Response to Reviewers

We thank all the reviewers for the time they have taken to provide their constructive and positive feedback. In the following we address the questions of all reviewers. The reviewer questions and comments are in *italic font* and our replies in **bold font**. Changes in the manuscript are shown in red.

Reviewer 1: (Gottfried Otting)

To explain the differences between Gd(DTPA) and Gd (DTPA-BMA): Could the charge of the Gd(DTPA) complex encourage binding to the protein, whereas the zero net charge of Gd(DTPA-BMA) is more likely to prevent specific binding? Inspection of the protein structure would tell the locations of positively charged amino acid residues (or overall positive electrostatic potential) in the vicinity of the tryptophan side chains.

We do not find a significant accumulation of positive charges (blue) in the vicinity of the tryptophans. To address this question further, we have measured backbone amide CSPs and amide ^{1}H T_2' rate constants of TET2 either without any dopant, or with either of the two dopants. These data, which are shown in the supplementary information of the revised version, point to more specific interactions of Gd(DTPA) than of Gd(DTPA-BMA), seen by both CSPs and ^{1}H T_2'. This new data aligns with the idea put forward by the reviewer. Thank you for the suggestion.

Line 170: Are the different water exchange rates in Gd(DTPA) and Gd(DTPA-BMA) the most plausible mechanism for the different PREs, i.e., is faster water relaxation the main driving source of accelerated longitudinal 19F relaxation? In principle, the importance of water could be determined by using D2O as the solvent during the crystallization but this would add much experimental work peripheral to the scope of the present article.

The water exchange rate was taken as one example of the properties that differ between the two complexes. This is, however, purely speculative. Experiments in D^2O buffer are certainly an interesting direction but would require new samples and experiment time, which is currently outside of the scope of this work. We clarified that this is just one example in the revised version.

Minor points:

Some of the data shown in Figures S3 and S4 seem to indicate slower relaxation in the presence of 2 or 4 mM Gd(DTPA-BMA) than in its absence (for some of the fluorotryptophan residues). Is this simply a matter of limited SNR?

We do not have a clear answer to this. Apart from SNR, we observed that the fits for the relaxation recovery experiments are partly dependent on the chosen list of delays.

In solution, the 13C relaxation of C-F groups in the tryptophan indole ring is subject to an intense TROSY effect (see, e.g., Maleckis et al., Org. Biomol. Chem., 19, 5133, https://doi.org/10.1039/D1OB00611H, 2021). Can the authors reveal anything about the 13C NMR spectra of the fluorotryptophans in the TET2 protein in solution (although this is definitely outside the scope of this article)?

We did not attempt to measure solution spectra. The size of the protein (almost 500 kDa) is probably too large for obtaining resolved spectra, due to the expected very short ^{19}F T_2.

Very minor points:

Line 10: it would be nice to report the name of the protein in the abstract.

Line 73: "which also comprises all plasmid details" – I presume this refers to AddGene rather than the plasmid, but is this part of the sentence necessary?

Lines 87 and 105: the names of chemicals are usually spelled with small characters.

Line 150 and elsewhere: please include the superscript 'opt' with tau(r.d.).

The references need to be double-checked. For example, the reference by Gronenborn appeared in Structure (line 264) and the reference by Jaroniec in Solid State Nuclear Magnetic Resonance (line 274).

Legend of Figure S8: for consistency, please provide the references in the Harvard style of referencing (not numbers) and the references themselves in the style of the main text.

We corrected the above points in the revised version of the manusctipt.

Please provide the commercial source of Gd(DTPA) and Gd(DTPA-BMA). Gd(DTPA-BMA) sold under the tradename of Omniscan contains also 5% NaCa(DTPA-BMA), which is a charged complex.

The Gd(DTPA-BMA) complex is indeed Omniscan. We added the sources of the two complexes in the methods section of the revised version. The sample indeed contains also NaCa(DTPA-BMA).

Reviewer 2:

1) The authors should show 19F DP spectra of the protein bound to 8 mM Gd(DTPA-BMA) and Gd(DTPA), compared with the apo protein spectra. These should be added to the main text. Currently Fig S7 shows the severely line-broadened spectrum (T2 PRE) of the Gd(DTPA) sample but not that of the better compound, GD(DPA-BMA).

We added the 19F DP spectra of 8mM Gd(DTPA-BMA) and 2mM Gd(DTPA) as Figure 4 to the revised version of the manuscript.

2) Likewise, the authors should show 13C spectra of the three samples: apo, 8 mM Gd(DTPA) and 8 mM Gd(DTPA-BMA). Both 13C CP and DP spectra should be shown, to illustrate the effects of the Gd3+ compounds on 13CT2 and 1H T1 relaxation.

We measured ^{13}C spectra to show the ^{13}C line broadening effect and compare CP and DP spectra. We assume that the review is referring to the ^{19}F T_1 (not ^{1}H) and the gain in SNR ratio obtained by measuring CP instead of DP spectra due to the shorter T_1 of ^{19}F than ^{13}C. We show these spectra in Figures S9 and S10 in the revised version of the manuscript.

3) Based on the 19F R1 and R2 relaxation enhancement factors measured for each Trp residue, can the authors deduce the tauc and the distance of the nearest Gd3+ dopant to each residue using equation 1? Moreover, assuming a reasonable tauc value, can the authors estimate the distance range where one can obtain significant T1 PRE but not T2 PRE to speed up experiments without suffering excessive line broadening?

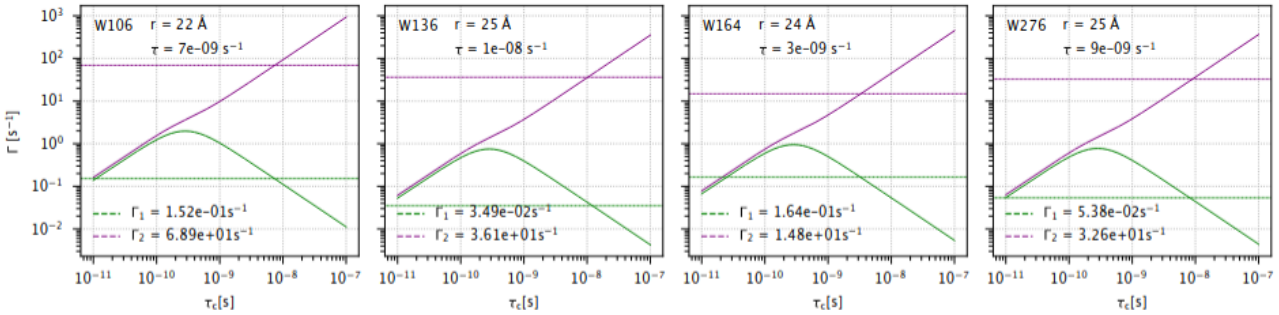
This is an interesting suggestion. Of note, the paramagnetic doping effect is not a result of just one but the combined effect of many dopant molecules and it is not clear if a single distance is meaningful in this context. Nonetheless, we have done calculations to estimate distances and tau_c values for each of the four Trp residues, which can be found in the figures that we uploaded in the Discussion, together with the scripts to generate them. These figures are shown below. We also plotted the distance dependence of equation 1. However, as one cannot influence the distance of the dopant complex to the protein, the relevance is not clear to us.

The three plots show the estimated distance and correlation times based on the longitudinal and transverse relaxation (Gamma1, Gamma2). The dashed lines show the experimentally observed values, and the solid lines the calculations for a given distance as a function of the correlation time. (A 2D grid search was done to find the distance, only 1D slices are shown along the correlation time.) The other plots estimates the distance between the dopant and a 19F site, assuming a correlation time that is in the range of the values found in the other two plots. The dashed lines show "acceptable" R2

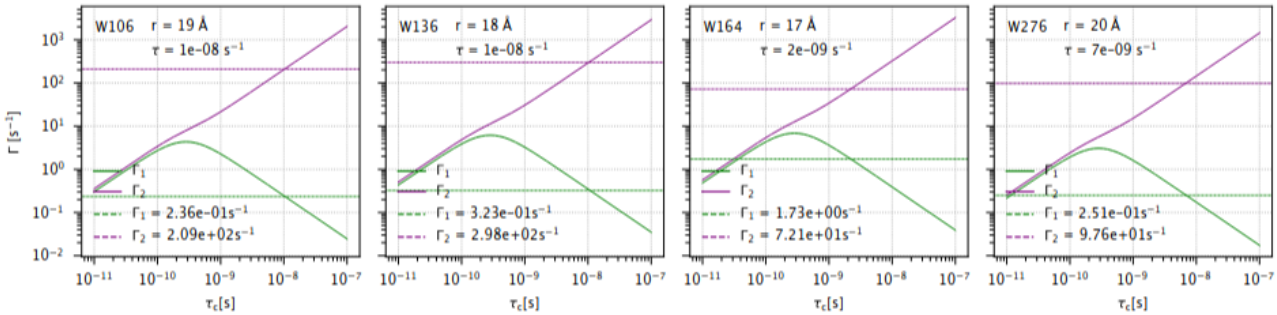
enhancement and "desired" R1 enhancement. For example, to reach an increase of R1 by 0.2 s-1 the distance should be of the order of 22 Å. This distance enhances R2 by 50 s-1.

In practice, these calculations are not overly relevant, we suppose, because experimentally one can only decide on the concentration of dopant to add, but one does not really choose the distance (although these two things are of course related).

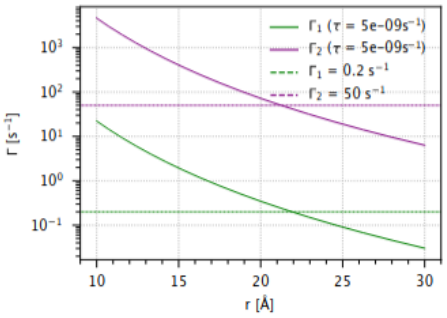
Estimation of correlation time and effective distance for Gd(DTPA-BMA), for each of the four Trp sites in TET2.



Same analysis for the data with Gd(DTPA)



Calculations of the induced relaxation for a fixed correlation time and a range of distances.



Reviewer 3: (Lauriane Lecoq)

Revision requested:

- Why are the 13C 1D spectra recorded in absence and in presence of Gd(DTPA-BMA) not shown? They should at least be included in the supplementary data.

Thank you for this constructive review.

We did not measure any ^{13}C spectra before, as the individual peaks are not resolved and ^{19}F detected spectra are more sensitive. For this revision, we followed the suggestion and recorded ^{13}C detected spectra in the revised version of the manuscript (see also the response to reviewer 2).

- Similarly, the 19F 1D spectrum with 8 mM Gd(DTPA-BMA) is not shown. Please add it to Figure S7 or in the main text.

We added the spectrum in the revised version as Figure 4.

Minor comments:

- Line 10: please add the name of the protein in the abstract

- Line 17: remove ':' after the references

- Line 26: replace 'capsides' by 'capsids'.

We corrected the above points.

- Line 79: it would be nice if a reference could be added for the synthesis of the compound, unless if not available at the time of the revision.

The reference to the synthesis will be published at a later point.

- Line 109: please add the temperature of the ultracentrifugation for rotor filling.

We added the temperature in the revised version.

- Line 202: the different behavior of W164 compared to other tryptophan residues is surprising. While this effect is not yet fully understood and could be due to dopant binding, are the authors aware of any examples of such behavior in the literature?

We are currently not aware of such a case from the literature.

- Python scripts could be included in the Supplementary data.

All scripts and data are publicly available in a data archive (DOI: 10.15479/AT-ISTA-21284).

Additional comment about the manuscript:

Figures S9 and S10 are new, as mentioned above.