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View Article Online
DOI: 10.1039/C7CP07248A

Journal Name

COMMUNICATION

Received 00th January 20xx,

Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Orientation selection in high-field RIDME and PELDOR experiments involving low-spin Co^{II} ions

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Orientation selective (OS) RIDME and PELDOR were conducted on a low-spin Co^{II} complex coordinated by two nitroxide (NO) labelled *2,2′:6′,2″*-terpyridine ligands. Co-NO RIDME at W- and Q-band gave insight into the relative orientation between the Co-NO interspin vector ($r_{\text{Co-NO}}$) and the NO moiety. This was further supported by W-band Co-NO PELDOR that also allowed elucidating the relative orientation of the Co^{II} and NO *g*-tensors. Differences to earlier predictions were confirmed by DFT calculations. Finally, NO-NO PELDOR allowed retrieving the mutual orientations between the NO-NO interspin vector ($r_{\text{NO-NO}}$) and the NO moieties. The results demonstrate that OS-RIDME and -PELDOR can provide geometric structure information on a system containing a Co^{II} ion and two nitroxides. Especially, the high sensitivity and ease of interpretation of RIDME at W-band opens avenues for new applications of Co^{II} as orthogonal spin label.

Over the past two decades pulse dipolar electron paramagnetic resonance (PD-EPR) has become valuable in resolving biomolecular architectures[1, 2] by allowing measurement of nanometre-range distances between intrinsic or chemically harboured unpaired electrons. Nitroxide (NO) labels and endogenous paramagnets are typically used in PD-EPR,[3] while other approaches involve the substitution of naturally occurring diamagnetic metal ions by paramagnets.[3] Pulsed electron-electron double resonance (PELDOR/DEER)[4-6] has been a cornerstone for measuring spin-spin distances (1-10 nm and beyond)[7] and relaxation induced dipolar modulation enhancement (RIDME)[8, 9] has recently emerged as an attractive alternative. PELDOR and RIDME employ microwave (mw) pulses to observe one electron spin (A) whilst inverting a coupled electron spin (B). Inversion of the B spin in PELDOR is induced by a coherent mw pulse, whilst in RIDME it is by longitudinal relaxation.[9, 10] The systematic variation of the timing of the B-spin inversion leads to a modulation of the A spin signal with the dipolar coupling frequency (ν_{dip}).

$$\nu_{dip} = \frac{\mu_0 \mu_B^2 g_A g_B}{4\pi\hbar} \frac{1}{{r_{AB}}^3} (1 - 3\cos^2\theta_{AB}) \qquad (1)$$

$\hbar$ is the reduced Planck's constant, μ_0 the permeability of vacuum, μ_0 is the Bohr Magneton, g_{i} is the *g*-factor of spin i, r_{AB} is the scalar distance between the two spins and θ_{AB} is the angle between the distance vector and the external magnetic field. In most cases, a random distribution of θ_{AB} can be assumed leading to the well-known frequency distribution, called Pake pattern. This distribution features "horns" and "edges" which correspond to the distance vector perpendicular to the magnetic field vector ($\nu_{\perp}$ at $\theta_{\text{AB}} = 90°$) and to a less probable distance vector parallel to the field ($\nu_{\parallel}$ at $\theta_{\text{AB}} = 0°$), respectively.

Interest in RIDME has been growing over the past three years[10-21] especially for measurements involving spectrally broad paramagnetic metal ions. Here, all spins can act as B spins and contribute to a larger modulation depth often leading to increased sensitivity.[11, 15, 22, 23] In PELDOR the limited excitation of broad EPR spectra by rectangular pulses can lead to selection of certain *g*-tensor components, a phenomenon referred as 'orientation selection' (OS).[4, 22-31] If the spin centres are connected with flexible linkers as is often the case in site directed spin labelling[32] OS will not affect the distance measurements. However, pronounced OS in combination with rigidly attached spin centres can lead to breakdown of the approximation of a Pake pattern as frequency response. In other words, selection of certain *g*-tensor values can lead to selection of certain molecular orientations which in turn conditions the values of θ_{AB} making ν_{dip} in eq. 1 dependent on the spectral components excited by the mw pulses. With the

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Electronic Supplementary Information (ESI) available: Primary data and details on data processing. See DOI: 10.1039/x0xx00000x

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DOI: 10.1039/C7CP07248A

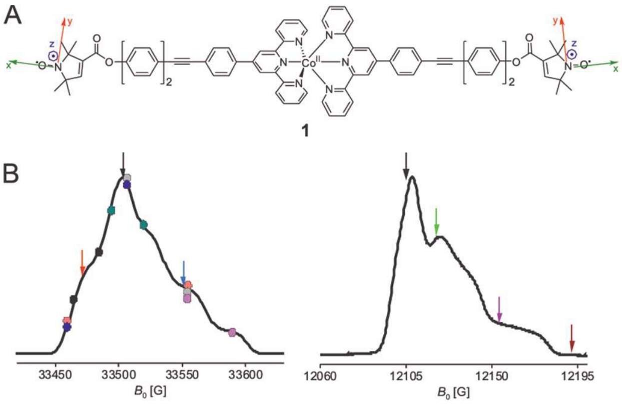

Fig. 1. Structure of complex **1** indicating the molecular axes of NO as investigated by our measurements (A); Field swept spectra of **1** optimized for NO at W- and Q-band (B, left and right, respectively). Circles indicate the detection/inversion positions of NO-NO PELDOR and arrows the detection and inversion position of Co-NO RIDME and PELDOR measurements, respectively.

special exception of Gd^{III} [33] and Mn^{II} [34] spins, OS of paramagnetic metals severely increases with high-fields, due to *g*-anisotropy, which can require measurements at multiple fields to reliably determine the distance(s). On the other hand, OS measurements can also yield atomic-level geometric and electronic structure information, as highlighted in OS-PELDOR of tyrosyl radicals in ribonuclease reductase.[30] Strikingly, the accuracy and precision of structural insight provided by OS-PELDOR allowed benchmarking DFT-based modelling parameters for nucleic acids.[35]

RIDME data also contains OS information with respect to the A spin. OS-RIDME effects were observed on a Cu^{II} system measured at Q-band frequencies; however, they were not analysed to extract geometric information.[19]

Cobalt spins have rarely been exploited in PD-EPR, except for one broadband PELDOR study on a Co^{II} porphyrin system at X-band.[36] In this work, a novel Co^{II} complex **1** having NO spins as the terminal parts of the terpyridine ligands[37] was synthesized (Fig. 1, A).

1 is expected to be rigid with negligible exchange coupling between the spins[23] and Co^{II} is known to form low-spin ($S = 1/2$) complexes with axial *g*-tensor with strong field ligands such as terpyridines.[38] Dependent on substitution, solvation and counter-ions spin-crossover is known to occur at temperatures above those commonly used for EPR distance measurements.[39, 40]

Here, we employed Co-NO OS-RIDME at W- and Q-band frequencies and Co-NO OS-PELDOR at W-band to derive structural information for **1**. The results were additionally supported by NO-NO OS-PELDOR at W-band.

Structural model and predicted results

From W-band measurements on similar but shorter and covalently linked bis-nitroxide chemical models[31, 41] we can predict the components of the Pake pattern that will dominate in OS-PELDOR and -RIDME measurements in **1**. To first order, one can approximate the NO bond to be along the backbone, but to fully rationalise[42] the OS- PELDOR data the NO moiety had to be modelled rotating freely on a cone with an opening angle of 25° about the molecular backbone.[41] In the Co-NO RIDME experiment the longitudinal relaxation of cobalt is assumed to be isotropic and ν_{dip} will solely be determined by the orientations of the NO selected by mw pulses observing the A spin. This will be highly complementary to Gd^{III}-NO PELDOR at W-band where ν_{dip} has been shown to be entirely determined by the NO orientations excited.[43] Using the common definition of the NO principal axes and the axial *g*-tensor of Co^{II} [38] we approximate NO g_x to be parallel to the Co-NO and NO-NO inter-spin vectors ($\boldsymbol{r}_{\text{Co-NO}}$ and $\boldsymbol{r}_{\text{NO-NO}}$, respectively) and therefore to the molecular backbone, while NO g_y and g_z to be perpendicular (Fig. 1, A). Accordingly, when exciting NO g_x spins we expect to select molecules with the NO bonds parallel to the magnetic field. Thus, $\boldsymbol{r}_{\text{Co-NO}}$ will be confined to be parallel to the field and modulation frequency is expected to correspond to “the parallel component” ($\nu_{\parallel}$). Substituting $\theta_{AB} = 0°$ in eq. 1 will yield in absolute values the double frequency of the $\nu_{\perp}$ case (“the perpendicular component” when $\theta_{AB} = 90°$). Detecting NO g_y and g_z is expected to select $\boldsymbol{r}_{\text{Co-NO}}$ perpendicular to the magnetic field vector. Furthermore, the geometry is assumed to fix the NO g_x components of both ligands collinearly, while g_y and g_z components can become interchangeable due to ester bond rotation. Comparison of NO-NO PELDOR experiments with previous results on a similar but shorter covalent bis-nitroxide allows to assess the overall formation and rigidity of the expected NO-Co-NO “yardstick”.

The Co-NO PELDOR experiment is expected to reveal information complementary to RIDME further correlating the component of Co *g*-tensor selected by the observer pulses with the different NO orientations as shown for a similar Cu^{II}-nitroxide model compound at X-band.[42] Due to instrumental bandwidth restrictions, only high fields (equivalently low *g*-values) of Co^{II} spectrally close to NO spins can be excited (see Supporting Information (SI), Fig. S1). According to literature[38] this component should correspond to a Co *g*-tensor orientation having the vector between Co^{II} and the central pyridine nitrogen parallel to the field. Thus, according to the approximations made above $\boldsymbol{r}_{\text{Co-NO}}$ should be parallel to the magnetic field vector ($\theta_{AB} = 0°$) and $\nu_{\parallel}$ should be observed when NO g_x spins are inverted, while no modulation is expected when NO g_y or NO g_z spins are inverted.

Nitroxide-nitroxide PELDOR

Measurements correlating the *xx* (denoting the detection and inversion positions, respectively, throughout the text; Fig. 2 A black), *yy* (dark cyan), *zz* (pink), *zx* (salmon), *zy* (light gray) and *xy* (blue) NO orientations (and details in SI) are in excellent agreement with previous experiments on shorter covalent bis-nitroxides,[31, 41] demonstrating that the expected complex is indeed formed and is equally rigid to a covalently bonded system.

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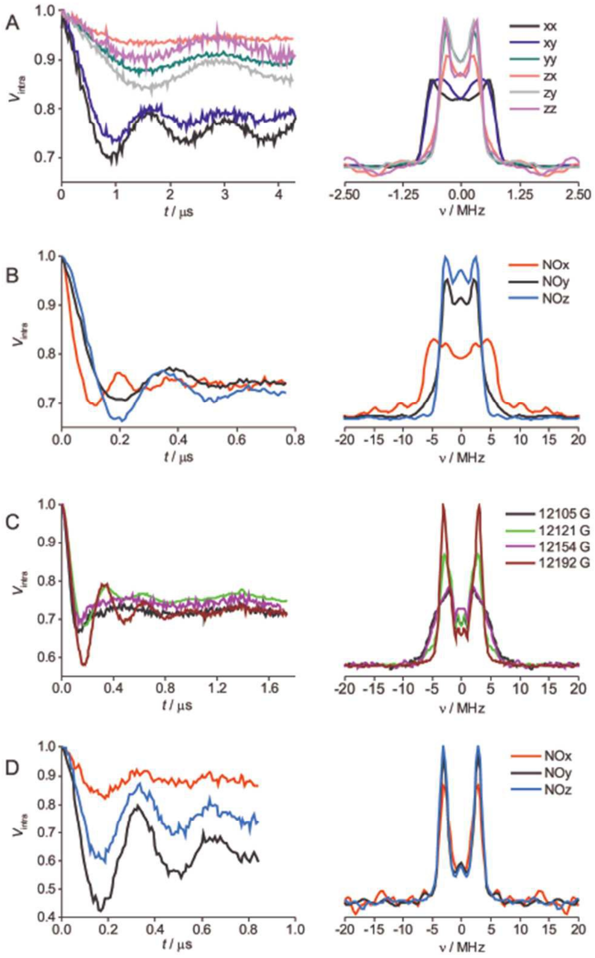

Fig. 2. Background corrected PELDOR and RIDME experiments (left) and corresponding dipolar spectra (right). NO-NO PELDOR at W-band (A); Co-NO RIDME at W- (B) and Q-band (C, here the field values are given due to admixture of NO g_x, g_y and g_z); Co-NO PELDOR at W-band (D). The colours correspond to the colours used in Fig. 1, B.

Co-nitroxide RIDME

Co-NO OS-RIDME was performed at W- and Q-band frequencies (Fig. 2, B and C, respectively) detecting NO spins at different positions of the spectrum (arrows on Fig. 1, B, left and right) with average measurement time 20 min. The W-band RIDME spectra exhibit significant double frequency contributions, $\nu_{\parallel}$, when observing on the NO g_x component (red arrow) indicating that this orientation must be parallel to $\boldsymbol{r}_{\text{Co-NO}}$. Performing RIDME at *y*- and *z*-orientations (black and blue arrows, respectively) gave a modulation frequency, $\nu_{\perp}$, corresponding to molecules with $\boldsymbol{r}_{\text{Co-NO}}$ perpendicular to the field. At Q-band the dependence of the RIDME signal on the position within the NO spectrum is less pronounced. To better resolve the parallel component of the Pake pattern at 12154 G more selective pulses were applied (SI, Fig. S21-S23). The effects observed in Q-band RIDME are similar to those observed on the analogous Cu^{II} complex[19] and results are in agreement with the NO-NO OS-PELDOR.

Beyond the ease of resolving NO orientation with respect to $\boldsymbol{r}_{\text{Co-NO}}$ providing structural information, performing Co-NO RIDME, itself, opens new routes to metal-based labelling strategies. Firstly, Co^{II} RIDME, in contrast to Co^{II} PELDOR, is orientation-free with respect to cobalt. Secondly, employing high-field ligands commonly used for Mn^{II} and Gd^{III} labelling strategies[33] are expected to yield low-spin Co^{II} tags that in contrast to Mn^{II} and Gd^{III} will not suffer from overtones[13, 17, 44] during the RIDME experiment since the free electron will undergo only one ($-1/2 \leftrightarrow +1/2$) transition. Therefore, low-spin Co^{II} tags seem promising for fast, high-sensitive RIDME applications at low-frequency bands.

Co-nitroxide PELDOR

Co-NO OS-PELDOR (Fig. 2, D) was conducted at W-band inverting NO (arrows in Fig. 1, B, left) while observing the low *g*-component of Co^{II}. The data show that exciting any orientation of NO selects molecules with $\boldsymbol{r}_{\text{Co-NO}}$ perpendicular to the field ($\nu_{\perp}$). It follows that if the low *g* component is assigned to the backbone parallel to the field as predicted above, Co-NO PELDOR would yield the parallel component, $\nu_{\parallel}$, while little or no modulation depth would be observed detecting NO g_y, g_z. However, for all orientations $\nu_{\perp}$ was observed with the largest modulation depths for Co-NO g_y and Co-NO g_z measurements (SI, Table S6) and only very little for Co-NO g_x. These data can only be rationalized if the low Co *g*-component corresponds to an orientation of the backbone perpendicular to the magnetic field. Now, inverting NO g_y or g_z will select complexes with the molecular backbone perpendicular to the magnetic field yielding $\nu_{\perp}$ and large modulation depth as observed experimentally. To validate this finding, we performed DFT calculations on the bis-terpyridine dication of Co^{II} (details in SI). The calculations show significant deviation from axiality. The eigenvalues were estimated to be 2.005, 2.087, 2.141, with the smallest *g*-value (highest field) corresponding to an eigenvector perpendicular to the Co^{II}-N bond of the central pyridine (corresponding to the backbone of our model system). Thus, the backbone is perpendicular to the magnetic field in excellent agreement with our Co-NO PELDOR data.

Conclusions

Overall, we have demonstrated the feasibility of performing Co-NO OS-RIDME allowing reconstruction of the relative orientation between $r_{\text{Co-NO}}$ and the NO *g*-tensor. Co-NO OS-PELDOR also gave insight into the Co *g*-tensor orientation, and was further supported by DFT calculations. NO-NO OS-PELDOR supported our structural model and comparison with earlier data on shorter covalent analogues confirmed the assumed rigidity of **1**. Through a combination of W-band OS-RIDME and -PELDOR we have demonstrated that low-spin Co^{II} can be straightforwardly employed for exploring the molecular structure of systems of unknown geometry. The high sensitivity of high power W- and Q-band RIDME make it a

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highly appealing choice among PD-EPR methods for measuring distances involving low-spin Co^{II} ions.

The research data supporting this publication can be accessed at https://doi.org/10.17630/86c5f65b-8c14-429e-97c1-8f8901290995

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ AG and CLM were supported by postgraduate fellowships by the EPSRC-funded doctoral training centre ‘integrated magnetic resonance’. This work was supported by the European Union Seventh Framework Programme (PCIG12-GA-2012-334496). The W-band instrument was developed under the U.K. Research Councils Basic Technology Program (grant EP/F039034/1) and the Q-band equipment is supported by the Wellcome Trust (099149/Z/12/Z). Computations were performed on a local Xeon cluster supported by EaStCHEM and maintained by Dr H. Früchtl.

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