

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 thoughtful comments. The following contains our detailed response in plain type given underneath the original comment in bold type.

(1) As was pointed out also in the public discussion, taking an average of structures from an ensemble could a simplification. NMR ensembles usually represent experimental uncertainty, which should be reflected in the uncertainty of the paramagnetic parameters derived for this ensemble.

Indeed, the point about ensemble averaging of NMR structures is important. Please

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see our public discussion reply to Claudio Luchinat's comment regarding the new and improved behaviour of the magnetic susceptibility tensor fitting algorithm.

(2) The standard Q-value definition is used here. We have introduced an alternative definition (Qa), with the sum of the absolute experimental and calculated values in the denominator rather than only the experimental values. The standard definition results in a bias in cases where the deviation between calculated and experimental data is large. This is relevant in particular for PRE data that can vary strongly with small changes in distance. In cases where the experimental PRE is much larger than the calculated, the Q value is low, in cases where the experimental value is low and the calculated high, the Q value is high, thus biasing the results toward fitting the low PREs better than the high ones. . . . The authors could consider whether the Qa is a better alternative for the Q factor.

The Q-factor reported in the main text is a widely accepted criterion that has been introduced for its inverse proportionality to the Pearson Coefficient (see Bax A.: Weak alignment offers new NMR opportunities to study protein structure and dynamics, 2002, Prot. Sci. 12:1-16, where also an alternative version of the Q-factor introduced by G.M. Clore is discussed). We acknowledge that the Q-factor does not behave symmetrically with respect to experimental or calculated inputs (unlike the Qa values of your examples). Paramagpy actually does not use the Q-factor as the score function to be minimised during fitting of the magnetic susceptibility tensor. This is instead achieved by a weighted sum of squares as outlined in equations 8, 23, 28 and 29. This score function was chosen for giving less weight to relatively large experimental values (as they are usually associated with a greater uncertainty in measurement), which is therefore less prone to a bias in the fitting. As a consequence, the best fit does not necessarily give rise to the smallest possible Q-factor. The new version of Paramagpy will include a routine for calculating the Qa-factor, but the more established Q-factor will still be displayed by default in the GUI. The following paragraph in blue has been added to section 8 of the main text.

Alternative Q -factors have been proposed (Clore 1999, Bashir 2010). The Q -factor proposed by Bashir et al. (Bashir 2010), which uses sums of experimental and calculated values in the denominator of equation 31 and therefore tends to be two times smaller, is supported by the scripted environment of Paramagpy. It is important to note that the fitting algorithm used by Paramagpy targets the minimal root-mean-square-deviation between experimental and calculated data rather than the Q -factor. It has been pointed out that Q -factor evaluations are meaningful only if the number of fitted data greatly exceeds the number of variables (Bax 2003).

References

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