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Review Article

Magnetic structure of manganese cluster in photosystem II investigated by electron paramagnetic resonance

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The electronic structure of manganese (Mn) cluster in photosystem II was investigated by electron paramagnetic resonance (EPR) spectroscopy. In order to determine the spin density distribution in magnetically coupled Mn in the S_2 state Mn cluster, pulsed electron–electron double resonance (PELDOR) measurement was performed. The local environment of the Mn cluster was investigated by electron-nuclear double resonance (ENDOR). Using spin projections determined by PELDOR, ENDOR signals were assigned to the water molecules ligated to the Mn cluster. The location of a high-affinity Mn^{2+} site in apo-photosystem II, which is the initial site of photoactivation of the Mn cluster, was determined by PELDOR.

Key words: photosystem II, manganese cluster, EPR, PELDOR, ENDOR

Photosynthesis is a series of the energy-conversion reactions from light energy to chemical energy. Photosynthetic oxygen evolution is one of the greatest inventions given by the creature on earth. The oxygen evolution mechanism has been a great mystery for human beings since the establishment of modern science. Oxygen evolution occurs in a manganese (Mn) cluster in photosystem II (PS II) protein complex. In 2011, Umena *et al.* revealed the X-ray crystal fine structure of PS II with 1.9 Å [1]. This work provided a significant clue to elucidate the chemical reactions. Although great advancements have been achieved, the mechanism of oxygen evolution is still under debate. Photosystem II, with complicated structures and reactions, is attractive as it can be used to explore many scientific methods, such as X-ray free-electron laser (XFEL), large-scale quantum calculations, Fourier transform infrared spectroscopy (FTIR), and electron paramagnetic resonance (EPR). In this mini review, we introduce our recent work on magnetic structure of Mn cluster using EPR.

The main functional unit of PS II consists of two proteins, D1 and D2, with a pseudo C_2 symmetry (Fig. 1A) (see reviews [2,3]). In the initial reaction, photon is collected at the reaction center chlorophyll pair P680. The unpaired electron, generated by charge separation in P680, is subsequently transferred to a plastoquinone molecule, Q_A, through pheophytin molecule on the D1 protein, and transferred to Cytochrome b/f complex via plastoquinone molecule, Q_B.

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◀ ***Significance*** ▶

The great advancements have been achieved to elucidate the photosynthetic oxygen evolution since the discovery of the X-ray crystal fine structure of Photosystem II. However, the mechanism is still under debate. In this review, we reported the electronic structure of manganese cluster in photosystem II investigated by electron paramagnetic resonance spectroscopy. PELDOR measurement was performed to determine the spin density distribution in magnetically coupled Mn in the S_2 state Mn cluster. ENDOR signals were assigned to the water molecules ligated to the Mn cluster. The location of a high-affinity Mn^{2+} site in apo-photosystem II was determined by PELDOR.

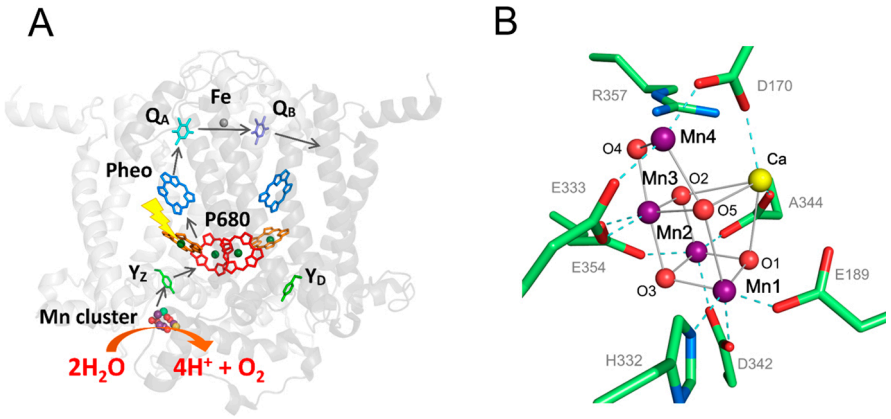

Figure 1 (A) Electron transfer chain for photosystem II (B) Structure model of Mn cluster (PDB: 4UB6).

The hole generated in $P680^+$ is transferred to the oxygen-evolving complex in the lumenal side of PS II via a redox-active tyrosine residue, D1-Tyr161, named as Y_Z. There is another redox-active tyrosine residue, D2-Tyr160, called Y_D, which is located at a symmetrical position to Y_Z on the D1/D2 complex. Y_D forms a stable neutral radical state, and therefore, is a signpost in EPR studies.

X-ray crystal structure analysis reveals the chemical formula of Mn_4CaO_5 for the oxygen evolving complex. In addition, ten amino acid residues, which ligate the metal cluster, are identified [1]. The structure of Mn_4CaO_5 cluster is called as a "distorted chair" structure (Fig. 1B). Three Mn ions, labeled as Mn1–Mn3, and one Calcium ion, are connected with four oxygen atoms, and the 4th manganese ion (Mn4) and 5th oxygen ion (O4) form the part of "backrest." The crystal structure revealed that there are four water molecules ligated to the cluster, labeled as W1–W4.

In the sequential reactions, two water molecules are oxidized to produce an oxygen molecule through a cyclic process of five distinct redox states, denoted as S_n states (n = 0–4) (Fig. 2). The S_0 state is the lowest redox state, and the S_1 state is the most stable state in the dark. Each S_n state advances to S_{n+1} by a single photon reaction in PS II. After advancement to the highest oxidation state S_4, the state is spontaneously converted to the lowest oxidation state S_0, with the release of an oxygen molecule [2,3].

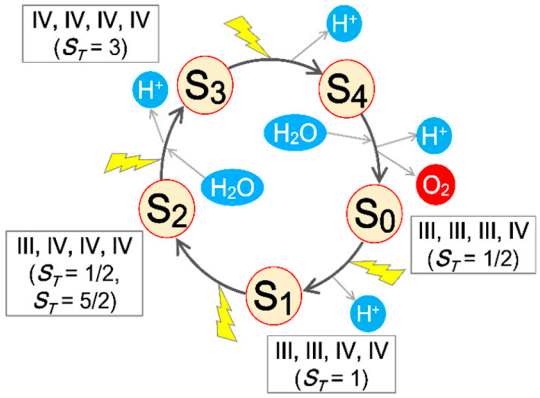

Figure 2 Schematic model for oxygen evolution cycle (Kok cycle).

1. Determination of spin projections on the Mn cluster using the PELDOR technique

EPR is a powerful tool to investigate the electronic structure on a molecule. EPR signals in the Mn cluster in the S_0–S_3 states can be detected. Generally, it is difficult to detect the EPR signal in an integer spin system. Therefore, it is difficult to detect the EPR signals in the S_1 and S_3 states. On the other hand, it is easier to detect the EPR signals in a half-integer spin system, such as the S_0 and S_2 states. Especially, the S_2 state multiline signal has been primarily studied as it is the main signal for the EPR studies. The S_2 state multiline signal is centered at $g = 2$ with an expansion over approximately 160 mT and is characterized by 19–21 hyperfine lines with a spacing of 8.5–9 mT between each pair of adjacent lines [4]. This signal has been ascribed to a spin state $\boldsymbol{S_T} = 1/2$ coupled magnetically with four Mn ions.

Generally, the spin Hamiltonian of the Mn cluster is written as follows:

$$H = \sum_i [\boldsymbol{B}\cdot\boldsymbol{g}_i\cdot\boldsymbol{S}_i + \boldsymbol{I}_i\cdot\boldsymbol{A}_i\cdot\boldsymbol{S}_i + \boldsymbol{S}_i\cdot\boldsymbol{D}_i\cdot\boldsymbol{S}_i] - \sum_{i<j} J_{ij}\boldsymbol{S}_i\cdot\boldsymbol{S}_j, \qquad (1)$$

where $\boldsymbol{S}_i$ and $\boldsymbol{I}_i$ are the operators of electron spin and nuclear spin of the i-th Mn ion, respectively, D_i is the zero-field splitting (ZFS) tensor for the i-th Mn ion, $\boldsymbol{g}_i$ is the g-tensor, and $\boldsymbol{A}_i$ is the effective hyperfine tensor of the i-th ion. J_{ij} is the exchange interaction between the i-th and j-th ions.

The determination of unique solutions of the six exchange interactions J among the Mn ions was experimentally difficult. Therefore, various models have been proposed. The effective hyperfine constants $A_{i,iso}$ in the S_2 state have been obtained by X- and Q-band ENDOR measurements, and the intrinsic hyperfine constants $a_{iso,}$ were estimated to be –165

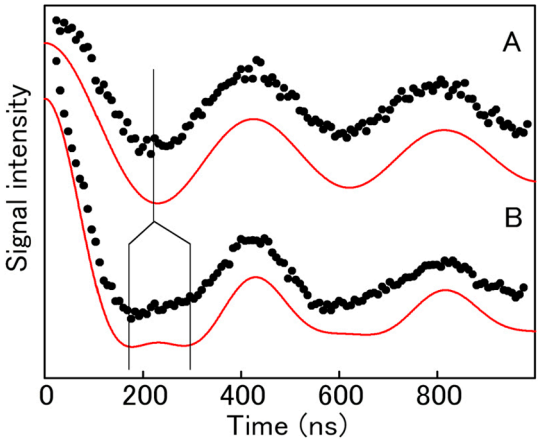

Figure 3 PELDOR signals between $Y_D^{\bullet}$ and S_2 state manganese cluster in the oriented PS II membrane. (Black circle) experimental and (red line) simulated signals. External magnetic field is applied (A) parallel and (B) perpendicular to the membrane normal [15].

to –225 MHz for Mn(III) and –187 to –253 MHz for Mn(IV) [5,6].

Pulsed EPR is a well-established method to manipulate electron spin by applying short and strong microwave pulses. Measurement in a short time range makes the spin relaxation effect negligible, indicating that a small magnetic interaction is detectable without irradiation of relaxation. Especially, pulsed electron-electron double resonance (PELDOR or DEER) is an excellent technique to detect electron spin-spin distance in the range of 10–100 Å with a resolution of 0.1 Å using two different microwaves [7]. We have investigated the magnetic structure of S_2 state Mn cluster.

PS II can be oriented two-dimensionally on a plastic film, such that the anisotropy of the magnetic interaction can be experimentally studied. Figure 3 shows the PELDOR signals between $Y_D^{\bullet}$ radical and S_2 state Mn cluster in the oriented membranes. The external magnetic fields were applied (A) parallel and (B) perpendicular to the membrane normal, $\boldsymbol{n}$. The circles and red lines show experimental and simulated signals, respectively. The oscillation patterns show magnetic dipole interaction between the two spin species. The PELDOR signals were obtained by a three-pulse sequence. The spin echo signal, formed by the 1st and 3rd pulses, is irradiated by the 2nd pulse with a different microwave frequency. The PELDOR signal amplitude $X(\tau')$ is a function of the time interval τ' between the 1st and 2nd pulses

$$X(\tau') \propto 1 - p[1 - \cos(2\pi D\tau')], \tag{2}$$

where p is the fraction of spin turned by the pumping pulse and D is the dipole interaction between the two spins.

In the case of PELDOR calculations of $Y_D^{\bullet}$ and the Mn cluster, the spin density distribution must be included. The dipole interactions are expressed as follows:

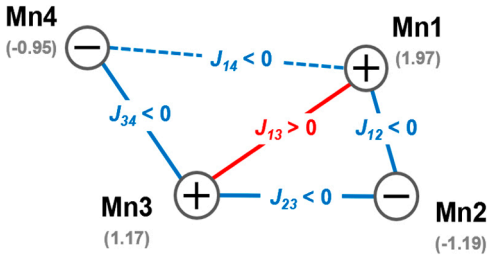

Figure 4 Spin projections on each manganese ion and the signs of exchange interaction J in the Mn cluster [15].

$$D = \sum_{i,j} \rho_i \rho_j \frac{g_1 g_2 \beta}{R_{ij}^3} (1 - 3\cos^2 \Theta_{ij}), \tag{3}$$

where ρ_i is the spin density at the i-th (i = 1–7) carbon/oxygen atom of the $Y_D^{\bullet}$ radical and ρ_j is the spin projection at the j-th (j = 1–4) Mn atom. R_{ij} is the length between the i-th (i = 1–7) carbon/oxygen atom of the $Y_D^{\bullet}$ radical and the j-th (j = 1–4) Mn atom. Θ_{ij} is the angle between the external magnetic field $\boldsymbol{H}$ and the distance vector $\boldsymbol{R}_{ij}$. The parameters g_1 and g_2 are the g-factors for the two electron spins, which were assumed to be 2.00 for $Y_D^{\bullet}$ and the S_2 multiline signal, and β is the Bohr magnetron.

In the oriented PS II sample, PELDOR intensity is given by:

$$I(\tau') = \iint X(\tau')\, G(\theta - \theta_0) \sin\theta d\theta d\varphi, \tag{4}$$

with

$$G(\theta - \theta_0) = \exp\left[-\frac{1}{2}\left(\frac{\theta - \theta_0}{\Delta}\right)^2\right], \tag{5}$$

where θ is the angle between $\boldsymbol{n}$ and external field $\boldsymbol{H}_0$, and $\boldsymbol{\Delta}$ is the distribution angle of the mosaic spread. $G(\theta - \theta_0)$ is the mosaic spread function which is assumed to be Gaussian. The z-axis was set to $\boldsymbol{n}$, along with the pseudo-symmetric C_2 axis of PS II. The coordinates of $Y_D^{\bullet}$ and the Mn atoms were obtained from a 1.9 Å resolution X-ray structure (PDB: 3ARC). The spin density distribution on $Y_D^{\bullet}$ was taken from density functional theory (DFT) calculation [8]. Based on these parameters, the spin density distribution on each Mn ion was evaluated. The simulated results show that the best fitting parameters were 1.97, –1.19, 1.17, and –0.95 for Mni (i = 1–4), respectively.

The obtained spin density on Mn1 is larger than the other Mn ions, indicating that the local spin at Mn1 is large. The valences of Mn in the S_2 state are believed to be 1 for Mn(III) and 3 for Mn(IV). Therefore, the virtual valence of Mn1 is derived as Mn(III). This is the first result where the location of Mn(III) in the S_2 state is experimentally obtained from the crystal structure. Figure 4 shows the relative signs of the exchange interaction J revealed by the PELDOR results, which connects the X-ray crystal structure with the magnetic structure.

2. Microenvironment of the Mn cluster using the ENDOR technique

It is essential to clarify the role of the surroundings of Mn_4CaO_5 in order to understand the oxygen evolving mechanism. Especially, the locations of the hydrogen atoms, which are essential to clarify the function of the Mn cluster because the hydrogen atoms are directly related to the water oxidation reaction and influence the DFT calculations. However, X-ray crystal analysis is difficult to resolve the protons.

Electron nuclear double resonance (ENDOR) is a powerful method to detect nuclear transitions surrounded in unpaired electrons. Figure 5A shows the proton matrix ENDOR of the S_2 state Mn cluster. Several pairs of signals, centered at about 15 MHz, are detected. Pair separation is determined by electron-nuclear magnetic dipole interaction and is proportional to $1/r^3$, where r is the distance between the Mn cluster and proton. For example, the maximum peak separation at 4 MHz is estimated to be 2.7 Å, using point-dipole approximation. However, the electron spin is actually distributed on the Mn cluster. Using the spin density distribution obtained by the PELDOR experiment and the crystal structure, we have assigned the ENDOR signals to the hydrogen atoms and W1–W4 water protons, connected to the Mn cluster. The H/D exchange ratios of W1 and W3 are high, showing the possibility of existence of the substrate water [9]. On the other hand, the exchange ratio of W2 is very low.

Some biochemical treatments are effective to understand the chemistry of the Mn cluster. Ca^{2+} depletion causes the loss of ENDOR signals assigned to the proton of W4 (Fig. 5B) [10], which connects Ca^{2+} and Y_Z directly. The role of Ca^{2+} involves the maintenance of the hydrogen-bond network near the Ca^{2+} site and the electron-transfer pathway to the Mn cluster.

It has been widely known that an alcohol molecule influences the magnetic structure of the S_2 state Mn cluster. Methanol has been proposed to bind to one or more Mn ions directly. Figure 6A shows the ENDOR spectra arising from a methanol molecule with (a) CH_3OH, (b) CD_3OH, and subtraction of (a) and (b). Two pairs of ENDOR signals, $A_{//}$ and $A_{\perp}$, are obtained for the methyl group of methanol in the subtraction spectrum. The obtained hyperfine constants show that the obtained distance between the methanol and the Mn ions is long, and therefore, methanol is not directly ligated to the Mn cluster. Figure 6B shows the isosurface plots of hyperfine couplings of the protons and carbon atoms. The blue and green surfaces show the possible locations for the methyl group of the methanol. The possible location for the methanol molecule is limited to the regions close to Mn1 (area I) and Mn4 (area II). The width of area I (located on Glu189) is approximately 4.0 Å perpendicular to the arc and 10 Å along the arc. The width of area II is approximately 3.5 Å. Glu189 and His332 amino acids near area I and Asp170 near area II are directly connected to the Mn cluster. Insertion of methanol near these amino acids might result in modification of the structure of the Mn cluster. Recent DFT calculations suggest that the S_2 state has two different isomers, which are called "open cubane" and "closed cubane" states [9,11]. The S_2 multiline signal is attributed to the $S = 1/2$ open cubane state in which O5 is connected to Mn4, Mn3, and Ca; Mn1 is in the five coordinated Mn(III) state. The g = 4 signal is attributed to the $S = 5/2$ closed cubane state, where O5 is coordinated to Mn1, and the closed cubane structure is composed of Mn1–3 and Ca. If methanol is located in area I, close to Mn1, the structural modification via the amino acids surrounding Mn1 might cause modification of the hyperfine anisotropy of Mn1 [12]. The interaction with the hydrogen-bonding network via W4 and Y_Z located nearby would lower the reaction rate [10]. On the other hand, modification of the spin structure is not necessary to be located close to Mn1 in a coupled spin system. Disruption of the hydrogen-bond network surrounding the Mn cluster would also cause the modification. Area II, near Mn4, would disrupt the whole hydrogen-bond structure and the equilibrium would be modified. In addition, Ser169 is in close proximity to Asn87, which is the only amino acid around the Mn cluster that differs between higher plants and cyano-

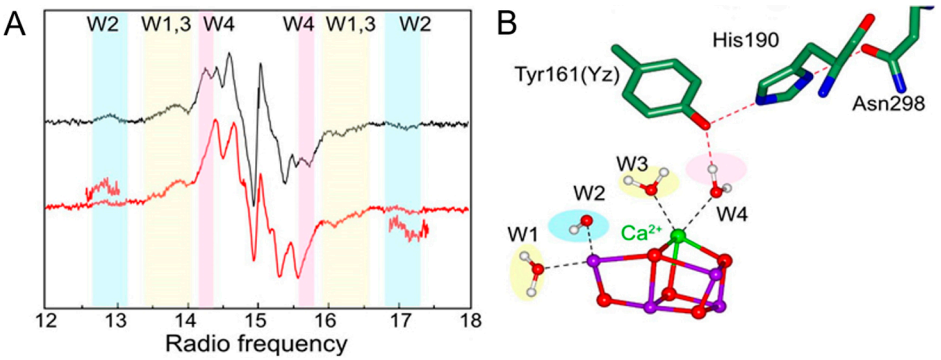

Figure 5 (A) ENDOR spectra of the S_2 state multiline in (black) untreated and (red) Ca^{2+}-depleted PS II and (B) locations of the corresponded protons [10,16].

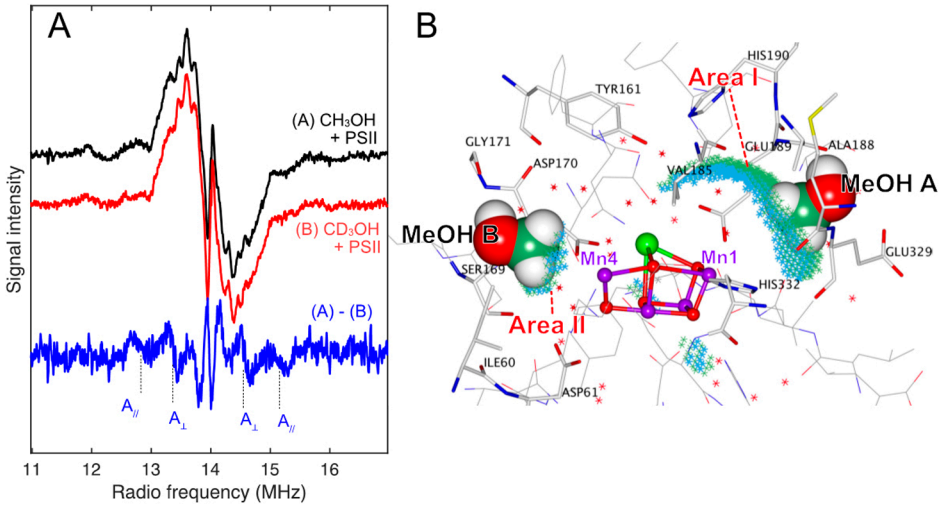

Figure 6 (A) Proton ENDOR spectrum spectra with (a) CH_3OH and (b) CD_3OH in the S_2 state Mn cluster. (B) Possible location of methanol molecule (areas I or II) near the Mn cluster [17].

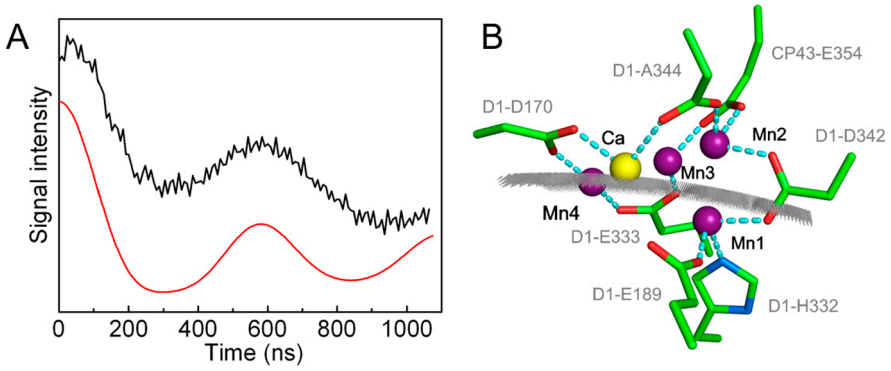

Figure 7 (A) Experimental (black) and simulated (red) PELDOR signals between (black) $Y_D^{\bullet}$ and Mn^{2+} at the high-affinity site. (B) Superposition of the possible Mn^{2+} site determined by PELDOR and the structure model of the native Mn cluster [18].

bacteria; the Asn87 in cyanobacteria is replaced by alanine in higher plants. If the difference is ascribed to the stability of the isomer multiline / $g = 4$ state through the hydrogen-bonded network, then the insertion of the methanol molecule in area II would disturb the network.

3. Unique affinity site of Mn^{2+} in apo-PS II for photoactivation using the PELDOR technique

The Mn cluster is easily removed by a reducing agent, such as NH_2OH. Under light, the Mn cluster is reassembled in the presence of Mn^{2+} and Ca^{2+}. This reaction is called "photoactivation". There is a high Mn^{2+} affinity site in the apo-PS II [13]. The high affinity site is believed to be the initial location for photoactivation. Figure 7A shows the PELDOR signal between the $Y_D^{\bullet}$ radical and Mn^{2+} located at the high affinity site in PS II. Assuming point-dipole approximation, the distance is estimated to be 30.5 Å. Figure 7B shows the superposition of the structure of the native Mn cluster and a possible high Mn^{2+} affinity site indicated in gray. The results show that the high Mn^{2+} affinity site is located near Mn4 in the structure of the native Mn cluster. Mn^{2+} would be fixed with axial ligands, Asp170 and Glu333, in the D1 polypeptide. The position of Mn1–3 in the native structure is difficult to bind axially to an amino acid, which is consistent with the PELDOR results. The structure connecting Asp170 and Glu333 with Mn^{2+} determines the orientation of the other C=O in the carboxylate of Asp170 and Glu333, which is the same as that in the structure of the native Mn cluster. Therefore, the structure would form a mold of the Mn cluster.

4. Summary

In this mini review, we have presented recent EPR studies on magnetic structure of Mn cluster. EPR results showed the magnetic structure of the Mn cluster, which complemented the molecular structure. The development of a highly-resolved

X-ray crystal analysis advanced the understanding of the oxygen evolution mechanism. Recently, the structure of the higher oxidation state S_3 state obtained using XFEL was reported [14]. With advancement in the X-ray crystal structure analysis, EPR would play an important role in future.

Conflicts of Interest

H. N., M. A. and H. M. declare that they have no conflict of interest.

Author Contribution

H. N. and M. A. performed EPR measurements. H. N., M. A. and H.M have analyzed the experimental results.

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