**Reviewer 1:**

*We would like to thank the reviewer 1 for his suggestions to improve the manuscript. Answers to the questions of reviewer 1are stated after each request.*

Reviewer 1: The manuscript entilted "An improved, time-efficient approach to extract accurate distance restraints for NMR2 structure calculation" by

Pokharna, Torres, Kadavath, Orts, and Riek describes a new approach for improving the precision of distances measured by NMR and used to calculate the position of ligand in protein pocket. The proposed method which consist to normalize the measured NOEs with the intensities of the corresponding diagonal element, has been proposed in the past, but is validated here in the frame of the NMR2 approach, used to determine poses of ligands in a protein pocket using NMR measurements. The normalisation with the diagonal intensity is shown to improve the precision of the determination of a ligand pose.

The content of the manuscript is significant and deserves publication. Nevertheless, at several places, the manuscript text should be worked
out to clarify the presentation of the work:

a) the last abstract sentence is very complicated and fuzzy.

The diagonal-normalised eNOE derived distance restraints NMR2 yielded the right orientation of the fragment in the binding pocket, and produced a structure that more closely resembles the benchmark X-ray structure (2XP6) (Potter et al., 2010) with an average heavy atom RMSD of 1.681 Å than the one produced with traditional NMR2 with an average heavy atom RMSD of 3.628 Å, attributed to the higher precision of the evaluated distance restraints.

*Answer: We followed the suggestion of the reviewer. The last sentence in the abstract reads now: "NMR2 calculations performed using the distances derived from diagonal-normalised eNOEs yielded the right orientation of the fragment in the binding pocket, and produced a structure that more closely resembles the benchmark X-ray structure (2XP6) (Potter et al., 2010) with an average heavy atom RMSD of 1.681 Å with respect to it, when compared to the one produced with traditional NMR2 with an average heavy atom RMSD of 3.628 Å. This is attributed to the higher precision of the evaluated distance restraints."*

Reviewer 1: b) line 90: the expression "(0%)" is a bit strange, as no experimental measure can be obtained at a infinite precision.

*Answer: We agree with the reviewer that no experimental measure can be obtained at infinite precision. The 0% must be read in the context of the implementation within the software CYANA which uses a harmonic potential as penalty function (i.e. target function TF) taken into account the lack of precision. In the revised version of the manuscript the following sentence is added around line 90: "It is noted that the software CYANA uses a harmonic potential for a its target function (TF) to accommodate the distance restraints and as such 0% means the harmonic potential only, while 20% distance tolerance means a flat potential from 0-20% distance followed by the harmonic potential beyond.*

Reviewer 1: c) in Figure A2, results of simulations are described. It is not clear on which system the simulations were performed.

*Answer: We adjusted the figure caption to "Figure A3: Effect of spin diffusion on the intensity build-up curves produced from eNORA-based approach (left) and diagonal-normalised approach (right). The build-up curves were fitted using artificially simulated peak intensities in a model system, Third Immunoglobulin Binding Domain of Protein G (GB3), which has been extensively studied using eNOE spectroscopy. The blue curve represents the intensity build-up in an isolated two-spin system with an inter-proton distance of 3.83 Å and the red curve represents the same two spins experiencing spin-diffusion due to presence of other spins in the system. The comparison between the plots highlight that it is easier to detect the influence of spin-diffusion with the diagonal-normalised approach (right), as it induces deviation from the expected linear fit."*

Reviewer 1:

d) caption of Figure 2: it is not clear that the colours purple and brown concern the ligand. The correspondence between the ligands shown in

Figure 2 and the lines of Table 1 should be given.

In addition, to which pose corresponds the ligands in which all atoms are coloured? (I don't understand this part of the question.)

In lines 130-147, the description of Figure 2 is not clear, the ligand colors should be quoted.

*Answer: Following the suggestions of the reviewer, we edited the manuscript as requested.*

Reviewer 1: e) What is the meaning of Total number of degenerate lowest energy conformers in Table 1?

*Answer: This is the total no. of distinct orientations of the ligand within the binding pocket that give a CYANA TF of 0 Å after NMR2 calculations. A number higher than 1 means that the experimental restraints were not sufficient in quality for the method to discern between these orientations. In footnote 3, the following sentence is added in the revised version of the manuscript: "The Total number of degenerate lowest energy conformers is the total number of distinct orientations of the ligand within the binding pocket that was obtained with a CYANA TF of 0 Å from NMR2 calculations."*

Reviewer 1: In addition, there is a more basic question about the method NMR2. The manuscript presents the use of NMR2 for the determination of very
precise position of ligand, which should correspond to an high affinity interaction? But, NMR is used for studying the interaction of low
affinity ligands for which the poses may much less precise. It would be interesting to insert comments about these points.

*Answer: This is an interesting point raised by the reviewer that we would like to answer from two points of view. We demonstrated here and by Torres et al. (2020) that the NMR2 approach presented is able to identify the binding pocket also of weak binders. On the other hand, there might be indeed as suggested multiple binding configurations and conformations present which we study currently with eNOE-based multi-state structure determination that requires the entire assignment and both inter- and intra-molecular eNOE-based distance restraints. Currently the NMR2 approach does not include multi-state structure determination at standard NMR resolution. We may implement that feature in the future, but that would most likely require even better input data.*

*For the determination of multiple binding configurations, namely multi-state structure determination, at high resolution, a detailed eNOE-based analysis would be required. In the revised version of the manuscript at the end of line 149, the following sentence is stated. It is noted that the presence of multiple configurations/conformations of the ligand in the binding pose will require detailed eNOE-based multi-state structure calculations (Vögeli et al. 2012, Ashkinadze et al. 2021)*