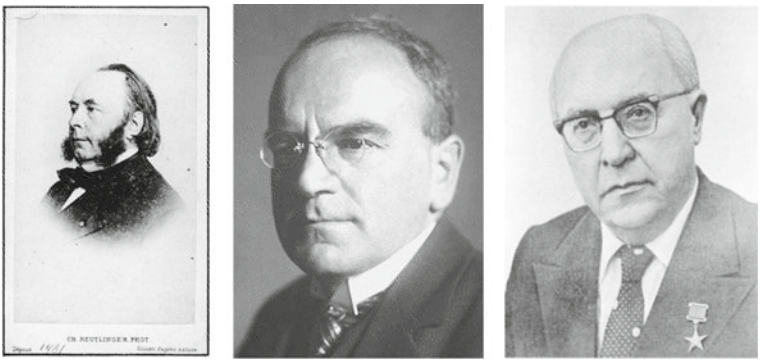


Fig. 1.1 Structures of pioneering nitroxides

1914 the synthesis of diaryl nitroxides in which the >N—O group is attached to two conjugated system (Fig. 1.1c) [3]. About 60 years after, stable di-*tert*-butyl nitroxide (Fig. 1.1d) was synthesized by Kentaro Hoffmann and Audrey T. Henderson [4]. In 1961, in the frame of G. A. Razuvaev chemical school, Lebedev et al. prepared 2,2,6,6-tetramethyl-1-piperidinyloxil from acetone and ammonia (Fig. 1.1e) [5]. Portraits of the first discovers of nitroxide chemistry are presented in Fig. 1.2. The synthesis of all nitroxides cited above was a remarkable achievement. However, the resulting compounds were not adapted to further modification.



Edmond Frémy Heinrich Otto Wieland Grigory A. Razuvaev

Fig. 1.2 Portraits of the first discovers of nitroxide chemistry

1.2 "Golden" Decade (1962–1972)

From period 1962–1972 practically all corner-stone ideas in the area of nitroxides were exposed and developed. These ideas were sustained by fundamental theoretical and experimental investigations in chemistry and physics of nitroxides. First nitroxides, presented in Fig. 1.1, though their principle importance, did not find wide application and were not be able to serve as a basis for synthesis of new paramagnetic compounds in a broad scale.

Until 1962, chemists adhered to the paradigm that the most chemically reactive portion of a radical can be a group bearing spin electron. This paradigm was broken by M. B. Neiman and E. G. Rozanzev (Figs. 1.3 and 1.4), who introduced nitroxide reactions with a nitroxide (Fig. 1.1e) without direct involvement of the spin center. A novel class of stable nitroxides radicals presented in publication [6, 7] was first met with skepticism, and even strong criticism, from qualified and very professional members of the scientific community. But, later, more and more young enthusiasts joined the ranks of scientists applying this new tool in their research, and ever increasing reports of nitroxides were published. These pioneering works have laid a chemical basis for the method of numerous nitroxide applications, spin labeling in particular. As a consequence, rapid and extensive progress in the area and a real burst of works on synthesis and application of nitroxide in chemistry, physics, biology, and even in medicine have been broken. The theoretical and experimental data presented in this book clearly demonstrate both history and the current progress within the nitroxide "empire" of about publications.

In parallel, the dependence of ESR spectra of nitroxides on their molecular dynamics in solutions was demonstrated. The radical motion leads to averaging of spin

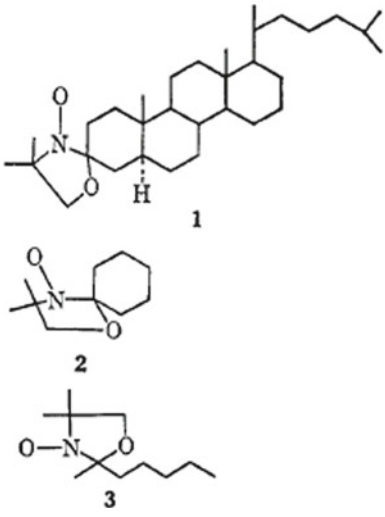
Fig. 1.3 Professor Moisey B. Neiman (1889–1967)

Fig. 1.4 Gertz I. Likhtenshtein, Anatoly L. Buchachenko, Eduard G. Rozanzev and Nikolay N. Semenov (1977)

electron spin nuclear interaction and to a drastic change of the ESR spectra. Correlation time for isotropic and anisotropic rotation τ_c for a nitroxide on a homogeneous media in the area of fast ($\tau_c = 10^{-9}$ 10^{-10} s) and slow ($\tau_c = 10^{-7}$ 10^{-8} s) motion can be estimated in the frame of theory developed by Kivelson [8] and Freed [9].

At the end of 1960 new classes of stable radicals, α-Nitronyl nitroxides [10] and Doxyl nitroxides performed by the J. F. W., Keana group Fig. 1.5 [11], possessing great potential for further wide applications, were synthesized.

Fig. 1.5 Doxyl nitroxides [11]



In the pioneering work of Golubev et al. [12], the first stoichiometric oxidation of alcohols to ketones mediated by an oxoammonium species with N^+-oxyl fragment was demonstrated.

Decisive breakthrough in the spin-labeling area was occurred owning a whole cascade of pioneering works of Howard McConnel and his collaborators. In the first publication in this series [13], a spin label, 2,2,6,5-tetramethyl-3-isocyanatopyrrolidine-1-oxyl, was covalently tethered to bovine serum albumin and to poly-L-lysine [13]. The nitroxide ESR spectra in solution indicated sensitivity of the nitroxide segment rotation to molecular motion of the labeled compound. Binding of a spin-labeled hapten, the 2,4-dinitrophenyl hydrazone of 2,2,6,6-tetramethyl 4-piperidone nitrogen oxide to antidinitrophenyl antibody was proved by the estimation of the rotational relaxation time of the bound hapten using analysis of the nitroxide ESR spectra [14] (Fig. 1.6).

Powerful potential capacity of spin-labeling method for study conformational transitions in proteins, which are necessary for its functional activity, was illustrated by the McConnell group on example of hemoglobin. Two reactive β-93SH groups in horse hemoglobin were modified with N-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolidinyl) iodoacetamide [15]. The ESR spectra of the labeled hemoglobin labeled were dependent on the degrees of oxygenation. The Hill constant n, a measure of the cooperativity of sigmoidal oxygen binding, was found to be $n = 2.3$ for the labeled hemoglobin, as compared to $n = 3$ for native hemoglobin. It was concluded that each of subunit of the protein tetramers undergoes a substantial conformational change when that subunit binds a molecule of oxygen. To tackle the problem of allosteric interactions in enzymes and proteins not having a quaternary structure, in works of G. I. Likhtenshtein group [16–18] the enzyme lysozyme was spin labeled by the hystidine-15

Fig. 1.6 Professor Harden McConnel (1927–2014)

group located at the distance 15 A from the substrate-binding center. Addition of specific inhibitors NAG and NAG–NAG induces distinct changes in ESR spectra, which were in a good quantitative agreement with the extent of the substrate binding. Similar transglobular effect was also detected in spin-labeled myoglobin [17].

The first work on the use spin labeling in enzyme catalysis has been published by L. J. Berliner, H. M. McConnell in 1966 [19]. It was shown that the nitroxide spin-labeled substrate, DL-2,2,5,5-tetramethyl-3-carboxypyrrolidine- *p*-nitrophenyl ester, can be used to study the activity of the proteolytic enzyme α-chymotrypsin. The most conclusive work in the investigation of active serine group in proteolytic enzymes based on phosphate and nitrobenzene derivatives was carried out by Hsia et al. [20]

One of remarkable achievement of the spin-labeling methods has been quantitative characterization of flexibility of model and biological membranes. Incorporation of hydrophobic nitroxide probe 2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-'y-carboline-3-oxyl into the sodium dodecyl sulfate remarkably decreased in the rate of tumbling of the probe as compare with its motion on solutions, which is quantitatively described by a rotational correlation time, τ_c [21]. Progress in the study of biological and model membranes with the use of the steroid and lipid spin probes was demonstrated in the works of Keana [11] and MacConnell [15] groups. For example, in sonicated phospholipid dispersions of the walking leg nerve fibers of *Homarus americanus,* and in erythrocytes oriented by hydrodynamic shear, the nitroxide probes motion with rotational diffusion frequencies of the order of 10^7 to 10^8 s^{-1} was revealed. The first approach for quantitative investigation of lateral diffusion in membranes with the use of spin labels was developed by McConnell and McFarland [15], Hubbell and McConnell [22]. In a typical experiment, small drops of a lipid spin probe were inserted to films of oriented multilayers of lecithin. Because of radical diffusion, the probe ESR spectrum changes from a singlet, which is the characteristic of large local concentration, to a triplet for diluted radicals. Analysis of the process kinetics allowed to calculate the coefficient of translational lateral diffusion.

The application of nitroxide radicals to covalent modification and the study of the structure, dynamic and conformational changes of nucleic acids (poly rA, poly rU, and poly rG) were based on the principles established for proteins [23–25]. The possibilities of non-covalently bound nitroxide probes were first demonstrated in [24]. As early as in 1968, A. M. Vasserman, A. L. Buchachenko, A. L. Kovarskii, and M. B. Neiman have shown powerful potentiality of nitroxide spin-label methods in investigation molecular dynamics and microstructure of high molecular mass compounds [26]. The first observation of effect of nitroxide on radical polymerization, that is inhibition with nitroxide mono- and biradicals, was made in 1966 by the M. B. Neiman group [27]. The inhibiting effect of the nitroxides on styrene polymerization at 50 °C, initiated by azodiisobutyronitrile, was interpreted as a cross-recombination of nitroxides and polymer radicals which led to the process termination.

Synthesis and investigation of the chelate complex Cu^{+2}-Schiff bases ligand derivative of TEMPO, which appeared to be the first transition metal complexes with paramagnetic ligands, were carried out in 1969 [28].

Method of double spin labeling, invented by Likhtenshtein in 1968 [29], is based on specific modification of chosen groups of the object of interest by two or several spin labels, nitroxides, or complexes of paramagnetic metal followed by the analysis of effects of the spin–spin interactions on the label ESR spectra. This approach allows to estimate the distance between the paramagnetic centers up to 2.5 nm [29–32]. Later, the higher sensitivity of spin–lattice relaxation time of a radical to interactions between the radical and paramagnetic ions up to 100 nm was demonstrated [17, 18, 33]. In parallel, the effects of spin-exchange interaction on the labels ESR spectra were used for establishment of structure of systems under investigation, such as iron–sulfur clusters in nitrogenase, ferredoxins, and non-heme protein [34].

The first version of method of spin label–spin probe method (SLSPM) proposed by Likhtenshtein and coworkers [35] was based on a dynamic exchange spin–spin interaction of a stable radical, mostly nitroxide, attached to molecular object of interest with a spin probe which are chemically inert paramagnetic species capable of diffusing freely in solution. The value of the dynamic exchange rate constant k_{ex} depends on microviscosity, steric hindrances, and distribution of electrostatic charges, and the method was intensively employed for investigating microstructure of object under interest [17, 18, 36–38]. In parallel, a variant of the SLSP method based on direct measurement of electron spin–lattice relaxation times was developed in Hide group [39].

Principles of application of nitroxide spin-label method as a tool for experimental investigation of proteins molecular dynamics ("breathing") have been formulated at the end of 1960 by Likhtenshtein [40, 41] and then were implemented in collaborative works. Parameters of motion of a nitroxide in the labeled protein may serve as characteristics of surrounding media dynamics. For example, the monitoring motion of hydrophobic nitroxide spin probe in binding site of human serum albumen revealed low amplitude wobbling of the probe with the correlation time $\tau_c \approx 10^{-8}$ s modulated by the binding site dynamics [41]

Protective antitumor activity of nitroxide in animals was demonstrated in pioneering work of N. P. Konovalova, M. B. Neiman, E. G. Rozanzev, and Emanuel N. M. in 1964 [42]. Later in 1970, G. Sosnovsky and M. Konieczny have performed syntheses anticancer drugs belonging to the class of alkylating agents containing aminoxyl radicals [43].

Continuous wave electron–electron double resonance (CW ELDOR) independently reported in 1968 by the Hyde and Freed [44], Bendersky and Blumenfeld [45] groups allowed to resolve problems that are not accessible in the CW-ESR. The ELDOR spectra were shown to be sensitive to very slow rotations which may provide unique information on the details of molecular dynamics and can be used for distance estimation between centers bearing spin electron.

Thus, chemical and physical contributions within the "golden" decade have formed the basis for subsequent progress in the area. In subsequent decades, fundamentals and tendencies laid out in the "golden" period were intensively developed and number of publications in the area accelerated almost in a geometric progression [46–49] and references therein.

1.3 New Era

Starting from the pioneering works of Ya. S. Lebedev and his colleagues [50], problems of poor resolution of the 3 cm ESR have been solved by the use of the high-field–high-frequency (148 GHz), high-resolution 2-mm EPR spectroscopy. The comprehensive review on advanced biomolecular EPR spectroscopy addresses both the EPR and NMR communities has been recently published [51]. New contributions of K. Mebius and W. Lubiz groups to high-field–high-frequency EPR were summarized.

Synthesis of imidazoline and imidasolidine nitroxides (Fig. 1.7) markedly expanded ability of nitroxides for its applications as spin labels, ligands for materials with ferromagnetic properties, inhibitors in polymer processing, and initiators for "living" radical polymerization [52, 53] and references therein.

Figure 1.8 shows photography of leaders of groups involved in the nitroxide spin labeling till 1979.

Measurement of distance between spin labels by analysis of line shape of ESR spectra using new computation methods was carried out for double and multiple labeled proteins and enzymes [54]. Various aspects of spin-label magnetic resonance studies on lipid–lipid and lipid–protein interactions with integral proteins were

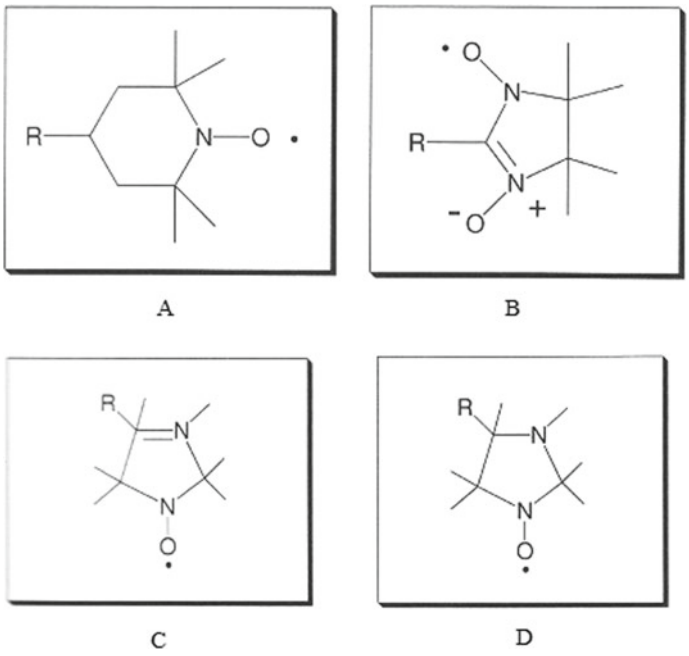


Fig. 1.7 Nitroxide derivatives: piperidine A, nitronyl B, imidasoline C, and imidasolidine D, and nitronyl D

Fig. 1.8 Leaders of groups involved in the nitrixide spin labeling till 1979. From left to right: rector of the Pech University, Leonid B. Volodarsky, Larry Berliner, Gertz Liktenshtein, R. Rassat, John Keena, George Sosnovsky, Kalman Hideg

reviewed [55–57]. Synthesis and determination of structural characteristics of magnetic all-organic radical liquid crystals were in focus of the program developed by R. Tamura with colleagues [58].

Works of 1970–1990 on chemical modification of DNA with nitroxide derivatives and ESR examination of structure and dynamics of the labeled biopolymer have laid the basis for further detail investigations in this extremely important area of molecular biology [59, 60]. After the first publication on labeling of linear polymers such as of cotton, silk, and wool by trichlorotriazine-based nitroxide [61], a series work on the investigation of cotton fibers and cellulose was reported by Marupov and Likhtenshein groups [62] and references therein.

Hundreds of complexes transition metals with nitroxide ligands were synthesized and investigated (for review see [63] and references therein).

The CW ESR saturation techniques were employed for the investigation of depth of immersions of nitroxide spin probes radicals up to 40 Å in lipid phases of biomembranes [64]. High sensitivity of spin–lattice relaxation parameters of nitroxide was also taken into consideration at development of theory of very slow motion ($\tau_c = 10^{-3}$–10^{-6} s) [65].

A new impact in nitroxide-mediated polymerization has started when P. G. Griffiths, E. Rizzardo, and D. H. Solomon showed that it was possible to prepare well-controlled and living (homo-, co) polymer by radical polymerization in the presence of nitroxyl radical as a controlling agent **[**66–68**]**. Since its discovery nitroxide-mediated radical polymerization (NMP) was proved to be a powerful method to synthesize well-defined macromolecular architectures with precisely controlled topologies, compositions, microstructures, and functionalities [69–73]. A significant contribution in the area has been brought by the groups of Solomon [69], Grishin [70], Fisher [71], and Bagryanskaya [72]. Kinetic aspects of the nitroxide-mediated radical polymerization were discussed in details in comprehensive review [73].

Significant progress in the high-field–high-frequency (HFHF) ESR has been achieved [74–76] especially owning to the design of millimeter-wave quasi-optic technique, permitting the construction of a 9-Tesla, 250-GHz (1.2 mm) [55]

After remarkable invention of echo-detected ELDOR (three-pulse ELDOR, PELDOR, double electron–electron resonance DEER) by Milov et al. [77] and its first application, a real burst of development of new ESR pulse methods has been broken [78–92]. Other methods used for the study of nitroxide labeling objects are four [80, 89, 90] and five-pulse techniques [91, 92], method to determine the effective saturation factor of nitroxide radicals for dynamic nuclear polarization (DNP) experiments in liquids [83], two-dimensional ELDOR [84]), high-frequency pulsed ENDOR/EPR [85], double and multiple quantum coherence pulsed ESR (DQC ESR) [79, 81], and ESR spectra hole burning [87].

The invention and use of site-directed mutagenesis in combination with modern ESR spectroscopy gave new breath to the nitroxide spin-labeling method. Site-directed spin-labeling (SDSL) designed by Hubbell group [93] is the substitution of a selected amino acid for cysteine via the site-directed mutagenesis technique following chemical modification with a sulfhydryl reactive nitroxide radical, *S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl) methyl methanesulfonothioate) (MTSL). The main advantage of the SDSL is the possibility to overcome the limitations of a choice of amino acids suitable for the labeling in native proteins. The efficiency of combination of the site-directed spin-labeling with the advance pulse techniques can be illustrated by numerous works, for example in studies of T4 Lysozyme [94] and mutants maltose-binding protein (MBP) 09-11 [95].

Recently, new spin-labeling approaches have been shown to be an attractive alternative to the traditional method of nitroxide spin labels for pulse dipolar ESR (PD ESR). The first one, based on high-spin Gd^{3+} ($S = 7/2$) complexes, was designed and developed in Goldfarb group [96]. A combined method, utilizing NMR and EPR spectroscopies, was employed to compare different types of nitroxide-based and Gd(III)-based spin labels attached to isolated RBDs of the polypyrimidine tract-binding protein 1 (PTBP1) and to short RNA fragments [97] in complexes with short

RNAs. An idea of the use of carbon-centered triarylmethyl (trityl) radicals instead of nitroxides for nanometer distance measurements was first introduced and realized in 2012 [98]. Nowadays, triarylmethyl radicals TMA are successively used as spin labels for studies on the structure of proteins and nucleic acids utilizing site-directed spin labeling (SDSL) and pulse dipolar EPR spectroscopy [99, 100].

Spin oximetry, first reported by Subczynski and Hide [101], is a version of spin label–spin probe method [35, 39] in which molecular oxygen plays role of spin probe. A method invented for measurement of the oxygen diffusion–concentration product was based on the dependence of the spin–lattice relaxation time T_1 of the spin label, detected by using saturation recovery (SR), on the bimolecular collision rate with oxygen. Various aspects of the spin oximetry applications have been reviewed [102–104].

As it was pioneered in [105], EPR spectra of stable nitroxides of the imidazoline and imidazolidine types are sensitive to pH and can serve as spin pH probes. Data on synthesis and application of a wide set of pH-sensitive nitroxides of different sensitivity, stability to reduction, lipophilicity and its covalent-binding macromolecules have been reported [106, 107]. To quantitatively determine SH groups in high- and low-molecular weight compounds, a disulfide biradical (RS-SR), where R is imidazoline residue, has been used [108, 109]. The biradical is shown to participate in a thioldisulfide exchange reaction with compounds containing SH groups. In this case, the ESR spectra of the biradical RS-SR and the resulting monoradical R-SH are different.

Nitroxide radicals have found various applications in the field of materials science. The landmark was the discovery by M. Kinoshita group et al. in 1991 [110] who prepared the first purely organic ferromagnet with respect to a nitronyl nitroxide, 2-(4-nitrophenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide Since then, stable nitric oxide structures have been widely used as the spin source and building block for the elaboration of organic or molecule-based magnetic materials [58, 111, 112]. In the last two decades, π-conjugated superparamagnetic organic compounds including polymer magnets with stability at ambient temperature and/or higher magnetic ordering temperatures have been attracting attention as models of multispin systems and potential magnetic devices [113]. Novel molecular magnets Cu(hfac)2LR with nitronyl ligands were prepared and investigated in details in Ovcharenko group [114].

The spin redox probe techniques utilizing ability of nitroxides to be reacted with reducing agent to corresponding hydroxyl amine are widely used for quantitative characterization of redox processes and protection from radical damage by CW ESR spectroscopy [115–118] and references therein.

Novel methods of fast and sensitive analysis of antioxidant status of biological systems, spin redox probing and spin trapping, investigation of molecular dynamics, models for studies of photophysical and photochemical processes, and construction of new magnetic light-sensitive materials are based upon the use of dual fluorophore–

nitroxide compounds were designed and developed [119–126]. In pioneering work of Likhtenshtein and colleagues [120], three fundamental effects were first demonstrated, namely (1) The nitroxide fragment is a strong quencher of the fluorescence. (2) The radical photoreduction can lead to the decay of the EPR signal and the drastic increase of the fluorescence intensity. (3) The photoreduction kinetics strongly depends on molecular dynamics of environment. Next principle step was a series of excellent papers by Blough and Simpson [123] in which the potential of these tethered, optically switching molecules as potent redox and radical trapping probes was realized. A significant contribution to the synthesis and use of the dual compounds was made by the groups of Bottle [124] and Braslau [125]. Dual fluorescence nitroxide compounds are effectively used as convenient photochemical and photophysical models and form the basis for photoswitching magnetic materials [126] and references therein.

Stable nitroxide free radicals were utilized as antioxidants in animal models and human diseases (e.g., cancer) to protect processes of formation reactive oxygen species, ROS ($O_2^{-\cdot}$, H_2O_2, ·OH) involving oxidative stress [127–130]. The works, in which the principle possibility of effectiveness of the spin trapping was demonstrated, came to the light in 1968 [131], later numerous theoretical and experimental studies of the spin trapping of inorganic and organic radicals were carried out [132, 133]. After sited above pioneering works [42, 43], biologically active spin-label molecules have been the focus of biophysical, biochemical, and synthetic and medicinal chemical studies [134–136]. A noticeable contribution to the theory of spin relaxation and its application was made in the works of Eaton and Eaton [137].

1.4 Concluding Remarks

Author of this review wrote in the first book on spin labeling:

> *Likhtenshtein G. I.: Spin-Labeling Method in Molecular Biology.* Moscow, Nauka (In Russian). 1974: "It is thus our hope that spin labeling will continue to be an effective tool for solving various complicated problems in molecular biology". Now after 45 years, it is evident that present-day reality has surpassed all optimistic expectations. As far as concern outlook for further developments, there are all reasons to believe that slow but permanent progress in the area would continue in the next decades. Nevertheless, who knows, new unexpected bright ideas would be launched and implemented and ensure vigorous success, unexpected today.

Portraits of scientists who significantly contributed in nitroxide chemistry, physics, and its miscellaneous applications are displayed in Fig. 1.9.

Fig. 1.9 Gallery of scientists who significantly contributed in nitroxide chemistry, physics and its miscellaneous applications

References

1. E. Fremi, Annales de Chimie et de Physique. Serie **3**(15), 408–488 (1845)
2. O. Piloty, B.G. Schwerin, Berichte den Deutschen Chemischen Gesselschaft **34**, 1870–1877 (1901)
3. H. Wieland, M. Offenbacher, Diphenylnitric oxide, a new organic radical with tetravalent nitrogen. Ber. Dtsch. Chem. Ges. **47**, 2111–2115 (1914)
4. A.K. Hoffmann, A.T. Henderson, A new stable radical: Di-t-Butyl nitroxide. Am. Chem. Soc. **83**, 4671–4672 (1961)
5. O.L. Lebedev, M.L. Khidekel, V.A. Razuvaev, Dokl. Acad. Nauk. **140**, 1327–1331 (1961)
6. M.B. Neiman, E.G. Rozantzev, Y.G. Mamedova, Free radical reactions involving no unpaired electrons. Nature **196**, 472–474 (1962)
7. E.G. Rozantzev, *Free Nitroxyl Radicals* (Plenum Press, New York, 1970)
8. D. Kivelson, Theory of EPR line widths of free radicals. J. Chem. Phys. **33**, 1094–1106 (1960)
9. J.H. Freed, Theory of the ESR spectra of nitroxids, in *Spin Labeling. Theory and Applications*, vol. 1, ed. by L. Berliner (Academic Press, New York, 1976)
10. J.H. Osiecki, E.F. Ullman, Studies of free radicals. I. alpha.-Nitronyl Nitroxides, a new class of stable radicals. J. Am. Chem. Soc. **90**, 1078–1079 (1968)
11. J.F.W. Keana, S.B. Keana, D. Beetham, New versatile ketone spin label. J. Am. Chem. Soc. **89**, 3055–3056 (1967)
12. V.A. Golubev, E.G. Rozantsev, M.B Neiman, Some reactions of free iminoxyl radicals with the participation of the unpaired electron. Bull. Acad. Sci. USSR, Div. Chem. Sci. **14**, 1898–1904 (1965)
13. T.J. Stone, T. Buckman, P.L. Nordio, H.M. McConnell, Spin-labeled biomolecules. Proc. Natl. Acad. Sci. **54**, 1010–1017 (1965)
14. L. Stryer, O.H. Griffith, A spin-labeled hapten. Proc. Natl. Acad. Sci. **54**, 1785–1791 (1965)
15. H.M. McConnell, B.G. McFarland, Physics and chemistry of spin labels. Q. Rev. Biophys. **3**, 91–136 (1970)
16. Y.D. Akhmedov, G.I. Likhtenshtein. L.V. Ivanov, Y.V. Kokhanov, Investigation of the lysozyme macromolecule by a spin-labeling method. Dokl. Acad. Nauk. SSSR **205**, 372–376 (1972)
17. G.I. Likhtenshtein, Spin labeling method in molecular biology (Moscow Nauka, 1974) (In Russian)
18. G.I. Likhtenshtein, Spin labeling method in molecular biology (N.Y., Wiley Interscience, 1976)
19. L.J. Berliner, H.M. McConnell, A spin-labeled substrate for α-chymotrypsin. Proc. Natl. Acad. Sci. **55**, 708–712 (1966)
20. J.S. Hsia, D.I. Kosman, J.S. Piette, Organophosphate spin-label studies of inhibited esterases, α-chymotrypsin and cholinesterase biochem. Biophys. Res. Commun. **36**, 75–78 (1969)
21. A.S. Waggoner, O.H. Griffith, C.R. Christensen, Magnetic resonance of nitroxide probes in micell-containing solutions, Proc. Natl. Acad. Sci. **57**, 1198–120 (1967)
22. W.L. Hubbell, H.M. McConnell, Orientation and motion of amfiphilic membranes. Proc. Natl. Acad. Sci. **64**, 20–27 (1969)
23. I.C.P. Smith, T. Yamane, Spin labeled nucleic acids. Proc. Natl. Acad. Sci. **58**, 884–887 (1967)
24. B.I. Sukhorukov, A.M. Wasserman, L.I. Kozlova, A.L. Buchachenko, Use the spin labeling method for study of the nucleic acid-water system. Dokl. Acad. Nauk SSSR. **177**, 454–457 (1967)
25. A.M. Bobst, Studies of spin-labeled polyrybo adenylic acids. Biopolymers **11**, 1421–1433 (1972)
26. A.M. Vasserman, A.L. Buchachenko, A.L. Kovarskii, M.B. Neiman, Study of molecular motion of polymers by method of paramagnetic probe. Vysok. Soedin., Seriya A, **10**, 1930–1936 (1968)
27. L.V. Ruban, A.L. Buchachenko, M.B. Neiman, Y.V. Kokhanov, Inhibition of radical polymerization with nitroxide mono-and biradicals. Vysok. Soedin. **8**, 1642–1646 (1966)

28. A.A. Medzhidov, L.N. Kirichenko, G.I. Likhtenshtein, Izvestiya akademii nauk SSSR, Seriya Khimicheskaya, 698–700 (1969)
29. G.I. Likhtenshtein, Determination of the topography of proteins groups using specific paramagnetic labels. Mol. Biol. (Moscow) **2**, 234–240 (1968)
30. J.C Taylor, S. Leigh, M. Cohn, The effect of dipole-dipole interaction between nitroxide radical and a paramagnetic ion on the line shape of the esr spectra of radical. Proc. Natl. Acad. Sci. **64**, 219–206 (1969
31. A.V. Kulikov, G.I. Likhtenshtein, E.G. Rozantzev, V. Suskina, A. Shapiro, Nitroxide Bi- and polyradicals as standard models for distance estimation between the nitroxide moieties. Biofizika **17**, 42–49 (1972)
32. A.I. Kokorin, K.I. Zamaraev, G.L. Grigoryan, V.P. Ivanov, E.G. Rozantsev, Distance estimation between nitroxyl radicals. Biofizika **17**, 34–41 (1972)
33. A.V. Kulikov, G.I. Likhtenshtein, The use of spin-relaxation phenomena in the investigation of the structure of model and biological systems by method of spin labels. Adv. Molecul. Relax. Proc. **10**, 47–78 (1977)
34. L.A. Syrtsova, L.A. Levchenko, E.N. Frolov, G.I. Likhtenshtein, T.N. Pisarscaya, L.V. Vorob'ev, V.A. Gromoglasova, Structure and function of the nitrogenase components from Azotobacter vinelandii. Mol. Biol. (Moscow) **5**, 726–734 (1971)
35. G.I. Likhtenshein, Y.B. Grebenshchikov, P. Bobodzhanov, Y.V. Kokhanov, Method of spin-labels spin probes. Molecul. Biol. (Moscow) **4**, 682–691 (1970)
36. G.I. Likhtenshtein, Study on the proteins microstructure by method of spin-label paramagnetic probe. Mol. Biol. (Moscow) **4**, 782–789 (1970)
37. G.I. Likhtenshtein, Y.B. Grebentchikov, E.G. Rosantev, V.P. Ivanov, Study on the electrostatic charges in proteins by method of paramagnetic probes. Mol. Biol. (Moscow) **6**, 498–507 (1972)
38. G.I. Likhtenshtein, *New Trends in Enzyme Catalysis and Mimicking Chemical Reactions* (Kluwer Academic/ Plenum Publishers, N.Y, 2003)
39. J.S. Hyde, H.M. Swartz, W.E. Antholin, The spin probe—spin label method, in *Biological Magnetic Resonance. Spin Labeling. Theory and Application*, vol. 8, ed. by L.J. Berliner J. Reubin (Plenum Press, New York, 1979), pp. 305–339
40. G.I. Likhtenshtein, A.P. Pivovarov, P.K. Bobodzhanov, E.G. Rozantsev, N.B. Smolina, Study of dynamic structure of proteins and enzymes by specific luminescent and paramagnetic labels. Biofizika **13**, 396–400 (1968)
41. E.N. Frolov, G.I. Likhtenshtein, N.V. Kharakhonycheva, Investigation of dynamic structure of human serum albumin by the method of spin-probe. Molek. Boil. (Moscow) **8**, 886–893 (1974)
42. N.P. Konovalova, G.N. Bogdanov, V.B. Mille, M.B. Neiman, E.G. Rozanzev, G. Emanuel, N.M. Doklady Akademii nauk SSSR **157**, 707–709 (1964)
43. G. Sosnovsky, M. Konieczny, Synthesis of phosphoorganic derivatives of nitroxide radicals, Naturforshung33b, 792–804 (1970)
44. J.S Hyde, J.C.W. Chien, J.H. Freed, Electron–electron double resonance of free radicals in solution. J. Chem. Phys. **48**, 4211–4226 (1968)
45. V.A. Benderskii, L.A. Blyumenfel'd, P.A. Stunzhas, F.A. Sokolov, Double electron-electron resonance of triplet excitons in ion-radical salts. Nature **220**, 365–367 (1968)
46. N. Kocherginsky, H.M. Swarts, *Nitroxide spin labels: Reactions in biology and chemistry* (CRC Press, 1995)
47. L.J. Berliner (ed.), *Spin Labeling: Next Millennium, Biological Magnetic Resonance* (Book 14) (Springer, 1998)
48. G.I. Likhtenshtein, *Biophysical Labeling Methods in Molecular Biology* (Cambridge University Press, Cambridge, N.Y., 1993)
49. G.I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A.I. Smirnov, R. Tamura, *Nitroxides: Application in Chemistry, Biomedicine, and Materials Science* (WILEY-VCH, Weinhem, 2008)

50. O.Y. Grinberg, A.A. Dubinskii, V.F. Shuvalov, L.G. Oranskii, V.I. Kurochkin, Y.S. Lebedev, EPR submillimeter spectroscopy of free radicals. Doklady Akademii Nauk SSSR **230**, 884–887 (1976)
51. K. Moebius, W. Lubitz, N. Cox, A. Savitsky, Biomolecular epr meets nmr at high magnetic fields. Magnetochemistry **4**, 50 (2018)
52. L.B. Volodarsky, V.A. Reznikov, V.I. Ovcharenko, *Synthetic Chemistry of Stable Nitroxides* (CRC Press Inc, Boca Raton, FL, USA, 1994)
53. L.B.Volodarsky, I.A. Grigor'ev, R.Z. Sagdeev, Stable imidazoline nitroxides, in *Biological Magnetic Resonance*, vol. 2, ed. by L. Berliner, J. Reuben (Plenum Press, 2000), p. 73
54. E.J. Hustedt, A.H. Beth, Structural information from CW-EPR spectra of dipolar-coupled nitroxide spin-labels, in *Biological Magnetic Resonance*, vol. 19, ed. by S.S. Eaton, G.R. Eaton, L.J. Berliner (2001)
55. J.H. Freed, New technologies in electron spin resonance. Ann. Rev. Phys. Chem. **51**, 655–689 (2000); D. Marsh, in *Handbook of Lipid Bilayers*, 2nd edn. (CRC Press, 2013)
56. A.K. Smith, J.H. Freed, Dynamics and ordering of lipid spin-labels along the coexistence curve of two membrane phases: an ESR study. Chem. Phys. Lipids **165**(3), 348–361 (2012)
57. M.A. Hemminga, L. Berliner, *ESR Spectroscopy in Membrane Biophysics* (Springer, 2007)
58. A.K. Vorobiev, N.A. Chumakova, D.A. Pomogailo, Y. Uchida, K. Suzuki, Y. Noda, R. Tamura, Determination of structural characteristics of all-organic radical liquid crystals based on analysis of the dipole—dipole broadened EPR spectra. J. Phys. Chem. B **118**, 1932–1942 (2014)
59. B.H. Robinson, C. Mailer, G. Drobny, Site-specific dynamics in experiments DNA. Annu. Rev. Biophys. Biomol. Struct. **26**, 629–635 (1997)
60. R.S. Keyes, Y.Y. Cao, E.V. Bobst, J.M. Rosenberg, A.M. Bobst, Spin-labeled nucleotide mobility in the boundary of the EcoRI endonuclease binding site. J. Biomol. Struct. Dyn. **14**, 163–172 (1996)
61. R.M. Marupov, P.K. Bobodzhanov, I.K. Usupov, E.N. Frolov, G.I. Likhtenshtein, Study of temperature stability of cotton fibers by spin labeling. Biofizika **24**, 519–523 (1979)
62. I.K. Ysupov, G.I. Likhtenshtein, Study of microstructure and molecular dynamics of cotton and cellulose fibers by methods of physical labels. Int. Res. J. Pure & Appl. Chemistry. **6**(3), 105–119 (2015)
63. S.S. Eaton, G.R. Eaton, Interaction of spin labels with transition metals. Coord. Chem. Rev. **83**, 29–72 (1988)
64. A.V. Kulikov, E.S. Cherepanova, V.R. Bogatyrenko, Determination of the closest distance between a radical and a paramagnetic Ion. Theor. Exper. Chem. **17**, 618–626 (1981)
65. J.S. Hyde, L. Dalton, Very slowly tumbling spin labels: adiabatic rapid passage. Chem. Phys. Lett. **16**, 568–572 (1972)
66. G.I. Likhtenshtein, Depth of immersion of paramagnetic centers. in *Magnetic Resonance in Biology*, vol. 19, ed. by L. Berliner, S. Eaton, G. Eaton (2000), pp. 309–347
67. D.H. Solomon, E. Rizzardo, P. Cacioli, Eur. Pat. Appl. (1985)
68. P.G. Griffiths, P.G.E. Rizzardo, D.H. Solomon, Initiation pathways in the polymerization of alkyl methacrylates with tert-butoxy radicals. J. Macromol. Sci., Chem. A **17**, 45–50 (1982)
69. D.H. Solomon, Genesis of the CSIRO polymer group and the discovery and significance of nitroxide-mediated living radical polymerization. J. Polym., Part A: Polym. Chem. **43**(23), 5748 (2005)
70. D.F. Grishin, I.D. Grishin, Controlled radical polymerization: prospects for application for industrial synthesis of polymers (Review) Russ. J. Appl. Chem. **84**(12), 2021–2028 (2011)
71. H. Fisher, M. Souaille, The Persistent radical effect in living radical polymerization –border cases and side-reactions. Macromol. Symp. **174**, 231–240 (2001)
72. M. Edeleva, G. Audran, S. Marque, E. Bagryanskaya, Smart control of nitroxide-mediated polymerization initiators' reactivity by pH, complexation with metals, and chemical transformations. Materials (Basel) **12**(5), 688 (2019)
73. E.G. Bagryanskaya, S.R.A. Marque, Kinetic aspects of nitroxide mediated polymerization. in *Nitroxide Mediated Polymerization: From Fundamentals to Applications in Materials Science*, ed. by Gigmes (RCS, 2015), pp. 45–113

74. V.I. Krinichny, *2-mm Wave band EPR Spectroscopy of Condensed Systems*. (CRC Press, Boca Raton, 1995)
75. S.K. Misra, J.H. Freed, Molecular motions, *Multifrequency Electron Paramagnetic Resonance*, ed. by S.K. Misra (2011), pp. 497–544
76. G.R. Eaton, S.S. Eaton, Multifrequency electron spin-relaxation times, in *Distance Measurements in Biological Systems by EPR Biological Magnetic Resonance*, vol. 19, ed. by L.J. Berliner, S. Eaton, G.R. Eaton (Springer, 2000), pp. 719–753
77. A.D. Milov, K.M. Salikhov, Y.D. Tsvetkov, Fiz. Tverd. Tela (Leningrad) **15**, 1187 (1973)
78. A.D. Milov, Y.D. Tsvetkov, A.G. Maryasov, M. Gobbo, C. Prinzivalli C.M. De Zotti, F. Formaggio, C. Toniolo, Conformational properties of the spin-labeled tylopeptin B and heptaibin peptaibiotics based on PELDOR spectroscopy. Appl. Magn. Res. **44**, 495–508 (2013)
79. P.P. Borbat, J.H. Freed, Multiple-quantum ESR and distance measurements. Chem. Phys. Lett. **313**, 145–154 (1999)
80. A. Schweiger, G. Jeschke, *Principles of Pulse Electron Paramagnetic Resonance* (Oxford University Press, Oxford, 2001)
81. P.P. Borbat, J.H. Freed, Double-quantum ESR and distance measurement. in *Biological Magnetic Resonance,* vol. 19, ed. by L.J. Berliner, S. Eaton, G.R. Eaton (Springer, 2001), pp. 383–460
82. A.D. Milov, R.P. Samoilova, Y.D. Tsvetkov, V.A. Gusev, F.M. Formaggio, M. Crisma, C. Toniolo, J. Raap, Spatial distribution of spin-labeled trichogin GA IV in the gram-positive bacterial cell membrane determined from PELDOR data. Appl. Magn. Reson. **23**, 81–95 (2002)
83. M.-T. Türke, M. Bennati, Saturation factor of nitroxide radicals in liquid DNP by pulsed ELDOR experiments. Phys. Chem. Chem. Phys. **13**, 3630–3633 (2011)
84. Y.-W. Chiang, A.J. Costa-Filho, B. Baird, J.H. Freed, 2D-ELDOR study of heterogeneity and domain structure changes in plasma membrane vesicles upon cross-linking of receptors. J. Phys. Chem. B **115**, 10462–10469 (2011)
85. T.I. Smirnova, A.I. Smirnov, S. Pachtchenko, O.G. Poluektov, Geometry of hydrogen bonds formed by lipid bilayer nitroxide probes: a high frequency pulsed ENDOR/EPR study. J. Am. Chem. Soc. **129**, 3476–3477 (2007)
86. S. Saxena, J.H. Freed, Theory of double quantum two-dimensional electron spin resonance with application to distance measurements. J. Chem. Phys. **107**, 1317–1134 (1997)
87. S.A. Dzuba, A. Kawamori, Selective hole burning in EPR: spectral diffusion and dipolar broadening. Concepts Magn. Reson. **8**, 49–61 (1996)
88. A.D. Milov, Y.D. Tsvetkov, A.G. Maryasov, M. Gobbo, C. Prinzivalli C, M. De Zotti, F. Formaggio, C. Toniolo, Conformational properties of the spin-labeled tylopeptin B and heptaibin peptaibiotics based on PELDOR spectroscopy. Appl. Magn. Res. **44**, 495–508 (2013)
89. G. Jeschke, M. Panier, H.W. Spies, Double electron-electron resonance, in *Distance Measurements in Biological Systems by EPR, in Biological Magnetic Resonance*, vol. 19, ed. by L.J. Berliner, S. Eaton, G.R. Eaton (Springer Verlag, 2001), pp. 493– 512
90. G. Jeschke, DEER distance measurements on proteins. Ann. Rev. Phys. Chem. **63**, 419–446 (2012)
91. I. Krstić, R. Hänsel, O. Romainczyk, J.W. Engels, V. Dötsch, T.F. Prisner, Long-range distance measurements on nucleic acids in cells by pulsed EPR spectroscopy. Angew. Chem. Int. Ed. Engl. **50**, 5070 (2011)
92. P.P. Borbat, E.R. Georgieva, J.H. Freed, Improved sensitivity for long-distance measurements in biomolecules: five-pulse double electron-electron resonance. J. Phys. Chem. Lett. **4**, 170–175 (2013)
93. W.L. Hubbell, R.L. Gross, M.A. Lietzow, Curr Opin Struct Biol **8,** 649–656 (1988)
94. P.P. Borbat, H. Mchaourab, J.H. Freed, Protein structure determination using long-distance constraints from double-quantum coherence ESR: study of T4 lysozyme. J. Am. Chem. Soc. **124**, 5304–5314 (2002)
95. B. Selmke, P.P. Borbat, N. Chen, V. Raghavan, J.H. Freed, W.E. Trommer, Open and closed form of maltose binding protein in its native and molten globule state as studied by electron paramagnetic resonance spectroscopy. Biochemistry **57**(38), 5507–5512 (2018)

96. A. Feintuch, G. Otting, D. Goldfarb, Gd3+ spin labeling for measuring. Distances in biomacromolecules: why and how? in *Methods in Enzymology*, vol. 563 (2015), pp. 416–457
97. C. Gmeiner, G. Dorn, F.H.T. Allain, G. Jeschke, M. Yulikov, Spin labelling for integrative structure modelling: a case study of the polypyrimidine-tract binding protein 1 domains in complexes with short RNAs. Phys. Chem. Chem. Phys. **19**, 28360 (2017)
98. G.W. Reginsson, N.C. Kunjir, S.T. Sigurdsson, O. Schiemann, Trityl radicals: spin labels for nanometer-distance measurements. Chemistry **18**, 13580–13584 (2012)
99. M.K. Bowman, C. Mailer, H.J. Halpern, The solution conformation of triarylmethyl radicals. J. Magn. Reson. **172**, 254 (2005)
100. O. Krumkacheva, E. Bagryanskaya, EPR-based distance measurements at ambient temperature. J. Magn. Reson. **280**, 117–126 (2017)
101. W.K. Subczynski, J.S. Hyde, The diffusion-concentration product of oxygen in lipid bilayers using the spin-label T_1 method. Biochim. Bioph. Acta **643**, 283–291 (1981)
102. R. Springett, H.M. Swartz, Measurements of oxygen in vivo: overview and perspectives on methods to measure oxygen within cells and tissues. Antioxid. Redox Signal. **9**, 1295–1301 (2007)
103. G. Ilangovan, J.L. Zweier, P. Kuppusamy, Oxygen sensing. Methods Enzym **381**, 747–762 (2004)
104. W.K. Subczynski, L. Mainali, T.G. Camenisch, W. Froncisz, J.S. Hyde, Spin-label oximetry at Q-and W-band. J. Magn. Reson. **209**, 142–148 (2011)
105. V.V. Khramtsov, L.M. Weiner, I.A. Grigor'ev, L.B. Volodarsky, Proton exchange in stable nitroxyl radicals. ESR study of the pH of aqueous solutions. Chem. Phys. Lett. **91**, 69–72 (1982)
106. I.A. Kirilyuk, A.A. Bobko, V.V. Khramtsov, I.A. Grigor'ev, Nitroxides with two p*K* values—useful spin probes for pH monitoring within a broad range". Org. Biomol. Chem. **3**, 1269 (2005)
107. S. Koda, J. Goodwin, V.V. Khramtsov, H. Fujii, H. Hirata, Electron paramagnetic resonance-based pH Mapping using spectral-spatial imaging of sequentially scanned spectra. Anal. Chem. **84**, 3833–3837 (2012)
108. V.V. Khramtsov, V.I. Yelinova, L.M. Weiner, T.A. Berezina, V.V. Martin, L.B. Volodarsky, Quantitative determination of SH groups in low- and high-molecular-weight compounds by an electron spin resonanc emethod. Anal. Biochem. **182**(1), 58–63 (1989)
109. L.M. Weiner, Quantitative determination of thiol groups in low- and high-molecular-weight compounds by an electron paramagnetic resonance. Methods Enzymol **251**, 87–105 (1995)
110. M. Tamura, Y. Nakazawa, D. Shiomi, K. Nozawa, Y. Hosokoshi, M. Ishikawa, M. Takahashi, M. Kinoshita, Bulk ferromagnetism in the β-phase crystal of the *p*-Nitrophenyl nitronyl nitroxide radical. Chem. Phys. Lett. **186**, 401–404 (1991)
111. D.B. Amabilino, J. Veciana, Nitroxide-based organic magnet, in *Magnetism: Molecules to Materials II*, ed. by J.S. Miller, M. Drillon (Wiley-VCH, Weinheim, Germany, 2001), pp. 1–60
112. R. Tamura, Organic functional materials containing chiral nitroxide radical units, in *Nitroxides: Application in Chemistry, Biomedicine, and Materials Science*, ed. by G.I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A. Smirnov, R. Tamura (Weinhem, WILEY-VCH, 2008), pp. 303–331
113. A. Rajca, From high-spin organic molecules to organic polymers with magnetic ordering. Chem. Eur. J. **8**, 4834–4841 (2002)
114. S.L. Veber, M.V. Fedin, K.Y. Maryunina, A. Potapov, D. Goldfarb, E. Reijerse, W. Lubitz, R.Z. Sagdeev, V.I. Ovcharenko, E.G. Bagryanskaya, Temperature-dependent exchange interaction in molecular magnets Cu (hfac) 2LR studied by EPR: methodology and interpretations. Inorg. Chem. **50**, 10204–10212 (2011)
115. H.M. Swartz, N. Khan, V.V. Khramtsov, Use of electron paramagnetic resonance spectroscopy to evaluate the redox state in vivo. Antioxid. Redox Signal. **9**, 1757–1771 (2007)
116. N. Kocherginsky, H.M. Swarts, in *Nitroxide Spin Labels. Reactions in Biology and Chemistry* (CRC Press, 1995)

117. P. Kuppusamy, M.C. Krishna, EPR imaging of tissue redox status. Curr. Top. Biophys. **26**, 29–34 (2002)
118. G.I. Likhtenshtein, Nitroxide redox probes and traps, nitron spin traps, in *Nitroxides: Application in Chemistry, Biomedicine, and Materials Science*, ed. by G.I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A. Smirnov, R. Tamura (WILEY-VCH, Weinhem, 2008), pp. 303–331
119. G.I. Likhtenstein, V.R. Bogatyrenko, A.V. Kulikov, K. Hideg, H.O. Hankovsky, N.V. Lukoianov, A.I. Kotelnikov, B.S. Tanaschelchuk, Study of superflow motion in solid solutions by the method of physical probes. Dokl. Akad. Nauk SSSR **253**, 481–484 (1980)
120. I.M. Bystryak, G.I. Likhtenshtein, A.I. Kotelnikov, O.H. Hankovsky, K. Hideg, The influence of the molecular dynamics of the solvent on the photoreduction of nitroxy-radicals. Russ. J. Phys. Chem. **60**, 1679–1983 (1986)
121. V.R. Vogel, E.T. Rubtsova, G.I. Likhtenshtein, K. Hideg, Factors affecting photoinduced electron transfer in a donor- acceptor pair (D-A) incorporated into bovine serum albumin. J. Photochem. Photobiol. A, Chem. **83**, 229–236 (1994)
122. E. Lozinsky, V.V. Martin, T.A. Berezina, A. Shames, A.L. Weis, G.I. Likhtenshtein, Dual fluorophore-nitroxide probes for analysis of vitamin C in biological liquids. J. Biochem. Biophys. Meth. **38**, 29–42 (1999)
123. N.V. Blough, D.J. Simpson, Chemically mediated fluorescence yield switching in nitroxide-fluorophore adducts, optical sensors of radical/redox reactions. J. Am. Chem. Soc. **110**, 1915–1917 (1988)
124. J.P. Allen, M.C. Pfrunder, J.C. McMurtrie, S.T. Bottle, J.P. Blinco, K.E. Fairfull-Smith, BODIPY-based profluorescent probes containing Meso- and β-Substituted Isoindoline Nitroxides European. J. Org. Chem. **3**, 476–483 (2017)
125. R. Braslau, F. Rivera III, E. Lilie, M. Cottman, Urushiol detection using a profluorescent nitroxide. J. Org. Chem. **78**, 238–245 (2013)
126. G.I. Likhtenstein, K. Ishii, S. Nakatsuji, Dual chromophore-nitroxides: novel molecular probes, photochemical and photophysical models and magnetic materials. Photochem. Photobiol. **83**, 871–881 (2007)
127. N. Khan, B.B. Williams, H.M. Swartz, Clinical applications of in vivo EPR: rationale and initial results. Appl. Magn. Reson. **30**, 185–199 (2006)
128. J.B. Mitchell, M.C. Krishna, P. Kuppusamy, J.A. Cook, A. Russo, Protection against oxidative stress by Nitroxides. Exp. Biol. Med. **226**, 620–621 (2001)
129. V.V. Khramtsov, In vivo spectroscopy and imaging of nitroxide probes. in *Nitroxides—Theory, Experiment and Applications*, ed. by A.I. Kokorin (September, 2012), pp. 317–346
130. G.I. Likhtenshtein, Biomedical and medical application of nitroxides, in *Nitroxides: Application in Chemistry, Biomedicine, and Materials Science,* ed. by G.I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A. Smirnov, R. Tamura (WILEY-VCH, Weinhem, 2008), pp. 371–401
131. E.G. Janzen, Y.Y. Wang, R.V. Shetty, Spin trapping with alpha-pyridyl 1-oxide N-tert-butyl nitrones in aqueous solutions. A unique electron spin resonance spectrum for the hydroxyl radical adduct. J. Am. Chem. Soc. **100**, 2923–2925 (1978)
132. F.A. Villamena, E.J. Locigno, A. Rockenbauer, C.M. Hadad, J.L. Zweier, Theoretical and experimental studies of the spin trapping of inorganic radicals by 5, 5-dimethyl-1-pyrroline N-oxide (DMPO). 2. Carbonate radical anion. J. Phys. Chem. A **111**, 384–391
133. R.P. Mason, Free using anti-5, 5-dimethyl-1-pyrroline N-oxide (anti-DMPO) to detect protein radicals in time and space with immuno-spin trapping. Radic. Biol. & Med. **36**, 1214–1223 (2004)
134. S. Schocha, V. Senb, S. Gajewskia, V. Golubevb, B. Straucha, A. Hartwiga, B. Köberlea, Activity profile of the cisplatin analogue PN149 in different tumor cell lines. Biochem. Pharmacol. **156**, 109–119 (2018)
135. M. Balog, T. Kálai, J. Jekő, H-J. Steinhoff, M. Engelhard, K. Hideg, Synthesis of New 2, 2, 5, 5-Tetramethyl-2, 5-dihydro-1H-pyrrol-1-yloxyl radicals and 2-Substituted-2, 5, 5-trimethylpyrrolidin-1-yloxyl radicals based a-amino acids. Synlett **14**, 2591 (2004)
136. O.D. Zakharova, T.S. Frolova, Y.V. Yushkova, E.I. Chernyak, A.G. Pokrovsky, M.A. Pokrovsky, S.V. Morozov, O.I. Sinitsina, I.A. Grigor'ev, G.A. Nevinsky, Antioxidant and