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# Recent Developments in Electron Paramagnetic Resonance for Spectroscopic Applications

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**Abstract:** This review investigated the developments of Electron paramagnetic resonance such as SDSL EPR, pulsed/CW ENDOR, high frequency/ high-field EPR, and ESEEM in biological systems such as photosynthesis, metalloproteins, radical enzymes, and phospholipid membranes. EPR spectroscopy act as a powerful tool to calculate the dynamics and structure of the biological systems from the paramagnetic centers. Applying the EPR measurements on the biomolecules like calmodulin, pyruvate kinase, nitrogenase, bromoperoxidase can also help understand their intermolecular behavior. The better insight into the detailed kinetics of the protein in the radical state can be evaluated quickly with the EPR spectroscopy than that of the XRD or NMR techniques. Hence, the specific potentials, principles, technicality, developments, and limitations, of EPR spectroscopy are highlighted here to understand its applications in the research and development of bio-medicine.

**Keywords:** EPR; photosynthesis RC; metalloproteins; radical enzymes; phospholipid membranes.



## 1. Introduction

X-ray crystallography is the gold standard when it comes to the study of structural geometry [1, 2]. It serves as the standard model to determine the structure of a crystal taken as the specimen. Nevertheless, it has limited use in the case of studying the species when present in intact cells [3]. In this context, researchers prefer electron paramagnetic resonance (EPR) to study biological systems out of many methodologies. EPR, also termed Electron spin resonance (ESR), has a unique feature of determining distances selectively in paramagnetic species [4], and it deserves more extensive usage than it has today. More focus on its broader utility will enhance its preference over other techniques.

An individual characteristic of EPR is its focus on unpaired electrons in the biological system, which attributes to its paramagnetic behavior. This method can unfold structural information about the immediate vicinity [5]. It helps visualize the dynamics of the paramagnetic specimen [6], which can startle other fundamental methods available. The large number of different proteins identified through the work of the human genome project (HGP) result in an equally large number of questions about protein interaction which can be answered using site-directed spin-labeling (SDSL) and EPR [7]. The result of the genome project paved a new scope for the EPR study. This progress came as a contradiction as it was predicted that EPR has low-range applications. The (SDSL)- EPR primarily depends upon the spin property

of electrons. It maps electron-electron spin-spin interactions to measure the distance between the two-electron spins [8]. Its application includes investigation of protein recombination, change in structural arrangements during protein function, the pattern of the subunits which make up the biological bodies, arrangements of membrane proteins, and geometry of the entire system [9, 10].

X-ray diffraction is well suitable for the species in crystal form. For a stable crystal, it can determine the interatomic protein distances. After the low-temperature cryogenic treatment, the Cryocooled specimen resolves about 1.2 Å of the protein [11]. There are two conditions that x-ray diffraction cannot explain- (a) The normal state structure of the solution protein and (b) the electronic configuration of metalloproteins [12]. Even if, in some instances, the protein can condense to the crystal form, which functions in a membrane, some parts remain inexplicable by the XRD [13]. Single particles of large complex species like the subunits of a multi-component device are easy to study due to the method's selectivity. In addition, when the anatomical and functional observation of these subunits is also available from the data of other techniques like NMR and XRD, it serves as an aid to the present information [14].

NMR, a nuclear phenomenon, unlike the EPR, is concerned with the inter proton distances. The basic principle remains the same in both methods, yet the primary difference is the higher magnetic moment [15]. In NMR, the pulse methods govern the field. In contrast, in the case of EPR, continuous wave (CW) remains a preference. The magnetic resonance study draws a clear line between the two methodologies. NMR spectra are characterized by samples with molar concentration, while EPR spectra typically prefer samples with a concentration of less than one nanomole. In NMR, relaxation time ranges from milliseconds to a second. For enhancing the signal-to-noise, NMR is using cryogenically cooled resonators to increase the QF of the resonator by improving S/N. EPR uses resonators with lower quality factors (QF). The imaging of both the methods also shows their peculiar difference indicating the time difference in various criteria like gradient rise time, echo time, and even variation in resolution, magnet and resonator orientation, magnetic homogeneity, susceptibility, and many more.

To apply any method, the main criteria are the nature of the sample or simply its state. To choose NMR to study the structural geometry of biomolecules like a protein, the specifications of the biological sample include the protein of high concentration with having minimal conductivity. Calculations and measurements from spectroscopic inferences show a lot more motion in protein than known. A sample is not definite with distinct features suitable for the above two methods. It varies from proteins in frozen, powders and, crystal forms. Unique features of EPR that stand as the specialty of the method in its application in biological systems include (a) detection of only unpaired electrons. (b) applicable to samples present in any phase and temperature. (c) due to large electron magnetic moment, EPR line shapes have long-range effects [16, 17]. Thus, EPR comes to rescue the researchers from the dilemma when the biological system in hand to study cannot be done with XRD or NMR method or is in an environment not suitable for both methods. It is suitable when the protein is present in membranes or whole cells and tissues with minimal sample perturbation [18].

The researchers studied broad applications of EPR in biological systems, such as (a) obtaining structural information of the biological systems from their paramagnetic centers [19]; (b) studying molecular dynamics with distance measurements by EP [20]; (c) measuring relaxation times of metal ions and organic radicals [21]; (d) EPR helps to analyze the intermolecular behavior of the biomolecules like calmodulin, pyruvate kinase, nitrogenase, bromoperoxidase, and many more; (e) the application of EPR in the field of biomedicine with

the advancement of the method with proper interpretation and analysis of data is a turning event of the research [22].

Hence, in this review article, we highlighted the range of applications of EPR technologies in biological systems like photosynthesis, metalloproteins, radical enzymes, and phospholipid membranes.

## 2. EPR Probe for Photosynthesis Biosystems

We all are aware that photosynthesis is of prime importance for its mark in enabling the existence of life. It transfers the sunlight into electrochemical energy [23]. It ultimately helps these organisms to grow, synthesize, and replicate. The photosynthetic process is associated with a vast range of time scales, starting from a few fs to more than 100Ms. As a result, photosynthesis is studied in various fields like radiation and condensed matter physics, photo and biochemistry, physiology, and botany [24, 25].

The primary process in photosynthesis includes the incoming sunlight (can be termed as light quanta). It is collected from 'Antenna' and then passed through the reaction centers (RC). Type 1 reaction centers (RC), such as photosystem1 (PS1), while in Type 2 RCs are bacterial reaction center (bRC) and photosystem 2 (PS2) [26].

Researchers can choose a suitable method from many available methods to study anatomy. Widely used x-ray crystallography was chosen to study the 3D RCs [27]. However, it has its limitations. The x-ray crystal structure solution for various RCs was obtained at different resolutions. This method is able to give fine measurements of distance and orientations of the proteins and cofactor molecules. Still, it lacks revealing information regarding dynamics and their conformational variations in RCs. Further, X-ray crystallography gives the dark states or ground states; hence difficult to have info about light-mediated variations. Application of NMR is difficult in this instance due to the large size RCs and the paramagnetic nature of the cofactor molecules.

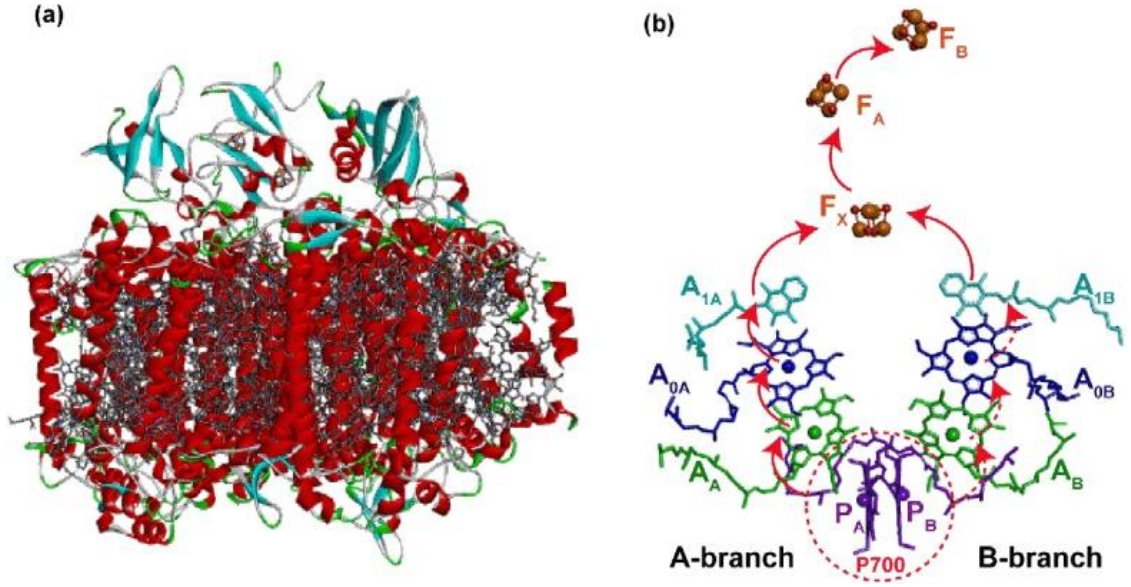


**Figure 1.** (**a**) Reaction center and (**b**) electron-transfer cofactors of PSI. (Reprinted with permission from reference [30]).

For its unique features and powerful interpretations, EPR is hailed as a suitable technique for examining photosynthetic RCs [28]. During photosynthesis, the light-mediated transfer in RCs results in known paramagnetic centers [29]. For these site-directed spin labeling

(SDSL), EPR proved to be a major achievement in research related to the study of Photosynthetic reaction centers (RCs) [30].

The locations in the protein selected for the covalent link of the spin-label are mostly solvent friendly. As a result, the spin-label accounts for less perturbation in structural and functional aspects of Proteins [31]. This new EPR approach, SDSL EPR, is now the preferable one. Proteins may be in different states such as soluble or intact to membranes or with any size and complexity can be easily studied by this technique leading to its application in various biological systems [32]. It has many critical applications like the determination of secondary structures of various proteins like bacteriorhodopsin [33], T4 lysozyme [34, 35], Rhodopsin [36, 37], and myosin [38]. The x-ray crystallography study of photosystem 1 (PS1) shows its clusters structure in Figure 1.

The main aim of investigating the same system using SDSL EPR spectroscopy was to test the PsaC-PsaB contacts with asymmetric C- terminal. Thus, SDSL EPR can determine the binding of a PsaC. Thus, this study's result can help understand the mechanism of PsaC. Furthermore, this precise result regarding the PsaC bioassembly in PS1 is well obtainable. This new approach of EPR, called site-directed spin-label SDSL EPR, shows how it is impossible to get this similar preciseness in measurement by applying the widely used NMR spectroscopy or Conventional EPR spectroscopy [39].

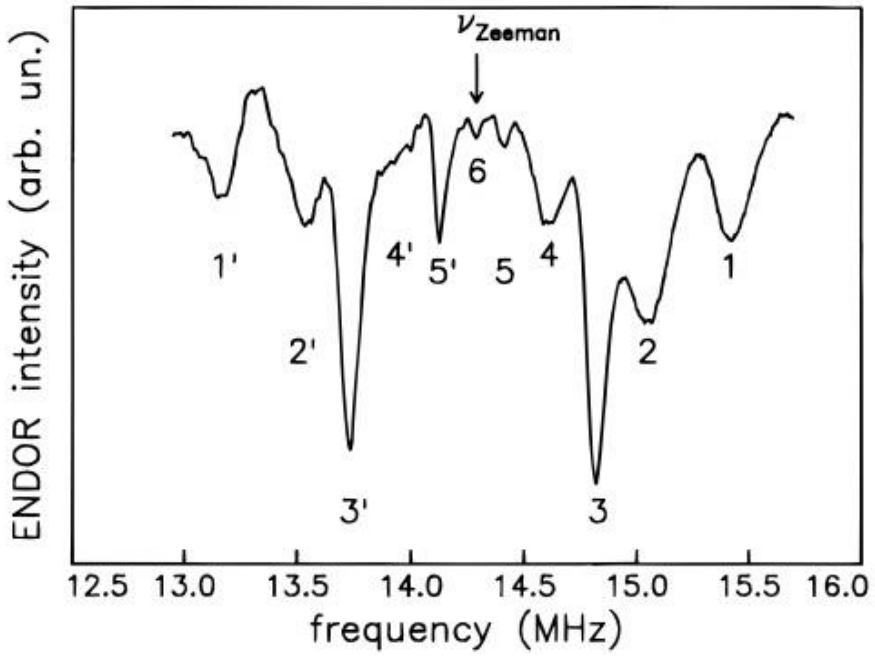


**Figure 2.** W-band ENDOR spectrum a 15N enriched single crystal with six coincidence [40]. Reprinted (adapted) with permission from ACS Journal of the American Chemical Society; https://doi.org/10.1021/ja962076u. Copyright (1996) American Chemical Society.

## 3. EPR probe for the detection of Metalloproteins in biological systems

Metalloproteins, proteins embedded with metal ions or metal clusters, have been an intriguing topic to study for past years. Metalloproteins consist of paramagnetic metal centers present in the functional part of the protein, which can be easily studied by using EPR spectroscopy [41]. EPR reveals information regarding the protein structure and its function. Metal complexes showing G-anisotropy are correctly investigated at the low EPR frequencies (Liquid helium or liquid Nitrogen) [42]. Furthermore, spectra lines become broad at high frequencies with that high sensitivity, and a higher concentration of samples becomes

mandatory. A few methods are being combined to get precise information that is easy to interpret and analyze to address this high-frequency effect.

Dipolar spectroscopy is employed to determine the paramagnetic metal centers. Hyperfine spectroscopy measures the interaction between an unpaired electron spin and nuclear spin. It, therefore, helps in characterizing the local structure of the metal center's ligand sphere precisely. However, the study becomes complex and hectic when many paramagnetic species are present in the protein, leading to an overlapped spectrum [43]. So, the EPR spectroscopy, with its different approach and suitable magnetic field strength and temperature, makes the detailed study clear. Thus, the collective study employing the mentioned spectroscopies with the High-frequency EPR spectroscopy leads to an excellent study of the structure and functions of metalloprotein complexes.

Many applications have been studied using this spectroscopy. It was found by applying dipolar spectroscopy and Hyperfine spectroscopy to metalloenzymes that the EPR method, with its high potential, has some limitations when applied to biological systems. This ultimately leads to high-field EPR spectroscopy based on time relaxation. HF-EPR spectroscopy as a probe in a biological system is widely studied in metalloproteins consisting of Manganese (Mn), Copper (Cu), and Iron (Fe).

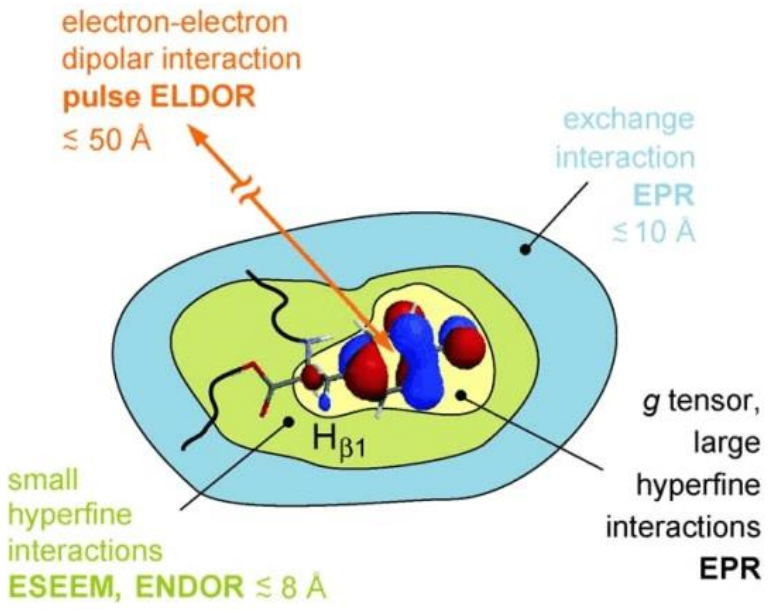


**Figure 3.** The determination methods, interactions, and the length scale with SOMO of radical enzymes. (Reprinted with permission from reference: [44]).

*3.1. HF-EPR spectroscopy on biological systems containing Mn proteins*

The High-frequency experiments such as the HF-EPR experiment with frequencies nearly 95 GHz need a resolution of the g-tensor anisotropy. In the case of metalloproteins, g-strain broadening increases with the field. Due to the dominance of the Zeeman interaction, high field and high frequency are required. The advantages include enhanced g-resolution and better sensitivity using HF-EPR. In addition, the multi-frequency approach is suitable for the proteins containing Mn.

Six lines were derived from the study for Manganese composition or Mn(II) due to the interaction. The narrow line increases the sensitivity of the EPR measurements. It serves as an anomaly to the precise measurement by making it an impurity while preparing the biological samples, and it becomes significant as the field increases. The study showed that the

cytochrome C oxidase has binding from the copper A site. Researchers performed HF-EPR at 139 GHz on the frozen protein [45].

### *3.2. HF-EPR spectroscopy on Cu proteins.*

Blue color proteins are found to be active in the bacterias' redox chains. The Cu in protein has five ligands. The ENDOR method was applied to both naturally abundant as well as to 15N enriched samples at 1.2K. The spectrum recorded for the enriched 15N sample can be seen in Fig. 2. The contribution coming from the 5 N can be seen here.

Further, accurate g-values can be calculated with the use of single-crystal EPR. The vital information regarding electron transfer (ET) can also be obtained. Other Cu centers with ET are binuclear copper A and mixed valence. Nitrous oxide reductase copper A center info can be obtained with pulsed ENDOR at 95 GHz [46].

### *3.3. HF-EPR spectroscopy on Fe proteins.*

Specific information on NO binding complexes, myoglobin, and hemoglobin containing $Fe^{3+}$ can be obtained by performing HF-EPR studies. Here the media to disentangle spectra can also be obtained. HF-EPR provides additional information. The iron site of this new species was identified using ENDOR with multi-frequency at 4 K.

The low-frequency X band part of the spectra is complicated to study the iron centers due to Zero-field splitting. But with the increase of the order of the frequency, the spectra become easier to analyze within 70 to 400 GHz. The origin of the paramagnetic metal center can be formulated with HF EPR on proteins. In some experiments, it was inferred that iron center is not the origin of paramagnetism from the g-anisotropy in EPR measurement at the W band. Instead, Fe was identified in the radical center at amino acid [47].

## 4. EPR probe for Biological Systems with Radical Enzymes

Paramagnetic centers are also present in the modified state of the amino acid known as the radical enzyme. Inactivation of the enzymes also can cause radical states. These paramagnetic centers present in the radical enzyme can be determined using the research method like EPR, NMR, x-ray diffraction [48-50]. Specifically, for the protein in the Radical state, HF- EPR spectroscopy is preferable, which provides better insight into the detailed kinetics [51, 52]. The isotropic hyperfine couplings with nuclei result in unpaired electrons here. The singly occupied molecular orbital (SOMO) shown in Figure 3 is a universal characteristic of the shift in g values. These g shifts decrease with the increasing perturbation energy between the excited and ground state. Some exceptions are also there, for instance, cysteine radicals because of the existence of the degenerate state. Higher frequencies and fields are required for the precise measurements of the radical enzymes [53].

The paramagnetic centers have a direct linkage with the hyperfine couplings. However, high precision ENDOR techniques are required to analyze if the coupling is small. These paramagnetic centers overlap to a larger extent in non-conducting matrices with an observable value of coupling.

The prototype of the radical enzyme is known as ribonucleotide reductase (RNR). In all living cells generation of deoxynucleotides from the nucleotides helps to catalyze the reaction. The mechanism can be understood in Figure 4. EPR spectroscopy helps to explore the class I RNR from tyrosyl radicals for the first time. The HF-EPR also can be a useful probe

to determine the substrate and protein intermediate radicals. The essential for catalysis RNRs can be of 4 types. But in all four types, the nucleotide reduction scheme has been observed with radical dependents.

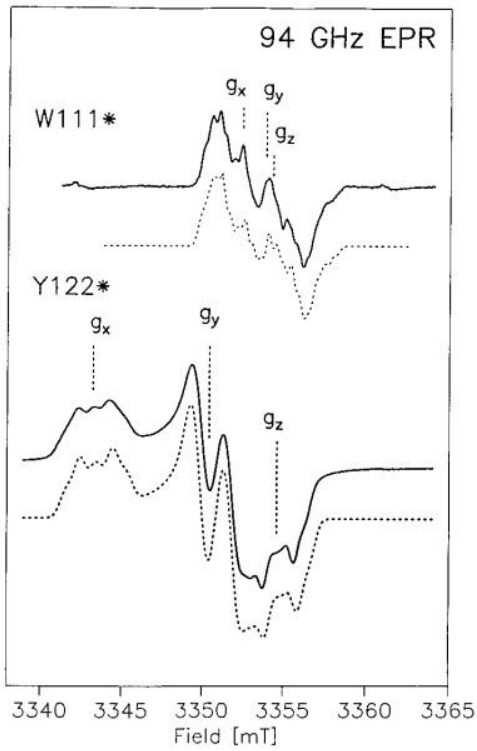


**Figure 4.** The tyrosine radical and tryptophan radicals 94 GHz HF- EPR. (Reprinted with permission from reference [54]).

## 5. EPR probe for Biological System of Phospholipid membrane

From times immemorial, it is evident that the living organisms depend upon the membranes which confine the individual cells and the organelles present within the cell. Study shows that around 30% of the human protein is membrane-bound. Membrane proteins are known to receive signals from the cell's exterior and transmit internally for intracellular action. It, therefore, enables a controlled material exchange, information, and energy transfer across the membrane [55]. It also accounts for the cell shape, stability, fusion, or division of membranes, organelles, and cells.

The SDSL EPR can be applied on the phospholipid membranes without any environmental aspects or size limitations. One can use the SDSL EPR to get the structural insights into the membrane-embedded protein by following the essential steps for obtaining information like dynamics, water or membrane accessibility, and the inter spin distances. First, the mutagenesis and the process of labeling are done. The commonly followed method introduces cysteines at different sites and attaches the Nitroxide label covalently through various functional groups shown in Figure 5 [56]. These probes have been using nitroxide spin labels for more than 30 years [57-59]. Anisotropic strong hyperfine couplings of N at low field, whereas G tensor part can be seen at higher magnetic fields [60-62]. But both the interactions are highly sensitive to the polarity and dynamics of the solvent [63-65]. Hence the pre-requisite part is the basic knowledge of the nitroxide spin-label of the molecular motions [66-69]. The CW-EPR with three lines for the nitroxide in water can be seen in Figure 5 (b) [56]. The present HF-EPR can provide more insight into the structural characterization with long-range interactions.

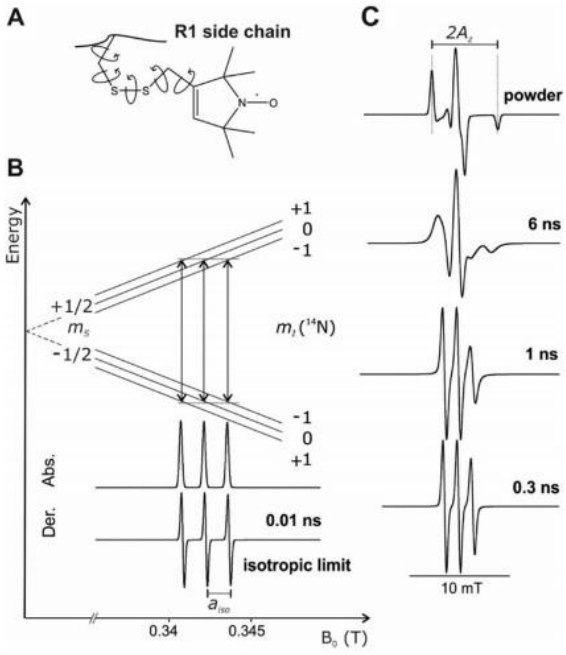


**Figure 5.** The dynamics encoded Nitroxide probes (**A**) scheme; (**B**) energy scheme; (**C**) CW EPR X-band spectra. (Reprinted with permission from reference [56]).

## 6. Summary and Perspectives

In summary, applications of electron paramagnetic resonance (EPR) technologies in biological systems like photosynthesis, metalloproteins, radical enzymes, and phospholipid membranes have been presented in this review work. The researcher demonstrates the effectiveness of the EPR techniques to obtain structural information of such biological systems from their paramagnetic centers. The distance measurements can also achieve the molecular dynamics of such systems through EPR. EPR techniques can also be implemented to the transition metal ions and organic radicals to calculate the relaxation mechanisms. Applying the EPR measurements on biomolecules like calmodulin, pyruvate kinase, Nitrogenase, Bromoperoxidase can also help understand their intermolecular behavior. For its unique features and powerful interpretations, EPR spectroscopy can be used as a suitable method for examining photosynthetic reaction centers. High-frequency EPR spectroscopy is also a suitable probe in the biological system to study the metalloproteins consisting of manganese (Mn), copper (Cu), iron (Fe). The better insight into the detailed kinetics of the protein in the Radical state can be evaluated easily with the EPR spectroscopy than that of the XRD or NMR techniques. The SDSL-EPR can also be applied on the membrane proteins to determine water accessibility and dynamics with higher sensitivity. Hence, the analysis and interpretation of the EPR data on the biosystems can act as a turning point for the research and development in biomedicine.

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## Conflicts of Interest

The authors declare no conflict of interest.

## References


1. Engh, R. A.; Huber, R. Accurate bond and angle parameters for X-ray protein structure refinement. *Acta Crystallographica Section A: Foundations of Crystallography* **1991,** *47*, 392-400, https://doi.org/10.1107/s0108767391001071.
2. Ladd, M.F.C.; Palmer, R.A. Palmer, R.A. Structure determination by X-ray crystallography. *Structure determination by X-ray crystallography*, 5th ed., Springer, **1977**, https://doi.org/10.1007/978-1-4615-7930-4.
3. Visser, J.; Amesz, J.; Van Gelder, B. EPR signals of oxidized plastocyanin in intact algae. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1974,** *333*, 279-287, https://doi.org/10.1016/0005-2728(74)90011-5.
4. Berliner, L.J.; Eaton, S.S.; Eaton, G.R. Distance measurements in biological systems by EPR. *Biological Magnetic Resonance*, Springer Science & Business Media, **2006**, *19*, https://doi.org/10.1007/b111467.
5. Prisner, T.; Rohrer, M.; MacMillan, F. Pulsed EPR spectroscopy: biological applications. *Annual review of physical chemistry* **2001,** *52*, 279-313, https://doi.org/10.1146/annurev.physchem.52.1.279.
6. Lindemann, W. R.; Ortony, J. H. Probing local molecular properties of self-assembled systems with electron paramagnetic resonance (EPR). *In Self-assembling Biomaterials*, Elsevier, **2018**, 275-289, https://doi.org/10.1016/b978-0-08-102015-9.00014-9
7. Braun, T.; Drescher, M.; Summerer, D. Expanding the genetic code for site-directed spin-labeling. *International journal of molecular sciences* **2019,** *20*, 373, https://doi.org/10.3390/ijms20020373.
8. Klare, J.P. Site-directed spin labeling EPR spectroscopy in protein research. *Biological chemistry* **2013,** *394*, 1281-1300, https://doi.org/10.1515/hsz-2013-0155.
9. Claxton, D.P.; Kazmier, K.; Mishra, S.; Mchaourab, H.S. Navigating membrane protein structure, dynamics, and energy landscapes using spin labeling and EPR spectroscopy. *Methods in enzymology* **2015,** *564*, 349-387, https://doi.org/10.1016/bs.mie.2015.07.026.
10. Berliner, L.J.; Reuben, J. Spin labeling: theory and applications. Springer Science & Business Media, **2012**, *8*, https://doi.org/10.1007/978-1-4613-0743-3.
11. Kim, C.U.; Wierman, J.L.; Gillilan, R.; Lima, E.; Gruner, S.M. A high-pressure cryocooling method for protein crystals and biological samples with reduced background X-ray scatter. *Journal of Applied Crystallography* **2013,** *46*, 234-241, https://doi.org/10.1107/s0021889812045013.
12. Moraes, I.; Evans, G.; Sanchez-Weatherby, J.; Newstead, S.; Stewart, P.D.S. Membrane protein structure determination—the next generation. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2014,** *1838*, 78-87, https://doi.org/10.1016/j.bbamem.2013.07.010.
13. Zheng, H.; Handing, K.B.; Zimmerman, M.D.; Shabalin, I.G.; Almo, S.C.; Minor, W. X-ray crystallography over the past decade for novel drug discovery–where are we heading next? *Expert opinion on drug discovery* **2015,** *10*, 975-989, https://doi.org/10.1517/17460441.2015.1061991.
14. Pandey, A.; Shin, K.; Patterson, R.E.; Liu, X.-Q.; Rainey, J. K. Current strategies for protein production and purification enabling membrane protein structural biology. *Biochemistry and Cell Biology* **2016,** *94*, 507-527, https://doi.org/10.1139/bcb-2015-0143.
15. Kaupp, M.; Buhl, M.; Malkin, V.G. Calculation of NMR and EPR Parameters. Wiley Online Library, **2004**, https://doi.org/10.1002/3527601678.
16. Spasojević, I.; Mojović, M.; Ignjatović, A.; Bačić, G. The role of EPR spectroscopy in studies of the oxidative status of biological systems and the antioxidative properties of various compounds. *Journal of the Serbian chemical society* **2011,** *76*, 647-677, https://doi.org/10.2298/jsc101015064s.
17. Hagen, W.R. Biomolecular EPR spectroscopy. CRC press, **2008**, https://doi.org/10.1201/9781420059588.
18. Sahu, I.D.; Lorigan, G.A. Electron paramagnetic resonance as a tool for studying membrane proteins. *Biomolecules* **2020,** *10*, 763, https://doi.org/10.3390/biom10050763.
19. Eaton, S.S.; Eaton, G.R. Excitement about Relaxation: Relaxation and the History of Electron Paramagnetic Resonance. *In Pioneers of Magnetic Resonance*, ACS Publications, **2020**, 197-225, https://doi.org/10.1021/bk-2020-1349.ch011.
20. Eaton, S.; Eaton, G. Measurements of interspin distances by EPR. *Electron Paramagnetic Resonance* **2004,** *19*, 318-337, https://doi.org/10.1039/9781847553560-00318.
21. Owenius, R.; Eaton, G.R.; Eaton, S.S. Frequency (250 MHz to 9.2 GHz) and viscosity dependence of electron spin relaxation of triarylmethyl radicals at room temperature. *Journal of magnetic resonance* **2005,** *172*, 168-175, https://doi.org/10.1016/j.jmr.2004.10.007.
22. Meyer, V.; Swanson, M.A.; Clouston, L.J.; Boratyński, P.J.; Stein, R.A.; Mchaourab, H.S.; Rajca, A.; Eaton, S.S.; Eaton, G.R. Room-temperature distance measurements of immobilized spin-labeled protein by DEER/PELDOR. *Biophysical journal* **2015,** *108*, 1213-1219, https://doi.org/10.1016/j.bpj.2015.01.015.
23. Abas, N.; Kalair, E.; Kalair, A.; ul Hasan, Q.; Khan, N. Nature inspired artificial photosynthesis technologies for hydrogen production: Barriers and challenges. *International Journal of Hydrogen Energy* **2020,** *45*, 20787-20799, https://doi.org/10.1016/j.ijhydene.2019.12.010.

24. Brown, W.M.; Gauger, E.M. Light harvesting with guide-slide superabsorbing condensed-matter nanostructures. *The journal of physical chemistry letters* **2019,** *10*, 4323-4329, https://doi.org/10.1021/acs.jpclett.9b01349.
25. Antonacci, A.; Scognamiglio, V. Photosynthesis-based hybrid nanostructures: Electrochemical sensors and photovoltaic cells as case studies. *TrAC Trends in Analytical Chemistry* **2019,** *115*, 100-109, https://doi.org/10.1016/j.trac.2019.04.001.
26. van Grondelle, R.; Boeker, E. Limits on natural photosynthesis. *The Journal of Physical Chemistry B* **2017,** *121*, 7229-7234, https://doi.org/10.1021/acs.jpcb.7b03024.
27. Igarashi, K.; Hagiwara, Y.; Sugishima, M.; Wada, K.; Fukuyama, K.; Ikeda, A.; Yano, N.; Kusaka, K.; Ostermann, A.; Unno, M. Crystal Growth of a Bilin Reductase PcyA I86D Mutant–Substrate Complex for Neutron Crystallography. *Crystal Growth & Design* **2018,** *18*, 5174-5181, https://doi.org/10.1021/acs.cgd.8b00607.
28. Nalepa, A.; Malferrari, M.; Lubitz, W.; Venturoli, G.; Möbius, K.; Savitsky, A. Local water sensing: water exchange in bacterial photosynthetic reaction centers embedded in a trehalose glass studied using multiresonance EPR. *Physical Chemistry Chemical Physics* **2017,** *19*, 28388-28400, https://doi.org/10.1039/c7cp03942e.
29. Mark, B.; Coates, C. S.; Sugimura, S.; Baldansuren, A.; Ku, J.; Debus, R.J.; Lakshmi, K. Determining the Electronic Structure of Paramagnetic Intermediates in membrane proteins: A high-resolution 2D 1H hyperfine sublevel correlation study of the redox-active tyrosines of photosystem II. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2020,** *1862*, 183422, https://doi.org/10.1016/j.bbamem.2020.183422.
30. Silva, K. I.; Jagannathan, B.; Golbeck, J. H.; Lakshmi, K. Elucidating the design principles of photosynthetic electron-transfer proteins by site-directed spin labeling EPR spectroscopy. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **2016,** *1857*, 548-556, https://doi.org/10.1016/j.bbabio.2015.08.009.
31. Le Nguyen Ngoc, L.; Pandey, R. B.; Sompornpisut, P. Dynamics and Environmental Characteristics of Spin Labels in a KvAP Voltage Sensor by Molecular Dynamics Simulations. *The Journal of Physical Chemistry B* **2021,** *125*, 748-756, https://doi.org/10.1021/acs.jpcb.0c08993.
32. García-Rubio, I. EPR of site-directed spin-labeled proteins: A powerful tool to study structural flexibility. *Archives of biochemistry and biophysics* **2020,** *684*, 108323, https://doi.org/10.1016/j.abb.2020.108323.
33. Brown, L.J.; Hare, J.E. ElECTRON PaRamagNETIC REsONaNCE: sITE-DIRECTED sPIN labElINg. *Pumps, Channels and Transporters: Methods of Functional Analysis* **2015**, *327*, https://doi.org/10.1002/9781119085126.ch12.
34. Pan, Y.; Li, H.; Li, Q.; Lenertz, M.; Schuster, I.; Jordahl, D.; Zhu, X.; Chen, B.; Yang, Z. Protocol for resolving enzyme orientation and dynamics in advanced porous materials via SDSL-EPR. *STAR protocols* **2021,** *2*, 100676, https://doi.org/10.1016/j.xpro.2021.100676.
35. Li, H.; Pan, Y.; Yang, Z.; Rao, J.; Chen, B. Emerging applications of site-directed spin labeling electron paramagnetic resonance (SDSL-EPR) to study food protein structure, dynamics, and interaction. *Trends in Food Science & Technology* **2021**, *109*, 37-50, https://doi.org/10.1016/j.tifs.2021.01.022.
36. Sahu, I. D.; Lorigan, G. A. Probing Structural Dynamics of Membrane Proteins Using Electron Paramagnetic Resonance Spectroscopic Techniques. *Biophysica* **2021,** *1*, 106-125, https://doi.org/10.3390/biophysica1020009.
37. Reichenwallner, J.; Liu, B.; Balo, A. R.; Ou, W.-L.; Ernst, O. P. Electron paramagnetic resonance spectroscopy on G-protein-coupled receptors: Adopting strategies from related model systems. *Current Opinion in Structural Biology* **2021**, *69*, 177-186, https://doi.org/10.1016/j.sbi.2021.06.003.
38. Arata, T. Myosin and Other Energy-Transducing ATPases: Structural Dynamics Studied by Electron Paramagnetic Resonance. *International journal of molecular sciences* **2020,** *21*, 672, https://doi.org/10.3390/ijms21020672.
39. Kazemzadeh, S.; Riazi, G.; Ajeian, R. Novel approach of biophotovoltaic solid state solar cells based on a multilayer of PS1 complexes as an active layer. *ACS Sustainable Chemistry & Engineering* **2017,** *5*, 9836-9840, https://doi.org/10.1021/acssuschemeng.7b01699.
40. Coremans, J.; Poluektov, O.; Groenen, E.; Canters, G.; Nar, H.; Messerschmidt, A. A W-band electron nuclear double resonance study of single crystals of 14N and 15N azurin. *Journal of the American Chemical Society* **1996,** *118*, 12141-12153, https://doi.org/10.1021/ja962076u.
41. Abdullin, D.; Schiemann, O. Localization of metal ions in biomolecules by means of pulsed dipolar EPR spectroscopy. *Dalton Transactions* **2021,** *50*, 808-815, https://doi.org/10.1039/d0dt03596c.
42. Hu, K.-N. Polarizing agents and mechanisms for high-field dynamic nuclear polarization of frozen dielectric solids. *Solid state nuclear magnetic resonance* **2011,** *40*, 31-41, https://doi.org/10.1016/j.ssnmr.2011.08.001.
43. Astashkin, A.V., Mapping the Structure of Metalloproteins with RIDME. *Methods in enzymology* **2015,** *563*, 251-284, https://doi.org/10.1016/bs.mie.2015.06.031.
44. Jeschke, G. EPR techniques for studying radical enzymes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **2005,** *1707*, 91-102, https://doi.org/10.1016/j.bbabio.2004.02.012.
45. Ubbink, M.; Worrall, J.; Canters, G.; Groenen, E.; Huber, M. Paramagnetic resonance of biological metal centers. *Annual review of biophysics and biomolecular structure* **2002,** *31*, 393-422, https://doi.org/10.1146/annurev.biophys.31.091701.171000.

46. Fittipaldi, M.; Steiner, R. A.; Matsushita, M.; Dijkstra, B. W.; Groenen, E. J.; Huber, M. Single-crystal EPR study at 95 GHz of the type 2 copper site of the inhibitor-bound quercetin 2, 3-dioxygenase. *Biophysical journal* **2003,** *85*, 4047-4054, https://doi.org/10.1016/s0006-3495(03)74818-8.
47. Barra, A.; Hassan, A.; Janoschka, A.; Schmidt, C.; Schünemann, V. Broad-band quasi-optical HF-EPR spectroscopy: Application to the study of the ferrous iron center from a rubredoxin mutant. *Applied Magnetic Resonance* **2006,** *30*, 385, https://doi.org/10.1007/bf03166208.
48. Qi, Q.; Hong, Y.; Zhang, Y.; Gu, Z.; Cheng, L.; Li, Z.; Li, C. Combinatorial effect of fermentation and drying on the relationship between the structure and expansion properties of tapioca starch and potato starch. *International journal of biological macromolecules* **2020,** *145*, 965-973, https://doi.org/10.1016/j.ijbiomac.2019.09.187.
49. Mekala, L.P.; Mohammed, M.; Chinthalapati, S.; Chinthalapati, V.R. Pyomelanin production: Insights into the incomplete aerobic l-phenylalanine catabolism of a photosynthetic bacterium, Rubrivivax benzoatilyticus JA2. *International journal of biological macromolecules* **2019,** *126*, 755-764, https://doi.org/10.1016/j.ijbiomac.2018.12.142.
50. Das, S.; Sahu, A.; Joshi, M.; Paul, S.; Shit, M.; Roy Choudhury, A.; Biswas, B., Ligand-Centered Radical Activity by a Zinc-Schiff-Base Complex towards Catechol Oxidation. *ChemistrySelect* **2018,** *3*, 10774-10781, https://doi.org/10.1002/slct.201801084.
51. Davis, I.; Koto, T.; Terrell, J. R.; Kozhanov, A.; Krzystek, J.; Liu, A. High-frequency/high-field electron paramagnetic resonance and theoretical studies of tryptophan-based radicals. *The Journal of Physical Chemistry A* **2018,** *122*, 3170-3176, https://doi.org/10.1021/acs.jpca.7b12434.
52. Davies, M. J. Detection and characterisation of radicals using electron paramagnetic resonance (EPR) spin trapping and related methods. *Methods* **2016,** *109*, 21-30, https://doi.org/10.1016/j.ymeth.2016.05.013.
53. Bracci, M.; Bruzzese, P. C.; Famulari, A.; Fioco, D.; Guidetti, A.; Liao, Y.-K.; Podvorica, L.; Rezayi, S. F.; Serra, I.; Thangavel, K. Paramagnetic species in catalysis research: a unified approach towards (the role of EPR in) heterogeneous, homogeneous and enzyme catalysis. In *Electron Paramagnetic Resonance: Volume 27*, **2020**, https://doi.org/10.1039/9781839162534-00001.
54. Bleifuss, G.; Kolberg, M.; Pötsch, S.; Hofbauer, W.; Bittl, R.; Lubitz, W.; Gräslund, A.; Lassmann, G.; Lendzian, F. Tryptophan and tyrosine radicals in ribonucleotide reductase: a comparative high-field EPR study at 94 GHz. *Biochemistry* **2001,** *40*, 15362-15368, https://doi.org/10.1021/bi010707d.
55. Melkikh, A. V.; Sutormina, M. Intra-and intercellular transport of substances: Models and mechanisms. *Progress in biophysics and molecular biology* **2020,** *150*, 184-202, https://doi.org/10.1016/j.pbiomolbio.2019.10.004.
56. Bordignon, E.; Bleicken, S. New limits of sensitivity of site-directed spin labeling electron paramagnetic resonance for membrane proteins. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2018,** *1860*, 841-853, https://doi.org/10.1016/j.bbamem.2017.12.009.
57. Manohar, A.; Krishnamoorthi, C. Photocatalytic study and superparamagnetic nature of Zn-doped MgFe2O4 colloidal size nanocrystals prepared by solvothermal reflux method. *Journal of Photochemistry and Photobiology B: Biology* **2017,** *173*, 456–65, http://dx.doi.org/10.1016/j.jphotobiol.2017.06.025 .
58. Manohar, A.; Krishnamoorthi, C. Magnetic and photocatalytic studies on Zn1−xMgxFe2O4 nanocolloids synthesized by solvothermal reflux method. *Journal of Photochemistry and Photobiology B: Biology* **2017**, *177*, 95–104, http://dx.doi.org/10.1016/j.jphotobiol.2017.10.009.
59. Manohar, A.; Krishnamoorthi, C. Structural, Raman, magnetic and other properties of co-substituted ZnFe2O4 nanocrystals synthesized by solvothermal reflux method. *Journal of Materials Science: Materials in Electronics* **2017** *29*, 737–45, http://dx.doi.org/10.1007/s10854-017-7967-2.
60. Manohar, A.; Vijayakanth, V.; Hong, R. Solvothermal reflux synthesis of NiFe2O4 nanocrystals dielectric and magnetic hyperthermia properties. *Journal of Materials Science: Materials in Electronics* **2019,** *31*, 799–806, http://dx.doi.org/10.1007/s10854-019-02588-z.
61. Manohar, A.; Krishnamoorthi, C.; Naidu, K.C.B.; Narasaiah, BP. Dielectric, Magnetic Hyperthermia and Photocatalytic Properties of $Mg_{0.7}Zn_{0.3}Fe_2O_4$ Nanocrystals. *IEEE Transactions on Magnetics* **2020,** *56*, 1–7, http://dx.doi.org/10.1109/tmag.2020.3024717.
62. Manohar, A.; Geleta, D.D.; Krishnamoorthi, C.; Lee, J. Synthesis, characterization and magnetic hyperthermia properties of nearly monodisperse CoFe2O4 nanoparticles. *Ceramics International* **2020**, *46*, 28035–41, http://dx.doi.org/10.1016/j.ceramint.2020.07.298.
63. Manohar, A.; Krishnamoorthi, C. Structural, optical, dielectric and magnetic properties of CaFe2O4 nanocrystals prepared by solvothermal reflux method. *Journal of Alloys and Compounds* **2017**, *722*, 818–27, http://dx.doi.org/10.1016/j.jallcom.2017.06.145.
64. A Manohar, A.; Krishnamoorthi, C. Low Curie-transition temperature and superparamagnetic nature of Fe3O4 nanoparticles prepared by colloidal nanocrystal synthesis. *Materials Chemistry and Physics* **2017**, *192*, 235–43, http://dx.doi.org/10.1016/j.matchemphys.2017.01.039.
65. Manohar, A. ;Krishnamoorthi, C.; Pavithra C,; Thota N. Magnetic Hyperthermia and Photocatalytic Properties of MnFe2O4 Nanoparticles Synthesized by Solvothermal Reflux Method. *Journal of Superconductivity and Novel Magnetism* **2020**, *34*, 251–9, http://dx.doi.org/10.1007/s10948-020-05685-x

66. Manohar, A.; Krishnamoorthi, C.; Naidu, K.C.B.; Narasaiah, B.P. Dielectric, Magnetic Hyperthermia and Photocatalytic Properties of $Mg_{0.7}Zn_{0.3}Fe_2O_4$ Nanocrystals. *IEEE Transactions on Magnetics* **2020**, *56*, 1–7, http://dx.doi.org/10.1109/tmag.2020.3024717.
67. Manohar, A.; Park, J.; Geleta, DD.; Krishnamoorthi, C.; Thangam, R.; Kang, H. Synthesis and characterization of ZnO nanoparticles for photocatalysis, antibacterial and cytotoxicity in kidney cancer (A498) cell lines. *Journal of Alloys and Compounds* **2021**, *874*, 159868, http://dx.doi.org/10.1016/j.jallcom.2021.159868.
68. Manohar, A.; Vijayakanth, V.; Pallavolu, MR.; Kim, KH. Effects of Ni - substitution on structural, magnetic hyperthermia, photocatalytic and cytotoxicity study of MgFe2O4 nanoparticles. *Journal of Alloys and Compounds* **2021**, *879*, 160515, http://dx.doi.org/10.1016/j.jallcom.2021.160515.
69. Manohar, A.; Chintagumpala, K.; Kim, KH. Magnetic hyperthermia and photocatalytic degradation of rhodamine B dye using Zn-doped spinel Fe3O4 nanoparticles. *Journal of Materials Science: Materials in Electronics* **2021**, *32*, 8778–87, http://dx.doi.org/10.1007/s10854-021-05549-7.