Reviewer 2 - Giuseppe Sicoli

The manuscript represents an interesting example of understanding to design and design for understanding related to the impact of the deuteration within the context of the relaxation processes. The message of the manuscript is clear and exhaustively delivered; however, for fulfilling the main 'take-home' message of the manuscript, some minor points can be revised:

Thank you.

- For the comparison of the values of $1/T_{m,f}$ and b_f for different samples and temperatures (figure 7 C, F; please notice into the text at page 20 such figure has been referred as '5'), the authors refer to Figure 10 (page 22) for describing the contribution of the fast component. Besides the *fairly constant behaviour* for the PyMTA, it would be interesting to provide further elements to the discussion on the behaviour of TPMTA, exhibiting a completely different behaviour.

  Thank you; we changed 5 to 7.

  As for the different behavior of TPMTA, we will add in p. 21 the following :

  The relative contribution of the two components is fairly constant in the temperature range tested for Gd-PyMTA, whereas for Gd-TPMTA the contribution of the fast component is constant for 1.6-4 K and thereafter, a significant increase with increasing temperature is observed in the range of 6-15 K **(Fig. 10)**. This trend seems to correlate with the relative intensity of the central transition (**Fig. 2**). Currently we do not have an explanation for this behavior.

- The general approach proposed does not mention the effect of the pH, which may have an impact into the affinity of the two main ligands described; such an effect on the relaxation is probably beyond the scope of the manuscript, but it can be worth to mention also that tuneable parameter (i.e., pH).

  We usually prepare the spin label at a pH where all carboxylates are deprotonated and able to coordinate the Gd(III), changing pH may lead to variation in the number of ligands and the ZFS, and will complicate things. We therefore think that this should be a completely different study.

- The assignment of dominating mechanism assigned for the two populations (*slow* and *fast*), as summarized on page 23 (lines 8-11) can eventually be reinforced by citing known structures where the T_1 and tZFS are distinctively contributing to the relaxation paths. It may support the effect of the

deuteration for ‘small’ molecules and validate the less pronounced effect on labelled proteins.

Unfortunately, as far as we know, there are no such studies, except the study of Raitsimring. It would be nice to have a correlation of T_1 with the ZFS, but currently, there is not enough data (measured at the same frequency and temperature) to support such a correlation.

- Please notice that the authors refer to Figure 2D (page 10) but the capital letter on the figure 2 (page 11) is missing. A-B-C-D on the four panel must be revised.

  This will be fixed.