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Perspectives in Magnetic Resonance

What are the methodological and theoretical prospects for paramagnetic NMR in structural biology? A glimpse into the crystal ball

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ABSTRACT

NMR spectroscopy is very sensitive to the presence of unpaired electrons, which perturb the NMR chemical shifts, *J* splittings and nuclear relaxation rates. These paramagnetic effects have attracted increasing attention over the last decades, and their use is expected to increase further in the future because they can provide structural information not easily achievable with other techniques. In fact, paramagnetic data provide long range structural restraints that can be used to assess the accuracy of crystal structures in solution and to improve them by simultaneous refinements with the X-ray data. They are also precious for obtaining information on the conformational variability of biomolecular systems, possibly in conjunction with SAXS and/or DEER data. We foresee that new tools will be developed in the next years for the simultaneous analysis of the paramagnetic data with data obtained from different techniques, in order to take advantage synergistically of the information content of all of them. Of course, the use of the paramagnetic data for structural purposes requires the knowledge of the relationship between these data and the molecular coordinates. Recently, the equations commonly used, dating back to half a century ago, have been questioned by first principle quantum chemistry calculations. Our prediction is that further theoretical/computational improvements will essentially confirm the validity of the old semi-empirical equations for the analysis of the experimental paramagnetic data.

1. Prospects for NMR of paramagnetic molecules

NMR of paramagnetic molecules – especially biomolecules - is a topic of increasing interest, because of the possibility offered by the paramagnetic data to provide long-range structural information on the overall displacement of different protein regions, accurate information on local conformational details, and information on the presence and amount of intramolecular mobility, under close to physiological conditions [1,2]. Therefore, paramagnetism-based NMR experiments are more and more exploited even for biological systems (proteins, nucleic acids, oligosaccharides, etc.) which are not natively paramagnetic, thanks to the possibility to substitute a diamagnetic metal, if present, with a paramagnetic metal, or to attach paramagnetic tags [3–10]. The increasing attention devoted to the NMR of paramagnetic systems is witnessed by the increasing number of publications appearing yearly on this subject (Fig. 1).

The presence of a paramagnetic center causes changes in the NMR shifts (called hyperfine shifts) and in the *J* splittings of the NMR peaks of nuclei connected with one another through chemical bonds (called residual dipolar couplings, RDCs) with respect to the shifts and *J* splittings measured for a diamagnetic analogue of the sample. The hyperfine shift is composed of the Fermi-contact and pseudocontact contributions, the former being proportional to the amount of unpaired electron spin density delocalized onto the detected nucleus, the latter to the dipolar field generated by the electron magnetic moment. For nuclei far away from the paramagnetic center, the hyperfine shift thus corresponds to the pseudocontact shift (PCS). Another major effect of the presence of a paramagnetic center is the increase in the nuclear relaxation rates, called paramagnetic relaxation enhancement (PRE) which also manifests itself into an increase of the linewidths of the NMR peaks.

PCSs, paramagnetic RDCs and PREs contain structural information of different types. In particular, PCSs contain long-range information on the macromolecular structure, as they depend on the position of each atom in a unique axis frame centered on the paramagnetic metal ion, and RDCs depend on the relative positions of coupled nuclei, again in a unique reference frame [11–15]. Paramagnetic RDCs can be measured for all coupled nuclei, independently of their distances from the paramagnetic metal, provided

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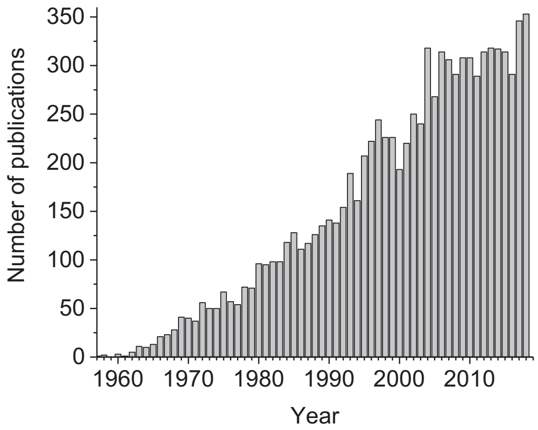

Fig. 1. Number of published papers dealing with paramagnetic NMR (as determined from a search with Scopus).

that their signal linewidth is small enough to determine the position of the peak with good accuracy, and PCSs, although depending on the inverse third power of the metal-nuclear distance, can be measured for ^{1}H distances as large as 50 Å from the paramagnetic ion for some lanthanoids, as shown in Fig. 2a. The figure also shows the radii of the ^{1}H blind spheres around the paramagnetic metal ions, for macromolecules with reorientation time of ca. 10 ns. Protons within this sphere can hardly be detected due to the large paramagnetic broadening resulting from the PREs. Since PREs depend on the squared magnetogyric ratio of the nucleus and on the sixth power of the inverse metal-nucleus distance, the radii of the blind spheres for ^{13}C nuclei are reduced by a factor $16^{1/6}$ ($\approx$1.6), and for ^{15}N nuclei by a factor $97^{1/6}$ ($\approx$2.1). In the case of systems with some degrees of internal mobility, the values of PCSs, RDCs and PREs (assuming for the latter that the time of interconversion is longer than the correlation time of the modulation of the electron-nucleus dipole-dipole interaction) are population-weighted averages of the values corresponding to the conformations sampled by the molecules, thus allowing for recovering information (although by far incomplete, due to the ill-posed nature of the problem) on the conformational freedom of the system. Paramagnetic data can thus provide a wealth of structural and dynamical information for proteins [2,16–19], oligosaccharides [20] and nucleic acids [21–23].

In summary, the relatively small set of paramagnetic data is rich of information on the local and global structure, as well as on the mobility of the molecule, in close to physiological conditions. The importance of this type of information is particularly relevant for the investigation of systems for which other techniques cannot

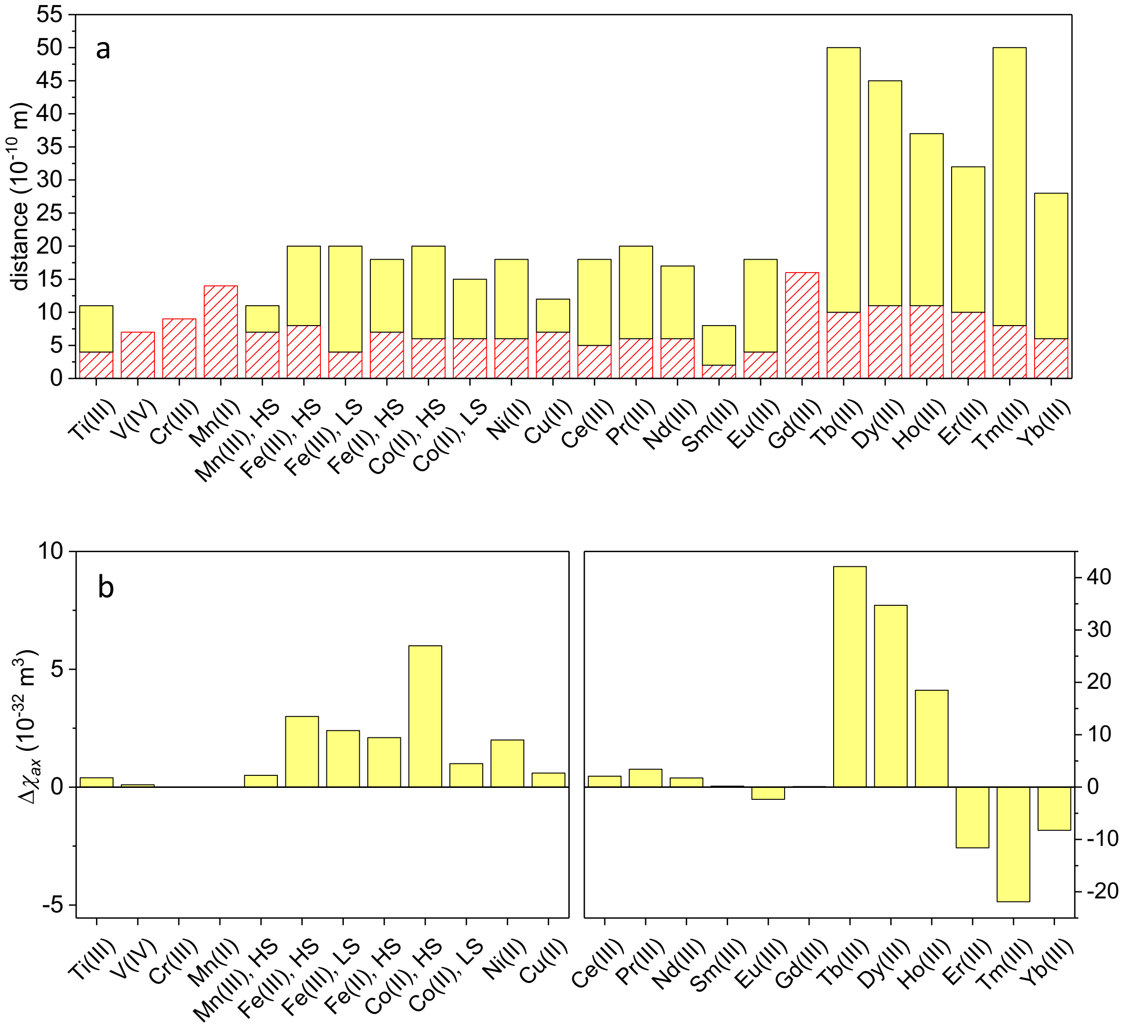

Fig. 2. Typical distance ranges of observability of ^{1}H PCSs (a) and paramagnetic susceptibility anisotropy (b) of paramagnetic first row transition metal and lanthanoid ions (HS: high spin; LS: low spin). The regions in red in (a) indicate the blind spheres caused by paramagnetic line broadening excessing 100 Hz.

be applied or are not informative enough [24–29]. If we could have a glimpse into a crystal ball we would see a brilliant future for paramagnetic NMR, also thanks to the availability of a collection of well suited paramagnetic tags which can allow full exploitation of the paramagnetic effects also for natively diamagnetic systems.

2. The future is in integrated approaches

On the other hand, paramagnetic data alone are insufficient for recovering a full structural characterization of a biomolecule. We thus need to analyze these data in conjunction with complementary restraints, such as those obtained from chemical shift index [30], or encoded in structural libraries based on residue sequences [31,32]. Indeed, the long-range nature of paramagnetic NMR data can be precious to validate, and possibly refine, structures obtained through X-ray data, cryo-electron microscopy, or by homology modeling. Recently, it has been shown that the paramagnetic data can be used together with X-ray reflections to refine protein structures. X-ray crystallography is usually the method of choice for solving protein structures when obtaining protein crystals is possible, because of the very short time required for measurement and data analysis and of the resulting precision, usually higher than that obtained by NMR because of the very large number of X-ray reflections. However, due to the presence of crystal packing forces in the solid state, crystal structures may not be accurate models for the macromolecular structures in solution, and the atomic coordinates may exhibit a non-negligible level of inaccuracy, called “structural noise” [33]. The joint refinement of protein structure against both X-ray reflections and NMR data provides two advantages: (1) validate the consistency of the molecular structures in solution and in the solid state [34,35], and (2) refine the structure of the protein in solution, possibly within the accuracy allowed by X-ray data (Fig. 3). This approach also allows for the identification of mobile regions in solution, as well as of overall rearrangements in the positions of protein domains on passing from the crystalline form to solution [36].

On this basis, it is easy to foresee that paramagnetic data will be more and more exploited in integrated structural biology protocols, in order to take full advantage of the complementary information contained in data collected from different techniques. Our crystal ball suggests that cryo-electron microscopy data will be the most interesting data to analyze together with NMR data, as they can be collected even for macromolecules that do not crystallize or are difficult to crystallize in specific functional states.

Furthermore, we have also proposed the possibility of exploiting PCSs and paramagnetic RDCs measured for nuclei far from the paramagnetic metal to refine the protein structure around the metal ion. In fact, in a close future we expect that it will be possible to calculate the tensor at the origin of these data, which strictly depends on the conformational details about the metal ligands positions. Such calculations require the knowledge of the relationship between the tensor and the EPR observables (**g** tensor and **D** tensor, see later) or, in the absence of a direct experimental access to these quantities under the same conditions at which the NMR data are collected, they require the use of quantum chemistry tools. While attempting this second route, an unexpected conflict popped up with the traditional semi-empirical theory. The quantum chemistry and semi-empirical approaches, and our prediction of how the conflict will be resolved, are summarized in the next section.

3. Paramagnetic restraints in the semi-empirical and the first principle quantum chemistry frameworks

The first descriptions of the PCSs were given by Bloembergen and Dickinson in 1950 [37] and by McConnell and Robertson in 1958 [38]. The equations that they derived are a function of the nuclear coordinates and of the anisotropy and orientation of the **g** tensor. The described dependence on the difference of the square of the *g* values along the three axes defining the main frame of the **g** tensor actually holds when the spin multiplet ground state is well isolated from excited electronic states and zero field splitting is absent or negligible. A more complete equation, valid also outside these conditions, was derived by Kurland and McGarvey [39] in 1970, in a research article published in the second volume of *J. Magn. Reson.* (not the first, alas, otherwise this contribution would fall exactly 50 years after!). Since then, PCSs are commonly described by Eq. (1):

$$\delta^{pc} = \frac{1}{12\pi r^3}\left[\Delta\chi_{ax}\left(3\cos^2\theta - 1\right) + \frac{3}{2}\Delta\chi_{rh}\sin^2\theta\cos 2\phi\right] \tag{1}$$

where $\Delta\chi_{ax} = \chi_{zz} - \frac{\chi_{xx}+\chi_{yy}}{2}$ and $\Delta\chi_{rh} = \chi_{xx} - \chi_{yy}$ are the axial and rhombic anisotropies of the paramagnetic susceptibility tensor $\boldsymbol{\chi}$, r is the metal-nucleus distance, and θ and ϕ are the angular coordinates of the nucleus in the principal frame of the $\boldsymbol{\chi}$ tensor. Kurland and McGarvey pointed out that the electron-nucleus hyperfine coupling, which is at the origin of the observed PCSs, depends on the interaction between the nuclear spin magnetic moment and the average total electron magnetic moment, which comprises both the spin and orbital components. In the presence of an external magnetic field, a nucleus far away from the paramagnetic metal thus senses an additional dipolar magnetic field generated by the average total electron magnetic moment, the intensity of which increases linearly with the field (until saturation conditions) with a proportionality constant described by the paramagnetic susceptibility $\boldsymbol{\chi}$. The symmetric tensorial quantity $\boldsymbol{\chi}$ is thus the proper and sole physical origin of the PCSs. The typical magnitudes of the axial anisotropies of the $\boldsymbol{\chi}$ tensors of first row paramagnetic transition metal ions and of paramagnetic lanthanoid ions are shown in Fig. 2b. By convention, the labeling of the main axes of the tensors are fixed in order to have rhombic anisotropies with absolute values ranging from 0 to $2\Delta\chi_{ax}/3$.

The same paramagnetic susceptibility tensor $\boldsymbol{\chi}$ is also at the origin of the paramagnetic RDCs. In fact, in the presence of a magnetic susceptibility anisotropy, the energy of a molecule changes as a function of its orientation with respect to the magnetic field direction, and orientations with lower energy will be favored with respect to orientations with higher energy. As a result, a partial

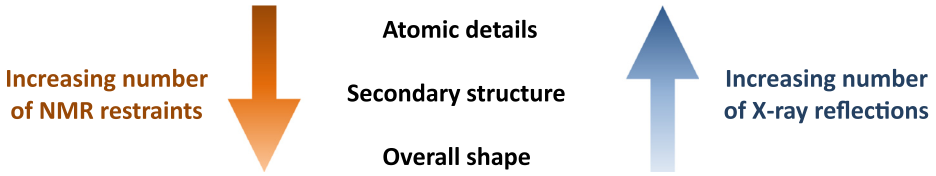

Fig. 3. Structural information available from X-ray increases from overall shape to atomic details with increasing number of X-ray reflections, extending to larger and larger values of reciprocal distances; conversely, structural information available from NMR increases from atomic details to overall shape with increasing number of collected restraints (reproduced from [1]).

self-alignment of the molecules occurs, which determine the additional contribution to the *J* splitting. The difference between the splitting in the paramagnetic molecule and in the diamagnetic analogue is the paramagnetic RDC, as the effect of the diamagnetic contribution to the magnetic susceptibility anisotropy is present also in the subtracted value of the diamagnetic sample. The effect was first described in 1981 [40] and measured for biomolecules in 1995 [41], being given by

$$\Delta\nu_{12}^{\mathrm{prdc}} = -\frac{1}{4\pi}\frac{B_0^2}{15kT}\frac{\gamma_{I1}\gamma_{I2}\hbar S_{LS}}{2\pi r_{12}^3}\left[\Delta\chi_{ax}(3\cos^2\alpha - 1) + \frac{3}{2}\Delta\chi_{rh}\sin^2\alpha\cos2\beta\right] \tag{2}$$

where the vector $\mathbf{r}_{12}$ is the distance between the coupled nuclei, α is the angle between $\mathbf{r}_{12}$ and the *z* axis of the diagonal $\boldsymbol{\chi}$ tensor, and β is the angle which describes the position of the projection of $\mathbf{r}_{12}$ on the *xy* plane of the $\boldsymbol{\chi}$ tensor, relative to the $\boldsymbol{\chi}$ axis. S_{LS} indicates the Lipari-Szabo order parameter accounting for the local mobility of the bond vector [42].

PCSs and RDCs were used for the first time as structural restraints depending on the paramagnetic susceptibility anisotropy tensor in papers from our lab in 1996 and 1998 [43,44]. Since then, PCSs and RDCs have been extensively analyzed to obtain information on structure and dynamics of biomolecular systems using Eqs. (1) and (2).

As summarized in Table 1, besides PCSs and paramagnetic RDCs, also Curie relaxation depends on the $\boldsymbol{\chi}$ tensor, as it also results from the interaction of the nuclear magnetic moment with the average total electron magnetic moment. The dipole-dipole nuclear-electron relaxation is instead related to the interaction between the nuclear magnetic moment and the total electron magnetic moment, and thus it depends on the **g** tensor, whereas the Fermi-contact shift and relaxation depend on the interaction between the nuclear magnetic moment and either the average electron spin magnetic moment or the electron spin magnetic moment, respectively.

Eq. (1) can be rewritten in matrix notation as

$$\delta^{\mathrm{pc}} = \frac{1}{12\pi r^3}\mathrm{Tr}\left[\boldsymbol{\chi}\cdot\left(\frac{3\mathbf{r}\mathbf{r}^T}{r^2} - \mathbf{1}\right)\right] \tag{3}$$

where **r** is the metal-nucleus vector. Very recently it was noticed that using the effective spin Hamiltonian parametrization, i.e. in the limit of isolated orbitally non degenerate ground states [45],

$$\boldsymbol{\chi} = \frac{\mu_0\mu_{\mathrm{B}}^2}{kT}\mathbf{g}\cdot\langle\mathbf{S}\mathbf{S}^T\rangle\cdot\mathbf{g}^T \tag{4}$$

where $\langle\mathbf{S}\mathbf{S}^T\rangle$ is the spin dyadic, which depends on the energy of the electronic states of the ground state manifold at zero magnetic field, and thus on the zero field splitting tensor **D**.

Almost 70 years after the first derivation of the equation describing the PCSs, its validity has been questioned by recent first principle quantum chemistry approaches [46–51]. Indeed, the development of theoretical approaches for the calculation of hyperfine shifts using quantum chemistry and the effective spin Hamiltonian parametrization is receiving an increasing interest after the 2004 paper by Moon and Patchkovskii [52]. If one derives the effect on the shift of the nuclei by treating this interaction as a nuclear spin-electron spin hyperfine coupling, a different equation for the PCSs results [53,54]

$$\delta^{\mathrm{sd}} = \frac{1}{12\pi r^3}\mathrm{Tr}\left[\boldsymbol{\chi}'\cdot\left(\frac{3\mathbf{r}\mathbf{r}^T}{r^2} - \mathbf{1}\right)\right] \tag{5}$$

with

$$\boldsymbol{\chi}' = \frac{\mu_0\mu_{\mathrm{B}}^2 g_e}{kT}\mathbf{g}\cdot\langle\mathbf{S}\mathbf{S}^T\rangle \tag{6}$$

which differs from Eq. (3) by a factor $\mathbf{g}^T/g_e$ [45]. This factor can determine a difference in the calculated PCS even larger than 2 [45], as the anisotropy of the $\boldsymbol{\chi}'$ tensor calculated from Eq. (6) can be smaller of a factor 2 or more than the anisotropy of the $\boldsymbol{\chi}$ tensor calculated from Eq. (4). However, in our view this new equation raises some concerns, one of them being that PCSs would depend on a non-symmetric tensor, which clashes with the very physical meaning of magnetic susceptibility [55]. From Eqs. (4)–(6), it results that the dependency of the PCSs from the $\boldsymbol{\chi}$ tensor is

$$\delta^{\mathrm{sd}} = \frac{g_e}{12\pi r^3}\mathrm{Tr}\left[\boldsymbol{\chi}\cdot\left[\mathbf{g}^T\right]^{-1}\cdot\left(\frac{3\mathbf{r}\mathbf{r}^T}{r^2} - \mathbf{1}\right)\right] \tag{7}$$

Differently from the PCSs, the Fermi-contact shift, depending only on the electron spin density delocalized onto the nucleus itself, results from the sole magnetic interaction between the nuclear magnetic moment and the fraction of the electron magnetic moment corresponding to the electron spin delocalized on the nucleus. Therefore, the Fermi-contact interaction does not involve the orbital magnetic moment, at variance with the dipolar interaction responsible for the PCS. The result is that the equation for the Fermi-contact shift derived in the first principle quantum chemistry framework [45,54] coincides with that derived in the semi-empirical framework [39,56] when formulated for isolated orbitally non degenerate ground states

$$\delta^{\mathrm{con}} = \frac{A^{\mathrm{con}}}{3\mu_0\hbar\gamma_I\mu_{\mathrm{B}}}\mathrm{Tr}\left[\boldsymbol{\chi}\cdot\left[\mathbf{g}^T\right]^{-1}\right] \tag{8}$$

where A^{con} is the constant of contact interaction. A symmetry is thus established between the equations for the Fermi-contact and the pseudocontact shifts (see Eqs. (7) and (8)) within the quantum chemistry framework. However, such a symmetry is not based on physical grounds, because, although expected when evaluating the electron-spin nuclear-spin interaction, it is broken for the different effect of the interaction between orbital magnetic moment and nuclear magnetic moment, which is affecting the pseudocontact shift and is absent in the Fermi-contact shift. In the quantum chemistry framework, the interaction between the nuclear magnetic moment and the orbital magnetic moment is accounted for by a spin-orbit contribution which is however expected sizably smaller than the value calculated from Eq. (7), and thus neglected altogether [47].

Furthermore, the use of Eq. (7) instead of Eq. (3) for the description of the PCSs breaks the symmetry between the PCS and the RDC equations, which in the semi-empirical framework depend on the same tensor and in the quantum chemistry framework would not.

Eqs. (5) and (7) are receiving increasing attention in the analysis of the experimental data [53,57–60]. Experimental validation has been attempted in the last years to assess the correct dependence of the PCSs from the $\boldsymbol{\chi}$ tensor. Theoreticians have shown that a cobalt containing protein and a small cobalt complex seem to indicate a better agreement between the experimental PCSs and Eq. (7) with the $\boldsymbol{\chi}$ tensor obtained from Eq. (4) and the **g** and **D** values

Table 1
Dependences of the paramagnetic NMR observables on the electron magnetic moment and on the **g** or $\boldsymbol{\chi}$ tensors in the semi-empirical framework.

$\delta^{\mathrm{dip}} \propto \langle\boldsymbol{\mu}_{\mathrm{el}}\rangle \propto \boldsymbol{\chi}$	dipolar shift
$\delta^{\mathrm{pc}} \propto \langle\boldsymbol{\mu}_{\mathrm{el}}\rangle \propto \boldsymbol{\chi} - \boldsymbol{\chi}_{\mathrm{iso}}$	pseudocontact shift
$\delta^{\mathrm{con}} \propto \langle\boldsymbol{\mu}_S\rangle \propto g_e\boldsymbol{\chi}\cdot\left[\mathbf{g}^T\right]^{-1}$	Fermi-contact shift
$\Delta\nu_{12}^{\mathrm{prdc}} \propto \langle\boldsymbol{\mu}_{\mathrm{el}}\rangle \propto \boldsymbol{\chi} - \boldsymbol{\chi}_{\mathrm{iso}}$	paramagnetic RDC
$R_{1,2M}^{\mathrm{dip}} \propto \boldsymbol{\mu}_{\mathrm{el}}^2 \propto \mathbf{g}\cdot\mathbf{g}^T$	dipole-dipole electron-nucleus relaxation
$R_{1,2M}^{\mathrm{Curie}} \propto \langle\boldsymbol{\mu}_{\mathrm{el}}\rangle^2 \propto \boldsymbol{\chi}\cdot\boldsymbol{\chi}^T$	Curie relaxation
$R_{1,2M}^{\mathrm{con}} \propto \boldsymbol{\mu}_S^2 \propto g_e^2$	Fermi-contact relaxation

calculated first principle quantum chemistry tools [53,58]. It was actually us who provided good quality PCSs for this cobalt-substituted protein, the structure of which we also refined in solution. However, the accuracy of the values calculated for the **g** and **D** tensors is hard to evaluate, and the possibility of motional averaging of the values calculated using a single molecular structure may largely reduce them, due to their high sensitivity to structural details. A more significant evaluation should be performed on pure experimental grounds, and it can be done for molecules containing an $S = 1/2$ metal ion (and thus with no zero field splitting), such as copper(II), or oxovanadium(IV), or low-spin cobalt(II), provided that the g values can be measured through EPR in the same experimental conditions (e.g. at room temperature) at which the PCSs data are obtained. Remarkably, the PCSs measured for blue copper(II) proteins [61,62] seem to be in much better agreement with the g values through Eq. (3) than through Eq. (5), thus confirming the validity of the old semi-empirical equation [63].

Another strong indication of the validity of the semi-empirical equation is offered by the similarity of the $\Delta\chi$ tensors obtained from the experimental PCSs and the experimental paramagnetic RDCs using Eq. (1) and (2), respectively. As we have seen, in the semi-empirical framework the two types of data should depend on the same tensor, i.e. the paramagnetic susceptibility anisotropy tensor, whereas in the quantum chemistry approach the PCSs depend on a much smaller tensor than that on which the RDCs depend. Therefore, the comparison of the tensors back-calculated from these data can indicate whether they are the same tensor or not. From the fit of the PCSs and paramagnetic RDCs measured for the same metal ion and the same protein, it is always found that the $\Delta\chi$ tensor obtained from the PCSs using Eq. (1) (or Eq. (3)) is similar to, although a bit larger than, the $\Delta\chi$ tensor obtained from the RDCs using Eq. (2), with $S_{LS} = 1$. A slightly smaller magnitude of the tensor back-calculated from the RDCs is expected as a result of the mobility of the protein nuclei, which is basically not affecting the coordinates of the detected nuclei with respect to a frame centered on the paramagnetic metal ion (on which the PCSs depend) but can sizably affect the orientations of the vectors passing through coupled nuclei (on which the RDCs depend). Actually, a good agreement is usually obtained both in terms of orientation and magnitude between the $\Delta\chi$ tensor back-calculated from PCSs and that back-calculated from the amide proton paramagnetic RDCs measured for folded protein residues, which are not expected to experience large mobility, and thus with S_{LS} around 0.9. On the contrary, if Eq. (7) were used to analyze the PCSs, the anisotropy of the best fit χ tensor would be sizably larger than the anisotropy of the tensor responsible for the RDCs, such as to require unrealistically small S_{LS} values.

In our view, PCSs must thus depend on the magnetic susceptibility as described in Eq. (3), where, in the presence of the isolated orbitally non degenerate ground states, the magnetic susceptibility tensor is given by Eq. (4).

The different description of the PCSs provided by quantum chemistry originates from the fact that they are calculated from the sole interaction between the nuclear spin magnetic moment and the electron spin magnetic moment. In reality, there is also the contribution from the interaction between nuclear spin magnetic moment and orbital electron magnetic moment to be considered [64]. Furthermore, the excited states may provide additional second-order contributions arising from the orbital part of the electron magnetic moment that are not taken into account even in Eq. (4) [65]. These contributions should be added as a further tensorial term to Eq. (4), and represent the temperature independent paramagnetism (TIP).

An interesting question that can be posed to the crystal ball is whether the hyperfine coupling measured for the paramagnetic electron(s), detectable with EPR, should be the same of that measured for nuclei, i.e. if it should coincide with the NMR hyperfine shift or not. “Yes” could turn out to be the most obvious, but possibly wrong, answer.

4. Conclusions

The future development of structural biology passes through smart integration strategies able to overcome the intrinsic limitations of each single technique, while taking advantage of the complementary information provided by the different techniques. In this respect, paramagnetic NMR data, which twenty years ago were used as restraints in programs for protein structural determination only in conjunction with classical NMR data (NOEs and dihedral angle restraints), are nowadays more profitably analyzed together with X-ray, SAXS and DEER data. Paramagnetic NMR brings a dowry of long-range distance information on the overall displacement of the structural domains present in the system, in close to physiological conditions. It also provides a wealth of accurate information about local conformational details, in the form of interatomic distances or orientations of vectors connecting chemically bound nuclei, together with information on the mobility present in the system. Therefore, new protocols will likely be developed in the next years to include paramagnetic NMR restraints together with other structural data in structural determination tools.

Recent theoretical and computational efforts for the *ab initio* calculation of PCSs from first principle quantum chemistry have raised some questions on the validity of the relationship between these observables and the coordinates of the nuclei to which they refer. Understanding the details of the origin of the PCSs is not only fundamental for the correct exploitation of their information content, and thus for the many researches dealing with paramagnetic NMR, but also for grasping the physical basis of the magnetic interaction between nuclei and electron(s). We seem to detect in the crystal ball that first principle quantum chemistry calculations require further theoretical advances to better evaluate all contributions to the PCSs and thus be able to reproduce the experimental data. Likely, the calculations will also require that molecular dynamics is taken into account in order to evaluate how the χ tensor averages over the different snapshots, provided that the small changes in the coordination geometry of the paramagnetic metal can determine sizable changes of the tensor.

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