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Interactive comment on "Paramagpy: Software for Fitting Magnetic Susceptibility Tensors Using Paramagnetic Effects Measured in NMR Spectra" *by* Henry William Orton et al.

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Thank you for the review and comment. The following contains our detailed response in plain type given underneath the original comment in bold type.

1) Could the authors analytically show that the Eqs. 17-21, 24-27 for the Curie-dipolar cross correlated relaxation (section 5) provide the well-known equation by Ghose- Prestegard (J. Magn. Reson. 1997, 128, 138) in its conditions of validity (isotropic susceptibility tensor)?

We agree that an analytical derivation of equations 24-27 from the original description

of Curie-dipolar cross-correlated relaxation theory by Ghose et al. is important for this publication as this is the first time such equations have been presented. Attached to this reply and proposed to be included in the revised supporting information of the manuscript is a detailed proof of their equivalence for the case of an isotropic magnetic susceptibility tensor.

2) When indicating that the multiple models in a solution NMR ensemble can be used to fit the data (section 7), a warning should be provided that ensemble averaging of the paramagnetic data may be incorrect because the NMR family reports on the experi- mental uncertainty of the structural model, and not on the real conformational ensem- ble sampled by the system.

Indeed, the majority of structures solved by solution NMR are presented by ensembles that capture the experimental uncertainty in the coordinates and are not meant to describe the conformational space. A new version of Paramagpy is in preparation to support fitting of magnetic susceptibility tensors to each model individually, followed by averaging of all such fitted tensors. Additionally, an error analysis will calculate the standard deviation of these fitted tensors to allow propagation of the structure uncertainty to the parameters of the magnetic susceptibility tensors. This fitting routine will be the default behaviour. Ensemble averaging will remain an additional option, which is useful for structures, where models describe the conformational space (such as from an MD simulation). Thank you for raising this important point. Section 7 of the main text has been amended to make this clear to the reader and is shown below in blue.

7 Molecular structures with multiple models

7.1 Structures with uncertainties represented by a family of models

Biomolecular structures in the PDB, which have been determined by solution NMR, usually report experimental uncertainty in the atomic coordinates by including multiple models, which individually fulfil the experimental restraints. The default behaviour of Paramagpy is to fit a magnetic susceptibility tensor to each model independently

and then report an average of all these tensors. The tensor averaging is achieved by equation 1 where the summation runs over the tensors fitted to each of the n models. This ensures no errors are introduced by averaging prolate/oblate tensors with different principal axes definitions. All other parameters involved in the fit, such as origin of the tensor position, rotational correlation time or electronic relaxation time, are averaged in the conventional way. Note that the final result is sensitive to different relative orientations of the models.

$$\chi_{average} = \frac{1}{n} \sum_{i}^{n} \begin{bmatrix} (\chi_{xx})_i & (\chi_{xy})_i & (\chi_{xz})_i \\ (\chi_{xy})_i & (\chi_{yy})_i & (\chi_{yz})_i \\ (\chi_{xz})_i & (\chi_{yz})_i & (\chi_{zz})_i \end{bmatrix} \tag{1}$$

7.2 Structures represented by a conformational ensemble

Some coordinate sets in the PDB have been determined by molecular dynamics, where the ensemble of models deposited fulfils the experimental restraints better than each individual model. For this case, Paramagpy has the option for calculation of ensemble-averaged paramagnetic effects at all stages of calculations and fitting. Ensemble-averaged fitting presents a subtle but important difference compared to the multiple-model method described in section 7.1 above. This is particularly noticeable for RDCs, where the ensemble average can be much smaller than the corresponding RDC of a single model, and therefore several models representing different bond orientations may be simultaneously required to fit an appropriate alignment tensor or $\Delta\chi$ tensor.

The implementation of ensemble averaging in Paramagpy averages the paramagnetic values calculated for each atom in the different models, identifying the specific atoms by identical atom numbers in the PDB file. Custom ensemble averaging behaviour can be changed by the user in the scripted environment. In the implementations of the SVD algorithm, ensemble averaging involves summation of rows for common atoms of the matrix A of equation 5 before calculation of the singular values. In the implementations

of the non-linear gradient descent algorithm, the values calculated for the common atoms are averaged prior to calculating the sum of squares of differences. This is shown in equation 2 where a^{cal} and a^{exp} are the calculated and experimental PCS, RDC, PRE or CCR values, respectively. The index m is for atoms that are common between models, and the index i runs over all atoms in the structure.

$$cost_{ensemble} = \sum_i \frac{(\sum_m \left[a_{m,i}^{cal} - a_i^{exp} \right])^2}{\sigma_{a(i)}^2} \qquad (2)$$

7.3 Fitting tensor parameters to multimers In the case of symmetric multimers composed of monomers with each containing a paramagnetic metal ion, the ensemble averaging feature of Paramagpy can be exploited to fit the $\Delta\chi$ tensor associated with a given monomer. This is achieved simply by defining the monomeric units in the PDB file as models of the same structure and applying the ensemble averaging routine to fit the $\Delta\chi$ tensor using the experimental PCSs, which reflect the average of the PCSs observed in each monomer. Note that, due to the averaging, the final fitted $\Delta\chi$ tensor must be scaled by the user n-fold, where n is the number of monomers. This feature can also be exploited in NMR crystallography (Kervern 2009).

References:

Kervern, G., D'Aléo, A., Toupet, L., Maury, O., Emsley, L., and Pintacuda, G.: Crystal-structure determination of powdered paramagnetic lanthanide complexes by proton NMR spectroscopy, Angew. Chemie Int. Ed., 48, 3082–3086, 5https://doi.org/10.1002/anie.200805302, 2009

Please also note the supplement to this comment:
https://www.magn-reson-discuss.net/mr-2019-3/mr-2019-3-AC2-supplement.pdf

Interactive comment on Magn. Reson. Discuss., https://doi.org/10.5194/mr-2019-3, 2019.